Resistance to Both Complement Activation and Phagocytosis in Type 3 Pneumococci Is Mediated by the Binding of Complement Regulatory Protein Factor H

CHRIS NEELEMAN,1,2 SIBYL P. M. GEELEN,1,3 PIET C. AERTS,1,3 MOHAMMED R. DAHA,4 TOM E. MOLLNES,3 JOHN J. ROORD,3 GEORGE POSTHUMA,6 HANS VAN DIJK,1 AND ANDRÉ FLEER1,3*

Department of Infectious Diseases, University Hospital for Children and Youth, "Het Wilhelmina Kinderziekenhuis," and Eijkman-Winkler Laboratory of Medical Microbiology1 and Department of Cell Biology,6 University Medical Centre, Utrecht, University Hospital Nijmegen, Nijmegen,2 and University Hospital Leiden, Leiden,4 The Netherlands, and Nordland Central Hospital, Bodø, Norway5

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To study the role of surface-associated proteins in the virulence of Streptococcus pneumoniae, we used two serotype 3 strains, ATCC 6303 and WU2, and two PspA-negative mutants of WU2, an encapsulated one, JY1123 (Caps+/PspA+), and an unencapsulated one, DW3.8 (Caps-/PspA-). ATCC 6303 and WU2 were highly virulent in mice, while the virulence of JY1123 was slightly decreased (50% lethal doses [LD50s], 24, 6, and 147 CFU/mouse, respectively); DW3.8 was avirulent (LD50, 2 × 10^8 CFU). In vitro, ATCC 6303, WU2, and JY1123 (Caps+/PspA+) strongly resisted complement activation and complement-dependent opsonophagocytosis, whereas DW3.8 (Caps-/PspA-) was easily phagocytized in fresh serum. Trypsin treatment of ATCC 6303, WU2, and JY1123 (Caps+/PspA+) resulted in enhanced complement activation and complement-dependent opsonophagocytosis. Trypsin had no deleterious effect on the polysaccharide capsule. In addition, trypsin pretreatment of ATCC 6303 strongly reduced virulence upon intraperitoneal challenge in mice. This indicated that surface proteins play a role in the resistance to complement activation and opsonophagocytosis and contribute to the virulence of type 3 pneumococci. In subsequent experiments, we could show that the modulation of complement activation was associated with surface components that bind complement regulator factor H; binding is trypsin sensitive and independent of prior complement activation. Immunoblotting of cell wall proteins of the virulent strain ATCC 6303 with anti-human factor H antibody revealed three factor H-binding proteins of 88, 150, and 196 kDa. Immunogold electron microscopy showed a close association of factor H-binding surface proteins with the outer surface of the cell wall. The role of these factor H-binding surface proteins in the virulence of pneumococci is interesting and warrants further investigation.

Despite the availability of effective antibiotics and a multifaceted capsular polysaccharide vaccine, Streptococcus pneumoniae remains an important pathogen to humans of all ages (4). This microorganism is the predominant causative agent of otitis media, community-acquired pneumonia, septicemia, and meningitis. Infections due to pneumococci are most frequently seen in infants, elderly persons, and immunocompromised patients (13, 25, 33).

It has been established that the polysaccharide capsule of the pneumococcus is an important virulence factor (1, 44). Watson and Musher (43) reported the production of pneumococcal mutant strains, lacking detectable polysaccharide capsule, from an encapsulated serotype 3 strain by Tn916 transposon insertion; the unencapsulated mutants had greatly reduced virulence in mice compared to the parental strain. However, it is clearly evident that among the approximately 90 known capsular serotypes, the degree of virulence varies widely and only a minority of the pneumococcal serotypes cause infections in humans (15, 42). Additionally, there is considerable difference in virulence among pneumococcal strains with identical capsular serotypes.

Several studies have demonstrated the existence of noncapsular virulence factors in pneumococci (6, 18, 31, 34). Briles and McDaniel established that the expression of a surface protein, pneumococcal surface protein A (PspA), is associated with the virulence of pneumococci in mice (8, 9, 26–28). In addition, strong evidence for an independent role of pneumolysin in the virulence of pneumococci has been presented (5, 24, 35). Thus, to date PspA and pneumolysin are the only well-characterized noncapsular virulence factors of pneumococci. It is also evident, however, that the spectrum of noncapsular virulence factors is still unknown, and their quantitative contribution to virulence is therefore poorly defined. Opsonophagocytosis is thought to play an important role in host defense against pneumococci (11, 12, 20, 40, 46). This process is initiated by complement activation via either antibody-dependent or antibody-independent pathways (11). Pneumococcal strains differ in their ability to activate the complement cascade (14, 39). The determinants for these differences, however, remain unclear, although the type of pathway and the extent of interaction of complement with the various pneumococcal capsular polysaccharides may, in part, explain these differences.

