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Characterization of Kinetics and Target Proteins for Binding of Human Complement Component C3 to the Surface-Exposed Outer Membrane of *Chlamydia trachomatis* Serovar L2

RAYMOND T. HALL,[1] TOD STRUGNELL,[1] XU WU,[1] DANA V. DEVINE,[2,3] AND H. GRANT STIVER[1]*

Division of Infectious Disease, Department of Medicine,[1] and Department of Pathology,[2] University of British Columbia, and the Canadian Red Cross Blood Transfusion Service,[3] Vancouver, British Columbia, Canada V5Z 1M9

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In order to characterize the interaction of human complement with *Chlamydia trachomatis*, flow cytometry was used to quantitate binding of complement component C3 to elementary bodies of *C. trachomatis* serovar L2 preincubated in fresh serum in the presence or absence of human polyclonal chlamydial antibody. Isolation of each of the complement activation pathways revealed that C3 was activated most effectively by the alternative pathway. The degree of binding by the classical pathway was proportional to the concentration of antibody, but dual-pathway-mediated binding was not greater than antibody-independent alternative pathway binding. Electrophoresis and immunoblotting of detergent-extracted outer membrane protein-C3b complexes indicated that the chlamydial major outer membrane protein was the primary cell surface moiety binding C3b in both the presence and absence of specific antibody. Hydroxylamine cleavage of outer membrane protein-C3b complexes provided evidence that the majority of C3b is bound to the major outer membrane protein by hydroxyl ester bonds. This result was also unchanged by the presence of specific antibody. An unexpected finding was the apparent binding of anti-C3 antibody to a 40-kDa protein of the chlamydial outer membrane complex, perhaps indicating C3 mimicry on the part of the chlamydial major outer membrane protein.

Chlamydia trachomatis is an obligate intracellular bacterium causing conjunctivitis, urethritis, and pelvic inflammatory disease. It is characterized by a dimorphic life cycle, consisting of the intracellular, metabolically active reticulate body and, after lysis of the host cell, the inert, infectious elementary body (EB), which is released into the extracellular environment (18). The interaction of EBs with host resistance factors in the extracellular milieu is an important area of chlamydial research in which there remain many unanswered questions.

The cell surface of *C. trachomatis* EBs has been studied extensively with monoclonal antibodies (MAb) to map and catalog the antigenic profiles of the outer membrane proteins (OMPs). Studies of these membrane antigens have revealed that the major OMP (MOMP) binds specific antibody and plays an important role both structurally and immunologically (20, 21). It is surface exposed at its four immunochemically variable domains (1), which bear the species and subspecies determinants for *C. trachomatis* serovars. Su and Caldwell (25) showed that Fab fragments of an MAb directed against the MOMP exerted a neutralizing effect by preventing chlamydial attachment to the host cell, indicating a possible role for the MOMP as an adhesin molecule in infectivity. Neutralization of chlamydial infectivity probably occurs both before and after entry into the host cell (4, 20) through a variety of mechanisms, some of which are complement dependent.

Several studies have shown that chlamydiae can activate the complement cascade in fresh serum in vitro (16) and that complement greatly enhances the neutralization of infectivity by antibody (13, 17). Although complement is activated by EBs in the absence of antibody, it does not neutralize chlamydiae without specific antibody as it does other "serum-sensitive" gram-negative bacteria (9). An early publication by Johnson et al. (8) reported that fresh serum alone was bactericidal for chlamydiae; however, the microimmunofluorescence (MIF) assay used may have been too insensitive to detect the small quantities of specific antibody thought necessary to interact with complement. The question of how complement and antibody interact in the neutralization of chlamydiae forms the basis of our studies.

To determine whether antibody functions to augment complement binding to the chlamydial outer membrane, we measured the deposition of C3b on the surface of whole EBs in situ by flow cytometry and compared the rate and magnitude of deposition in the presence and absence of specific antibody. We then isolated the two complement activation pathways, comparing the binding of C3b by each. Last, we extracted the OMP-C3b complexes from whole EBs in order to define the C3b-binding proteins exposed on the chlamydial cell surface and to elucidate the nature of the bond.

