The Respiratory Pathogen

**Moraxella catarrhalis** Binds to Laminin via Ubiquitous Surface Proteins A1 and A2

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**Moraxella catarrhalis** is one of the leading causes of exacerbations in chronic obstructive pulmonary disease (COPD). In the present article, we show that *M. catarrhalis* binds to the major basement-membrane glycoprotein laminin, which is thickened in the airways of smokers. Using clinical strains of *M. catarrhalis* and their corresponding ubiquitous surface protein (Usp) A1/A2 mutants, we demonstrate that UspA1 and UspA2 are important for the laminin interaction. Binding assays with recombinant proteins demonstrated that the binding regions are localized within the N-terminal fragments, where both proteins form a globular head. Thus, UspA1/A2–dependent interactions with laminin might promote bacterial adhesion, particularly in smokers with COPD.

*Moraxella catarrhalis* is an important cause of lower-respiratory-tract infections in adults with chronic obstructive pulmonary disease (COPD). Its major outer membrane proteins (OMPs) and their interactions with the human host have been intensely studied and are reviewed elsewhere [1]. The OMPs ubiquitous surface protein (Usp) A1 and UspA2 have highly conserved epitopes and are of considerable interest as potential vaccine candidates [1]. These proteins are multifunctional in nature and are essential for the attachment of *M. catarrhalis* to epithelial cells [2–4]. UspA1 also targets the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) found on epithelial cells [5]. Moreover, UspA1 and UspA2 are involved in serum resistance by binding C3, C4BP, and vitronectin [6–9].

In the majority of patients with COPD, *M. catarrhalis* UspA1 and UspA2 are targets for antibodies that clear the organism [10]. However, the role that these proteins may play in the pathogenesis of *M. catarrhalis* infection in this group of patients is unclear. It is known that chronic smoking is associated with lower epithelial membrane integrity, which exposes the basement membrane. Interestingly, the laminin layer in the basement membrane is significantly thicker in smokers than in nonsmokers [11]. In the present study, we show that *M. catarrhalis* binds laminin and that UspA1 and UspA2 are responsible for the interaction. This may be especially important in smokers with COPD.

**Materials and methods.** The clinical *M. catarrhalis* strains BBH18 and RH4 and their corresponding mutants were as previously described [7]. The mutants expressed amounts of *M. catarrhalis* IgD-binding protein (MID) equal to those expressed by wild-type strains (data not shown). Another random selection of 15 clinical strains of *M. catarrhalis* were used: S6-4404, S6-4307, S6-4195, S6-4036, S6-3979, S6-3992, S6-3806, and S6-3761 were clinical isolates from the Department of Medical Microbiology, Malmö University Hospital; Perez 112 and RH1 have been described elsewhere [12]; and Bro1, Japan 6, BBH15, Riley 118, and RH2 were isolates obtained from J. J. Christensen (State Serum Institute, Copenhagen, Denmark). Bacteria were routinely cultured in brain-heart infusion (BHI) agar plates at 37°C overnight and resuspended in buffer for the experiments. The UspA1-deficient, UspA2-deficient, and double mutants were cultured in BHI supplemented with antibiotics as described elsewhere [7].

Recombinant UspA¹⁵⁰–⁷⁷⁰ (aa 50–770 of UspA1) and UspA²¹⁰–⁵³⁹, which are devoid of their hydrophobic C-termini, were manufactured [7]. In addition, recombinant proteins corresponding to multiple regions spanning UspA¹⁵⁰–⁷⁷⁰ and UspA²¹⁰–⁵³⁹ were used [4].

Rabbit anti-MID polyclonal antibodies (PAs) were used [7, 12]. Rabbit anti-laminin PAb was obtained from Sigma, and swine horseradish peroxidase (HRP)–conjugated anti-rabbit PAb and fluorescein isothiocyanate (FITC)–conjugated swine anti-rabbit PAB were obtained from Dakopatts.

Laminin binding to whole bacteria was analyzed by incubation of bacteria (10⁶) with 5 μg of laminin in PBS with 2% fish gelatin (hereafter referred to as “PBS-gelatin”) for 1 h at 4°C. After washings, the bacteria were incubated with a rabbit anti-laminin PAB for 30 min. Thereafter, the bacteria were

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washed and incubated for 30 min with FITC-conjugated anti-rabbit PAb. After washes, bacteria were analyzed by flow cytometry. All incubations were kept in a final volume of 100 μL of PBS-gelatin. Inhibition experiments were performed by preincubating 2 μg of laminin with various concentrations of UspA fragments (0–10 μmol/L). Inhibition appeared saturable and was evident at a ≥1 μmol/L concentration of UspA protein. Repeated experiments were performed with 1 μmol/L. The residual-free amount of laminin that bound to M. catarrhalis was determined by flow cytometry as described above.

