NOTES
Anti-Teichoic Acid Antibodies and Non-treponemal Serological Tests for Syphilis

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Antibodies specific for the polyglycerophosphate "backbone" of glycerol teichoic acids act as reagin in the Kolmer complement fixation test for syphilis.

Many of the nontreponemal or serological tests for syphilis involve the detection of serum antibodies (reagin) which are not directed against a component of the causative organism itself but may arise as a result of pathological changes brought about by proliferation of Treponema pallidum in host tissue. Test antigens for the detection of reagin are lipoidal, the main active component being the phospholipid, cardiolipin. It is known that reagin may be produced not only as a result of infection with T. pallidum but also by a wide variety of other organisms and pathological conditions (3, 8). False-positive reactors have been defined as patients with no history or clinical evidence of syphilis or other treponematoses whose sera react with nontreponemal but not with treponemal antigens (5). In studies on the specificity of antibodies to bacterial cell membrane glycerol teichoic acids (11), we have used glycerol-phosphoryl-glycerol-phosphoryl-glycerol (G₃P₂), derived from cardiolipin by the removal of the fatty acid residues, as a specific inhibitor of antibodies directed at the common polyglycerophosphate "backbone" of glycerol teichoic acids. It was therefore of interest to determine whether teichoic acid antibodies would react with cardiolipin itself in the serological tests used for the detection of reagin. Membrane glycerol teichoic acids appear to be ubiquitous in their occurrence in gram-positive bacteria (1), and this group of organisms is associated with many of the pathological conditions that may give rise to false-positive biological reactions for syphilis (8).

Antisera to teichoic acids may show serological specificities directed towards sugar substituents or to the polyglycerophosphate backbones of the polymer (11). The rabbit antisera used in this study were those previously prepared against membrane glycerol teichoic acids (lipoteichoic acid) or cell wall ribitol teichoic acids from various lactobacilli; the specificities of these sera are summarized and documented in Table 1. Human syphilitic sera were purchased (470-1) from the Commonwealth Serum Laboratories, Australia, or were a gift (C-007) from M. F. Garner (Institute of Clinical Pathology and Medical Research, Lidcombe, N.S.W. Australia). The Kolmer (KCF) and Reiter protein (RPCF) complement fixation tests used were standard procedures (9), all reagents being commercially available (Commonwealth Serum Laboratories). Fluorescent treponemal antibody absorption (FTA-ABS) and treponema immobilization (TPI) tests were kindly performed by M. F. Garner. In the case of FTA-ABS tests on rabbit sera, a goat anti-rabbit gamma globulin fluorescein conjugate was used in place of the usual anti-human conjugate. For complement fixation tests with glycerol teichoic acid (GTA-CF), the lipoteichoic acid from Lactobacillus fermenti NCTC 6991 (10) at a concentration of 10 μg/ml in saline was used in place of diluted Kolmer cardiolipin antigen, all other reagents and their proportions being identical to the standard Kolmer procedure. For studies on the inhibition of complement fixation, sera were diluted to 4× their KCF titer and incubated for 1 hr at 37°C with either 1 μg of cardiolipin or 1 μmole of G₃P₂ per ml of diluted serum. Absorbed sera were centrifuged, and the supernatant fluids were used immediately in KCF and GTA-CF tests. Diluted sera were also absorbed with glycerol teichoic acid by successive treatments with sheep red blood cells coated with L. fermenti membrane lipoteichoic acid (4) until no further agglutination occurred. Absorbed sera were clarified by centrifugation as before.
A comparison of the results obtained for the cardiolipin (KCF) and glycerol teichoic acid (GTA-CF) complement fixation tests with various rabbit anti-teichoic antisera is shown in Table 1. Positive reactions in both tests were obtained when the antiserum showed, at least in part, a specificity to the polyglycerophosphate "backbone" of a glycerol teichoic acid, but the reactions were negative with sera having specificities to sugar substituents alone. Where this has been determined, the titers in both tests for a particular serum were identical. Antisera specific to the "backbone" of ribitol teichoic acids were negative in both tests. Serum 213, which gave positive reactions in both tests, had antibodies specific to both the "backbone" of glycerol and ribitol teichoic acids. Preabsorption of this serum with homologous ribitol teichoic acid did not affect the complement fixation activity of the serum; preabsorption with glycerol teichoic acid rendered the serum negative in both tests. All rabbit sera, including normal serum, gave positive reactions in the RPCF test, and a high incidence of positive reactions in this test with sera from healthy rabbits has been noted previously (2).

Human syphilitic antisera gave titers in the KCF test comparable to those of rabbit antisera but did not react in the GTA-CF test even when the KCF titer was high (C-007).

### Table 1. Comparison of the reactions of antisera of known specificity in the Kolmer (KCF) and glycerol teichoic acid (GTA-CF) complement fixation tests