MATERIALS AND METHODS

Chlamydial EBs. *C. trachomatis* L2/434/Bu was used. Chlamydiae were harvested from McCoy cell monolayers by standard methods, and EBs were purified on discontinuous Renografin gradients as described elsewhere (3). Preparations contained >90% EBs, as determined by electron microscopy. This observation was supported by uniformity of particle size in scattering laser light during flow cytometry. Samples were found to contain approximately 4×10^8 inclusion-forming units per ml by iodine staining of McCoy

* Corresponding author.

cell cultures. EBs were resuspended in phosphate-buffered saline, pH 7.4 (PBS), divided into portions, and stored at −70°C until needed.

Human serum. Blood used as a complement source was collected from healthy volunteers, the serum was separated, and aliquots were frozen at −70°C. On the day of the binding experiments, a portion of the thawed serum aliquot was tested for complement activity and found to contain, on average, 200 classical hemolytic 50 (CH50) and 146 alternative pathway hemolytic 50 (APH50) units per ml. Western immunoblots against chlamydial OMPs failed to detect any antibody-reactive bands. This serum was designated normal human serum (NHS).

Immune human serum (IHS) was pooled from three donors. Frozen serum samples from two patients with acute chlamydial genital infections were kindly provided by W. R. Bowie, University of British Columbia. These sera were reactive with all 15 serovars of *C. trachomatis* as well as with *Chlamydia psittaci* and *Chlamydia pneumoniae* by MIF. The MIF titers of these sera for serovar L2 were 1:32 and 1:8. The third serum sample was from a laboratory worker who has no history of chlamydial infection but has worked with lymphogranuloma venereum strain L2 for many years. This serum was also broadly reactive by MIF, with a titer of 1:8 for serovar L2. Two of these sera had no higher titers against the other serovars, including *C. pneumoniae* TW183 and *C. psittaci* 6BC. One of the sera (reacting at 1:8 to L2) reacted at 1:32 to serovars B, D, and E and at 1:16 to serovars C, H, I, J, and A.

A Western blot against the serovar L2 OMP demonstrated the specificity of these sera for epitopes of 8 to 12 proteins, including the MOMP, the 60- and 62-kDa cysteine-rich proteins, and other proteins of 10, 29, 32, 75, and 92 kDa. A chlamydial complement fixation test (24) with a genus-specific antigen performed on IHS yielded a titer of 1:32. A chlamydial neutralization test (26) with this serum demonstrated that 10% IHS with fresh NHS as the complement substrate resulted in a 99% reduction in inclusion-forming units of the lymphogranuloma venereum L2 strain in HaK cells, compared with only 50% reduction with heated IHS alone.

IHS and some aliquots of NHS were heated for 30 min at 56°C and found to be negative for complement activity by the CH50 assay. Some aliquots of NHS were run through a protein A-Sepharose column to remove serum immunoglobulin G (IgG). This serum, called IgG-depleted NHS, was found to be negative for IgG by Western blot with goat anti-human IgG antiserum (Sigma Chemical Co., St. Louis, Mo.).

Measurement of C3 binding by flow cytometry. In order to optimize the parameters of the flow cytometer (EPICS Profile; Coulter Corporation), EBs were incubated with a fluorescein isothiocyanate (FITC)-conjugated murine MAb to *C. trachomatis* (Kallestad, Chaska, Minn.) for 30 min at room temperature. After being washed three times in PBS, EBs were run on the flow cytometer, and the optimum parameters for detection of EBs were set and stored in the onboard computer. Events processed was set at 10,000 particle counts; sheath pressure was set at maximum in order to count only particles approximately 300 nm in diameter.

To determine the time course of binding, EBs were incubated with NHS or NHS supplemented with 10% IHS for 1, 5, 15, 30, 45, and 60 min at 37°C with gentle shaking. Controls were incubated for 30 min. Cold PBS was added to samples to stop the reaction, and samples were then held at 4°C until all reactions were stopped. Samples were washed three times with cold PBS and centrifuged at 41,000 × *g* for 60 min at 4°C to pellet the EBs. To detect bound complement, samples were incubated with FITC-conjugated goat anti-human C3 (Atlantic Antibodies, Scarborough, Maine), diluted 1:500 in PBS, for 30 min at 37°C with gentle agitation. Heat-inactivated NHS was used as a negative complement control, and normal goat serum followed by FITC-conjugated rabbit anti-goat IgG was used as a control for nonspecific binding of goat IgG. After the samples were washed and centrifuged as described above, EB-associated fluorescence was measured by flow cytometry.

