Moraxella catarrhalis Binding to Host Cellular Receptors Is Mediated by Sequence-Specific Determinants Not Conserved among All UspA1 Protein Variants[▽]

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The *Moraxella catarrhalis* ubiquitous surface proteins (UspAs) are autotransporter molecules reported to interact with a variety of different host proteins and to affect processes ranging from serum resistance to cellular adhesion. The role of UspA1 as an adhesin has been confirmed with a number of different human cell types and is mediated by binding to eukaryotic proteins including carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs), fibronectin, and laminin. A distinct difference in the ability of prototypical *M. catarrhalis* strains to adhere to CEACAM-expressing cell lines prompted us to perform strain-specific structure-function analyses of UspA1 proteins. In this study, we characterized CEACAM binding by a diverse set of UspA1 proteins and showed that 3 out of 10 UspA1 proteins were incapable of binding CEACAM. This difference resulted from the absence of a distinct CEACAM binding motif in nonadhering strains. Our sequence analysis also revealed a single *M. catarrhalis* isolate that lacked the fibronectin-binding motif and was defective in adherence to Chang conjunctival epithelial cells. These results clearly demonstrate that UspA1-associated adhesive functions are not universally conserved. Instead, UspA1 proteins must be considered as variants with the potential to confer both different cell tropisms and host cell responses.

Moraxella catarrhalis is a gram-negative, unencapsulated bacterium whose natural existence is generally restricted to the respiratory tract of humans. Previously considered to be a commensal organism, it is now recognized as an important mucosal surface pathogen responsible for causing many cases of acute otitis media in children (9, 26). *M. catarrhalis* is also an important cause of exacerbations of chronic obstructive pulmonary disease (COPD) in adults (28, 37). These facts have led to increased efforts to identify bacterial factors that contribute to mucosal colonization and disease pathogenesis (16). Of these, the interaction between the surface-exposed proteins of *M. catarrhalis* and their receptors in the respiratory tract has become an important focus of research to define vaccine targets (23, 27) and novel therapeutics to treat infection caused by this organism (12).

A particular interest concerning *M. catarrhalis* interactions with its human host is the ubiquitous surface proteins (UspAs). The UspA proteins are subdivided into three basic groups based on conserved amino acids motifs within the N and C termini of these macromolecules: UspA1 (~88 kDa) (2, 7), UspA2 (~62 kDa) (2, 7), and the hybrid UspA2H (~92 kDa) (18). The UspA proteins are homologues of the YadA protein of *Yersinia* sp. (14, 17) and, as such, are predicted to be auto-

transporter proteins (11) that are present on the bacterial cell surface. Each protein consists of three distinct structural domains (14, 17). The N-terminal region is considered to form a β -sheet-based globular head, while the C-terminal region is predicted to form a membrane-spanning β -barrel structure (17). The stalk region that connects the head to the membrane-anchoring domains consists of a coiled-coil structure (6, 17). Although predicted to be structurally conserved, UspA1 and UspA2 proteins are distinct in that they are made up of a variable assortment of polypeptide modules that alter the size and composition of both the stalk and globular regions of each different protein (4a).

UspA1 and UspA2 have been shown to interact with a variety of human-derived targets normally found on certain eukaryotic cell surfaces or in the extracellular matrix, including fibronectin (42), laminin (41), and carcinoembryonic antigen (CEA)-related cellular adhesion molecule 1 (CEACAM1) (13). Each binding function contributes to *M. catarrhalis* interactions with different cell types to various degrees (13, 18, 24). In addition to its role as an adhesin, UspA1 was previously reported to confer serum resistance by interacting with both C3 (32) and C4b (31), components of the complement cascade. Most recently, UspA1 was shown to be associated with the development of apoptosis in epithelial cells exposed to *M. catarrhalis* (30) and to be involved in the invasion of human epithelial cells in vitro (39, 40). Remarkably, the interaction with each of these host proteins has been localized to a different region of UspA1, suggesting a potential for this bacterial macromolecule to simultaneously coordinate multiple ligands. However, the studies that have attributed each individual phe-

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TABLE 1. *M. catarrhalis* strains used in this study

