Latex-Rickettsia rickettsii Test Reactivity in Seropositive Patients

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In correlating results obtained from a new latex-Rickettsia rickettsii test with results obtained from a reference microimmunofluorescence test for Rocky Mountain spotted fever, we found that for seropositive patients each microimmunofluorescence titer (up to 4,096) was usually double the median titer obtained from the latex-R. rickettsii test. The pattern of immunoserological response indicated that latex-R. rickettsii is reactive with sera containing either anti-R. rickettsii immunoglobulin M (IgM) or IgG or both. The agglutination efficiency of the new test was greater when the anti-rickettsial IgM/IgG ratio was ≥1. Sera from late-convalescent patients were generally minimally reactive or nonreactive in the latex-R. rickettsii test unless anti-R. rickettsii IgM was present.

Recently, Hechemy et al. (4) reported on the first-year evaluation of a latex-Rickettsia rickettsii test. During this evaluation sera from 2,656 patients were received from nine collaborating laboratories (see Acknowledgments) in states where Rocky Mountain spotted fever (RMSF) is endemic. These sera were tested in our laboratory by the newly developed latex-R. rickettsii test (2) and by the standard microimmunofluorescence (micro-IF) test (6, 7). Results from both tests were in qualitative agreement for 2,607 patients (98%): 382 patients were seropositive, and 2,225 patients were seronegative. Results were not in agreement for the remaining 49 patients.

For the 382 seropositive patients, latex-R. rickettsii titers did not always parallel micro-IF titers with anti-human immunoglobulin conjugate. Some latex-R. rickettsii titers were lower than micro-IF titers; others were higher. Since the varying immunoglobulin composition of the sera could have caused this variability in titers (8), we determined the anti-R. rickettsii immunoglobulin M (IgM) and IgG concentrations in sera from 382 seropositive patients. We then examined the effect of the anti-rickettsial IgM/IgG ratio on the agglutination efficiency of the latex-R. rickettsii test relative to that of the micro-IF test and on the time course of reactivity.

MATERIALS AND METHODS

Patient sera, sources of reagents, and procedures for performing the latex-R. rickettsii test have previously been described (4). However, preparation of the latex-R. rickettsii reagent has been modified to a forced adsorption technique (K. E. Hechemy and Robert L. Anacker, J. Immunoassay, in press). In this technique the erythrocyte-sensitizing substance from R. rickettsii is precipitated on latex particles with 0.5% sodium acetate in ethanol, and the mixture is centrifuged. Ethanolic supernatant is discarded, latex particles are suspended in twice the original volume of glycine-buffered saline (pH 8.1), and supplemental erythrocyte-sensitizing substance is added (ca. 20 to 30% of the volume used to precipitate on latex). Finally, glycine-buffered saline (pH 8.1) containing 0.1% fatty acid-free bovine albumin (Sigma Chemical Co., St. Louis, Mo.) is added.

The micro-IF test (7) was performed with three conjugates. Fluorescein isothiocyanate-conjugated anti-human immunoglobulin (IgA + IgG + IgM) heavy and light chains were used in the micro-IF-immunoglobulin test. Anti-human IgM (μ) was used in the micro-IF-IgM test. Anti-human IgG (γ) was used in the micro-IF-IgG test.

For the 382 patients found seropositive by the latex-R. rickettsii and micro-IF–immunoglobulin tests, sera from 374 were available for further testing by the micro-IF–IgG and micro-IF–IgM tests. Sera from the remaining eight patients were unavailable for further testing.

RESULTS AND DISCUSSION

Correlation of median latex-R. rickettsii titer and micro-IF–immunoglobulin titer. A total of 659 sera from the 382 seropositive patients were grouped by micro-IF titer, and for each group the median latex-R. rickettsii titer was determined (Table 1). With one exception (a group too small [1 sample] for meaningful trend analysis) the median latex-R. rickettsii titer remained constant or increased stepwise within a micro-IF–immunoglobulin titer range of 8 to 16,384.

Each micro-IF–immunoglobulin titer (up to 4,096) was usually double the median titer obtained from the latex-R. rickettsii test. For each
group, the correlation between micro-IF and median latex-\textit{R. rickettsii} titers could be conveniently expressed as a doubling factor, i.e., the number of times a median latex-\textit{R. rickettsii} titer had to be doubled to equal the corresponding micro-IF titer.