Determination of complement activation pathway. To isolate the alternative complement activation pathway, NHS was incubated in a final concentration of 20 mM EDTA for 5 min at 37°C with gentle shaking. EBs were added to all samples, a final concentration of 25 mM $MgCl_2$ was added to appropriate samples, and all samples were incubated for 30 min at 37°C with gentle shaking (7). Control EB samples were incubated in either NHS or heat-inactivated NHS without preincubation in EDTA. After the samples had been washed three times with PBS, bound C3 was detected by incubation in FITC-conjugated human C3 antiserum as described above.

In the next set of experiments, designed to isolate the classical complement pathway, EBs were incubated in complement factor B-deficient serum (Quidel, San Diego, Calif.) with and without 10, 20, and 40% heat-inactivated IHS (as a source of specific antibody) for 30 min at 37°C and washed three times in PBS. Complement factor B-deficient serum was tested for complement activity and found to be negative for APH50 but to have a normal CH50 value (119 units per ml). Bound C3 was detected and measured in the flow cytometer as described in the previous section. NHS and heat-inactivated NHS were included as positive and negative controls, respectively.

Extraction of chlamydial OMPs and OMP-C3b complexes. Purified EBs in PBS were incubated with either NHS, NHS with 10% IHS, heat-inactivated NHS, or PBS at a 1:1 (vol/vol) ratio for 45 min at 37°C with gentle agitation. They were then washed twice in PBS to remove excess serum and centrifuged at 41,000 × *g* for 60 min at 4°C to pellet the EBs. OMP was extracted by the method of Caldwell et al. (3). Briefly, EBs were incubated in PBS, pH 8.0, containing 2% (wt/vol) Sarcosyl (sodium lauroyl sarcosine) and 1.5 mM EDTA for 60 min at 37°C, centrifuged at 100,000 × *g* for 60 min at 25°C, resuspended in Sarcosyl, and centrifuged again under the previous conditions. The insoluble pellet was washed and centrifuged three times at 100,000 × *g* for 30 min at 4°C in PBS, pH 8.0, and then incubated in reducing Laemmli SDS sample buffer (11) for 1 h at 37°C before being boiled for 2 min, until solubilized. Aliquots were frozen at −70°C prior to analysis. Samples were designated OMP, OMP-C3b (OMP preincubated with NHS), OMP-C3b-Ab (OMP-C3b preincubated with NHS supplemented with 10% IHS), or OMP-heated NHS.

Electrophoresis and immunoblotting. Samples of IgG-depleted NHS and samples of OMP complexes (described above) were electrophoresed under reducing conditions on 7.5% polyacrylamide gels by the method of Laemmli (11). Silver staining demonstrated protein banding patterns characteristic of *C. trachomatis* L2 outer membrane complexes (20). The samples were then transferred to nitrocellulose membranes by the method of Towbin et al. (27) and immunoblotted to detect either human complement C3 (C3 probe) or the chlamydial MOMP (MOMP probe).

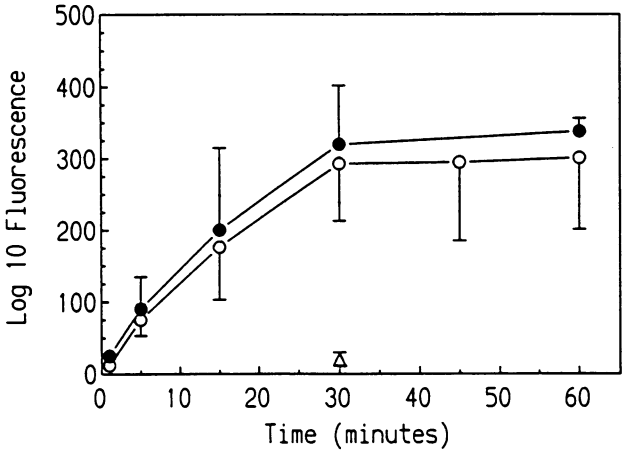

FIG. 1. Deposition of C3 on EBs of *C. trachomatis* as determined by flow cytometry. EBs were incubated in fresh NHS (○), NHS supplemented with 10% IHS (●), or heat-inactivated (56°C for 30 min) NHS (△). Bound C3 was detected by incubation in FITC-conjugated goat antibody to human C3. The data shown are the means ± standard deviation for four experiments.