Hostetter previously showed that although both cell wall and capsular polysaccharide of type 3 pneumococci activate complement, leading to C3b deposition on both cell wall and capsule, type 3 pneumococci strongly resist phagocytosis (21). Angel et al. subsequently demonstrated that type 3 pneumococci express C3-degrading activity associated with the cell wall...
(3). The underlying mechanism, however, was not further explored. The purpose of the present paper is to define the role of surface-associated proteins of type 3 pneumococci in resistance to complement activation and opsonophagocytosis and to identify the mechanisms involved in this resistance.

MATERIALS AND METHODS

Animals. Male outbred Swiss mice were used for 50% lethal dose (LD₅₀) determinations. They were obtained from Harlan CPB (Zeist, The Netherlands), maintained in the animal facilities of Utrecht University, and used at 8 to 14 weeks of age.

LD₅₀ determination. Groups of five mice were injected intraperitoneally (i.p.) with 0.5 ml of a 10-fold dilution series of bacterial suspensions (1 × 10⁶ CFU/ml) in saline. Deaths were recorded over an 8-day period. LD₅₀ values were calculated by the method of Reed and Muench (32).

Buffers. Phosphate (20 mM)-buffered saline (PBS) (pH 7.4) was used for washing bacteria. Veronal (5 mM)-buffered saline (pH 7.4) containing 0.15 mM Ca²⁺ and 5.5 mM Mg²⁺ (VSB⁺) and veronal (5 mM)-buffered saline containing 10 mM EDTA (EVA-B) or 8 mM EGTA and 2.5 mM magnesium (EGTA-VB) were used as incubation buffers in complement assays. All buffers were prepared from a 5× stock solution (41).

For the trypsin treatment of bacteria, VSB⁺ was used. Hanks balanced salt solution (HBSS) containing 0.02% gelatin (GHBISS) was used for the dilution of serum and bacteria in the phagocytosis assay. Alsever's old solution (114 mM citrate, 27 mM glucose, 72 mM sodium chloride [pH 6.1]) served to store chicken blood.

Rabbit polyclonal anti-serotype 3 pneumococcal serum. ATCC (ATCC 6303) was obtained from the American Type Culture Collection (Rockville, Md.). Wild-type 3 strain WU2 and its encapsulated PsPα-negative mutant JY1123 was provided by L. S. McDaniel (Birmingham, Ala.). Strain DW3.8 is generated by conjugal transfer of transposon Tn916 from donor strain Enterococcus faecalis CG110 to the genome of WU2 (43). The pneumococci were grown to mid-logarithmic phase at 37°C in Todd-Hewitt broth (Difco, Detroit, Mich.) supplemented with 0.5% yeast extract in a 5% CO₂ atmosphere. After incubation, the bacteria were washed three times with PBS.

Pneumococci were heat killed by being incubated for 60 min at 56°C. Subsequently, the bacteria were washed three times in PBS and stored until use.

The strains are designated according to their phenotypic characteristics with respect to the presence or absence of capsule and PsPα; i.e., ATCC 6303 and WU2 are designated (Caps+/PsPα⁺), DW3.8 is designated (Caps⁻/PsPα⁻), and JY1123 is designated (Caps⁻/PsPα⁻).

Enzyme treatment of pneumococci. A total of 10⁹ CFU of heat-killed pelleted bacteria was suspended in 1 ml of PBS containing 1 mg of trypsin (Boehringer GmbH, Mannheim, Germany) purified by crystallization. The bacterial suspensions were incubated at 37°C for 1 h. After being washed, the bacteria were resuspended in VSB⁺ to 10⁶ CFU per ml.

Choline chloride treatment of pneumococci. A total of 10⁹ pelleted pneumococci were resuspended for 30 min at room temperature on a rotator in 1 ml of PBS containing 2% choline chloride (Riedel-de Haen, Seelze, Germany). After treatment, the bacteria were washed three times and subsequently resuspended to 10⁹ CFU/ml for analysis in test buffer (either Veronal buffer [VSB⁺] or GHBISS) (10⁹/ml).

Normal human serum. Human serum was collected from 30 healthy adults and pooled. The pool is hereafter referred to as normal human serum (NHS). NHS was stored in 1-ml aliquots at −20°C until use.