Strain	Adult or child	Disease	Location of isolation	Geographical location	GenBank accession no. for UspA	Reference, source
ATCC 25238	Adult	Unknown	Unclear; nasopharynx (<i>Neisseria</i> isolate)	University of Rochester	AAD43465	7, ATCC
O35E	Child	Otitis media	Tympanocentesis-derived isolate	Dallas, TX	AAB96359	2, John D. Nelson
O35E.118						19
O35EΔUspA1						2
O12E	Child	Otitis media	Tympanocentesis-derived isolate	Dallas, TX	AAF40118	18, John D. Nelson
O12EΔUspA1						18
V1145	Child	Healthy	Nasopharynx	North Carolina	ABY64882	3, Frederick Henderson
V1145 ΔUspA1						3
V1156	Child	Healthy	Nasopharynx	North Carolina	ACC44785	3, Frederick Henderson
V1156 ΔUspA1						3
V1118	Child	Healthy	Nasopharynx	North Carolina	ACB58308	3, Frederick Henderson
7169	Child	Otitis media	Tympanocentesis-derived isolate	Buffalo, NY	ACB59385	21, Anthony Campagnari
7169 ΔUspA1						3
TTA37	Adult	COPD	Transtracheal aspirate	Tennessee	AAF40122	18, Steven Berk
FIN2344	Child	Unclear	Unknown	Finland	ACC44784	3, Merja Helminen
FIN2344 ΔUspA1						3
ATCC 43617	Adult	COPD	Transtracheal aspirate from coal miner with chronic bronchitis	Belgium	ACB58310	45, ATCC

notype to UspA1 have typically been performed using different strains, and each binding activity is frequently considered in isolation, making it difficult to reconcile sometimes contradictory observations regarding UspA1 function. This prompted us to perform a sequence-based functional analysis with a diverse set of *M. catarrhalis* isolates, thereby allowing us to demonstrate a previously unappreciated link between the variability in UspA1 primary amino acid sequence and cellular tropism.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The *M. catarrhalis* wild-type strains and mutants employed in this study are listed in Table 1. The UspA1 mutants used in this study were previously described (2, 3, 18) and were designated ΔUspA1 in the present study because they contain an internal deletion in the UspA1 open reading frame. For the preparation of whole-cell lysates, *M. catarrhalis* strains were grown at 37°C on brain heart infusion (BHI) agar (Difco/Becton Dickinson, Sparks, MD) in an atmosphere containing 5% CO₂ or in broth. Recombinant *Escherichia coli* DH5α strains containing pACYC184-based recombinant plasmids were grown on Luria-Bertani (LB) medium containing chloramphenicol (10 μg/ml) at 37°C. *E. coli* strain EPI300 cells (Epicentre Biotechnologies, Madison, WI) expressing recombinant UspA1 proteins were grown at 37°C on LB medium with standard antibiotic supplementation and in LB medium with chloramphenicol and CopyControl induction solution (Epicentre) as required.

General DNA methods. *M. catarrhalis* genomic DNA was isolated from agar plate-grown cells using the Easy-DNA kit (Invitrogen, Carlsbad, CA). The preparation of plasmid DNA and the purification of PCR products were performed using kits manufactured by Qiagen (Santa Clarita, CA). Nucleotide sequence data obtained from automated sequencing systems were analyzed using the MacVector analysis package (version 6.5; Oxford Molecular Group, Campbell, CA). Mult-Align (8) was used to generate sequence alignments.

DNA cloning. Plasmid pELU1-10G, which encodes the *uspA1* gene from *M. catarrhalis* O35E, was described previously (18). To clone *uspA1* genes into the CopyControl vector (Epicentre), PCR was used to amplify DNA encoding the entire *uspA1* open reading frame and approximately 40 to 200 nucleotides of DNA 5' from the *uspA1* translational start codon using bacterial chromosomal DNA as the template for PCR. This PCR product was then cloned into the Blunt Cloning-Ready pCC1 vector (Epicentre) to generate recombinant plasmids expressing each different UspA1 protein. Each cloned *uspA1* gene was fully sequenced and confirmed to be identical to that in the chromosome of the relevant *M. catarrhalis* strain. A kanamycin resistance cassette was cloned into pCC1 to generate the pCC-Kan negative control plasmid. These CopyControl-based plas-

mids were electroporated into Electrocompetent TransformMax *E. coli* EPI300 as described for the Epicentre CopyControl cDNA, gene, and PCR cloning kit.