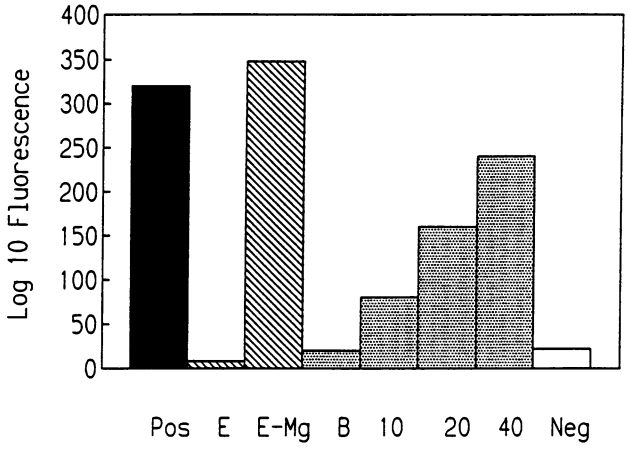

FIG. 2. Determination of the complement activation pathway. C3 bound to EBs was detected with FITC-conjugated goat antibody to human C3. NHS preincubated in 20 mM EDTA (E) inhibited activation of both complement pathways. Adding back 25 mM $MgCl_2$ (E-Mg) selectively restored the alternative pathway to a level comparable to that of the fresh-NHS positive control (Pos). Substituting complement factor B-deficient serum for normal serum (B) decreased binding to the level in the heat-inactivated serum negative control (Neg). Supplementing factor B-deficient serum with 10, 20, or 40% heat-inactivated IHS (10, 20, and 40, respectively) selectively promoted the classical pathway; however, binding was not restored to the level with E-Mg.

C3 was detected by using a goat antibody to human C3 (Sigma) followed by horseradish peroxidase-labeled rabbit antibody to goat IgG (Sigma). MOMP was detected by using a mouse monoclonal antibody directed towards a species-specific epitope on MOMP (kindly provided by I. W. Maclean, University of Manitoba) followed by horseradish peroxidase-labeled rabbit antibody to mouse IgG (Dako Corp., Santa Barbara, Calif.). Control blots substituted normal goat serum (C3 probe) and normal mouse serum (MOMP probe) for the primary antibodies. An additional control blot for nonspecific binding of goat antibody included goat anti-mouse IgG antiserum (Sigma) as a primary antibody. Blots were probed with anti-C3 and then with anti-MOMP and also in the reverse order. On one blot, an MAb to the 57-kDa OMP2 chlamydial protein was used (kindly provided by I. W. Maclean, University of Manitoba). The sensitivity of the Western blots was increased by the use of enhanced chemiluminescence (ECL; Amersham Inc.). This technique also allowed extinguishing of the first probe followed by sequential reprobing of the same blot with a different primary antibody (ECL protocol; Amersham).

Hydroxylamine cleavage of OMP-C3b complexes. Samples containing OMP-C3b complexes were incubated for 45 min at 37°C in freshly prepared 2 M hydroxylamine, pH 10.5. Hydroxylamine was removed by dialysis overnight at 4°C against 0.1% sodium dodecyl sulfate (SDS) in distilled water. Samples were then separated by SDS–7.5% polyacrylamide gel electrophoresis (PAGE) as described above. Control samples were treated with distilled water instead of hydroxylamine.

RESULTS

Deposition of C3b on EBs and effect of specific antibody. C3 binding to EBs of serovar L2 in NHS increased abruptly within 5 min, reaching a plateau at 30 min (Fig. 1). When 10% IHS (specific antibody) was introduced, neither the rate of increase nor the level of maximal binding ($\log_{10}$ 320 ± 81.9 fluorescence units with antibody, and $\log_{10}$ 292 ± 80.4 fluorescence units without antibody) was altered. Control FITC-conjugated normal goat serum showed negligible fluorescence (data not shown). EBs incubated in heat-inactivated NHS had a mean fluorescence intensity of $\log_{10}$ 20.1 ± 8.2 fluorescence units, indicating minimal C3b binding. Subsequent experiments with NHS preincubated in EDTA revealed no detectable fluorescence. This method is therefore a better negative control for activation and binding of C3b than heat inactivation.