Laminin was labeled with 0.05 mol of iodine (Amersham Biosciences) per mole of protein by use of the chloramine-T method. M. catarrhalis strains BBH18 and RH4, together with their derived mutants (108), were incubated for 1 h at 37°C with [125I]-labeled laminin (1000 kcpm/sample) in PBS and 2% bovine serum albumin (BSA). After 3 washings with the same buffer, [125I]-labeled laminin bound to bacteria was measured in a gamma counter (Wallac).

To test the binding of M. catarrhalis to immobilized laminin, microtiter plates (Nunc-Immuno Module; Nunc) were coated with Engelbreth-Holm-Swarm mouse sarcoma laminin (30 μg/mL) (Sigma), BSA (30 μg/mL), or type IV collagen (30 μg/mL) (Sigma) in Tris-HCl, pH 9.0, at 4°C overnight. The plates were washed with PBS and 0.05% Tween 20, pH 7.2, and subsequently blocked with 2% BSA in PBS and 0.1% Tween 20, pH 7.2. M. catarrhalis RH4 and BBH18 (108) in 100 μL of PBS-gelatin were then added, followed by incubation for 1 h. Unbound bacteria were removed by washing. Residual bound bacteria were detected by use of an anti-MID PAb, followed by detection with HRP-conjugated anti-rabbit PAb. Both recombinant UspA1 50–770 and UspA2 30–539 proteins were tested for laminin binding, by use of flow cytometry. Positive binding was arbitrarily defined as a mean fluorescence intensity ≥10. Interestingly, the majority of the strains (93.3%) significantly bound laminin (data not shown). To study the laminin binding in detail, 2 clinical M. catarrhalis isolates (RH4 and BBH18) and their specific mutants lacking UspA1, UspA2, or both proteins were analyzed by use of a whole-cell ELISA. The wild-type M. catarrhalis RH4 strongly bound to immobilized laminin (figure 1A). The M. catarrhalis RH4ΔuspA1 mutant showed laminin binding similar to the wild type. In contrast, the M. catarrhalis RH4ΔuspA2 mutant and the double mutant RH4ΔuspA1/A2 bound significantly less to laminin (15.2% and 18.1%, respectively), compared with the wild type. The binding of M. catarrhalis RH4ΔuspA2 and RH4ΔuspA1/ A2 to laminin did not significantly differ from background binding to wells coated with BSA or type IV collagen. Similar results were obtained with M. catarrhalis BBH18 (figure 1B). In M. catarrhalis BBH18 and RH4 wild-type bacteria, UspA2 is the predominant protein expressed, compared with UspA1, which constitutes only 8% of the total UspA proteins found at the bacterial surface [7]. This explains the minimal difference in laminin binding between the wild-type and UspA1 mutant bacteria.

To validate the importance of UspA1 and UspA2 in laminin binding, a radioimmunoassay was performed with [125I]-labeled laminin. Both M. catarrhalis RH4 and BBH18 strongly bound soluble [125I]-labeled laminin, whereas mutants devoid of UspA1 and UspA2 showed minimal binding. In figure 1C, results from experiments with M. catarrhalis RH4 and Escherichia coli, which was included as a negative control, are shown. Considered together, the results of the present study show that UspA1 and UspA2 were the predominant Moraxella proteins responsible for bacterial laminin binding.

To further analyze the binding between UspA1/A2 and laminin, truncated UspA150–770 and UspA250–539 proteins were produced in E. coli. Recombinant proteins were coated on microtiter plates and incubated with increasing concentrations of laminin. Bound laminin was detected by use of a rabbit anti-laminin PAb, followed by incubation with an HRP-conjugated anti-rabbit PAb. Both recombinant UspA150–770 and UspA250–539 strongly bound soluble laminin, and the binding was dose dependent and saturable (figure 2A and 2B).

