Opsonization of *Treponema pallidum* Is Mediated by Immunoglobulin G Antibodies Induced Only by Pathogenic Treponemes

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Rabbit antisera to *Leptospira interrogans*, *Borrelia hermsii*, and *Treponema phagedenis* biotype Reiter, reactive to shared spirochetal antigens, failed to enhance phagocytosis of *Treponema pallidum* by macrophages, while immunoglobulin *G* to *Treponema pallidum* subsp. *pertenue* and *Treponema paraluiscuniculi* promoted phagocytosis. Opsonic antibodies are directed to pathogen-restricted, not shared spirochetal antigens.

During primary infection in rabbits, *Treponema pallidum* is cleared soon after infiltration of the local site by macrophages (27), and treponemes have been identified within macrophages of healing lesions (26, 39), further implicating these phagocytic cells in bacterial clearance. In vitro, *T. pallidum* is phagocytized by rabbit macrophages, and ingestion and killing are enhanced by *T. pallidum*-immune serum (1, 2, 9, 28). Earlier studies (7, 8, 24, 25) have demonstrated the molecular basis for antigenic cross-reactivity between *T. pallidum*, the cultivable nonpathogenic treponemes, and related spirochetes (e.g., *Leptospira interrogans* and *Borrelia hermsii*). To identify an antigen(s) that mediates opsonization of *T. pallidum*, we examined the opsonic capacities of antibodies directed against shared spirochetal or treponemal antigens and pathogen-restricted antigens.

Rabbit peritoneal macrophages were cultured as previously described (28) for 18 h prior to phagocytosis assays. *T. pallidum* (28) was harvested in M199 (without serum or antibiotics) from rabbits infected intrathecally for 9 to 10 days and adjusted to 1.25 × 10⁷ to 2.5 × 10⁹ spirochetes per ml. Macrophage culture medium was replaced with 0.4 ml of treponemal suspension (final ratio, 10 to 20 bacteria per macrophage), 50 μl of heated (56°C, 30 min) normal rabbit serum (NRS), and 50 μl of heated test serum. Test sera included NRS, immune rabbit serum (IRS), IRS absorbed with *Treponema phagedenis* biotype Reiter, and rabbit antisera directed against *B. hermsii*, *L. interogens*, *T. phagedenis* biotype Reiter, *Treponema paraluiscuniculi*, and *Treponema pallidum* subsp. *pertenue*. Additionally, NRS and opsonic antitreponetal antisera were fractionated into purified immunoglobulin G (IgG) and IgM-enriched fractions by protein G separation of the saturated ammonium sulfate-cut globulin fraction. By immunodiffusion, IgG fractions were pure and IgM-enriched fractions contained trace albumin but no IgG. Globulin, purified IgG, and IgM-enriched fractions were adjusted to equivalent fluorescent treponemal antibody absorption (FTA-ABS) titers by using the appropriate NRS fraction.

Phagocytosis assays were performed in triplicate, stained coverslips were coded for unbiased scoring, and the percentage of macrophages containing phagocytosed treponemes was determined (28). Nonspecific background staining (macrophages without *T. pallidum*) was 1.0 ± 1.4% (mean ± standard error). Opsonic capacities were compared by using a two-tailed Student's *t* test, and *n* was conservatively defined as the number of macrophage populations tested with each serum rather than the total number of replicate cultures.

We examined the ability of antibodies directed against antigens shared by *T. pallidum* and more distantly related spirochetal pathogens, *L. interrogans* and *B. hermsii*, to opsonize *T. pallidum* (Table 1). Both sera failed to enhance phagocytosis of *T. pallidum*: the percentage of macrophages containing ingested *T. pallidum* in the presence of either antiserum (18.0 ± 5.3 and 18.0 ± 6.7, respectively) was not significantly different from the percentage obtained with NRS controls (20.5 ± 5.1). As expected, phagocytosis in the presence of IRS (81.0 ± 0) was significantly higher (*P* < 0.001).

