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Immune Evasion of *Moraxella catarrhalis* Involves Ubiquitous Surface Protein A-Dependent C3d Binding

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The complement system plays an important role in eliminating invading pathogens. Activation of complement results in C3b deposition (opsonization), phagocytosis, anaphylatoxin (C3a, C5a) release, and consequently cell lysis. *Moraxella catarrhalis* is a human respiratory pathogen commonly found in children with otitis media and in adults with chronic obstructive pulmonary disease. The species has evolved multiple complement evasion strategies, which among others involves the ubiquitous surface protein (Usp) family consisting of UspA1, A2, and A2 hybrid. In the present study, we found that the ability of *M. catarrhalis* to bind C3 correlated with UspA expression and that C3 binding contributed to serum resistance in a large number of clinical isolates. Recombinantly expressed UspA1 and A2 inhibit both the alternative and classical pathways, C3b deposition, and C3a generation when bound to the C3 molecule. We also revealed that the *M. catarrhalis* UspA-binding domain on C3b was located to C3d and that the major bacterial C3d-binding domains were within UspA1$^{299–452}$ and UspA2$^{165–318}$. The interaction with C3 was not species specific since UspA-expressing *M. catarrhalis* also bound mouse C3 that resulted in inhibition of the alternative pathway of mouse complement. Taken together, the binding of C3 to UspAs is an efficient strategy of *Moraxella* to block the activation of complement and to inhibit C3a-mediated inflammation. ***The Journal of Immunology*****, 2011, 186: 000–000.**

Moraxella catarrhalis is a Gram-negative diplococcus that during the last two decades has increasingly gained recognition as a respiratory pathogen (1, 2). After pneumococci and *Haemophilus influenzae*, *M. catarrhalis* is the third most common pathogen isolated from middle ear fluid cultures from children with acute otitis media (3). Fifteen to 20% of all otitis media cases are caused by *M. catarrhalis* (4). *M. catarrhalis* is also an important cause of lower respiratory tract infections in patients with chronic obstructive pulmonary disease (5). The carrier rates of *M. catarrhalis* among chronic obstructive pulmonary disease patients were 5–32% when sputum samples were analyzed from patients that did not have any clinical signs of infection (Ref. 6 and references therein). Additionally, *M. catarrhalis* is one of the most common inhabitants of the pharynx of healthy preschool children, and within this group it becomes pathogenic upon simultaneous viral infection. During the first year of life, the carrier rate of *M. catarrhalis* is ~67% of all children and the species colonizes oropharynx and nasopharynx (7). This implies that *M. catarrhalis* as a commensal has numerous strategies to survive in the host (1). In recent years, focus has been on both its outer membrane protein composition and interactions with the human host (8, 9). Several virulence determinants of *M. catarrhalis* have been identified, including *M. catarrhalis* IgD-binding protein/hemagglutinin, protein CD, *M. catarrhalis* adherence protein, and the ubiquitous surface proteins (Usp) (10–15).

The UspA family consists of UspA1 (88 kDa), UspA2 (62 kDa), and the hybrid protein UspA2H (92 kDa) (16, 17). All strains express UspA1 in combination with either UspA2 or UspA2H. UspA1 and A2 are closely related and share significant sequence similarity in a region comprising 140 aa (16). UspA1 and UspA2 are multifunctional proteins, have highly conserved epitopes, and thus are of considerable interest as potential vaccine candidates (9, 18). The hybrid UspA2H consists of UspA1 and UspA2 with its N-terminal (head region) and C-terminal (near-end stalk and membrane-anchored region) having shared sequence similarity with UspA1 and UspA2, respectively (17, 19, 20). The stalk region is highly conserved between UspA1, UspA2, and UspA2H of different strains (17, 19, 21). The UspAs bind the extracellular matrix proteins laminin and fibronectin and are essential for attachment of *M. catarrhalis* to epithelial cells (17, 22–24), and they play important roles in *M. catarrhalis* serum resistance by interacting with C3, C4b-binding protein (C4BP), and vitronectin (25–28). Moreover, naturally acquired Abs to UspA1 and UspA2 are bactericidal (29, 30).

The complement system is a major part of the innate immune defense against pathogenic microorganisms, and activation of this system leads to a cascade of protein deposition on the bacterial

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Abbreviations used in this article: BHI, brain heart infusion; C4BP, C4b-binding protein; C3met, methylaminetreated C3; DGVB, dextrose gelatin veronal buffer; Efb, extracellular fibrinogen-binding protein; MAC, membrane attack complex; NHS, normal human serum; pAb, polyclonal Ab; Sbi, staphylococcal binder of IgG; Usp, ubiquitous surface protein.

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surface resulting in opsonization of pathogens, which facilitates phagocytosis, release of chemoattractants, as well as formation of the membrane attack complex (MAC). The classical pathway of the complement system is activated by target-bound Abs and C-reactive protein (31), the lectin pathway by binding of mannose-binding lectin, whereas the alternative pathway is spontaneously activated through direct contact with foreign particles or cells (32). All pathways lead to the formation of the C3 convertases, with subsequent cleavage of C3 to C3a and C3b. The complement protein C3 is one of the most abundant proteins in serum (1–2 mg/ml) and one of the most important components in the complement system. It is a large molecule composed of two chains, an α-chain (110 kDa) and a β-chain (75 kDa), which are held together with disulfide bonds (33). Upon activation, C3 undergoes marked conformational changes and proteolytic cleavages, which results in amplification of complement, anaphylatoxin (C3a) release, cell lysis, opsonization, and phagocytosis (34). The presence of an internal thioester bond is required for C3 deposition on molecular surfaces. C3 forms a link between all three pathways of complement activation and in addition it is also involved in the interaction between the innate and acquired immune systems (35). Patients with C3 deficiency have shown increased susceptibility to infections of a variety of bacterial pathogens (36). The C3 convertases cleave C3 to C3b and C3a and these fragments have important functions (37). C3a is an anaphylatoxin that induces proinflammatory activities. C3b binds factor B, which allows factor D to cleave factor B, resulting in a surface-bound C3 convertase, C3bBb. This alternative pathway C3 convertase will cleave more C3 in an amplification loop. The classical and lectin pathways form a C3 convertase from activation of C4 and C2, resulting in C4b2b (37). The alternative pathway functions as an amplification loop for the classical and lectin pathways and can also be spontaneously activated by covalent attachment of C3 to the surface of a microbe in the absence of complement inhibitors. Thereafter, C3b can bind covalently to the complement-activating surface, C4bC2b or C3bBb, forming the C5 convertases of the classical/lectin (C4bC2bC3b) and alternative (C3bBbC3b) pathways (37). The C5 convertases bind and cleave C5 to the proinflammatory analphylatoxin C5a and C5b. C5b initiates the assembly of the late complement components (C5b, C6, C7, C8, and C9), forming pores in the membrane. These channels result in the MAC (C5b–9), which reduces the osmotic pressure and causes lysis of the target cell. Lysis of the microorganism is one way for the complement system to clear the pathogen.