Erythrocytes. Chicken blood, diluted 1:1 in Alsever's old solution, was obtained from bioTrading (Wilnis, the Netherlands) and used as the source of chicken erythrocytes. Before use, the cells were washed three times with saline (0.85%).

PMN. Polymorphonuclear leukocytes (PMN) were isolated from heparinized human blood. A volume of blood was mixed 1:1 with PBS and layered on a Ficoll-Histopaque gradient consisting of 10 ml of Ficoll-Paque (Pharmacia, Uppsal, Sweden) and 12 ml of Histopaque (Sigma, St. Louis, Mo.). After centrifugation at 400 × g and room temperature for 20 min, PMN were collected from the upper part of the Histopaque and ice-cold RPMI was added. The cells were centrifuged at 250 × g, and the residual erythrocytes were lysed by resuspending the cells in 9 ml of ice-cold distilled water. After 30 s, the cell suspension was rendered isotonic with 10× PBS. Next, RPMI was added. The PMN fraction was centrifuged and resuspended in RPMI at 10⁷/ml. The cell concentration consisted of >95% PMN. Viability always exceeded 95% as determined by propidium iodide uptake.

ELISA for capsular polysaccharide content. A sandwich enzyme-linked immonosorbent assay (ELISA), performed in polyvinyl chloride microtiter plates, was used to determine the amount of capsule per pneumococcus (in femtograms/bacterial cell) from the optical density values obtained with the bacterial suspension.

Complement activation microassay. The complement activation assay was performed as described by Geelen et al. (17). Briefly, in the wells of U-shaped microtiter plates, 20 μl of pneumococcal suspension (1 × 10⁹ CFU/ml) was mixed with either a suspension of pneumococci (5 × 10⁸ CFU/ml) or purified type 3 capsular polysaccharide (a gift of A. F. M. Verheul, Eijkman-Winkler Institute, Utrecht, The Netherlands) at a starting concentration of 1.25 μg/ml, both serially diluted in saline, was added to the microtiter plates before incubation at 37°C for 1 h. Mouse polyclonal IgG against serotype 3 type strain ATCC 6303 polysaccharide (50 μl; 1:500 in PBS; 0.1% Tween 20) was used for the detection of serotype 3 capsular polysaccharide (incubation at 37°C for 1 h). The plates were developed by adding 50 μl of peroxidase-labeled goat anti-mouse IgG (heavy plus light chains; H+L⁺) (Nordic, Tilburg, The Netherlands), diluted 1:60,000 in PBS; 0.1% Tween 20, at 37°C for 1 h followed by extensive washing and then by the addition to each well of a mixture of tetramethylbenzidine (Sigma) and hydrogen peroxide (incubation at 37°C for 10 min). The plates were developed by adding 0.1 M sodium acetate–citric acid buffer (pH 5.5) containing 200 μl of a stock solution of tetramethylbenzidine (6.0 mg/ml of dimethyl sulfoxide) and 2 μl of a 30% hydrogen peroxide solution per 12 ml of buffer. Substrate conversion was stopped by the addition of 50 μl of 1 M sulfuric acid. Absorbance values at 450 nm were determined by using a ELISA reader (no. 3550; Bio-Rad, San Francisco, Calif.). The calibration curve was obtained by plotting optical density values against the concentration of purified type 3 capsular polysaccharide (in micrograms per milliliter) to calculate the amount of capsule per pneumococcus (in femtograms/bacterial cell) from the optical density values obtained with the bacterial suspension.

Fluorescence-activated cell sorter assay for PsPα expression. Eppendorf vials (1.5 ml) were coated with BSA (Boslar; Organon Teknika, Bostel, The Netherlands) by incubation with a 1% solution for 1 h at 37°C. The vials were washed three times with phosphate-buffered saline (PBS), and 50 μl of 10⁹ CFU/ml PBS was added. The vials were centrifuged at 15,000 × g for 7 min. The pellets were washed with 200 μl of PBS-specific mouse monoclonal antibody
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Table 1. Virulence of S. pneumoniae type 3 (ATCC 6303 and WU2) and type 3 mutant strains (JY1123 and DW3.8) in micea

| Pneumococcal strain | LD50 (CFU/mouse) |
|---------------------|------------------|
| ATCC 6303 (Caps+ /PspA−) | .......................... 24 |
| WU2 (Caps− /PspA−) | .......................... 0 |
| JY1123 (Caps− /PspA−) | .......................... 147 |
| DW3.8 (Caps− /PspA−) | .......................... 2.24 × 108 |

*a The virulence was determined by i.p. injection (see Materials and Methods).

