Early Complement Components Enhance Neutralization of
*Chlamydia trachomatis* Infectivity by Human Sera

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Immunoglobulin G in human serum neutralizes chlamydial infectivity in vitro. Complement-intact, C5-depleted, and C8-depleted human serum all have significantly more neutralizing activity than serum heated to inactivate early components of complement. Cobra venom factor, an analog of human C3b, enhances neutralization of antichlamydial immunoglobulin G in the absence of early complement components.

*Chlamydia trachomatis* infects mucous membranes of human eyes and genital tracts and causes chronic inflammation with the following resultant clinical syndromes: trachoma (serovars A, B, Ba, and C); conjunctivitis, urethritis, cervicitis, and pelvic inflammatory disease (serovars D, E, F, G, H, I, J, and K); and lymphogranuloma venereum (serovars L1, L2, and L3) (22). The immunology and pathogenesis of these diseases currently are not well understood. Because *C. trachomatis* infection induces diverse inflammatory reactions, the concept that chlamydial infection manifests different immunopathologic features has emerged (9, 13). As a step toward examining immune interactions of *C. trachomatis* with the human host, we have begun to examine the interaction of human antibody and complement with this organism in vitro.

The prevalence of chlamydial antibodies in sexually active individuals is high. A number of studies utilizing the micro-immunofluorescence test have reported seroprevalence rates ranging from 50 to 80% for individuals who are culture negative for *C. trachomatis* to 80 to 100% for individuals who are culture positive (21, 27). More specifically, human sera have been shown to contain antibodies to proteins of 15 serovars of *C. trachomatis* (14). In the absence of functional complement (heat inactivation), only high-titered human sera neutralize *C. trachomatis* (12), but in the presence of complement, lower-titered sera (for example, fresh normal human sera) are capable of diminishing the infectivity of certain *C. trachomatis* serovars (5, 12, 15).

(This work was presented in part at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif., October 1988 [10a].)

*C. trachomatis* serovar A, strains G-17 and OT; serovar E, strains UW-5 and Cx; serovar K, strains UW-31 and Cx; serovar L2, strain 434; and serovar L3, strain 404, were grown in McCoy monolayers, and elementary bodies (EBs) were purified as previously described (24); the infectivity titers of EB preparations were expressed by enumerating inclusion-forming units (IFUs), and yields were usually 1 × 10^7 to 6 × 10^7 IFUs/μg of protein. Aliquots of EBs were stored at −70°C in HEPES-N(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid)-sucrose-calcium buffer (10) and were used after a single thaw as antigens in a neutralization or dot enzyme-linked immunosorbent assay (ELISA) (see below). Two individual freshly frozen human serum specimens (JN and PR) and a separate pool of fresh human serum, marketed as human complement by Sigma (St. Louis, Mo.), were used. JN was from a hypogammaglobulinemic patient, and PR was from a healthy laboratory worker. Neither had a prior history of chlamydial infection. Cobra (Naja naja kaouthia) venom factor (CVF) and human serum depleted of terminal complement components C5 (CSD) and C8 (CSD) were purchased from Quidel (San Diego, Calif.). Immunoglobulin (Ig) isotypes were quantitated by nephelometry (Array Protein System; Beckman). The functional integrity of each complement pathway was determined by hemolytic assay (7, 11).

Ig isotype-specific antibody titers to *C. trachomatis* antigens were detected with a whole-cell dot ELISA as previously described (1). Fourfold serial dilutions of sera were reacted with formalinized EBs (10⁶ IFUs) placed on nitrocellulose squares (BA 85/20-SD; Schleicher & Schuell, Keene, N.H.). Reactions were developed with peroxidase-conjugated goat anti-human IgG, IgM, or IgA (Sigma; affinity purified), followed by hydrogen peroxide used as the substrate and tetramethylbenzidine as the indicator (8). Titers were expressed as the reciprocal of the highest dilution that gave a blue dot when read at 5 min. Antigen controls (reacted without buffer) and serum controls (reacted with mock-infected McCoy cell extracts prepared as described above) were included as negative controls in each run.

Twenty-five microliters of organisms in HEPES-sucrose-calcium buffer (10⁵ to 10⁶ IFUs/ml, predetermined) was added to 25 μl of test sera or HEPES-sucrose-calcium buffer containing 10% heated (56°C, 30 min) fetal calf serum (free of chlamydial antibody), vortexed, and sonicated in an ultrasonic cleaner (RAI Research Corp., Hauppauge, N.Y.) for 1 min.