Opsonization by antiserum raised against the cultivable *T. phagedenis* biotype Reiter (22.8 ± 5.4) was equivalent to opsonization by IRS (20.5 ± 5.1) and was significantly less effective than opsonization by IRS (81.0 ± 0) (Table 1). In a complementary study, IRS extensively absorbed with Reiter treponemes, removing all antibodies directed against shared treponemal antigens, showed undiminished opsonic activity (74.8 ± 5.2) compared with unabsorbed IRS (81.0 ± 0).

Antiseras raised against the human pathogen *T. pallidum* subspecies *pertenue* (anti-Yaws) and the rabbit pathogen *T. paraluiscuniculi* (anti-Tpc) are significantly more effective than NRS in enhancing phagocytosis of *T. pallidum* (69.3 ± 6.2 and 62.8 ± 11.3, respectively). These sera were not different from IRS in opsonic activity, particularly when adjusted to equivalent FTA-ABS titers (Table 2). IgG and IgM fractions of IRS, anti-Tpc, and anti-Yaws, adjusted to equivalent FTA-ABS titers, were tested; all opsonic activity is clearly mediated by IgG (Table 2); none was detected when the IgM fraction was used.

Immunoblot analysis (6) of the test antisera (data not shown) confirmed our published findings (8) that the major antigens shared by *T. pallidum* and other spirochetes are on the 80- and 69-kDa molecules and the 37-, 35-, 33-, and 30-kDa endoflagellar components. *T. pallidum* and *L. interrogans* share a 47- to 48-kDa molecule as well, and faint reactivity is seen at 41 and 28 kDa. These molecules also represent antigens shared by *T. pallidum* and Reiter (25).
Because antibodies to these antigens are not opsonic, shared epitopes on these molecules are not targets for opsonization of *T. pallidum*. Conversely, antibodies remaining in IRS following absorption with Reiter clearly are opsonic. In addition to Venereal Disease Research Laboratory (VDRL) antibody, the major molecular specificities demonstrable by immunoblot in this absorbed antisera are 47, 37, 14, and 12 kDa, although other minor specificities may also be involved. These molecules contain pathogen-specific determinants also found on *T. pallidum* subsp. *pertenue* (7) and *T. paralucisuniculicti* (8), consistent with the opsonic capabilities of these antisera, although no direct evidence yet links any individual molecules with opsonic activity.

Our demonstration that *T. pallidum* opsonic antibody is directed against pathogen-restricted antigens is consistent with failure of immunization with the nonpathogenic treponemes (5, 17, 22) or purified endoflagella of *T. pallidum* or *T. phagedenis* (11, 19) to protect against *T. pallidum* infection. Complete protection against syphilis in rabbits has been achieved convincingly only by Miller (31), whose immunization strategy was based upon the hypothesis that the immunoprotective component of *T. pallidum* is a fragile surface component, shed rapidly upon manipulation or storage in vitro. The identity of the immunoprotective antigen is unknown. As hypothesized by Medici (30), the outer membrane is now considered to be relatively inert immunologically (12, 13, 34, 35), with few surface-exposed protein molecules (37, 40, 46). Earlier claims of surface locations of the 47-kDa (23), 37-kDa (called 39-kDa [33]), 190-kDa “4D” (15, 36), and other (4, 29, 32) antigens have not been substantiated, and currently no surface antigen has been identified.

Similarly, location of the opsonic target antigen is unknown, but the lengthy time (3 to 4 h) required for phagocytosis of *T. pallidum* (2, 28) suggests several possibilities. First, the opsonic target may be on the surface but masked by host material, including mucopolysaccharides (12, 41-43, 47) or serum-derived proteins (3, 45), thus delaying binding of antibody (14, 16, 45). Alternatively, as suggested by Blanco and coworkers (10), antibody may bind to the surfaces of the bacteria very rapidly yet require time for antigens to aggregate and trigger effector functions such as complement activation (10) or, hypothetically, phagocytosis. Lastly, the target antigen may be subsurface, requiring deterioration or mobility of the outer membrane to allow antibody access to subsurface molecules. In vivo, the outer membrane may be fluid, momentarily exposing interior structures to the external (antibody-containing) environment. Alternatively, complement may serve to disrupt the outer membrane (20, 21, 36), thus exposing subsurface structures to antibody binding.