Determination of complement activation pathway. Figure 2 combines the data from two sets of experiments, each designed to isolate one of the complement activation pathways and to determine its role in the deposition of C3b on chlamydial EBs. When normal serum was preincubated with EDTA, C3 bound to EBs was reduced to a minimal level. However, when the Mg^{2+} ion was added back, selectively restoring the alternative pathway, binding of C3b returned to the level of the fresh-serum positive control.

To examine the contribution of the classical pathway, factor B-deficient serum was substituted for normal serum, preventing the alternative pathway from being activated. Binding was reduced to the level in heat-inactivated serum. When factor B-deficient serum was enriched with 10, 20, or 40% heat-inactivated IHS, to promote the antibody-dependent classical pathway, binding was restored only partially, but proportional to the antibody concentration, compared with that of the NHS positive control.

Immunoblotting of OMP-C3b complexes. Extracted OMP-C3b complexes were electrophoresed under reducing conditions on 7.5% polyacrylamide gels and transferred to nitrocellulose membranes. Probing with goat antiserum to the human complement C3 α-chain revealed protein bands of various apparent sizes (Fig. 3A). Lane 1, IgG-depleted NHS, demonstrates bands of 110 and 67 kDa, the α-chains of C3 and C3bi, respectively. On blots probed with anti-C3 reactive with both α- and β-chains, the β-chain was also seen at 75 kDa (data not shown). In lane 2, anti-C3 antibody

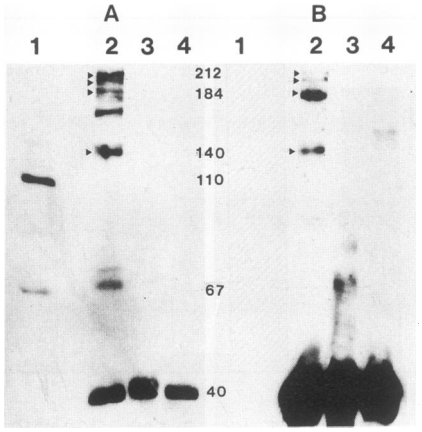

FIG. 3. Detection of chlamydial OMP-C3b complexes by immunoblot. EBs were incubated in either serum or PBS, and the outer membranes were extracted as described in Materials and Methods. (A) Probing with an antibody to complement C3 reveals binding complexes of 140, 166, 184, 202, and 212 kDa, representing the α-chain of C3b bound to OMPs (lane 2). Lane 1, NHS, demonstrates the α-chain of C3 at 110 kDa and a 67-kDa fragment of C3bi. Lanes 3 and 4 show EBs incubated in heat-inactivated serum and PBS, respectively. No complexes are visible. Bands at 40 kDa in lanes 2, 3, and 4 were unexpected and are discussed in the text. (B) After the C3 probe was extinguished, the same blot was reprobed with an MAb to the chlamydial MOMP. Lane 1, NHS, does not cross-react. Lane 2 demonstrates four of the five complexes seen in panel A (arrowheads). Lanes 2, 3, and 4 all demonstrate the intense signal of native MOMP at 40 kDa.

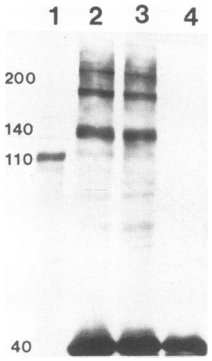

FIG. 4. Chlamydial OMP-C3b complexes in the presence of immune serum. EBs were incubated in either NHS (lane 2) or NHS supplemented with 10% IHS (lane 3). The outer membrane complexes were immunoblotted with an antibody directed against human complement C3. Lane 1, NHS; lane 4, OMP in the absence of serum. The bands at 40 kDa in lanes 2, 3, and 4 are discussed in the text.

detected OMP-C3b complexes of several sizes. As the α-chain of C3b normally migrates at approximately 100 kDa (after cleavage of the 10-kDa C3a fragment), the bands seen at 140, 166, 184, 202, and 212 kDa represent binding complexes of chlamydial proteins and C3b. Other bands detected at 40 kDa in lanes 2, 3, and 4 are discussed below.