The mixtures were incubated at 37°C for 30 min in a water bath shaker and sonicated again to disperse aggregates, and aliquots were diluted to 10⁻² or 10⁻³ with buffer and inoculated in triplicate to McCoy monolayers to determine remaining infectivity: the number of inclusions on each slide was counted. Neutralization assays were performed in two separate experiments, and the combined results (six measurements) are presented. Differences in neutralization were assessed for significance by using a Student's *t* test by using the number of inclusions counted in each reaction mixture. For comparisons among different serovars and different sera, neutralization of *C. trachomatis* infectivity...
TABLE 1. Specific IgG antibody titers and neutralization of *C. trachomatis* infectivity by human sera (50%)  

<table>
<thead>
<tr>
<th>Chlamydia serovar</th>
<th>Mean no. of Chlamydia IFUs ± SD (10⁵)</th>
<th>Buffer</th>
<th>Heat-inactivated serum</th>
<th>Fresh serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1,024</td>
<td>6.18 ± 1.20</td>
<td>3.00 ± 1.13*</td>
<td>0.02 ± 0.02**</td>
</tr>
<tr>
<td>A</td>
<td>1,024</td>
<td>12.96 ± 2.02</td>
<td>7.48 ± 0.78*</td>
<td>3.87 ± 0.89**</td>
</tr>
<tr>
<td>L2</td>
<td>1,024</td>
<td>12.49 ± 1.83</td>
<td>6.12 ± 0.87*</td>
<td>3.64 ± 0.70**</td>
</tr>
<tr>
<td>E</td>
<td>256</td>
<td>2.91 ± 0.33</td>
<td>2.36 ± 0.81</td>
<td>1.35 ± 0.57</td>
</tr>
<tr>
<td>L3</td>
<td>256</td>
<td>1.89 ± 0.58</td>
<td>1.63 ± 0.54</td>
<td>1.72 ± 0.44</td>
</tr>
</tbody>
</table>

* *, for heat-inactivated serum (50°C, 30 min) versus buffer control, P < 0.05 by Student's t test; **, for fresh serum versus heat-inactivated serum, P < 0.025.

was also expressed as follows: % neutralization = \((1 - \frac{\text{no. of inclusions in serum/no. of inclusions in buffer}}{100})\)

In the first experiment, the two individual human serum specimens (complement intact) were tested at a 50% concentration to determine neutralization activity: one serum specimen (PR) had normal levels of Igs, and the other (JN, obtained from a hypogammaglobulinemic patient) had one-half of the normal level of IgG (537 mg/dl) and 10% of that of IgM (15 mg/dl) and was deficient in IgA (<7 mg/dl). IgG antibody titers to *C. trachomatis* were the same in each serum (1:1,024 against serovars K, A, and L2 and 1:256 against E and L3 [Table 1]), but JN serum had little or no detectable IgM or IgA antibody titers (≤8). IgM and IgA antibody titers in PR serum were lower than that of IgG; IgM antibody titers were 1:64 against the five serovars, and IgA antibody titers were 1:32 against serovars K, A, and L2 and 1:8 against serovars E and L3. The 50% classical hemolytic complement was 130 and 122 U and the 50% alternative hemolytic complement was 15 and 21 U in PR and JN sera, respectively. Results of neutralization with each serum specimen were similar, and the combined experimental results are shown in Table 1. Heat-inactivated or complement-deficient sera neutralized serovars K, A, and L2 (reciprocal antibody titers, 1,024) significantly: 51, 42, and 51%, respectively (Table 1; P < 0.05). Fresh or complement-intact sera enhanced neutralization of these three serovars more than heat-inactivated sera: serovar K was neutralized 99.7%, serovar A was neutralized 69%, and serovar L2 was neutralized 71% (P < 0.025 compared with heated sera; Table 1). Neither serum neutralized serovars E and L3 significantly, even in the presence of complement. Notably, both sera contained lower IgG antibody titers against these serovars (1:256; Table 1). JN serum lacked IgG and IgA chlamydial titers yet maintained neutralization equivalent to PR serum; hence, IgG and IgA chlamydial antibodies did not contribute to neutralization.

In the presence of antibody and complement, bacteriolysis takes place by membrane attack complexes (C5b-9) which require the late components of complement (6, 25). To determine the possible contribution of the direct bacteriolytic effect of antibody and complement to neutralization, we examined the ability of C8-depleted (CSD) and C5-depleted (CSD) sera to neutralize *C. trachomatis* infectivity; neither serum had detectable hemolytic activity (50% classical hemolytic complement, <10 U; 50% alternative hemolytic complement, <5 U). As shown in Table 2, fresh CSD serum significantly neutralized more infectivity than heat-inactivated CSD serum; 99% neutralization versus 56% for serovar K (P < 0.01), 77% versus 27% for serovar A (P < 0.01), and 64% versus 30% for serovar L2 (P < 0.05), respectively. Fresh CSD serum neutralized serovars K and A, but not serovar L2, better than heat-inactivated CSD serum: 99.9% neutralization versus 37% for serovar K (P < 0.01) and 51% versus 22% for serovar A (P < 0.05). To ensure that residual amounts of C5 or C8 in these sera were not involved, anti-C5 or anti-C8 serum was added to the respective reaction mixtures, and similar results were obtained (data not shown). These results indicate that extracellular killing is not essential for enhancement of neutralization to occur; furthermore, early complement components enhance this neutralization.