Human pathogens have evolved mechanisms to escape the innate immunity and complement. One frequent strategy used by microbes is to use the inhibitors of complement, including factor H, factor H-like protein 1, factor H-related protein 1, C4BP, and vitronectin for protection against complement-mediated killing (38–41). Importantly, the complement inhibitors are functionally active when bound to the surface of the pathogen and thus inhibit the complement-mediated attack. In fact, a number of microorganisms, including *H. influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Neisseria meningitidis* have all been reported to bind factor H and C4BP (42–49). Another strategy used by some pathogens is to directly inactivate C3 by producing C3-binding proteins. *Staphylococcus aureus*, *Porphyromonas gingivalis, Candida albicans*, and *Pseudomonas aeruginosa* are pathogens able to bind and/or degrade C3 to impair the immune response (39, 50–54). Staphylococcal complement inhibitor, extracellular fibrinogen-binding protein (Efb), Efb homologous protein, and staphylococcal binder of IgG (Sbi) all expressed by *S. aureus* and pH-regulated Ag 1 expressed by *C. albicans* are microbial proteins that bind C3 and its fragments and thereby inhibit the C3 convertases and further activation of complement (52, 55–59). Additionally, interpain A expressed by *Prevotella intermedia* efficiently degrades the α-chain of C3 and thereby inhibits all complement pathways (60).

M. catarrhalis has been shown to interact with C4BP, the inhibitor of the classical pathway, via UspA1 and UspA2, which bind to the α-chain of C4BP via complement control protein domains 2, 5, and 7 (26). C4BP bound to the *M. catarrhalis* cell surface retains its cofactor activity as determined by analysis of C4b degradation. A more intriguing strategy to combat complement is that *Moraxella* binds and neutralizes C3 (27). *M. catarrhalis* readily absorbed C3 from human serum in which complement was inactivated and noncovalently bound purified methylamine-treated C3 (C3met) via the UspAs. An interesting finding was that preincubation of serum with recombinant UspA1$^{50–770}$ and UspA2$^{30–539}$ results in absorption of C3 and increases the survival of a *M. catarrhalis* UspA1/A2H-deficient mutant. In contrast to *M. catarrhalis*, several other species including *Moraxella* subspecies lack the capacity to bind C3 or C3met (27).

In the present study we found that *Moraxella*-dependent C3 binding correlated with UspA expression and that the ability of *M. catarrhalis* to bind C3 contributed to serum resistance. Both UspA1 and UspA2 were able to inhibit activation of the alternative and classical pathways and C3a generation in activated human serum and thereby they most likely contribute to survival of *M. catarrhalis* in the human host. Interestingly, the UspA1 and A2 bound the C3d fragment of C3, and the C3d binding domains were located within UspA1$^{299–452}$ and UspA2$^{165–318}$. *M. catarrhalis* also bound murine C3, indicating that the interaction with C3 is not species specific. Taken together, UspA-mediated C3d binding of *M. catarrhalis* inhibits all complement pathways and thus contributes to the survival of the complement-mediated attack.

Materials and Methods

Bacterial strains and culture conditions

The clinical *M. catarrhalis* isolates ($n = 20$) were described earlier (61). *M. catarrhalis* were routinely cultured in brain heart infusion (BHI) liquid broth or on BHI agar plates at 37°C. *M. catarrhalis* RH4 mutants were made previously (26). The UspA1-deficient mutant was cultured in BHI supplemented with 1.5 μg/ml chloramphenicol (Sigma-Aldrich, St. Louis, MO), and the UspA2H-deficient mutant was incubated with 7 μg/ml zeocin (Invitrogen, Carlsbad, CA). The UspA2H of *M. catarrhalis* RH4 was formerly known as UspA2 (26, 28, 62). Both chloramphenicol and zeocin were used for growth of the UspA1/A2H double mutants.

Proteins and Abs

C3b-like molecules (C3met) were prepared by incubation of purified C3 with 100 mM methylamine (pH 7.6) for 1 h at 37°C and subsequent dialysis against 100 mM Tris-HCl, 150 mM NaCl (pH 7.5). C3, C3b, C3c, and C3d were purchased from Complement Technology (Tyler, TX). Recombinant C3d was expressed and purified as described previously (63). *S. aureus* Efb was cloned in the expression vector PET200D and expressed as an N-terminal His-tagged protein in *Escherichia coli*. Human factor H and C4BP were from Complement Technology. The manufacture of recombinant UspA1$^{50–770}$ and UspA2$^{30–539}$, which are devoid of their hydrophobic C termini and recombinant proteins spanning the entire UspA1$^{50–770}$ and UspA2$^{30–539}$, were described previously (24, 26).

The anti-UspA1/A2/A2H (anti-UspA) polyclonal Ab (pAb) was produced and purified as described (26). The rabbit anti-human C3d pAb, the mouse anti-human C5b–9 mAb, the FITC-conjugated swine anti-rabbit pAb, the FITC-conjugated rabbit anti-mouse pAb, the HRP-conjugated swine anti-rabbit pAb, and the HRP-conjugated rabbit anti-mouse pAb were purchased from Dakopatts (Glostrup, Denmark), the C3b mAb was from AbD Serotec (Düsseldorf, Germany), and the goat anti-human C3 and the rabbit anti-C3a pAb were from Complement Technology. The HRP-conjugated donkey anti-goat pAb was obtained from Serotec (Oxford, U.K.).

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Flow cytometry analysis

The capacity for different *M. catarrhalis* strains to bind C3met was analyzed by flow cytometry. Bacteria were grown on solid medium overnight and washed once in PBS containing 2% BSA (PBS-BSA) (Saveen Werner, Malmö, Sweden). Bacteria (10^8) were incubated with C3met (40 μg/ml) in PBS-BSA for 1 h at 37˚C. After two washings, the bacteria were incubated with FITC-conjugated goat anti-rabbit C3 pAb (Dakopatts). After two additional washes, bacteria were analyzed by flow cytometry (Epics XL-MCL; Coulter, Hialeah, FL). All incubations were kept in a final volume of 100 μl PBS-BSA and washings were done with the same buffer. The FITC-conjugated anti-C3 pAb was used as a negative control for each strain analyzed. To analyze UspA1/A2/A2H expression, bacteria were incubated with the anti-UspA pAb and washed as described above. FITC-conjugated goat anti-rabbit pAb was used for detection. To determine the efficiency of *M. catarrhalis* binding to C3, the wild-type strain and the UspA1/A2H-deficient double mutant were incubated with increasing concentrations of C3met (range, 1–750 μg/ml) or normal human serum (NHS) with 10 mM EDTA (NHS-EDTA) (0–60%). After washes the bacteria were incubated with a FITC-conjugated rabbit anti-human C3 pAb. After two final washes, bacteria were analyzed by flow cytometry.

To analyze the *M. catarrhalis* interaction with mouse C3, the wild type and the UspA1/A2H-deficient mutants were incubated with increasing concentrations of EDTA-treated mouse serum (0–10%) (Innovative Research, Novi, MI), followed by incubation with FITC-conjugated goat anti-mouse C3 pAb (ICN Biomedicals, Irvine, CA).

Serum bactericidal assay

M. catarrhalis strains were diluted in a buffer allowing activation of all complement pathways; that is, dextrose gelatin veronal buffer ($DGVB^{2+}$) (2.5 mM veronal buffer [pH 7.3], containing 70 mM NaCl, 140 mM glucose 0.1% [w/v] gelatin, 1 mM $MgCl_2$, and 0.15 mM $CaCl_2$). Bacteria (10^4 CFU) were incubated in 10% of pooled NHS diluted in $DGVB^{2+}$ in a final volume of 100 μl. After 30 min, 10-μl aliquots were removed and spread onto chocolate agar plates. After 18 h incubation at 37˚C, CFU were determined. NHS was obtained from healthy blood donors with informed consent.