**Figure 1.** Survival of mice after intraperitoneal challenge with S. pneumoniae ATCC 6303 (Caps+ /PspA−) pretreated with trypsin or buffer as a control (see Materials and Methods). The curves show survival time (days) of mice after an i.p. injection of 20 CFU/mouse (15 mice per group). Trypsin-treated pneumococci were significantly different (*P < 0.01) from pneumococci treated with buffer as estimated by Kaplan-Meier survival analysis.
WU2 activated the complement cascade poorly. In contrast, the avirulent (Caps$^+/PspA^-$) mutant DW3.8 and the intermediate virulent (Caps$^+/PspA^-$) mutant of WU2, JY1123, were powerful and intermediate complement activators, respectively.

The role of the classic and alternative pathways in complement activation by the different pneumococcal strains was investigated by using EGTA- and EDTA-containing buffers during the activation step. Complement activation by all strains was completely blocked in the presence of Mg$^{2+}$-EGTA or EDTA-serum (results not shown).

To investigate at which level the virulent strains interfered with complement activation, the generation of soluble TCCs in 8% serum was estimated after incubation with the strains. Figure 3 shows that the formation of TCC was high in serum incubated with (Caps$^+/PspA^-$) mutant DW3.8 while significantly fewer TCC were generated in serum after incubation with ATCC 6303, WU2 (both Caps$^+/PspA^-$), and JY1123 (Caps$^+/PspA^-$).

**TABLE 2. Complement activation induced by S. pneumoniae type 3 (ATCC 6303 and WU2) and type 3 mutant strains (JY1123 and DW3.8)**

| Pneumococcal strain | Z (no. of active sites/erythrocyte) |
|---------------------|----------------------------------|
| ATCC 6303 (Caps$^+/PspA^-$) | $0.11 \pm 0.03$ |
| WU2 (Caps$^+/PspA^-$) | $0.13 \pm 0.01$ |
| JY1123 (Caps$^+/PspA^-$) | $0.24 \pm 0.02**$ |
| DW 3.8 (Caps$^+/PspA^-$) | $0.51 \pm 0.03***$ |

$^a$ Complement activation was measured as lysis of bystander cells (chicken erythrocytes) and is expressed as the Z value, representing the number of active hemolytic sites per erythrocyte (mean ± standard errors of three separate experiments). Hemolysis was measured in the presence of $6 \times 10^9$ pneumococci in a reaction volume of 150 µl in a microtiter plate (see Materials and Methods).

$^b$ JY1123 and DW3.8 were significantly different from WU2 (**, $P < 0.01$; ****, $P < 0.001$ [Student’s t test]).

**FIG. 4. Effect of trypsin treatment of pneumococci on complement activation by type 3 strains ATCC 6303 and WU2 (both Caps$^+/PspA^-$) and unencapsulated and PspA-deficient mutants of WU2. Complement activation, determined by the hemolytic assay, is expressed as a Z value, representing the number of active hemolytic sites per erythrocyte (y axis). Bars represent mean ± standard errors of three independent experiments. S3 (ATCC 6303) and WU2 are fully encapsulated parent strains, JY1123 is a PspA-deficient mutant of WU2 (Caps$^+/PspA^-$), and DW3.8 is an unencapsulated mutant of WU2 (Caps$^+/PspA^-$). Trypsin-treated bacteria (open bars) were significantly different from untreated bacteria (hatched bars), and both buffer-treated JY1123 and DW3.8 were significantly different from buffer-treated WU2 (**, $P < 0.01$; ****, $P < 0.001$ [Student’s t test]).
shows that the pretreatment of ATCC 6303, WU2 (both Caps/PspA), or JY1123 (Caps/PspA) with trypsin considerably increased complement activation by these strains whereas trypsin treatment of DW3.8 (Caps/PspA) only moderately increased complement activation. Table 3 demonstrates that the capsule content of ATCC 6303, WU2, and JY1123 was not affected by trypsin pretreatment.

The effect of trypsin pretreatment on opsonophagocytosis of the virulent strain ATCC 6303 is shown in Fig. 5. Trypsin treatment strongly enhanced the phagocytosis of ATCC 6303 by human PMN after opsonization in human serum. Similar results were obtained after trypsin treatment of strains WU2 and JY1123, while trypsin treatment did not affect the opsonophagocytosis of DW3.8 (data not shown).