The two pathways of complement converge at C3, resulting in the cleavage of C3 to C3b by C3 convertases. C3 is the most abundant complement protein in blood and plays a central role in the activation sequence of the complement system (26). Neutralization of *C. trachomatis* infectivity in CSD serum, as shown above, may result from binding of C3b or its cleavage products (e.g., iC3b, C3c, or C3dg) to *C. trachomatis*-antibody complexes (19, 26). We used CVF as an analog of human C3b (cobra C3b, structurally resembling the C3c fragment [21]) to stimulate the direct contribution of human C3b in neutralizing *C. trachomatis*. When CVF (50 U/ml) was added to heat-inactivated human serum, neutralization of serovar K but not of serovar L2 was enhanced significantly (Table 3). No neutralization was observed when we used a lower concentration of CVF (12.5 U/ml). Neutralization of serovar K was similarly enhanced when CVF was added to immunoglobulin fractions isolated from human serum by ammonium sulfate precipitations (data not shown).

We have examined interactions of *C. trachomatis* EBs and human antibodies and have demonstrated neutralization of chlamydial infectivity in vitro. This neutralization occurs in the absence of aggregation in normal human serum (10b) and

**TABLE 3. Enhancement of the neutralization of *C. trachomatis* infectivity by human antibodies with CVF**

<table>
<thead>
<tr>
<th>Chlamydia serovar</th>
<th>CVF added*</th>
<th>Mean no. of Chlamydia IFUs ± SD (10⁵)</th>
<th>Buffer</th>
<th>Heat-inactivated serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>+</td>
<td>2.33 ± 0.30</td>
<td>0.09 ± 0.07**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2.40 ± 0.30</td>
<td>1.07 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>+</td>
<td>2.67 ± 0.35</td>
<td>0.80 ± 0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2.65 ± 0.35</td>
<td>1.16 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

* 50 U/ml (no effect at 12.5 U/ml).
* For CVF(+) versus CVF(-), P < 0.01 by Student’s t test.
in immune sera as shown previously (3, 28). Neutralization of \textit{C. trachomatis} infectivity by antibody may occur by preventing attachment, internalization, or replication after ingestion (4) or combinations of these factors. Thus, different antibodies may neutralize \textit{C. trachomatis} by different pathways. A previous study has shown that a monoclonal antibody that reacts to a species-specific epitope of the major outer membrane protein does not inhibit attachment and internalization of EBs to HeLa 229 cells. Nevertheless, it still neutralizes chlamydial infectivity (16). Another study showed that a serovar-specific monoclonal antibody to the major outer membrane protein of serovar L2 capable of neutralizing chlamydial infectivity abrogated ATPase activity of EBs (17). Monoclonal antibodies to serovar- and subspecies-specific epitopes of the major outer membrane protein also neutralize chlamydial infectivity (of serovar B) by blocking chlamydial attachment to hamster kidney cells (23). Identification of epitopes recognized by these antibodies may help to clarify biologic functions of these target antigens.

We have shown here that the late complement components were not required to enhance neutralization of chlamydial infectivity. Neutralization by the early complement components in vitro may be similar to neutralization of viral infectivity that results from the encapsulation of viral particles with antibody and complement (4). This occurs upon activation of complement via the classical pathway of complement through C4 and occurs in the absence of detectable viral agglutination or lysis (4). In our studies, serovar L2 appears less sensitive than serovar K to complement-enhanced neutralization of normal human sera, as previously shown to be the case with sera from patients infected with serovar L2 (18).

The protective role of antibody in host defense against the mouse pneumonitis agent (MoPn; murine \textit{C. trachomatis}) has been shown previously (28). Immune serum given intranasally delayed or prevented death in mice, and IgG or IgA fractions from immune serum or immune bronchial lavage fluid were effective in producing protection when used to opsonize MoPn. Furthermore, C5D mice were not more sensitive to MoPn than the appropriate control mice. Injection of CVF, which also depletes terminal components of complement, does not increase susceptibility to MoPn (28). Late complement components therefore may not be required in the host defense against chlamydial infection, as demonstrated by these in vivo experiments. Interactions of early complement components and antibody may facilitate interactions with inflammatory cells via Fc or C3 cleavage products (20, 26) or sustain viable EBs in noninfectious forms in vivo. Quantitative and qualitative studies of C3 depositions should provide a framework for further studies to define protective or immunopathological effects of human antibodies and complement in vivo.

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REFERENCES