Figure 3B shows the same blot as in Fig. 3A reprobed with an MAb to the MOMP after the C3 probe was extinguished. Blots exposed after being extinguished showed no bands prior to reprobing. Lane 1, IgG-depleted NHS, does not cross-react. Lanes 2, 3, and 4 (OMP-C3b, OMP-heated NHS, and OMP, respectively) react intensely at approximately 40 kDa, illustrating MOMP in its uncomplexed state. Lane 2 alone, OMP-C3b, shows bands of 140, 184, 202, and 212 kDa, corresponding to four of the five bands detected with the anti-C3 antibody. The MOMP of *C. trachomatis* has a molecular mass of approximately 40 kDa (3), and when covalently bound to the α-chain of C3b, it would migrate at 140 kDa. The bands seen at 184, 202, and 212 kDa may contain multimers of MOMP bound to C3b. These protein complexes, which line up at identical positions when the original ECL films are superimposed, have bound antisera to both C3 and MOMP, in both cases at masses higher than in their native states but consistent with complexes of C3b α-chain and MOMP or multimers of MOMP. Probing the blots first with anti-C3 antibody and then with anti-MOMP antibody, or in the reverse order, did not affect the result.

The binding complex appearing at 166 kDa in the C3 probe (lane 2, Fig. 3A) is unidentified, as it did not cross-react with an MAb to the MOMP or with an MAb to the chlamydial 57-kDa OMP2 (data not shown). In Fig. 3B, the MOMP probe in lanes 3 and 4 (OMP-heated NHS and OMP) does not react other than at 40 kDa, as expected, since these samples were preincubated in heat-inactivated serum and PBS, respectively (the band at 70 kDa, lane 3, was seen inconsistently and is probably a multimer of MOMP, and the faint band seen at 150 kDa, lane 4, was seen only in this particular blot). However, in the C3 probe, lanes 3 and 4 (panel A), as well as in lane 2 (OMP-C3b), reactivity was also detected at 40 kDa. A normal goat serum control blot and a goat anti-mouse IgG control blot did not show bands at 40 kDa or elsewhere (data not shown). The normal mouse serum control blot (MOMP probe control) showed only faint bands at 40 kDa (data not shown), most likely representing nonspecific binding of IgG by the MOMP, as has been shown previously (19).

Using previously published amino acid sequences, we looked for homology between the serovar L2 MOMP (29) and human complement C3 (6). Of interest was an aspartate-leucine-lysine (DLK) sequence that appeared in both the variable domain 3 of MOMP and the α-chain of C3. The significance of this finding has yet to be determined.

Immunoblots of OMP-C3b-Ab (EBs preincubated in NHS supplemented with 10% IHS before OMP extraction) were probed with anti-C3 and anti-MOMP as in the previous section, with OMP-C3b lanes included for reference. The results (Fig. 4) demonstrated identical binding patterns in both the presence and absence of specific antibody.

Hydroxylamine cleavage of OMP-C3b complexes. To determine whether the reactive thioester of C3b had bound to free hydroxyl or amino groups, we attempted to dissociate the OMP-C3b complexes with 2 M hydroxylamine. Figure 5 shows an immunoblot probed with antiserum to human complement C3. Lane 1 (NHS) demonstrates reactivity with the α-chain of C3 at approximately 110 kDa and a 67-kDa α-chain fragment of C3bi (as described above, Fig. 3A). In lanes 2 and 3, the OMP-C3b complexes were treated exactly the same except that in lane 2 distilled water was substituted for hydroxylamine. Lane 2 demonstrates the high-mass OMP-C3b binding complexes seen previously. Lane 3, hydroxylamine treated, shows greatly diminished high-mass binding complexes along with strong bands at approximately 100 kDa, the α-chain of C3b, and 67 kDa, a fragment of the α-chain of C3bi containing the C3b binding site. Residual high-mass binding complexes are still seen faintly in lane 3, suggesting minimal binding of C3b by hydroxylamine-resis-

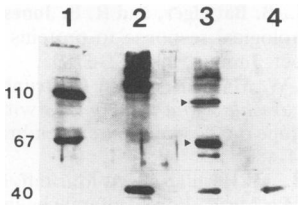

FIG. 5. Hydroxylamine cleavage of the OMP-C3b complexes. Complexes were incubated for 45 min in 2 M hydroxylamine, electrophoresed on 7.5% polyacrylamide gels, and probed with an antibody to complement C3. Lane 1, NHS, shows the α-chain of C3 at 110 kDa and a 67-kDa fragment of C3bi. Lanes 2 and 3 are samples of OMP-C3b complexes untreated (lane 2) and treated (lane 3) with hydroxylamine. Lane 2 demonstrates the high-mass complexes described previously. Lane 3 reveals greatly diminished high-mass complexes, along with the α-chain of C3b at 100 kDa and a 67-kDa fragment of the α-chain of C3bi (arrowheads). Lane 4, OMP alone, shows no binding complexes. The 40-kDa bands in lanes 2, 3, and 4 were unexpected and are discussed in the text.

