MAJOR ARTICLE

# Physiologic Cold Shock Increases Adherence of *Moraxella catarrhalis* to and Secretion of Interleukin 8 in Human Upper Respiratory Tract Epithelial Cells

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***Moraxella catarrhalis*, a major nasopharyngeal pathogen of the human respiratory tract, is exposed to rapid and prolonged downshifts of environmental temperature when humans breathe cold air. In the present study, we show that a 26°C cold shock up-regulates the expression of UspA1, a major adhesin and putative virulence factor of *M. catarrhalis*, by prolonging messenger RNA half-life. Cold shock promotes *M. catarrhalis* adherence to upper respiratory tract cells via enhanced binding to fibronectin, an extracellular matrix component that mediates bacterial attachment. Exposure of *M. catarrhalis* to 26°C increases the outer membrane protein–mediated release of the proinflammatory cytokine interleukin 8 in pharyngeal epithelial cells. Furthermore, cold shock at 26°C enhances the binding of salivary immunoglobulin A on the surface of *M. catarrhalis*. These data indicate that cold shock at a physiologically relevant temperature of 26°C affects the nasopharyngeal host-pathogen interaction and may contribute to *M. catarrhalis* virulence.**

*Moraxella catarrhalis* colonizes the surface of the human nasopharynx and is a major cause of acute otitis media in children and exacerbations of chronic obstructive pulmonary disease (COPD) in adults [1, 2]. Clinical studies have revealed that the prevalence of both colonization and infections caused by this pathogen display seasonal variation and are greatest in winter [3–6]. This phenomenon is incompletely understood. Viral infections occurring during the cold season pave the way for subsequent secondary bacterial infection by T cell–mediated release of interferon γ, which inhibits bacterial phagocytosis by macrophages [7] and increases the expression of adhesion receptors on epithelial cells (eg, carcinoembryonic antigen-related cell adhesion molecule 1) [8]. Also, virus-induced inflammation results in exposure of extracellular matrix proteins, which facilitates adherence of bacterial pathogens.

In addition—and this has received little attention in the literature—the human nasopharyngeal flora is repeatedly exposed to rapid downshifts of environmental temperature. Breathing cold air (eg, −1°C at 10–20 L/min) reduces the nasopharyngeal temperature from 34°C at room temperature to ~25°C within several minutes and for extended periods of time [9]. Temperature is a critical environmental factor, and cold shock (as it has been characterized for *Escherichia coli*) affects the bacterial transcriptome in a concerted attempt to maintain essential cellular functions [10]. Our previous findings established that a 26°C cold shock results in up-regulation of the UspA1 adhesin of *M. catarrhalis* and enhances adherence to human conjunctival cells [11]. This response occurs in both phylogenetic lineages of *M. catarrhalis* and entails adaptive events in multiple outer membrane (OM) components. Cold shock, which occurs when humans inspire cold air [9], is a physiologic phenomenon during the cold season and can affect the host-pathogen interaction in

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several ways. First, enhanced adherence may increase the bacterial density on the nasopharyngeal surface. Studies in children have indicated that the density of *M. catarrhalis* in the nasopharynx is positively associated with prolonged respiratory tract symptoms [12] and a greater likelihood of otitis media [13]. Second, temperature-induced variation in OM composition, including OM proteins (OMPs) and lipooligosaccharide (LOS), can affect the recognition of bacteria by cell-surface receptors (eg, cell-associated fibronectin) [14], influencing the adherence of *M. catarrhalis* to host cells. Third, cold shock–induced alteration of the OM may affect the inflammatory responses in both respiratory epithelial and monocytic cells [15, 16] and influence the mucosal immune response. Secretory immunoglobulin A (sIgA) against *M. catarrhalis* are directed against a small number of major OMPs, including UspA1 [17, 18].

In the present study, we investigated the effect of a 26°C cold shock on the ability of *M. catarrhalis* to adhere to and induce an inflammatory response in human upper respiratory tract cells. Cold shock enhanced bacterial adherence to pharyngeal and laryngeal epithelial cells and induced an elevated interleukin 8 (IL-8) response in comparison with those for bacteria incubated at 37°C. Furthermore, cold shock increased the binding of salivary immunoglobulin A (IgA) to the surface of *M. catarrhalis*.