Measurement of bacterial attachment to human cells. Attachment of wild-type and mutant *M. catarrhalis* strains and recombinant *E. coli* strains expressing *M. catarrhalis* UspA1 proteins to Chang human conjunctival epithelial cells (ATCC CCL20.2) and to A549 human lung epithelial cells (ATCC CCL-185) was measured by using a modification of a previously described attachment assay (1). It must be noted that these Chang cells, purchased from the American Type Culture Collection (ATCC), were accompanied by an ATCC notice that this line has been contaminated with HeLa cells. Briefly, tissue culture medium was removed from human cells grown overnight in 24-well plates and replaced with 0.5 ml of fresh tissue culture medium to which 25 μl of *M. catarrhalis* cells (~10⁶ CFU) or recombinant *E. coli* cells (~10⁶ CFU) was then added. These bacterial suspensions were obtained by harvesting growth from agar plates that had been grown overnight and suspending these cells in either BHI (for *M. catarrhalis*) or LB (for *E. coli*) medium to a density of approximately 300 Klett units as measured in a Klett-Summerson colorimeter (VWR Scientific, West Chester, PA). The tissue culture plates were then subjected to centrifugation at 200 × g for 5 min and incubated at 37°C in an atmosphere of 95% air–5% CO₂ for 30 min or 1 h, after which the wells were washed five times with BHI or LB medium. The human cells and attached bacteria were removed from the wells by treatment with trypsin (0.05% to 0.25%), subjected to vigorous mixing, and plated onto either BHI or LB agar to determine the number of attached bacteria.

Additional bacterial attachment experiments with Chang cells examined the effect of specific antibodies or serum components on attachment. To determine the effect of CEACAM antibodies on bacterial attachment, tissue culture medium was removed from Chang cells grown overnight in 24-well plates and replaced with 1 ml of fresh tissue culture medium containing 50 μl of rabbit antiserum to CEACAM (catalog number A0115; Dako North America, Carpinteria, CA), normal rabbit serum (catalog number X0903; Dako), or phosphate-buffered saline (PBS). These two antisera had been previously dialyzed overnight against PBS to remove azide. These plates were subjected to centrifugation at 200 × g for 5 min and then incubated at 37°C for 1 h. Next, the medium was removed from each well and replaced with fresh tissue culture medium to which 25 μl of recombinant *E. coli* cells containing plasmid pACYC184 or pELU1-10G (suspended to 250 Klett units) was then added, and the attachment assay was carried out as described above except that attachment was allowed to proceed for 2 h.

To determine the effect of serum components on attachment, Chang cell monolayers grown in the presence of 10% (vol/vol) fetal calf serum (FCS) were washed once with serum-free tissue culture medium, and tissue culture medium with or without 10% FCS was then added to each well. Recombinant *E. coli* cells containing *M. catarrhalis* *uspA1* genes cloned into the CopyControl vector were induced for 3 h with CopyControl induction solution and adjusted to a density of 300 Klett units,

FIG. 1. Cellular tropism of the *M. catarrhalis* O35E UspA1 protein. (A) Attachment of wild-type strain O35E.118 and mutant strain O35EΔUspA1 to A549 and Chang cells in vitro using a 30-min incubation period for attachment. (B) Expression of CEACAMs by Chang and A549 cells determined by Western blot analysis. Whole-cell lysates

25-μl portions of this suspension were then added to the wells, and the attachment assay was performed as described above, with a 1-h incubation period.

Western blot and overlay analyses. Bacterial whole-cell lysates (2) were boiled and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 12.5% (wt/vol) polyacrylamide separating gels, transferred onto Immobilon-P membranes (Millipore, Bedford, MA), and probed with the appropriate antibody. UspA1 was detected using monoclonal antibody (MAb) 24B5 (7). Whole-cell lysates of Chang and A549 cells were prepared from confluent monolayers grown in 24-well tissue culture plates. These monolayers were trypsinized, suspended in PBS, and then solubilized in sodium dodecyl sulfate-polyacrylamide gel electrophoresis digestion buffer. Rabbit antiserum to CEACAM (described above) and mouse antiserum to glyceraldehyde-3-phosphate dehydrogenase were used as the primary antibodies. CEACAMs were detected using CEA-specific rabbit polyclonal antisera (Dako, Glostrup, Denmark). In overlay experiments, the blots were probed with soluble CEACAM5 (Fitzgerald, Concord, MA) or soluble CEACAM1 (36), followed by the CEA-specific antiserum, which was then detected using secondary antiserum. The secondary antibodies were goat anti-mouse immunoglobulin G or anti-rabbit immunoglobulin G conjugated to either Bodipy or Cy5 fluorophores (Jackson ImmunoResearch, West Grove, PA). Antigen-antibody complexes were visualized by immunofluorescence using a Typhoon 9400 instrument (Amersham Biosciences).