Hemolytic assay

Sheep erythrocytes were washed with $DGVB^{2+}$ and rabbit erythrocytes with MgEGTA buffer (2.5 mM veronal buffer [pH 7.3] containing 70 mM NaCl, 140 mM glucose, 0.1% [w/v] gelatin, 7 mM $MgCl_2$, and 10 mM EGTA). The latter buffer allows study of exclusively the alternative pathway due to lack of calcium. After washings, the erythrocytes were resuspended at a concentration of 10^9 cells/ml. Sheep erythrocytes were preincubated with amboceptor (Dade-Behring, Marburg, Germany) (64) for 20 min at 37˚C, and thereafter washed twice with $DGVB^{2+}$. After washings, the IgG-sensitized sheep erythrocytes (1.25×10^7) were incubated with 40 μl NHS diluted in $DGVB^{2+}$ (1:80), and rabbit erythrocytes were incubated with 5% mouse serum diluted in MgEGTA buffer, in a volume of 150 and 100 μl, respectively. After 1 or 3 h at 37˚C, erythrocytes were centrifuged and the amount of lysed erythrocytes was determined by spectrophotometric measurement of released hemoglobin at 414 nm. For inhibition with UspA1 and UspA2, 40 μl NHS (1:80) was preincubated with 2.5–10 μg/ml recombinant $UspA1^{50–770}$ or $UspA2^{30–539}$ for 15 min at 37˚C, and thereafter added to the erythrocytes. For inhibition of the mouse complement system, 5% mouse serum was preincubated with 10–100 μg/ml recombinant $UspA1^{50–770}$ or $UspA2^{30–539}$ for 30 min at 37˚C.

Complement activation ELISA

The inhibitory effect of UspA1 and UspA2 on the alternative and classical pathways was analyzed in an ELISA. This assay is based on activation of the alternative or classical pathways with specific activators and detection of C3b or MAC (C5b–9) deposition as previously described (65). Microtiter plates (F96 Maxisorb; Nunc-Immuno Module, Roskilde, Denmark) were coated with LPS (10 μg/ml) (activating the alternative pathway) or IgM (2 μg/ml) (activating the classical pathway) overnight at 4˚C, washed four times with PBS with 0.1% Tween 20 (PBS-Tween), and blocked for 1 h at 37˚C with PBS containing 2% BSA. NHS was diluted in MgEGTA buffer (20 mM HEPES, 144 mM NaCl, 7 mM $MgCl_2$, and 10 mM EGTA [pH 7.4]) or gelatin veronal buffer (Complement Technology) and incubated in plates for 1 h at 37˚C. After washings, complement activation was measured using mouse anti-human C3b mAb or mouse anti-human C5b–9 mAb and HRP-conjugated rabbit anti-mouse pAbs. The reaction was developed with 1,2-phenylenediamine dihydrochloride (Dako). Complement activity was quantified by measuring the absorbance at 492 nm. The complement activation of NHS alone was set to 100%. For inhibition, $UspA1^{50–770}$ (5–50 μg/ml), $UspA2^{30–539}$ (5–50 μg/ml), factor H (5–50 μg/ml) (alternative pathway), C4BP (5–50 μg/ml) (classical pathway), or BSA (5–50 μg/ml) were preincubated with 20% NHS (alternative pathway) or 2% NHS (classical pathway) for 15 min at 37˚C and thereafter added to the wells.

Trypsin sensitivity assay

C3 (6 μg) was incubated with or without 15 μg recombinant $UspA1^{50–770}$, $UspA2^{30–539}$, or Efb in PBS containing 0.01 μg/ml trypsin at 37˚C for 0, 5, 10, 15, and 20 min. At each time point, aliquots (15 μl) were withdrawn and the reactions were inhibited with soybean trypsin inhibitor (1:1). The samples were thereafter separated by SDS-PAGE (10%) and visualized by Coomassie blue staining.

Analysis of C3a generation

For activation of the alternative pathway, NHS (5%) was incubated with zymosan (100 μg/ml) with or without increasing concentrations (0-50 μg/ml) of recombinant $UspA1^{50–770}$ or $UspA2^{30–539}$ in a buffer only allowing alternative pathway activation (PBS containing 3 mM $MgCl_2$ and 0.01 M EGTA) for 30 min at 37˚C. This MgEGTA-containing buffer inhibits the classical and lectin pathways and therefore allows separate analysis of the alternative pathway. The incubations were kept in a final volume of 100 μl. After incubation, the supernatants (7.5 μl/lane) were subjected to 12% SDS-PAGE. Electrophoretical transfer of protein bands from the gel to a nitrocellulose membrane was done in semidry conditions. After transfer, the Immobilon-P membrane was blocked in PBS-Tween containing 1% BSA and 4% milk powder (Carl Roth, Karlsruhe, Germany). After several washings in PBS-Tween, the membrane was incubated with rabbit anti-C3a pAb, followed by a HRP-conjugated swine anti-rabbit pAb (Dakopatts). Development was performed with ECL Western blotting detection reagents (Applichem, Darmstadt, Germany).

ELISA

To estimate which fragments of C3 were involved in the binding to *M. catarrhalis*, microtiter plates (F96 Maxisorb; Nunc-Immuno Module) were coated with live *M. catarrhalis* or purified recombinant full-length UspA1 (5 μg/ml) or UspA2 (5 μg/ml) in 0.1 M Tris-HCl (pH 9.0) overnight at 4˚C. Plates were washed with PBS-0.05% Tween and blocked for 1 h at room temperature with AppliChem blocking buffer. After washings, the wells were incubated for 1 h at room temperature with C3 (5 μg/ml), C3b (5 μg/ml), C3c (5 μg/ml), or C3d (5 μg/ml) in PBS. Thereafter, the plates were washed and incubated with goat anti-human C3 pAb or rabbit anti-human C3d pAb for 1 h. After additional washings, HRP-conjugated anti-rabbit pAb or HRP-conjugated anti-goat pAb were added and incubated at room temperature for 40 min. The wells were washed, developed, and the absorbance was measured at $OD_{492\ nm}$ with a Multiscan Ascent (Thermo Labsystems, Helsinki, Finland).

To determine which part of UspA1 and UspA2 was responsible for the C3d binding, UspA1 and UspA2 fragments (20 μM) were immobilized on microtiter plates overnight at 4˚C. The wells were blocked using AppliChem blocking buffer and thereafter washed four times. C3d (5 μg/ml) was added to the wells and incubated for 1 h at room temperature. Thereafter, the plates were washed and incubated with rabbit anti-human C3d pAb for 1 h followed by HRP-conjugated anti-rabbit pAb. The wells were washed, developed, and the absorbance was measured at $OD_{492\ nm}$ with a Multiscan Ascent (Thermo Labsystems).