For six of the nine micro-IF–immunoglobulin titers ranging from 16 to 4,096, the doubling factor was 1.0 (Table 1). With this value we could confirm that a titer of 64 is the minimum significant level of reactivity in the latex-\textit{R. rickettsii} test (2) and is comparable to a micro-IF titer of 128, the minimum significant level of reactivity in the micro-IF–immunoglobulin test (6).

A doubling factor of 1 could not be applied to our data for micro-IF–immunoglobulin titers >4,096. From our experience (unpublished data), sera with high micro-IF–immunoglobulin titers (≥16,384) do not necessarily have high latex-\textit{R. rickettsii} titers (>2,048). Usually such high-titer sera by micro-IF are collected in late-convalescent RMSF and have a low anti-\textit{R. rickettsii} IgM/IgG ratio.

\textbf{Effect of IgM/IgG ratio on the efficiency of the latex-\textit{R. rickettsii} test.} Even after adjustment with the doubling factor, titers obtained from the latex-\textit{R. rickettsii} test for seropositive patients did not always coincide with those obtained from the micro-IF–immunoglobulin test for the same patients. To determine the extent of this difference, we calculated equivalent titers for both tests for each of 374 specimens. In this calculation (3), the actual test titer was divided by the minimum significant level of reactivity in the test (128 for micro-IF and 64 for latex-\textit{R. rickettsii}).

The ratio of equivalent titers (latex-\textit{R. rickettsii}/micro-IF) was then computed for each serum sample, and the sera were grouped into three categories: ratio <1, ratio = 1, and ratio >1. These groups indicated, respectively, lower, the same, or greater efficiency of antibody detection by the latex-\textit{R. rickettsii} test relative to that by the micro-IF test.

To determine the effect, if any, of anti-\textit{R. rickettsii} IgM or IgG on both the efficiency of the latex-\textit{R. rickettsii} test, we regrouped the specimens in each category by ratio of micro-IF–IgM titers to micro-IF–IgG titers (IgM/IgG ratio) (Table 2) and found that the efficiency of the test was directly proportional to the IgM/IgG ratio. This correlation was statistically significant (chi-square test, X = 77.15, P < 0.001).

We next measured the efficiency of antibody detection by the latex-\textit{R. rickettsii} test in sera that contained either anti-\textit{R. rickettsii} IgM or IgG, but not both, as determined by the micro-IF–IgM or –IgG test. We associated greater efficiency with the presence of anti-\textit{R. rickettsii} IgM (Table 3), although the test was qualitatively accurate in either case. Greater efficiency of

\begin{table}
\begin{tabular}{cccc}
\hline
Micro-IF–immunoglobulin titer & Latex titer & Doubling factor & No. of specimens \\
\hline
8 & 8 & (8–64) & 0 & 127 \\
16 & 8 & (8–128) & 1 & 43 \\
32 & 16 & (8–256) & 1 & 22 \\
64 & 32 & (8–1,024) & 1 & 41 \\
128 & 64 & (8–2,048) & 0 & 109 \\
256 & 128 & (8–4,096) & 1 & 104 \\
512 & 192 & (8–8,192) & 1.415 & 52 \\
1,024 & 512 & (32–2,048) & 1 & 56 \\
2,048 & 512 & (32–2,048) & 2 & 39 \\
4,096 & 2,048 & (32–2,048) & 1 & 28 \\
8,192 & 256 & — & 5 & 1 \\
16,384 & 2,048 & (64–2,048) & 3 & 37 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\begin{tabular}{cccc}
\hline
IgM/IgG ratio & No. of specimens with indicated ratio of equivalent titers
\hline
<0.02 & 15 & 6 & 4 \\
0.02 & 3 & 0 & 0 \\
0.03 & 11 & 5 & 2 \\
0.06 & 10 & 3 & 4 \\
0.13 & 23 & 5 & 7 \\
0.25 & 12 & 11 & 11 \\
0.50 & 19 & 9 & 14 \\
1.00 & 10 & 13 & 23 \\
2.00 & 13 & 10 & 22 \\
4.00 & 6 & 8 & 24 \\
>4.00 & 12 & 10 & 49 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} See text for calculation of equivalent titers.
agglutination in sera containing IgM has been reported by Pike (8). Anacker et al. (1) also used an indirect hemagglutination system for RMSF antibodies to link efficiency of the hemagglutination system with IgM antibodies.