CEACAM-binding assays. Cultures of *E. coli* cells expressing *M. catarrhalis* UspA1 proteins were grown overnight in LB chloramphenicol (12.5 μg/ml) and Copycontrol induction solution (Epicentre) for 5 h. Cells were washed with PBS-Mg-Ca (10 mM MgCl₂ and 5 mM CaCl₂). For bacterial dot blots, bacterial density was calculated by measuring the optical density at 550 nm and then diluting to 10⁶ bacteria/ml before spotting onto nitrocellulose. Cells were dried overnight at room temperature, and the membranes were blocked in PBS containing 4% skim milk. These membranes were probed with soluble CEACAM5, which was subsequently detected using CEA-specific antiserum and horseradish peroxidase (HRP)-coupled secondary antibodies. For enzyme-linked immunosorbent assays (ELISAs), Nunc plates were coated with heat-killed bacteria and dried at 37°C overnight. Wells were blocked using PBS containing 4% skim milk, followed by incubation with soluble CEACAM5 or CEACAM1. CEACAM was detected as described above for the dot blot experiments except that the HRP-based colorimetric detection system (Sure Blue) was used to measure CEACAM binding by detection at 650 nm.

RESULTS

UspA1 proteins differ in cellular adherence through CEACAM. Recent work clearly demonstrates that the UspA1 protein from *M. catarrhalis* strain ATCC 25238 mediates bacterial binding to CEACAM1 (13). However, the cellular tropism of prototypical *M. catarrhalis* strain O35E did not correlate with CEACAM expression. Specifically, UspA1-expressing *M. catarrhalis* strain O35E demonstrated effective binding to Chang conjunctival epithelial cells (Fig. 1A), which express relatively little CEACAM (Fig. 1B), while demonstrating little association with A549 respiratory epithelial cells (Fig. 1A) ($P < 0.05$) that express relatively large quantities of several different CEACAMs (Fig. 1B) (43, 44). An isogenic UspA1-deficient strain of *M. catarrhalis* O35E (O35EΔUspA1) did not adhere to Chang cells, allowing us to at-

of these two human cells lines were probed with antibody to CEACAM (panel 1) or glyceraldehyde-3-phosphate dehydrogenase (panel 2), followed by HRP-conjugated secondary antibody. (C) Attachment of wild-type *M. catarrhalis* O35E.118 and recombinant *E. coli* DH5α strains containing either pELU1-10G, which expresses the O35E UspA1 or the empty pACYC184 vector to A549 and Chang cells using a 30-min incubation period for attachment. (D) Effect of rabbit antibody to CEACAM (αCEACAM) and normal rabbit serum (NRS) on the binding of the two recombinant *E. coli* strains to Chang cells. Representative experiments are shown (A, C, and D). Statistical analysis of attachment data involved the use of one-way or two-way ANOVA with a Bonferroni posttest.

tribute O35E adherence to UspA1 expression, yet the *uspA1* mutant binding to the A549 cells was greater than that of the wild-type strain ($P < 0.05$) (Fig. 1A), suggesting that another adhesin(s) contributed to this interaction. This prompted us to express the UspA1 protein (UspA1_{O35E}) from *M. catarrhalis* O35E in *E. coli* so that we could characterize host cellular binding by UspA1 in the absence of other *M. catarrhalis* adhesins.

Consistent with the phenotype of the parental *M. catarrhalis* strains, Chang cell binding of *E. coli* cells expressing UspA1_{O35E} was much greater than that of A549 cells (Fig. 1C) ($P < 0.05$), suggesting that CEACAM receptors are not the primary determinants of A549 binding by this UspA1 variant. Next, we determined the contribution of CEACAM to adherence by performing attachment assays in the presence of a CEACAM-specific polyclonal antiserum, which effectively blocks the binding of *M. catarrhalis* ATCC 25238 (13). There was no effect of the CEACAM antiserum on Chang cell adherence conferred by UspA1_{O35E} ($P > 0.05$) (Fig. 1D), suggesting that CEACAM binding was not essential for adherence to this cell line.