Direct binding assay

Recombinant C3d was labeled with 0.05 mol iodine (GE Healthcare, Buckinghamshire, U.K.) per mol protein, using the chloramine-T method (66). The *M. catarrhalis* wild type (RH4) and UspA1/A2H-deficient mutants were grown on solid medium overnight and washed in PBS containing 1% BSA (Saveen Werner). Bacteria (2×10^7) were incubated with [^{125}I]-labeled C3d at 37˚C for 1 h. After incubation, the bacteria were centrifuged ($10{,}000 \times g$) through a 20% sucrose column. The tubes were frozen and cut, and radioactivity in the pellet supernatant was measured in a gamma counter. Binding was calculated as amount of bound radioactivity (pellet) versus total radioactivity (pellet plus supernatant).

Statistical analysis

Most results were analyzed by a Student *t* test ($*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$) where $p \leq 0.05$ was considered to be statistically significant. C3met binding and UspA1/A2/A2H expression were assessed by Pearson correlation analysis.

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Results

Binding of C3 is related to UspA expression of M. catarrhalis *in clinical isolates and correlates with serum resistance*

We have previously shown that *M. catarrhalis* binds and neutralizes C3 at the bacterial surface, which protects against the complement-mediated attack and contributes to serum resistance (27). UspA1 and UspA2/A2H are the main outer membrane proteins that are involved in the interaction between *M. catarrhalis* and C3 (27). To analyze whether the UspA expression of different *M. catarrhalis* strains correlated with C3 binding, bacteria were incubated with a rabbit anti-UspA pAb recognizing all UspAs or C3met, followed by FITC-conjugated swine anti-rabbit or rabbit anti-human C3 pAb, and analyzed by flow cytometry. A correlation between UspA expression and C3met binding (Pearson correlation coefficient, 0.57; $p \leq 0.01$) was found (Fig. 1*A*). Thus, most of the UspA-expressing *M. catarrhalis* interacts with C3.

To further analyze whether there was a correlation between serum resistance of different clinical *M. catarrhalis* isolates and the capacity to bind C3, C3met binding to *M. catarrhalis* was analyzed by flow cytometry and correlated with the ability of the different strains to survive in NHS. Native C3 was purified from human serum and treated with methylamine, which converts C3 to a C3met molecule equivalent to C3b without the capacity to covalently bind to microbes. In the bactericidal assay, the different *M. catarrhalis* isolates were incubated in the presence of NHS for 30 min of incubation and surviving bacteria (CFU) were determined. Serum-resistant strains bound more C3met; that is, the high C3met-binding strains were more resistant to NHS than were the low C3met-binding strains (Fig. 1*B*). More than 75% survival after 30 min of incubation was classified as serum-resistant strains. Additionally, the serum-sensitive *M. catarrhalis* isolates bound less C3met as compared with the more serum-resistant strains. Less than 25% survival after 30 min of incubation was classified as serum-sensitive strains.

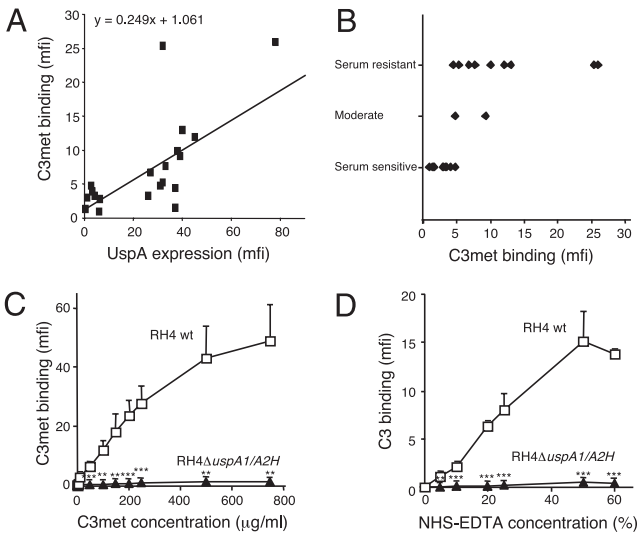

FIGURE 1. UspA expression of *M. catarrhalis* correlates with C3 binding, and serum resistance of different *Moraxella catarrhalis* strains corresponds to C3met binding. *A*, Bacteria were incubated with an anti-UspA pAb, followed by a FITC-conjugated swine anti-rabbit IgG pAb and flow cytometry analysis. To determine C3 binding, *M. catarrhalis* was incubated for 60 min with 40 μg/ml C3met (*A*, *B*) or increasing concentrations of C3met (1–750 μg/ml) (*C*). C3 bound to the bacteria was analyzed by a FITC-conjugated anti-C3 pAb and flow cytometry. In *B*, the clinical *M. catarrhalis* isolates were incubated in the presence of 10% NHS for 30 min. Thereafter, the suspensions were spread on BHI agar plates to allow determination of surviving bacteria. The number of bacteria (CFU) at the initiation of the experiment was defined as 100%; >75% survival and <25% survival was classified as serum-resistant or serum-sensitive strains, respectively. *D*, *M. catarrhalis* binds C3 directly from NHS via UspAs, and the binding is dose-dependent. *Moraxella* was incubated with increasing concentrations of NHS-EDTA (5–60%) and analyzed by flow cytometry. The mean values out of three independent experiments are shown. Error bars indicate SD. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

Binding of C3met to *M. catarrhalis* wild type and the Usp double mutant was analyzed by flow cytometry using a polyclonal anti-C3 antiserum. The *M. catarrhalis* RH4 wild type bound C3met in a dose-dependent manner at C3met concentrations ranging from 1 to 750 μg/ml (Fig. 1*C*). In contrast, no significant binding of C3met was observed with the UspA1/A2H-deficient double mutant (RH4 Δ*uspA1/A2H*) even at the highest C3met concentration (750 μg/ml) used. The UspA2H of *M. catarrhalis* RH4 was formerly known as UspA2, but the C3met binding capacity of UspA2 and UspA2H is similar (26, 28, 62). The experiments shown in Fig. 1*C* were performed using purified C3met in the absence of serum. To analyze whether the *M. catarrhalis* RH4 wild type also bound C3 directly from serum, we incubated bacteria with increasing concentrations of NHS with addition of EDTA (0–60%) and analyzed C3 binding using a polyclonal anti-C3 antiserum and flow cytometry. EDTA inhibits all complement pathways by absorbing divalent cations (Mg^{2+} and Ca^{2+}). When the complement system was inactivated in the presence of EDTA, *M. catarrhalis* still bound C3 (Fig. 1*D*). Thus, *M. catarrhalis* bound C3 regardless of complement activation. The RH4 wild type bound C3 in a dose-dependent manner and the binding was saturated at 50% NHS-EDTA, which corresponded to a C3 concentration of ~500 μg/ml (Fig. 1*D*). As expected, the UspA1/A2H-deficient *Moraxella* mutant did not show any significant binding to C3 when incubated with NHS-EDTA. A weaker intensity of the C3 binding was detected when serum was used as a source of C3 (mean fluorescence intensity, ~15) compared with the purified C3met (mean fluorescence intensity, ~40) when binding at 500 μg/ml C3met was analyzed. Taken together, *M. catarrhalis* dose-dependently bound C3met or C3 directly from serum via the UspAs.