tant imidoester bonds or incomplete cleavage by hydroxylamine. The majority of binding appears to be due to C3b binding to free hydroxyl groups, resulting in hydroxylamine-sensitive ester linkages (12). These experiments were also repeated with OMP-C3b-Ab, and the results (not shown) indicated no change when binding occurred in the presence of specific antibody.

DISCUSSION

These results demonstrate that specific chlamydial antibody does not mediate the infection-neutralizing enhancement effect of complement by augmenting binding of C3. The mechanism operative in antibody-complement neutralization is unknown. It is thought that the terminal membrane attack complex is involved in part, and antibody may facilitate membrane attack complex effects by configuring C5b-9 in the host cell membrane, as has been demonstrated for *Borrelia burgdorferi* (10). It is clear from recently published reports, however, that *C. trachomatis* neutralization may occur without the participation of C5b-9. For example, antibody neutralization of serovar K by heat-inactivated human serum in phagocyte-free medium is augmented only if cobra venom factor, a surrogate for C3, is added. This effect appears to be serovar dependent, as it did not occur with the L2 serovar (14).

Our findings indicate that C3 binds to chlamydial EBs primarily through the alternative complement pathway. These results are consistent with previous studies demonstrating that the alternative pathway-mediated neutralization of different *C. trachomatis* serovars is more efficient than that by the classical pathway, especially at higher serum concentrations (13).

Chlamydial lipooligosaccharide may be the initial complement-activating moiety, but this is as yet unclear. Probes of intact EBs with colloidal-gold-labeled lipooligosaccharide MAb have failed to demonstrate any surface-exposed lipooligosaccharide epitopes (5). However, an earlier study by Maclean et al. (15) reported a surface-exposed protein of 10 kDa that was presumed to be chlamydial lipooligosaccharide. Caldwell and Hitchcock (2) have also described a surface-exposed MAb to chlamydial lipooligosaccharide.

The MOMP appears to be a major target protein for C3 binding, likely in the surface-exposed variable domains. Amino acid mapping has revealed residues with free hydroxyl groups in all four variable domains (1, 29). These free hydroxyl groups seem to be the major binding sites for C3 on EBs, as evidenced by disruption of the binding by hydroxylamine. Other OMPs may also be involved, as an additional OMP-C3b complex was detected that did not react with the MOMP or 57-kDa protein MAb probes.

An unexpected and intriguing finding is the binding of goat anti-human C3 antibody to a 40-kDa EB protein after incubation of EBs in PBS, although the intensity of the band was not as great as after incubation in fresh serum. Normal goat serum and the goat anti-mouse IgG control antibodies did not react with this protein. If this band appeared because of nonspecific binding of goat IgG by the MOMP, binding would also be expected to be seen with these goat antibody controls. This was indeed the case with the control for the MOMP probe, normal mouse serum, although the intensity was faint compared with that of the mouse MAb against the MOMP. As this observation with the C3 probe was made consistently, the binding of anti-C3 at 40 kDa raises the possibility of C3-like antigenicity in the MOMP. The possibility that this binding is due to a degradation fragment of bound C3bi comigrating at 40 kDa with the MOMP is discounted by the fact that this observation was made in the OMP lane also, i.e., chlamydial EBs preincubated in PBS, not serum. Thus, we interpret this to suggest the possibility of antigenic mimicry of C3 in MOMP.

The finding of the identical amino acid sequence (DLK) in the variable domain 3 region of the L2 MOMP and in the α-chain of C3 and C3bi may or may not be relevant to this observation. This sequence has positively and negatively charged amino acids flanking a neutral amino acid, and in this aspect is similar to the RGD sequence which binds to the CR3 receptor (23, 28). The fact that, at least in *C. psittaci* infection of monocyte-derived macrophages, EBs utilize, in part, the CR3 and CR4 receptors for entry (22) argues for further study of the significance of this finding to *C. trachomatis*.

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