On the basis of our findings, cross-reactive or shared treponemal and spirochetal antigens should not be considered potential opsonic targets. The ability of antisera directed against other pathogenic treponemes to significantly promote phagocytosis of *Treponema pallidum* subsp. *pallidum* is consistent with the substantial cross-protection provided by these infections (18, 38, 44). However, cross-protection is not absolute. In Miller’s immunization studies (31), immunized rabbits were completely protected against the homologous strain of *T. pallidum* but not against *T. pallidum* subsp. *pertenue*. As with protection, some differences in opsonic capacity between species and subspecies may exist, but they may not be detectable with the sensitivity of our assay system.

Clearly, species- and/or subspecies-specific antibodies may participate in opsonization and may determine the fate of treponemes within macrophages. Theoretically, augmentation or selection of this antibody response could result in earlier clearance of bacteria, and subsequently a shorter infectious stage. Optimally, prior immunization with the target antigen(s) might prevent infection and provide the key to the search for an effective vaccine for syphilis.

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### TABLE 1. Opsonization of *T. pallidum* by various antisera

<table>
<thead>
<tr>
<th>Opsonin source</th>
<th>n</th>
<th>% Phagocytosis</th>
<th>P vs. value for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NRS</td>
</tr>
<tr>
<td>NRS</td>
<td>4</td>
<td>20.5 ± 5.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IRS</td>
<td>3</td>
<td>81.0 ± 6.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-B. henselae&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>18.0 ± 6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-L. interrogans&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>18.0 ± 5.3</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-Reiter&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>22.8 ± 5.4</td>
<td>NS</td>
</tr>
<tr>
<td>IRS absorbed with Reiter&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>74.8 ± 5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-Tpc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>62.8 ± 11.3</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Anti-Yaws&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>69.3 ± 6.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of macrophages with phagocytized *T. pallidum* (mean ± standard error).
<sup>b</sup> NS, not significant.
<sup>c</sup> IRS pooled from seven rabbits infected with *T. pallidum* subsp. *pallidum* Nichols for 4 to 12 weeks; VDRL R32; FTA-ABS, 4+.
<sup>d</sup> Anti-B. henselae serotype 7 (VDRL WR; FTA-ABS, 4+) and anti-L. interrogans serogroup Canicola (Moulton strain) (VDRL WR; FTA-ABS, B) prepared as described in reference 8.
<sup>e</sup> Anti-T. phagedenis biotype Reiter (VDRL R1; FTA-ABS, 3 to 4+) and IRS absorbed with Reiter (VDRL R32; FTA-ABS, 4+) prepared as described in reference 25.
<sup>f</sup> Anti-Tpc pooled from four rabbits naturally or experimentally infected with *T. paralucisuniculicti*; VDRL R2; FTA-ABS, 4+.
<sup>g</sup> Anti-Yaws pooled from four rabbits infected with *T. pallidum* subsp. *pertenue* Gauthier for 10 to >60 days; VDRL R16; FTA-ABS, 4+.

**Notes:**
- All whole antisera and IgG fractions were adjusted to a 1:512 equivalent FTA-ABS titer, within groups, prior to the assay. Undiluted IgM fractions were 1 to 2+ reactive.
- Percentage of macrophages with phagocytized *T. pallidum*. Values are means ± standard errors of assays using three separate macrophage preparations; triplicate cultures per test serum per macrophage.
- NS, not significant.

### TABLE 2. Opsonic activities of antisera directed against pathogenic treponemes

<table>
<thead>
<tr>
<th>Opsonin source&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Phagocytosis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P vs. value for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NRS</td>
</tr>
<tr>
<td>Whole antisera&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IRS</td>
<td>54 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-Tpc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45 ± 4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-Yaws&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRS</td>
<td>42 ± 4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-Tpc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-Yaws&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgM fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRS</td>
<td>17 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-Tpc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-Yaws&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 ± 1.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup> All whole antisera and IgG fractions were adjusted to a 1/512 equivalent FTA-ABS titer, within groups, prior to the assay. Undiluted IgM fractions were 1 to 2+ reactive.
<sup>b</sup> Percentage of macrophages with phagocytized *T. pallidum*. Values are means ± standard errors of assays using three separate macrophage preparations; triplicate cultures per test serum per macrophage.
<sup>c</sup> NS, not significant.
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REFERENCES


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