UspAs inhibit the hemolytic activity of the classical pathway

Recombinant UspA1 and UspA2 inhibit the alternative pathway when analyzed in a hemolytic assay using rabbit erythrocytes and a buffer only allowing the activation of the alternative pathway (27). To establish the role of UspA1 and UspA2 as inhibitors of the classical pathway, we included a classical hemolytic assay consisting of NHS and amboceptor-coated sheep erythrocytes (64). NHS was preincubated with recombinant $UspA1^{50–770}$ or $UspA2^{30–539}$ followed by addition of erythrocytes. These two UspAs were derived from the UspA1/A2-expressing strain *M. catarrhalis* Bc5. After 1 h incubation, the amount of erythrocyte lysis was measured. Maximum hemolysis (NHS without inhibitor) in each experiment was defined as 100%. Significantly decreased hemolysis was observed when NHS was preincubated with $UspA1^{50–770}$ or $UspA2^{30–539}$ as compared with untreated NHS (Fig. 2). UspA1 and UspA2 (both at 10 μg/ml) inhibited hemolysis by 82 and 50%, respectively. Thus, in addition to inhibit the alternative pathway (27), the UspAs also reduced the classical pathway-mediated hemolysis of sheep erythrocytes.

UspAs inhibit complement activation at the C3 level

To confirm that UspA1 and UspA2 inhibit both the alternative and classical pathways and to determine at what level UspAs inhibit complement activation, analysis of complement activation was performed with ELISA. The alternative and classical pathways

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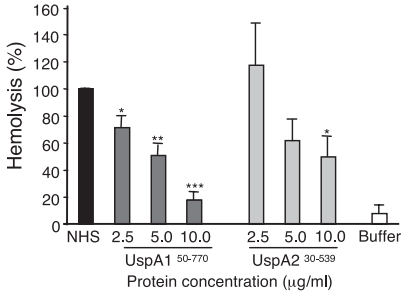

FIGURE 2. Recombinant UspA1 and UspA2 decrease hemolysis of sheep erythrocytes by inhibiting the classical pathway. NHS was incubated with or without recombinant UspA1$^{50–770}$ (2.5–10 μg/ml) or UspA2$^{30–539}$ (2.5–10 μg/ml) for 15 min at 37°C and thereafter added to the amboceptor-coated erythrocytes. After incubation for 60 min, the suspension was centrifuged and the supernatants were measured by spectrophotometry at 414 nm. Maximum hemolysis in each experiment was defined as 100%. The mean values out of three separate experiments are shown and error bars correspond to SD. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

were activated with LPS and IgM, respectively, NHS was pre-incubated with UspA1 and UspA2, and activation was followed by detection of C3b and MAC (C5b–9) deposition. As can be seen in Fig. 3, UspA1 and UspA2 inhibited activation of the alternative pathway. In the alternative pathway, recombinant UspA2 at low concentrations was more efficient in inhibiting C3b and C5b–9 deposition compared with UspA1 (Fig. 3*A*, 3*B*), but at higher concentrations both UspA1 and UspA2 showed an efficient inhibition. At a concentration of 50 μg/ml, UspA1 lowered both C3b and C5b–9 deposition by 50% and UspA2 by $>$ 70%, respectively, when the alternative pathway was analyzed (Fig. 3*A*, 3*B*). Thus, both bacterial proteins were efficient inhibitors of the alternative pathway and already inhibited on the level of the C3 convertase. In parallel to inhibiting the alternative pathway, the microbial proteins also inhibited the activation of the classical pathway. At 50 μg/ml, UspA1 and UspA2 inhibited the C3b deposition by 61 and 48%, respectively (Fig. 3*C*). As expected, when the bacterial proteins blocked complement at the C3 level, the C5b–9 deposition was also inhibited (Fig. 3*B*, 3*D*). The positive controls factor H (alternative pathway inhibitor) and C4BP (classical pathway inhibitor) inhibited C3b and/or C5b–9 deposition. In contrast to a C4BP-dependent blockage of C5b–9 deposition (Fig. 3*D*), C4BP did not block C3b deposition (Fig. 3*C*). The reason for this is presently unknown. In conclusion, UspA1 and UspA2 inhibited activation of both the alternative and classical pathways. Our results further proved that the bacterial outer membrane proteins inhibited the C3b deposition and therefore acted at the C3 level in the complement cascade.

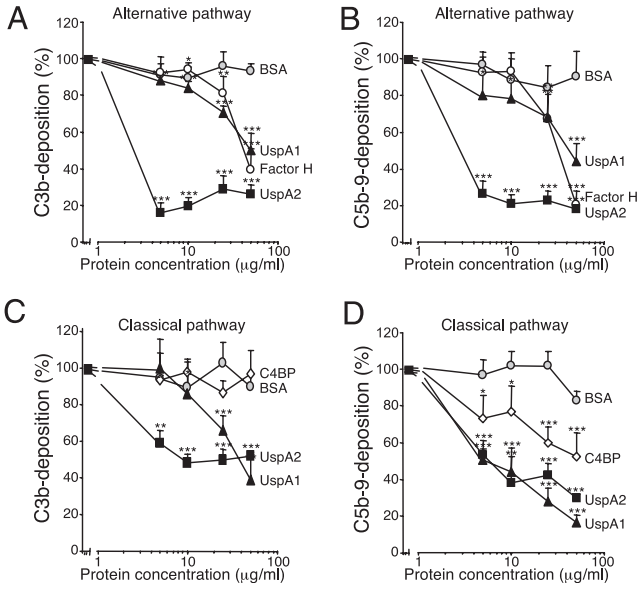

FIGURE 3. UspA1 and UspA2 inhibit classical and alternative pathways. *A* and *C*, C3b deposition via the alternative (*A*; 20% NHS) and the classical (*C*; 2% NHS) pathways. *B* and *D*, C5b–9 deposition via the alternative (*B*; 20% NHS) and the classical (*D*; 2% NHS) pathways. NHS was incubated with UspA1 (5–50 μg/ml) (▲), UspA2 (5–50 μg/ml) (■), BSA (5–50 μg/ml) (gray shaded circle), and factor H (5–50 μg/ml) (○), or C4BP (5–50 μg/ml) (◇), and activation was recorded by using an anti-C3b mAb or anti-C5b–9 mAb and HRP-conjugated rabbit anti-mouse pAb. Maximum complement activation (NHS without inhibitor) in each experiment was defined as 100%. The mean values out of three separate experiments are shown and error bars correspond to SD. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

UspA1 and UspA2 do not induce conformational changes of C3

Protease sensitivity assays can be used to determine whether a protein induces changes in the conformation of a particular protein (57). Interestingly, it has been shown that the C3d-binding Efb of *S. aureus* changes the conformation of C3, and that C3 bound to Efb is degraded into several low-molecular mass fragments in the presence of trypsin (57). To determine whether binding of UspA1 and A2 to C3 would give similar result as Efb, a trypsin sensitivity assay was performed. C3 was incubated with UspA1, UspA2, or Efb in the presence of trypsin. At different time points, aliquots were removed and inhibited by soybean trypsin inhibitor, separated by SDS-PAGE, and visualized by Coomassie blue staining. When a bacterial protein changes the conformation of C3 and induces trypsin sensitivity, the intensity of the C3 α′-chain is reduced and low-molecular mass fragments appear (57). No significant decrease in the intensity of the α′-chain in the presence of UspA1 or A2 could be detected compared with C3 alone (Fig. 4). However, additional bands with a molecular mass between 40 and 50 kDa appeared in the presence of UspA1 or UspA2. Western blot analysis using anti-human C3 pAbs was performed to determine whether these extra bands were degradation products of C3. The anti-C3 pAb did not detect these bands, suggesting that the extra bands were degradation products of UspA1 and UspA2 (data not shown). Thus, UspA1 and UspA2 did not induce a conformational change in C3, making it susceptible to trypsin. Since it previously has been shown that Efb makes C3 susceptible to proteolysis in the presence of trypsin (57), Efb was included as a positive control. A reduced intensity of