## MATERIALS AND METHODS

***Cell lines and growth conditions.*** Detroit 562 pharyngeal cells (ATCC CCL-138) were maintained in Eagle minimal essential medium (Invitrogen), supplemented with 10% of heat-inactivated fetal calf serum (FCS), 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate (Sigma), 1% nonessential amino acids (Sigma), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% $CO_2$. Hep-2 laryngeal cells (ATCC CCL 23) were grown in the Eagle minimal essential medium supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin.

***Bacterial strains and culture conditions.*** *M. catarrhalis* strain O35E, its isogenic *uspA1* (O35E.*uspA1*) and *lpxA* (O35E.*lpxA*) mutants, and the clinical isolates 300, 415, and 420 have been described elsewhere [11, 19]. Bacteria were cultured at 37°C and 200 rpm in brain-heart infusion (BHI) broth (Difco) or on BHI agar plates in an atmosphere containing 5% $CO_2$. Cold shock experiments were performed as described elsewhere [11]. Bacteria were grown overnight at 37°C, resuspended in fresh BHI medium, and grown to an optical density at 600 nm ($OD_{600}$) of 0.3. Subsequently, bacteria were exposed to 26°C or 37°C, respectively, for 3 h (unless otherwise stated), harvested by centrifugation, resuspended in cell culture medium, adjusted to an $OD_{600}$ of 1 (~$5 \times 10^8$ colony-forming units/mL), and used for infecting epithelial cells at indicated multiplicities of infection. To investigate inactivated *M. catarrhalis*, bacteria were resuspended in phosphate-buffered saline (PBS) and heat-killed by incubation at 60°C for 1 h.

***Preparation of* M. catarrhalis *OM vesicles and LOS.*** OM vesicles (OMVs), composed of OMPs and LOS [20], and purified LOS from strain O35E exposed for 3 h to either 26°C or 37°C, respectively, were prepared as described elsewhere [19, 21].

***Cell infection.*** Cells were seeded onto 24 well plates at a density of $2.5 \times 10^5$ cells/well in medium without antibiotics 24 h before infection. After adhesion, cells were starved in serum-free medium for 18 h to avoid increased adherence of bacteria, considering the ability of serum components (such as fibronectin) to facilitate binding [22, 23] and to get rid of the serum growth factors that can influence cytokine secretion. Cells were infected with *M. catarrhalis* at the indicated multiplicity of infection, centrifuged for 5 min at 165 *g*, and incubated at 37°C in 5% $CO_2$. To assess the proinflammatory effects of OMPs or LOS, cells were stimulated with purified OMVs or LOS at the indicated concentrations. Lipopolysaccharide (LPS) from *Salmonella enterica* (Sigma) was used as a positive control. Cell viability was evaluated morphologically and by trypan blue exclusion.

***IL-8 enzyme-linked immunosorbent assay.*** Cells were infected as described above. Growth media were collected, centrifuged, and stored at −80°C. IL-8 was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (R&D Systems).

***Adherence assay.*** The ability of *M. catarrhalis* exposed to 26°C or 37°C, respectively, to adhere to epithelial cells was measured, as described elsewhere [11, 24].

***Assessment of messenger RNA stability.*** Messenger RNA (mRNA) stability was determined as described elsewhere [25, 26]. An overnight culture of strain O35E was resuspended in fresh BHI broth and grown to an $OD_{600}$ of 0.3. Subsequently, bacteria were exposed to 26°C or 37°C, respectively, for 1 h. Portions (5 mL) were then removed and mixed with an equal volume of 50 mmol/L sodium azide and kept on ice. Rifampicin (Sigma) was added to the rest of the bacterial cultures at a final concentration of 150 μg/mL. Additional 5-mL portions of the cultures were removed after 2, 5, and 10 min and treated as described above. RNA was isolated and used for complementary DNA synthesis as described elsewhere [11]. To assess for DNA contamination, RNA samples were also run without reverse transcriptase. Quantitative real-time polymerase chain reaction (PCR) was performed in triplicate for both target (*uspA1* and *recA*) and normalizer (16S ribosomal RNA [rRNA]) genes. Primers and probes for *uspA1*, *recA*, and 16S rRNA were used as described elsewhere [11, 27]. Relative quantification of gene expression was performed using the comparative threshold method. The ratios obtained after normalization were expressed