To define the laminin-binding domains, recombinant UspA1 and UspA2 spanning each entire molecule was manufactured. Laminin was incubated with immobilized truncated UspA1 and UspA2 fragments, followed by quantification by use of ELISA. UspA150–441 bound to laminin almost as efficiently as UspA150–770 did, suggesting that the binding domain was within this part of the protein (figure 2A). However, among the other truncated UspA1 fragments spanning this region, no other fragment appeared to bind laminin. The N-terminal frag-
Figure 1. Binding of *Moraxella catarrhalis* to laminin, via ubiquitous surface protein (Usp) A1 and UspA2. A and B, Binding of wild-type *M. catarrhalis* RH4 and BBH18 to laminin, compared with mutants devoid of UspA1 (ΔuspA1), UspA2 (ΔuspA2), or both proteins (ΔuspA1/A2). Laminin (30 μg/mL) was coated on microtiter plates, followed by blocking, incubation with bacteria, and, finally, washes. Bound bacteria were detected by use of an anti- *M. catarrhalis* IgD-binding protein polyclonal antibody (PAb) and a horseradish peroxidase–conjugated anti-rabbit PAb. Control experiments were performed by coating plates with bovine serum albumin (BSA) and type IV collagen at the same concentration. C, Radioimmunoassay showing *M. catarrhalis* UspA1/A2–deficient mutants, which did not bind [125I]-labeled laminin. *M. catarrhalis* RH4 wild-type isolates were compared with mutants devoid of UspA1, UspA2, or both UspA1 and UspA2. *Escherichia coli* BL21 was used as a negative control. Background indicates no bacteria. Bacteria were incubated with [125I]-labeled laminin, followed by 3 washes and analysis in a gamma counter. The mean values of 3 experiments are shown. Error bars indicate SDs.

**Discussion.** *M. catarrhalis* is a common cause of infectious exacerbations in patients with COPD. The success of this species in patients with COPD is probably related in part to its large repertoire of adhesins. In addition, there are pathological changes, such as loss of epithelial integrity, which results in exposure of basement membrane, where the laminin layer itself is thickened in smokers [11]. Interestingly, other pathogens causing respiratory-tract infections, such as the *Haemophilus influenzae*, also possess laminin-binding proteins [13].

We recently demonstrated that both UspA1 and UspA2 bind fibronectin [4]. The fibronectin–binding domains are located within UspA1299–452 and UspA2 165–318. In the present study, the N-terminal halves of UspA130–491 and UspA2 30–539 (containing the fibronectin domains) were shown to bind laminin (figure 2). However, the smallest fragments that bound fibronectin—
that is, UspA$^{1299-452}$ and UspA$^{165-318}$—did not bind laminin to any appreciable extent. In fact, fragments smaller than the N-terminal half of UspA (UspA$^{100-471}$) lost all of their laminin-binding ability, whereas, in the case of UspA2, only UspA2$^{30-170}$ bound laminin, albeit at a lower level than the whole recombinant protein (UspA$^{30-539}$). Inhibition experiments with recombinant fragments were able to confirm that UspA1 residues within the N-terminal half were responsible for laminin binding. The inability of the smaller UspA2 fragments to inhibit could be explained by the relatively weak binding found even by ELISA. It is likely that recombinant fragments may not constitute the tertiary structure necessary for their biological activity. Nevertheless, the results of the present study support the notion that various parts of UspA1 and UspA2 molecules play different functional roles (figure 2D).

When the shortest laminin-binding regions of UspA1 and UspA2 are compared, there is, however, little amino acid homology between UspA2$^{30-170}$ and UspA1$^{30-471}$ (data not shown). This is not surprising, because it is known that both proteins have a “lollipop”-shaped globular head structure, despite having only 22% amino acid identity in their N-terminal halves [14, 15]. The localization of the binding domains at the N-
terminal ends is logical, because this is where these OMPs come into contact with the basement membrane.

Bacterial factors mediating adherence to tissue and extracellular matrix components are grouped together in a single family named “microbial surface components recognizing adhesive matrix molecules” (MSCRAMMs). Only fibronectin and vitronectin bound UspA proteins, whereas other glycoproteins, such as type IV collagen, fetuin, and heparin, did not [2]. Because UspA1 and UspA2 bind both fibronectin and laminin, these proteins can be designated MSCRAMMs. Interestingly, both UspA1 and UspA2 bind C4b-binding protein, UspA21527–458 interacts with the complement protein C3, and UspA1527–667 binds to CEACAM [5, 7, 9]. In the light of recent findings that UspA1 and UspA2 interact with various human proteins, it is evident that UspA1 and UspA2 are multifunctional proteins with multiple domains interacting with different ligands that the bacteria encounter in the respiratory tract. Similar broad-spectrum binding profiles have been reported for other bacterial proteins, such as YadA of Yersinia enterocolitica, to which UspA1 and UspA2 bear a structural relationship. Notably, YadA also binds both fibronectin and laminin [14].

In summary, we have shown that UspA1 and UspA2 are crucial for the M. catarrhalis interaction with the basement-membrane glycoprotein laminin. UspA1/A2–dependent laminin binding may play an important role in the pathogenesis of M. catarrhalis infection in patients with COPD.

References