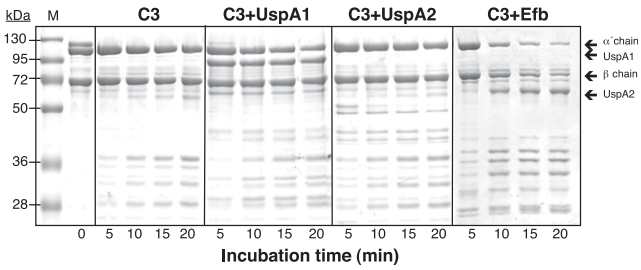

FIGURE 4. UspA1 and UspA2 do not induce a conformational change of C3 in the presence of trypsin. C3 was incubated with UspA1, UspA2, Efb, or buffer alone in the presence of 0.1% trypsin. At different time points aliquots were removed, inhibited by soybean trypsin inhibitor, separated by SDS-PAGE, and proteins were stained with Coomassie blue. C3 without trypsin was used as a control (*lane 2*, 0 min). A typical experiment out of three is demonstrated.

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the α′-chain of C3 and the appearance of additional degradation fragments were found in the presence of recombinant Efb (Fig. 4).

UspA1 and UspA2 are efficient inhibitors of C3a generation

Complement activation results in generation of the anaphylatoxins, including C3a and C5a, and these proteins are an important part of the inflammatory process (37). As UspA1 and UspA2 bound to C3b and inhibited complement activation at the level of the C3 convertase, we wanted to analyze whether the bacterial proteins would affect the C3a formation and thereby the inflammatory response. The C3 convertase cleaves C3 into C3b and C3a. The effect of UspA1 or UspA2 upon the formation of C3a was examined in zymosan-activated NHS. Zymosan was used to activate NHS with or without recombinant UspA1 and UspA2, and aliquots of the supernatants were analyzed for C3a production by SDS-PAGE and Western blotting. When C3a was detected with an anti-human C3a pAb, detectable levels of C3a were observed in the control samples without recombinant UspA1/A2 (Fig. 5, *lane 1*). In contrast, 25 μg/ml both UspA1 and UspA2 reduced the amount of C3a generated, and UspA1 and UspA2, both at 50 μg/ml, completely inhibited the C3a generation (Fig. 5, *lanes 4–5*). Factor H (Fig. 5, *lane 6*) and BSA (Fig. 5, *lane 7*) were used as positive and negative controls, respectively. Thus, both UspA1 and UspA2 inhibited the C3a generation that may lead to a decreased inflammatory reaction.

M. catarrhalis *binds to the C3d domain of the C3 molecule*

C3 is comprised of one α-chain and one β-chain, which are connected by disulfide bonds and associated by noncovalent forces (35). During complement activation, C3 undergoes multiple cleavage events that generate various fragments, including C3a, C3b, iC3b, C3c, C3dg, and C3g. To analyze which part of the C3 molecule mediates the interaction between *M. catarrhalis* and C3, a series of different experiments were performed. To identify the binding fragment of C3, binding of C3, C3b, C3c, or C3d to *M. catarrhalis* RH4 wild type was analyzed in a whole-cell ELISA. *M. catarrhalis* significantly bound C3d (Fig. 6*A*), whereas no binding of the C3c fragment was seen. Binding of C3 and C3b, which both contain the C3d fragment, was also demonstrated. Binding of C3d to the wild type and the different UspA mutants was analyzed by a direct binding assay with [^{125}I]-labeled C3d (Fig. 6*B*). Interestingly, the binding of C3d correlated with UspA1/A2H expression; both of the *M. catarrhalis* Δ*uspA1* and Δ*uspA2H* isogenic mutants bound less C3d, whereas C3d binding to the *M. catarrhalis* Δ*uspA1/A2H* mutant was further decreased and nearly abolished. To further analyze the interaction between

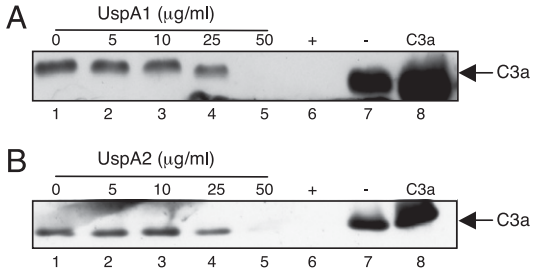

FIGURE 5. UspA1 and UspA2 inhibit C3a formation in a zymosan activation assay. Human serum was incubated with or without recombinant UspA1$^{50–770}$ (*A*) and UspA2$^{30–539}$ (*B*) and activated by zymosan. The supernatants were analyzed by Western blots using anti-human C3a pAb and HRP-conjugated swine anti-rabbit pAb. A typical experiment out of three is demonstrated. Factor H (20 μg/ml) that inhibits C3a formation and BSA (50 μg/ml) were included as positive (+) and negative (−) controls, respectively.

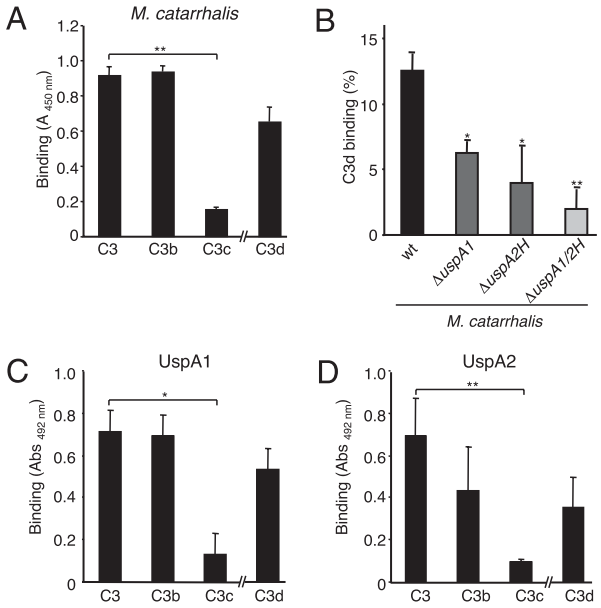

FIGURE 6. *M. catarrhalis* UspAs interact with C3d. *M. catarrhalis* wild type (*A*), UspA1$^{50–770}$ (*C*), and UspA2$^{30–539}$ (*D*) were immobilized on microtiter plates and incubated with C3, C3b, C3c, or C3d, followed by incubation with goat anti-human C3 pAb or rabbit anti-human C3d pAb. HRP-conjugated anti-goat pAb or anti-rabbit pAb was used as a secondary layer. Because two different pAbs were used, the absolute absorbance obtained is not directly comparable. *B*, The wild type and UspA1-, UspA2H-, and UspA1/A2H-deficient *Moraxella* mutants were incubated with [^{125}I]-labeled C3d. Binding was determined as percentage of bound radioactivity versus added radioactivity measured after separation of free and bound [^{125}I]-labeled protein over a sucrose column. The mean values of these experiments with duplicates are shown with error bars indicating SD. *$p \leq 0.05$, **$p \leq 0.01$.