**Factor H binding by pneumococcal strains.** The above experiments indicated that virulent pneumococcal strains ATCC 6303, WU2, and the (Caps/PspA) mutant of WU2, JY1123, interfered with complement activation at the level of the generation of either the C3 or C5 convertase. For this reason, we investigated whether the pneumococcal strains bound one of the known complement-regulatory proteins. The experiments showed that the encapsulated pneumococcal strains, upon incubation in human serum, did not react with polyclonal antibodies against human decay-accelerating factor or complement receptor 1 or with antibodies against the soluble inhibitors C1 inhibitor and C4-binding protein (results not shown). However, significant reactivity with polyclonal anti-factor H serum was observed after incubation of strain ATCC 6303, WU2, or JY1123 with NHS or purified factor H under conditions similar to those in the bystander hemolysis assay (17) (Fig. 6). Figure 6 demonstrates that considerably less factor H bound to the surface of DW3.8 (Caps/PspA). This was the case not only under conditions permitting complement activation but also in the presence of EDTA. Upon pretreatment of bacteria with trypsin, factor H binding by WU2, ATCC 6303, and JY1123 was strongly decreased (Fig. 6). It should be noted, however, that trypsin treatment also removed PspA from strains ATCC 6303 and WU2. Figure 7 also confirms the virtual absence of PspA from JY1123, which vigorously bound factor H (Fig. 6). Moreover, selective removal of PspA from WU2 and ATCC 6303 by pretreatment of the bacteria with choline chloride did not influence factor H binding (data not shown).

**Immunoblotting of factor H-binding proteins of pneumococci.** The results of the immunoblotting experiments with cell wall proteins from *S. pneumoniae* ATCC 6303 are presented in Fig. 8. After SDS-PAGE the electrophoresed proteins were blotted onto a nitrocellulose membrane and subsequently probed for factor H-binding proteins by sequential incubation with NHS, polyclonal rabbit anti-human factor H, and, finally, horseradish peroxidase-labeled goat anti-rabbit IgG antibody. The immunoblot clearly shows two sharply demarcated bands of 88 and 196 kDa. In addition, a somewhat fainter band of 88 kDa appeared.

**Immunoelectron microscopy of factor H-binding sites on the pneumococcal cell wall.** The results of immunoelectron microscopy of pneumococci after sequential incubation with hu-
man serum, anti-human factor H antibody, and gold-labeled protein A are depicted in Fig. 9. The pneumococcal cells appeared morphologically intact, with clearly distinguishable plasma membranes and cell walls. The mild fixation in combination with ultrathin cryosectioning of the bacteria allowed a very accurate localization of factor H binding as visualized by gold particle deposition. It is evident that gold particles were associated almost exclusively with the outer surface of the pneumococcal cell wall, either directly adjacent to it or embedded in or superimposed on the “fuzzy” layer covering the cell wall (Fig. 9). In some instances, there appeared to be an association of gold particles with structures extending from the cell wall (Fig. 9). However, these structures could not be visualized any better, despite attempts at higher magnification.

DISCUSSION

The results of the present study show that virulent type 3 S. pneumoniae strains bind the complement-regulatory protein factor H. In addition, our findings strongly suggest that the binding of factor H by virulent type 3 pneumococci is a key factor in the resistance of these strains to complement activation and complement-dependent opsonophagocytosis, which is an essential part of the host defense against these pathogenic bacteria (10, 13, 20, 40, 46). This is the first study that shows that virulent pneumococci are capable of binding a complement-regulatory protein, in this case factor H, independent of the previous activation of complement.

The binding of complement-regulatory proteins is a natural process during complement activation by autologous cells to protect these cells, notably blood cells, from complement-induced lysis. In this process, fixing of complement-regulatory proteins such as factor H is strictly complement activation dependent. Factor H binding occurs to both C3 and C5 convertases, strongly restricting the formation of lytic C5b-C9 complexes. Indeed, we could show that the generation of these terminal complement complexes by the virulent type 3 strains ATCC 6303 and WU2 was greatly diminished compared with that by the nonvirulent mutant strain DW3.8, which showed diminished binding of factor H and was therefore a potent activator of complement.

It has been shown previously that in type 7 pneumococci both capsule and cell wall may fix factor H, but this binding was found to be strictly C3b dependent (10). In contrast, in both ATCC 6303 and WU2, factor H binding was EDTA resistant and therefore independent of C3b deposition on either cell wall or capsule.