<table>
<thead>
<tr>
<th>Serum</th>
<th>Specificity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Anti-body&lt;sup&gt;b&lt;/sup&gt; content (mg/ml)</th>
<th>KCF reaction (cardiolipin antigen)</th>
<th>GTA-CF reaction (lipoteichoic acid antigen)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Antiserum prepared against</th>
<th>Organism from which teichoic acid derived</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>146</td>
<td>S + pGP</td>
<td>0.70</td>
<td>R (1:8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>R</td>
<td>+</td>
<td><em>Lactobacillus fermenti</em> NCTC 6991</td>
<td>6, 11</td>
</tr>
<tr>
<td>218</td>
<td>pGP</td>
<td>4.90</td>
<td>R (1:128)</td>
<td>R (1:128)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td><em>L. casei</em> NCTC 6375</td>
<td>11</td>
</tr>
<tr>
<td>236</td>
<td>pGP</td>
<td>0.97</td>
<td>R (1:32)</td>
<td>R (1:32)</td>
<td>+</td>
<td><em>L. casei</em> NIRD RO94</td>
<td>11</td>
</tr>
<tr>
<td>176</td>
<td>S + low pGP</td>
<td>1.50</td>
<td>R (1:4)</td>
<td>R</td>
<td>+</td>
<td><em>L. helveticus</em> NCIB 8025</td>
<td>7</td>
</tr>
<tr>
<td>183</td>
<td>pGP</td>
<td>2.30</td>
<td>R (1:32)</td>
<td>R (1:32)</td>
<td>+</td>
<td><em>L. plantarum</em> NCIB 7220</td>
<td>11</td>
</tr>
<tr>
<td>202</td>
<td>S + low pGP</td>
<td>4.00</td>
<td>R (1:32)</td>
<td>R (1:32)</td>
<td>+</td>
<td><em>L. fermenti</em> NCTC 6991</td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>S</td>
<td>4.50</td>
<td>NR</td>
<td>NR</td>
<td>+</td>
<td><em>L. plantarum</em> NCTC 7220</td>
<td></td>
</tr>
<tr>
<td>213</td>
<td>pRP + pGP</td>
<td>0.64</td>
<td>R (1:8)</td>
<td>NR</td>
<td>+</td>
<td><em>L. plantarum</em> ATCC 10241/R1</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human 470-1</td>
<td>Anti-<em>Treponema pallidum</em></td>
<td></td>
<td>R (1:32)</td>
<td>NR</td>
<td>+</td>
<td>Sera from human syphilitic patients</td>
<td></td>
</tr>
<tr>
<td>Human C-007</td>
<td>Anti-<em>T. pallidum</em></td>
<td></td>
<td>R (1:320)</td>
<td>NR</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Specificity of antibodies to sugar substituents (S), polyglycerophosphate backbone (pGP) or polyribitol phosphate backbone (pRP) of teichoic acid.
  * Measured as the maximum amount of antibody protein precipitated by homologous glycerol teichoic acid antigen.
  * Extracted from *Lactobacillus fermenti* NCTC 6991 and utilized at a concentration of 10 μg/ml.
  * R = reactive; NR = nonreactive; titers expressed as Kolmer dilutions (a Kolmer dilution of 1:2 = an actual serum dilution of 1:30).
  * Positive reactions in Reiter protein complement fixation, fluorescent treponemal antibody absorption, and treponema immobilization tests for syphilis.
  * Unpublished data, Wicken and Knox.

<sup>a</sup> A competition of the results obtained for the cardiolipin (KCF) and glycerol teichoic acid (GTA-CF) complement fixation tests with various rabbit anti-teichoic antisera is shown in Table 1. Positive reactions in both tests were obtained when the antiserum showed, at least in part, a specificity to the polyglycerophosphate "backbone" of a glycerol teichoic acid, but the reactions were negative with sera having specificities to sugar substituents alone. Where this has been determined, the titers in both tests for a particular serum were identical. Antisera specific to the "backbone" of ribitol teichoic acids were negative in both tests. Serum 213, which gave positive reactions in both tests, had antibodies specific to both the "backbone" of glycerol and ribitol teichoic acids. Preabsorption of this serum with homologous ribitol teichoic acid did not affect the complement fixation activity of the serum; preabsorption with glycerol teichoic acid rendered the serum negative in both tests. All rabbit sera, including normal serum, gave positive reactions in the RPCF test, and a high incidence of positive reactions in this test with sera from healthy rabbits has been noted previously (2).

Human syphilitic antisera gave titers in the KCF test comparable to those of rabbit antisera but did not react in the GTA-CF test even when the KCF titer was high (C-007).
Rabbit antisera 218 and 236 were used in further studies of the cross-reaction. Both sera were negative in FTA-ABS and TPI tests, indicating that neither rabbit had been exposed to treponemal infection. Preabsorption of these sera with cardiolipin rendered them anticomplementary although a similar absorption of serum 176 gave inhibition of both of the previously positive KCF and GTA-CF tests. Inhibition of both tests with sera 218 and 236 was obtained by preabsorption with G3P2 or sheep red blood cells sensitized with glycerol teichoic acid. Human syphilitic sera were rendered negative in the KCF test by preabsorption with cardiolipin or G3P2, but not glycerol teichoic acid.

From these results it is evident that antibodies specific to the polyglycerophosphate "backbone" of glycerol teichoic acids react in the KCF test as reagin, in that they will cross-react with the polyglycerophosphate moiety of cardiolipin. On the other hand, reagin produced in cases of syphilitic infection does not cross-react with glycerol teichoic acid. Here the antibodies appear to exhibit a tighter specificity with respect to the size of the polyglycerophosphate moiety of the cardiolipin molecule than do teichoic acid antibodies. That both cardiolipin and G3P2 inhibited the KCF test with syphilitic sera suggests that the fatty acid residues of the cardiolipin molecule do not play an important role in serological specificity.

It is of interest to speculate that high titers of anti-teichoic acid antibodies in the sera of human patients with a history of recent gram-positive bacterial infection may be associated with false-positive reactions for syphilis. Considerable serological testing of human false-positive reactors associated with a wide range of infections and pathological conditions would be required to establish the validity of this suggestion. Such serological surveys are often routinely conducted by many laboratories, and we would commend our suggestion to such institutions for their consideration.

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**LITERATURE CITED**