CEACAM binding by UspA1 protein variants. Previous studies confirmed that UspA1 proteins are expressed in recombinant *E. coli* strains and that their function reflects that in the parental *M. catarrhalis* strain (18). In an attempt to compare CEACAM binding by a diverse set of UspA1 proteins, we expressed a total of 10 UspA1 proteins in *E. coli* (Table 1). To confirm UspA1 expression, bacterial lysates were probed with UspA1-specific antibodies. Consistent with data from previous reports (25), individual UspA1 proteins were expressed at various levels (Fig. 2A). In addition, all of these cloned UspA1 proteins could be detected on the surfaces of their respective recombinant *E. coli* strains when these strains were probed with UspA1-specific MAb 24B5 by flow cytometry (data not shown).

It was previously demonstrated that denatured UspA1 bound to purified soluble CEACAM (13). The blots containing the lysates were therefore reprobed using soluble CEACAM5. Seven of 10 UspA1 proteins tested revealed CEACAM5 binding, while the other three (UspA1_{ATCC 43617}, UspA1_{TTA37}, and UspA1_{O35E}) did not (Fig. 2A). To confirm that the binding in the Western blot overlay assays reflected the phenotype of UspA1 in the recombinant bacterial membrane, the *E. coli* strains were spotted onto nitrocellulose (Fig. 2B) and adsorbed onto wells of an ELISA plate (Fig. 2C). In each case, binding reflected that of the overlay assays, with the same seven UspA1 variants conferring CEACAM5 binding in each assay (compare Fig. 2A, B, and C). These results suggest that CEACAM binding is not an inherent property of all UspA1 variants, as certain strains express UspA1 variants that do not bind CEACAMs.

In order to confirm that the CEACAM-binding phenotype of UspA1 expressed by *E. coli* reflected that of the parental *M. catarrhalis* strains, we used a panel of UspA1-deficient *M. catarrhalis* strains (1, 3, 18). Lysates derived from the wild-type and UspA1-deleted strains were probed with MAb to the UspA1 protein to confirm the expected UspA1 expression (Fig. 3A and B, top). These blots were subsequently reprobed with soluble CEACAM1 or CEACAM5 to assess the CEACAM-binding activity of blotted proteins. CEACAM binding colocalized with the UspA1 variants of *M. catarrhalis*

FIG. 2. CEACAM binding by recombinant *E. coli* expressing UspA1 variants. (A) *E. coli* whole-cell lysates were Western blotted and then probed to detect UspA1 protein (top) with MAb 24B5, and soluble CEACAM5 binding (bottom) was detected with anti-CEACAM (Dako). CEACAM5 binding was associated with UspA1 in 7 of 10 strains, while no binding was evident in the other three strains. (B) *E. coli* cells expressing the indicated UspA1 variants were spotted onto nitrocellulose and then incubated with soluble CEACAM5, followed by antibodies to detect bound CEACAM5. Ig, immunoglobulin. (C) *E. coli* cells expressing the indicated UspA1 variants were adsorbed to ELISA plates and then incubated with soluble CEACAM5, which was then detected using specific antibodies.

strains O12E, FIN2344, V1145, and V1156 but not with that from strain O35E (Fig. 3A and B, middle). To confirm that overlay-based experiments reflect the phenotypes of the intact *M. catarrhalis* strains, wild-type and mutant *M. catarrhalis* strain binding to soluble CEACAM1 and CEACAM5 was assessed using the ELISA-based assay (Fig. 3C). While different variants of the adhesins from other pathogenic bacteria may be specific for either CEACAM1 and/or CEACAM5 (10), the *M. catarrhalis* UspA1 proteins appear to bind either both CEACAMs or neither one. Altogether, these results demonstrate that CEACAM binding is conferred by certain UspA1 protein variants and that no other CEACAM-binding adhesins are expressed by *M. catarrhalis* that are detectable in this assay system.

