Occurrence of Infection with Group B Coxsackievirus in Rheumatic and Nonrheumatic Filipino Children

Benjamin M. Limson, Veronica F. Chan, Santiago V. Guzman, Marieta R. Maaba, and Myrna T. Mendoza

From the Research Division, Philippine Heart Center for Asia, Quezon City; and the Department of Medical Microbiology, Institute of Public Health, University of the Philippines, Manila, Philippines

The pathogenesis of rheumatic fever has been linked to various immune mechanisms involving streptococcal antibodies and heart tissue antigens. Latent myocarditis due to group B coxsackievirus has also been considered as a possible conditioning factor. The validity of the role of infection with group B coxsackievirus in rheumatic fever was tested by determining the incidence of type-specific neutralizing antibodies in sera of Filipino children. Analysis of the results by means of a normal Z-test showed that the incidence in rheumatic children was not statistically significant in comparison to the incidence in asymptomatic children.

Cross-reaction of streptococcal antibodies with heart tissue [1, 2], immune complex-mediated tissue injury [3], and a cell-mediated immune mechanism [4] are possible links in the immunopathogenesis of rheumatic fever, while there has been no satisfactory explanation for the relative infrequency of a rheumatic sequel after pharyngitis due to group A streptococci. Latent viral carditis as a possible conditioning factor has been suggested. Burch et al. have produced "rheumatic-like" heart lesions in experimental animals by injection of group B coxsackievirus [5, 6] and have demonstrated group B coxsackievirus antigens in human autopsy material in association with rheumatic lesions in the heart [7]. The viral carditis has been presumed to be latent because a clinical relationship with rheumatic fever has not been documented.

Pongpanich et al. [8] reported serial rises in titers of neutralizing antibodies to group B coxsackievirus in 13 of 14 children with active rheumatic heart disease, whereas four other children with inactive, chronic rheumatic heart disease had no rise in titers. In this study, we compared the incidence of neutralizing antibodies to group B coxsackievirus in rheumatic and nonrheumatic Filipino children to test the validity of infection with group B coxsackievirus as a factor in the pathogenesis of rheumatic fever.

Materials and Methods

From July 1976 to February 1977, children aged six to 13 years were studied for evidence of infection with group B coxsackievirus, rheumatic fever, and streptococcal pharyngitis. The children were classified in three groups: asymptomatic control subjects and patients with pharyngitis from Barangka Elementary School, Metro Manila, and hospitalized children with active rheumatic heart disease in the Philippine Heart Center for Asia, Quezon City, and in the Philippine General Hospital, Manila. Of 806 children, 112 were asymptomatic, 650 had uncomplicated acute pharyngitis, and 44 had rheumatic fever, as diagnosed with the modified Jones criteria [9].

Throat cultures were inoculated in sheep blood agar plates, and streptococcal isolates were serologically grouped by the Lancefield method [10, 11]. Blood samples were allowed to clot for 1 hr and centrifuged for 10 min at 500 g to separate the sera, which were then stored in deep freeze until titrated for antistreptolysin O (ASO) antibodies and for neutralizing antibodies to group B coxsackievirus. Whenever possible, serial specimens were obtained 10 to 14 days apart for tests of rises in titers.
The laboratory criteria for a streptococcal etiology in pharyngitis were a serial rise in ASO titer by at least 100 units (with or without a positive culture) or a single ASO titer of at least 300 units with a positive throat culture for group A streptococci. ASO titers were determined by a standard method [12]. The mean ASO titer had been previously established to be 119.8 units in asymptomatic Filipino children, 413.4 units in patients with uncomplicated streptococcal pharyngitis, and 668 units in patients with rheumatic fever [13].

Infection with group B coxsackievirus was determined by means of a standard neutralization test [14] in tube cultures of Vero cell monolayers of the six types of group B coxsackieviruses. Two-fold dilutions of serum samples were prepared either in Hanks' balanced salt solution or in maintenance medium of the cell cultures. Viral cultures were diluted to contain 100 TCID<sub>50</sub> in a volume of 0.1 ml (as determined by previous titration of the virus), with the same diluent used for the serum sample. Equal volumes of the serum dilution and the test virus dilution were mixed and incubated at room temperature (about 24 C) for 1 hr and then inoculated in a volume of 0.2 ml into cell monolayer tube cultures. Each serum-virus mixture was cultured in triplicate. Serum controls (the lowest dilution of serum sample mixed with an equal volume of diluent) and a homologous control system (test virus dilution mixed with an equal volume of previously titrated specific antisera and test virus dilution mixed with an equal volume of diluent) were likewise inoculated into cell monolayer tube cultures.