C3 and UspA1/A2, recombinant UspA1$^{50–770}$ and UspA2$^{30–539}$ were immobilized in microtiter plates, followed by incubation with different C3 fragments as indicated in Fig. 6*C* and 6*D*. The ELISA demonstrated that both UspA1 and UspA2 bound C3d and the C3d-containing fragments (i.e., C3 and C3b). Addition of C3c, which does not contain the C3d region, showed a much weaker binding to both recombinant UspA1 and UspA2. Our experiments revealed that *M. catarrhalis* bound C3d via the outer membrane proteins UspA1 and UspA2/A2H.

The C3d binding domains are located within UspA1$^{299–452}$ and UspA2$^{165–318}$

To define the specific C3d-binding domains of UspA1 and UspA2, a series of recombinant proteins spanning the entire UspA1$^{50–770}$ and UspA2$^{30–539}$ molecules were manufactured. C3d was incubated with equimolar amounts of the immobilized full-length proteins and the truncated UspA1 and UspA2 fragments. Thereafter, the binding was quantified by ELISA. UspA1$^{299–452}$ and UspA2$^{165–318}$ efficiently bound C3d, suggesting that a binding domain was located within these amino acid residues (Fig. 7). However, UspA1$^{50–321}$ was most likely also involved in the C3d binding, albeit this sequence had a lower binding capacity compared with UspA1$^{299–452}$.

UspAs interact with C3 derived from mice

To determine whether C3 binding of UspA1/A2H-expressing *M. catarrhalis* is species specific, the binding of C3 from murine serum was analyzed. Bacteria were incubated with increasing concentrations of EDTA-treated mouse serum (0–10%). When the mouse complement system was inactivated in the presence of

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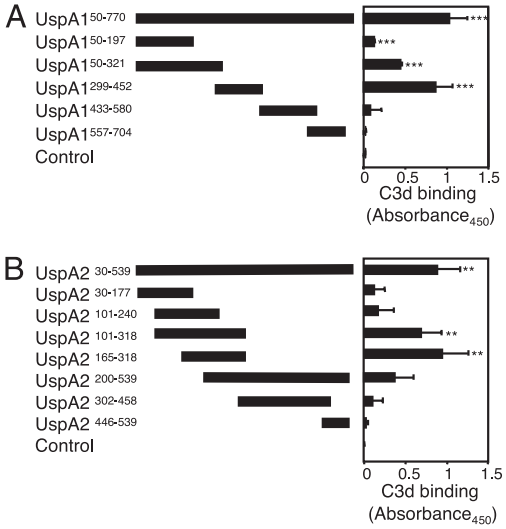

FIGURE 7. Active C3d binding regions are located within UspA1$^{299–452}$ and UspA2$^{165–318}$. Truncated proteins derived from UspA1 and UspA2 are shown. All fragments were tested for binding to C3d by ELISA; 20 μM each fragment was immobilized on microtiter plates and and incubated with 5 μg/ml C3d. Bound C3d was detected with rabbit anti-human C3d pAb followed by HRP-conjugated anti-rabbit pAb. Mean values are shown with error bars indicating SD. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

EDTA, *M. catarrhalis* still bound murine C3 in a dose-dependent manner (Fig. 8*A*). In contrast, murine C3 showed a significantly decreased (~80%) binding to the RH4Δ*uspA1/A2H* double mutant. Both the UspA1- and UspA2H-deficient mutants displayed a reduced binding; UspA1- and UspA2H-deficient mutants bound ~20 and 40% less C3, respectively, as compared with the wild

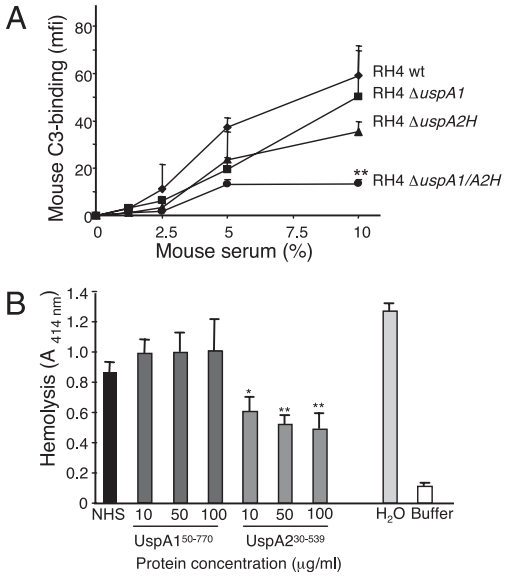

FIGURE 8. *Moraxella* UspAs interact with mouse C3, and UspA2 decreases hemolysis of rabbit erythrocytes by inhibiting the alternative pathway in mouse serum. *A*, *M. catarrhalis* bound C3 from mouse serum. The wild type and corresponding UspA1/A2H-deficient mutants were incubated with increasing concentrations of mouse serum (0–10%), followed by detection with a FITC-conjugated rabbit anti-mouse C3 pAb and flow cytometry analysis. *B*, Mouse serum was incubated with or without 10–100 μg/ml UspA1$^{50–770}$ or UspA2$^{30–539}$ at 37°C for 30 min. The preincubated mouse serum was added to the erythrocytes. After incubation for 3 h, the suspension was centrifuged and the supernatants were measured by spectrophotometry. The mean values out of three separate experiments are shown and error bars correspond to SD. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

type when incubated with 10% mouse serum (Fig. 8*A*). Thus, *M. catarrhalis* bound murine C3 regardless of complement activation, and both UspA1 and UspA2 contributed to the binding.

To analyze whether the inhibition of the alternative pathway was species specific, a hemolytic assay with murine serum was also performed. Serum was preincubated with recombinant UspA1$^{50–770}$ or UspA2$^{30–539}$, followed by addition to rabbit erythrocytes. After 3 h incubation, the amount of erythrocyte lysis was measured. Interestingly, significantly decreased hemolysis was observed when mouse serum was preincubated with UspA2$^{30–539}$ compared with untreated mouse serum (Fig. 8*B*). However, when the mouse serum was preincubated with UspA1$^{50–770}$, no inhibition was detected. Taken together, in parallel with the experiments comprising NHS, *M. catarrhalis* UspA2 inhibited the alternative pathway in mouse serum by binding mouse C3.

Discussion

The pathogenesis of *M. catarrhalis* relies on its capacity to resist the human host defense, including complement (1). The complement system is very harmful for Gram-negative pathogens, including *M. catarrhalis*, and bacterial complement resistance is one of the most important virulence mechanisms (67). *M. catarrhalis* has thus developed several efficient strategies to circumvent complement. It has been demonstrated that UspA1 and UspA2 interact with and inhibit the alternative pathway of complement by noncovalently binding C3 (27). In the present study we show a correlation between C3met binding and *Moraxella*-dependent UspA expression. Furthermore, C3met binding corresponded with serum resistance of *M. catarrhalis*. Because C3 is a key molecule in the complement system, the binding of C3 most likely results in the regulation of all three pathways and may contribute to serum resistance. In fact, UspAs inhibit the activation of the alternative and the classical pathways and block generation of C3b and MAC (C5b–9) deposition. The reduction of the C3b deposition shows that UspAs act early in the complement cascade and at the C3 level by binding C3. Because the bacterial proteins act and interfere with this early level of complement activation, the inhibitory capacity of UspAs is not restricted to one single pathway. By blocking the C3b deposition, the downstream steps, including formation of the C5 convertase and generation and deposition of MAC, are also inhibited. In fact, *Moraxella* UspAs inhibit both MAC deposition and hemolysis of erythrocytes when activated via the alternative and classical pathways (27). Inhibition of the classical pathway is most likely caused by simultaneous recruitment of both C4BP and C3 (26). By inhibition of the different complement pathways resulting in increased survival, *M. catarrhalis* can colonize, infect the human host, and eventually cause disease.