Time course of latex reactivity. Since evidence of a recent infection is usually dependent on a ≥fourfold rise in titer, we compared the antibody change for 265 pairs of sera in both tests without considering the time of onset and date of collection. The tests showed agreement in 240 pairs (90.6%). Titers changed <4-fold for 21 pairs and ≥4-fold for 219 pairs in both tests; titers changed <4-fold for 11 pairs and ≥4-fold for 14 pairs in the latex-<i>R. rickettsii</i> test only; and titers changed <4-fold for 14 pairs and ≥4-fold for 11 pairs in the micro-IF test only.

To determine latex-<i>R. rickettsii</i> reactivity throughout the course of RMSF, we tested a total of 277 sera from patients with known dates of onset and of collection with the latex-<i>R. rickettsii</i> and micro-IF tests. Equivalent titers were determined, and the geometric mean for each 3-day interval was computed (Fig. 1A). A moving average (Fig. 1B), based on the mean for each interval plus the means for the preceding and following intervals, was also computed to smooth out variations among time intervals (9).

Reactivity at or above the threshold value was found at a 7- to 9-day interval for both tests. This substantiates the findings of Kleeman et al. (5). The moving average reached its peak at 25 to 45 days for the latex-<i>R. rickettsii</i> test and at 31 to 35 days for the micro-IF test. After a 55- to 57-day interval, latex-<i>R. rickettsii</i> titers dropped below significant levels of reactivity (data not shown).

Sera were collected from three patients up to 160 days after onset (Fig. 2). Assays of these sera indicated that latex-<i>R. rickettsii</i> reactivity in late convalescence correlates mainly with the presence of anti-<i>R. rickettsii</i> IgM. In two patients (Fig. 2A and B), latex-<i>R. rickettsii</i> reactivity dropped as IgM levels dropped. In another patient (Fig. 2C), IgM levels remained high, as did latex-<i>R. rickettsii</i> reactivity. This pattern suggests that in a patient with only anti-<i>R. rickettsii</i> IgG response, the reactivity of the latex-<i>R. rickettsii</i> test would be short-lived.

In the early stages of RMSF the latex-<i>R. rickettsii</i> test shows reactivity with either or both of the anti-<i>rickettsial</i> antibody classes, but the degree of reactivity is greater with high concentrations of anti-<i>R. rickettsii</i> IgM. In these early stages the results of both tests appear parallel. However, late-convalescent sera are
TABLE 3. Efficiency of the latex-\textit{R. rickettsii} test relative to that of the micro-IF test for sera with either anti-\textit{R. rickettsii} IgM or IgG\textsuperscript{a}

<table>
<thead>
<tr>
<th>Antibody class</th>
<th>No. of specimens</th>
<th>No. of nonties (n)</th>
<th>No. of times latex-\textit{R. rickettsii} equivalent titers were greater than micro-IF equivalent titers (T)</th>
<th>Critical range for $\alpha = 0.05$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>36</td>
<td>30</td>
<td>24</td>
<td>$&lt;10, &gt;21$</td>
</tr>
<tr>
<td>IgG</td>
<td>38</td>
<td>30</td>
<td>8</td>
<td>$&lt;10, &gt;21$</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Cutoff for statistical significance, $t = (n \pm Z\sqrt{n})/2$, where $t$ is the range of critical values, $n$ is the number of nonties (number of specimens not in agreement between the two tests), and $Z$ is 1.96 when the total number of specimens is $>30$. If $T$ is outside of $t$, $\alpha = 0.05$.

less likely than early sera to register positive in the latex-\textit{R. rickettsii} test, because late sera usually contain less anti-\textit{R. rickettsii} IgM. The use of the latex-\textit{R. rickettsii} test as a diagnostic tool is promising, although its application to seroepidemiological studies may be limited.

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LITERATURE CITED