Inoculated cultures were incubated at 35 C and examined microscopically for ability of the serum to inhibit the viral CPE at intervals over a seven-day period. A titer of $\geq 1:40$ in single tests or a fourfold change in titer in serial tests was considered significantly positive for neutralizing antibodies against the six types of group B coxsackieviruses.

Results

Table 1 shows the results of serologic tests for neutralizing antibodies to group B coxsackievirus. In the group of 112 asymptomatic control subjects, 21 (18.7%) had significant titers of antibody to coxsackievirus B1, B2, and B3 (in order of prevalence), but were negative for B4, B5, and B6.

Of 44 children with acute or reactivated rheumatic heart disease, 13 (29.5%) had significant titers of antibody to all of the six types of group B coxsackieviruses.

Of 306 children with signs and symptoms of acute pharyngitis, 170 (55.6%) had significant titers of antibody to group B coxsackievirus and were negative for laboratory evidence of streptococcal infection.

A total of 650 children with acute pharyngitis had throat cultures and ASO tests. In 172 (26.5%), a streptococcal etiology was considered; this total included 149 children who had significant ASO titers and whose throat cultures were positive for group A streptococci and 23 children whose cultures were negative but who had a significant rise in ASO titer in serial tests. Six additional patients with pharyngitis whose cultures were positive for group A streptococci and 10 others whose cultures were positive for hemolytic streptococci other than group A had negative or low ASO titers.

In the 156 remaining children with acute pharyngitis, results of tests for infection with streptococci and group B coxsackievirus were negative.

![Table 1. Titer of antibody to six types of group B coxsackievirus in Filipino children.](https://example.com/table1.png)

**Table 1.** Titer of antibody to six types of group B coxsackievirus in Filipino children.

<table>
<thead>
<tr>
<th>Condition of subjects (no.)</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic (112)</td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21 (18.7)</td>
</tr>
<tr>
<td>Active rheumatic Heart disease (44)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td>Acute pharyngitis (306)</td>
<td>39</td>
<td>25</td>
<td>32</td>
<td>30</td>
<td>33</td>
<td>11</td>
<td>170 (55.6)</td>
</tr>
</tbody>
</table>

*Defined as a titer of $\geq 1:40$ for single test or a fourfold change in titer in serial tests.

Statistical analysis. As shown in table 2, the normal Z-test was used to compare the incidence of group B coxsackievirus-positive tests among the children with active rheumatic heart disease with the incidence among asymptomatic control
Table 2. Statistical difference in the incidence of antibodies to group B coxsackievirus in rheumatic and asymptomatic Filipino children.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. significantly positive/no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active rheumatic heart disease</td>
<td>15/44 (29.5)</td>
</tr>
<tr>
<td>Asymptomatic controls</td>
<td>21/112 (18.7)</td>
</tr>
<tr>
<td>Total</td>
<td>34/156 (21.8)</td>
</tr>
</tbody>
</table>

NOTE. Z = difference/se of difference = 1.47; critical value for significance of a normal Z-test = 1.64.

*se for each mean was 0.011.

The present study on the role of infection with group B coxsackievirus in rheumatic heart disease compared the incidence of significant titers of neutralizing antibodies to the six types of group B coxsackievirus in rheumatic and non-rheumatic Filipino children. Subjects with uncomplicated acute pharyngitis due to group A streptococci or group B coxsackievirus were identified so as not to influence the statistical analysis of the rheumatic group as compared with the asymptomatic control group.

Infection with group B coxsackievirus was established in 18.7% of the asymptomatic group, in 29.5% of the rheumatic group, and in 55.6% of the group with uncomplicated pharyngitis. A streptococcal etiology was found in 26.5% of the group with pharyngitis.

By means of the normal Z-test, the statistical significance of the incidence of infection with group B coxsackievirus in rheumatic children as compared with the incidence in asymptomatic children was determined. The computed value of 1.47 falls short of the critical value of 1.64 for significance. Therefore, our study failed to show a significant correlation between infection with group B coxsackievirus and rheumatic heart disease.

References

4. Yang, L. C., Soprey, P. R., Wittner, M. K., Fox, E. N.


Addendum

We have recently performed tests for HAI antibody to type 2 dengue virus and to chikungunya virus in sera from the 44 rheumatic subjects; only two had dengue virus infection, and none had chikungunya virus infection.