Sequence analysis of CEACAM binding in UspA1 proteins. To reveal the basis of the differences in CEACAM binding for UspA1, the UspA1 amino acid sequences for each of the 10

FIG. 3. *M. catarrhalis* wild-type (wt) or UspA1-deficient (Δ) strain binding to CEACAMs. *M. catarrhalis* whole-cell lysates were separated by electrophoresis and were immunoblotted with MAb 24B5 to detect UspA1 (A and B, top) or binding to soluble CEACAM5 (A, bottom) or CEACAM1 (B, bottom). UspA proteins were detected using Cy5-conjugated secondary antibodies, while bound CEACAM was using Bodipy-conjugated antisera. *M. catarrhalis* ferric binding (Fbp) was probed using mouse anti-Fbp antiserum as a loading control (bottom). (C) *M. catarrhalis* strains were adsorbed into ELISA plates and then incubated with soluble CEACAM1 or CEACAM5. The bound CEACAMs were then detected using CEACAM cross-specific antisera (Dako CEA), followed by HRP-conjugated goat anti-rabbit immunoglobulin. Abs, absorbance.

strains tested were aligned. Previous work by Hill et al. (12) localized CEACAM1 binding to a 141-amino-acid polypeptide fragment derived from the C-terminal stalk region of UspA1_{ATCC 25238}. Peptides derived from this region are able to

bind CEACAM1 and block UspA1-dependent *M. catarrhalis* binding to CEACAM1-expressing cells (12). Our sequence alignments show that each UspA1 variant that lacks CEACAM-binding activity is missing a portion of the CEACAM-binding region (Fig. 4A). Consistent with the loss of these sequences not being an isolated event, the size of the deletion varied among the three proteins. Of these, UspA1_{TTA37} had the smallest deletion, lacking only 33 amino acids of the sequence corresponding to the CEACAM-binding region described previously by Hill et al. (12).

Fibronectin and Chang cell binding. In addition to CEACAM binding, various UspA1 proteins have been shown to bind extracellular matrix protein fibronectin (24). In particular, a 153-amino-acid peptide derived from the UspA1 protein from *M. catarrhalis* strain Bc5 was shown to be sufficient to mediate fibronectin binding and could inhibit UspA1-dependent binding to Chang cells (42). The minimal sequence capable of binding fibronectin spans a region containing a so-called “NINNY” repeat flanked on either side by a “VEEG” repeat (Fig. 4B), which was conserved between the UspA1 and UspA2 proteins expressed by strain Bc5 (42). Whether or not this entire region was necessary for fibronectin binding has remained undetermined. In order to understand whether the presence of these fibronectin-binding motifs correlated with CEACAM binding, we compared our UspA1 sequences (Fig. 4B). Based on this analysis, we observed that UspA1_{ATCC 43617} was unique in that it lacked the complete VEEG-NINNY-VEEG repeat. While the NINNY repeat is considered to represent the core of the fibronectin-binding sequence, the flanking regions were suggested to play a role in binding. The ordered VEEG-NINNY-VEEG sequence is highly conserved among UspA1 proteins but is less well conserved in UspA2 proteins. Subsequently, UspA2 proteins lacking portions of this ordered repeat were recognized to be deficient in adherence to fibronectin and in Chang cell binding (18, 24). Based upon these analyses, we predicted that UspA1_{ATCC 43617} would be unable to bind to Chang cells.

In order to determine whether the absence of one VEEG repeat would affect fibronectin-based Chang cell binding, we compared Chang cell binding by recombinant *E. coli* bacteria expressing UspA1_{ATCC 43617} to that of the prototypical UspA1_{O35E} variant. While UspA1_{O35E} expression conferred binding to Chang cells, bacteria expressing UspA1_{ATCC 43617} mirrored that of the background control *E. coli* strain (Fig. 4C). The adherence of the UspA1_{O35E} bacteria was further increased in the presence of serum, consistent with the ability of serum components such as fibronectin to facilitate binding (42). These results indicate that the first VEEG repeat is essential for UspA1-dependent binding to Chang epithelial cells and that naturally occurring variants of UspA1 can lack this binding function.