Thus, our data indicate that virulent type 3 pneumococci carry specific receptors or binding sites for factor H. Factor H binding was strongly decreased by the pretreatment of strains ATCC 6303 and WU2 with trypsin, indicating that the putative factor H receptors are proteinaceous. In addition, trypsin pretreatment strongly enhanced complement activation and com-
lement-dependent phagocytosis by ATCC 6303 and WU2. Finally, trypsin pretreatment strongly decreased the virulence of ATCC 6303 upon i.p. challenge in mice. Together, these data indicate that the binding of factor H, complement activation, opsonophagocytosis, and the virulence of type 3 pneumo-
cocci are modulated by the same surface-associated proteins. These findings also exclude the capsule of type 3 pneumococci as a major target of factor H binding, since the extent of the capsule was not affected by trypsin treatment. In this respect, i.e., by the binding of factor H, the putative protein receptors on three pneumococci act similarly to M protein, an important virulence factor of group A streptococci (16, 19). Collectively, these data suggest that the putative factor H-binding proteins constitute an independent virulence factor of type 3 pneumo-
cocci and, possibly, other serotypes as well.

This conclusion is further supported by the fact that the binding of factor H appears to be independent of the well-
established virulence factor PspA. PspA is an important deter-
mnant of pneumococcal virulence independent of capsular type. It is interesting that PspA has structural properties sim-
ilar to streptococcal M protein (47). However, the PspA-defi-
cient mutant of WU2, JY1123, bound similar or even larger amounts of factor H than its parent strain did. Together, these findings clearly indicate that PspA is not a major candidate for the binding of factor H by virulent type 3 pneumococci. Nev-

ertheless, a minor role of PspA in interfering with complement activation and opsonophagocytosis may be based on a mecha-
nism different from factor H binding.

The factor H-dependent inactivation of C3 appears to be independent of the C3 degradation activity displayed by pneumo-
coccal serotypes 3, 4, and 14 as described by Angel et al. (3). These investigators showed that the degradation of purified C3 by these pneumococcal serotypes occurred in the absence of any other serum or complement proteins, notably in the ab-
sence of complement-regulatory proteins.

Hypothetically, C3 inactivation and degradation by type 3 pneumococci could be a two-stage process. First, bound C3 is inactivated by receptor-bound factor H, and subsequently, bound C3 may be degraded by the putative C3 protease as described by Angel et al. (3). Finally, the immunoblotting and ultrastructural studies revealed that there are probably multi-
ple factor H-binding proteins present on the pneumococcal cell wall. Specifically, the immunoblotting experiments clearly showed three bands of 88, 150, and 196 kDa. Whether these bands represent different polymerizations of the same basic protein molecule or completely different proteins must await structural analysis. Sequence analysis should also indicate whether the factor H-binding proteins of type 3 pneumococci are structurally related to the M proteins of group A strepto-
cocci, which possess factor H-binding domains (16, 19).

The ultrastructural studies involving immunogold electron microscopy revealed two basic modes of factor H binding to the pneumococcal cell wall, either directly adjacent to the outer surface of the cell wall or apparently associated with projections extending from the cell wall. The structural features of these apparent surface projections could not be ana-
lyzed any further in the present study despite attempts at better visualization at higher magnification. Together, these observations suggest that factor H binding by type 3 pneumococci is probably not dependent on a single molecule but is a result of a variety of proteins present on different sites on the pneumococcal surface. Apparently, these factor H-binding proteins are all sensitive to trypsin, and in this connection we made the interesting observation that trypsin treatment of ATCC 6303 considerably reduced virulence upon i.p. infection in mice. This is possibly explained by assuming that the syn-
thesis of surface proteins is apparently sufficiently delayed in the peritoneal cavity to permit efficient complement activation and opsonophagocytosis to proceed.

In conclusion, our results indicate that the virulence of the type 3 pneumococcal strains ATCC 6303 and WU2 is strongly related to their ability to resist complement activation and complement-mediated phagocytosis. This modulation of com-
plement activation appears to be associated with trypsin-sensitive surface components that are responsible for the comple-
ment activation-independent binding of factor H to the bacterial surface. Immunoblotting and immunogold electron microscopic studies suggested that factor H binding may be dependent on several proteins, which may be expressed on the pneumococcal surface in two different ways, i.e., either directly adjacent to the outer surface of the cell wall or associated with projections extending from the cell wall. The role of these interesting putative factor H-binding surface proteins in the virulence of pneumococci warrants further investigation.

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