A very common evasion mechanism used by most pathogens involves the acquisition and utilization of human complement inhibitors, such as C4BP, factor H, and vitronectin (38). The different complement inhibitors are functionally active when bound to the surface of the pathogens and are capable of inhibiting different steps of the complement cascade. In addition to binding the complement inhibitor C4BP, *M. catarrhalis* binds and utilizes vitronectin for protection against the terminal pathway (25, 28). These interactions contribute to serum resistance and most *M. catarrhalis* isolates from patients with lower respiratory tract infections are resistant to complement-mediated killing (68). It is a well-known fact that both complement factors and inhibitors are present in the respiratory tract (69–71). Therefore, respiratory pathogens must protect themselves from complement in similarity to blood-borne pathogens, and consequently these microbes also have access to complement inhibitors. The surface-expressed

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UspA1 and/or UspA2/A2H of *M. catarrhalis* have been shown to be responsible for C4BP and vitronectin binding and thus are important for bacterial survival in human serum (10, 22, 25, 26, 28). The use of more than one strategy to evade the complement system is common and shared by several pathogens (38–40). One pathogen can express multiple proteins contributing to the escape of the complement attack. It has been shown that *M. catarrhalis* is equally resistant to both the classical and alternative pathways (27). What exact mechanism that is the most important in the clearance of *M. catarrhalis* in the human host is at present unknown. However, *M. catarrhalis* uses several evasion strategies for protection against the complement attack involving the expression of different surface proteins, including the UspAs, CopB, protein CD, and protein E (13, 26–28, 72, 73). The contribution of the different complement evasion mechanisms to the pathogenesis of *M. catarrhalis* needs to be further studied and clarified. However, every mechanism that provides protection from complement is certainly beneficial for bacterial survival. By interfering with the complement system at the C3 level, which is common for all pathways, C3d-dependent complement inhibition of *M. catarrhalis* is a very efficient mechanism that contributes to serum resistance and thereby the virulence of the bacteria.

During complement activation the C3 convertases cleave C3 into the opsonin C3b and the anaphylatoxin C3a. UspA1 and UspA2 bind C3 and inhibit C3b deposition, and by inhibiting C3b deposition *M. catarrhalis* may prevent opsonization and phagocytosis. Furthermore, the UspAs inhibited the generation of the potent anaphylatoxin C3a when human serum was used as a source of the C3 convertase. C3a belongs to the group of the most powerful immunopharmacological substances known and is an important part of the inflammatory process. This group of small peptides causes histamine release from mast cells, smooth muscle contraction, and increases vascular permeability (74, 75). Additionally, C3a recruits inflammatory cells to the site of inflammation and activates their effector mechanisms. Thus, UspA1 and UspA2 both inhibit the C3b deposition and C3a generation, suggesting that these interactions may inhibit phagocytosis and reduce the inflammatory response.

During complement activation, C3 undergoes multiple cleavage events that generate various fragments, including C3a, C3b, iC3b, C3c, C3dg, and C3g. We show that *M. catarrhalis* interacts with the C3d fragment of C3. By analyzing the UspA1 mutant, the UspA2H mutant, and the double mutant (*M. catarrhalis* Δ*uspA1/A2H*) in a direct binding assay, we found the UspAs to be the major C3d-binding proteins of *M. catarrhalis*. Additionally, the C3d-containing fragments C3, C3b, and C3d bound both recombinant UspA1 and UspA2 in ELISA, whereas C3c, which lacks the C3d region, did not bind. Defining the *M. catarrhalis* and UspA-binding site to the C3d fragment is important since this fragment has binding sites for both factor H and the complement receptor 2 (CD21) (35). CD21 is expressed on B cells and, together with CD19 and CD81, forms the B cell coreceptor complex, which lowers the threshold for B cell activation substantially (76). Binding of pathogen-bound C3d is therefore regarded as an essential link between the innate and adaptive immune responses. In addition to inhibiting complement, UspA1 and UspA2 may also affect the interaction of C3d with its major receptor and to attenuate the initiation of downstream immune responses. Several pathogens have been shown to bind C3 and its fragments, including *C. albicans, S. pneumoniae*, and *S. aureus* (52, 57, 77, 78). *S. aureus* Efb binds native C3 and some of its fragments (i.e., C3b and C3d), and binding of C3 induces a conformational change of C3 so that the protein is unable to participate in successful activation of the complement cascade (57). In contrast to Efb, neither UspA1 nor UspA2 induced a conformational change in C3 when analyzed in a trypsin sensitivity assay. Therefore, UspA1 and UspA2 inhibit the complement system in a different way compared with Efb from *S. aureus*. Additionally, Efb and Ehp, which is another complement inhibitory protein of *S. aureus,* both bind to C3 and reduce its availability at the bacterial surface, thus reducing the opsonization of the bacteria (51, 56, 57, 79). Furthermore, *S. aureus* Sbi binds C3d and inhibits the alternative pathway of complement (55, 80). Interestingly, Efb, Ehp, and Sbi bind the C3d fragment and inhibit the interaction between C3d and its receptor CD21, which plays an important role in B cell activation and maturation (55, 81, 82). In addition to binding human C3, UspAs also bind C3 from mouse serum. UspA2 inhibits the alternative pathway of mouse complement, suggesting that the interaction with C3 and inhibition of complement activation are not species specific.

The C3d-binding regions UspA1$^{299–452}$ and UspA2$^{165–318}$ from the clinical isolate *M. catarrhalis* Bc5 were found to contain the C3d binding sites. Intriguingly, UspA1$^{299–452}$ and UspA2$^{165–318}$ share a sequence of 31 identical amino acids residues, including the 23-aa NNINNIYELAQQQDQHSSDIKTL (NNINNIY sequence) (24). This sequence contains the epitope for the protective mAb 17C7, for which there is universal reactivity (16, 83). In a mouse model, passive immunization with mAb 17C7 provided protection and improved pulmonary clearance of *M. catarrhalis* (83). Additionally, these two fragments (UspA1$^{299–452}$ and UspA2$^{165–318}$) also contain the fibronectin-binding domains of UspA1/A2 from *M. catarrhalis* and they have been shown to be important in adhesion to epithelial cells (24). The capacity of the UspAs to interact with several human proteins shows that they indeed are multifunctional adhesins.

In conclusion, *M. catarrhalis* UspAs are highly important virulence factors that interfere with complement in several ways to protect the species from the complement-mediated attacks (1, 38). To our knowledge, this is the first report describing that *M. catarrhalis* does not only avoid killing by complement but also modulates complement activation at the level of ther C3 converatse and thus may also inhibit the inflammatory response by neutralizing C3 and reducing C3a generation.

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Disclosures

The authors have no financial conflicts of interest.

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