DISCUSSION

The surface protein UspA1 has been shown to play a primary role in *M. catarrhalis* attachment to a variety of different cell types (1, 13, 24). This association appears to result from its ability to bind host-derived molecules including fibronectin (42), laminin (41), and members of the CEACAM family of intercellular adhesion molecules (13). While not considered in

FIG. 4. UspA1 protein sequence alignments. (A) Alignment of variant sequences spanning the CEACAM-binding region of UspA1, defined by Hill et al. (12), which is delineated with a horizontal black line above the sequences. CEACAM binding by *M. catarrhalis* and/or recombinant *E. coli* strains, as determined in the aforementioned studies, is indicated. +, CEACAM binding; –, does not bind CEACAM. (B) Alignment of UspA1 variant sequences spanning the region reported to contribute to fibronectin and Chang cell binding (42). Relevant peptides from the UspA1 and UspA2 proteins of *M. catarrhalis* strain Bc5, which was originally used to define the fibronectin-binding sequences, are included for comparison with the sequences considered in this study. (C) Attachment of recombinant *E. coli* cells expressing UspA1_{O35E} or UspA1_{ATCC 43617} to Chang conjunctival epithelial cells in the presence or absence of FCS. The negative control is *E. coli* containing the pCC1-Kan construct (Kan).

this study, UspA1 also interacts with components of both the classical and alternative complement pathways by interacting with both C3 (32) and C4b (31).

The diversity of functions attributed to UspA1 is remarkable. However, the various binding activities have been largely considered to be independent of each other and often with different strains of *M. catarrhalis*. While it was generally assumed that each function would be conserved among UspA1 variants, the current study was prompted by our finding that prototypical *M. catarrhalis* strain O35E does not bind to certain CEACAM-expressing cell lines. Since the sequence of variant UspA1 proteins is frequently unavailable, we cloned and sequenced several additional *uspA1* genes to obtain a total of 10 genes encoding different UspA1 proteins. Three of the 10 UspA1 variants tested did not bind to CEACAMs (Fig. 2), and each of these variants lacked a portion of the sequence that has been shown to confer CEACAM1 binding (12). While *M. catarrhalis* expresses a number of different adhesins (4, 5, 15, 18, 20, 22, 33, 34), our UspA1-deficient mutants clearly demonstrated that UspA1 was the only adhesin with the potential to confer CEACAM binding.

The lack of fibronectin binding exhibited by the UspA1 protein from *M. catarrhalis* ATCC 43617 (Fig. 4C) is also a clear demonstration of the variant nature of individual UspA1 proteins. Fibronectin binding has been shown to mediate the adherence of *M. catarrhalis* to Chang cells and, unlike CEACAM binding, has been associated with both UspA1 and UspA2 (42). The amino acid sequence considered to contribute to fibronectin binding is present in UspA1, UspA2, and the naturally occurring chimera UspA2H; however, its association with each UspA protein is strain specific. The fact that both

UspA1 and UspA2 can confer fibronectin-mediated binding to Chang cells makes it difficult to test the relative contribution of each one in the context of *M. catarrhalis*. However, our nucleotide sequence analyses indicated that UspA1 from strain ATCC 43617 contained a deletion within the region that was previously shown to contribute to fibronectin binding (42), and our binding assays demonstrated that this variant could not adhere to Chang epithelial cells.

The existence of three different *M. catarrhalis* isolates (O35E, TTA37, and ATCC 43617) that are unable to bind CEACAM receptors makes it intriguing to speculate how this phenotype may contribute to *M. catarrhalis* colonization of the nasopharynx or the production of disease in the respiratory tract. *M. catarrhalis* can be found as a member of the commensal flora in healthy individuals or associated with distinct diseases in either children (i.e., otitis media) or adults (i.e., exacerbations of COPD). The specific location (nasopharynx, trachea, or middle ear) and manner (swab, tympanocentesis, or aspirate) in which *M. catarrhalis* isolates are obtained must be considered when strain-specific differences in phenotype are analyzed. It is interesting in this regard that two of the three strains that lack the CEACAM-binding ability were isolated from adult tracheal aspirates.

While much remains to be learned about how each UspA1-associated function contributes to colonization or disease production (or both), it is clear that the ability to bind CEACAM and fibronectin is not a universally conserved function among *M. catarrhalis* strains. Sequence analyses and functional assays must be performed before the spectrum of potential functions can be deduced. We must also consider that other functions, including complement binding, may also vary among *M. ca-*

tarrhalis isolates (3). It is therefore enticing to consider that the ability of UspA1 to engage different combinations of host receptors and serum components will elicit different cellular responses (29, 35, 38) and that the different combination of activities may define the colonization ability and virulence potential of each strain.

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