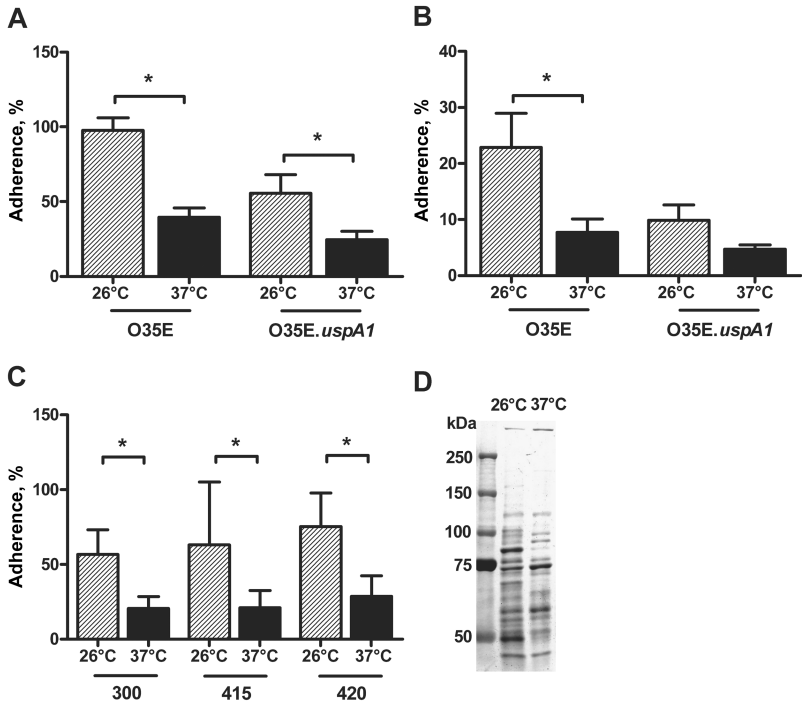


**Figure 1.** Increases in adherence of *Moraxella catarrhalis* to epithelial cells of the upper respiratory tract due to cold shock. Shown is adherence of strain O35E, its isogenic mutant O35E.*uspA1*, and isolates 300, 415, and 420 to Detroit 562 (*A* and *C*) and Hep-2 (*B*) cells in vitro after exposure to 26°C or to 37°C for 3 h, using a 30-min incubation period for attachment. The level of adherence is expressed as the percentage of bacteria attached to human cells relative to that for the original inoculum added to the well. Means ± 1 standard deviation for 2 or 3 independent experiments performed in triplicate are shown. The corresponding OMP profiles of *M. catarrhalis* strain O35E exposed to 26°C or 37°C for 3 h were visualized by Coomassie brilliant blue staining (*D*). $^*P < .05$ for 26°C versus 37°C (1-way analysis of variance [*A* and *B*] or *t* test [*C*]).

as folds of change compared with untreated samples (the amount of mRNA at $t = 0$ min was considered 100%). Half-life was determined by plotting the percentage of mRNA left versus the time elapsed after addition of rifampicin. The equation describing the best-fitting line (Excel software; Microsoft) was used to determine the time at which 50% of the original mRNA had been degraded.

***Flow cytometry.*** The capacity of *M. catarrhalis* to bind to fibronectin was analyzed by flow cytometry [22]. Bacteria grown to mid-logarithmic phase were exposed to either 26°C or 37°C for 3 h. Subsequently, the $OD_{600}$ was adjusted to 0.2, and 200-μl aliquots were centrifuged, washed in PBS–1% bovine serum albumin (BSA), resuspended, and incubated in 200 μL of 50 μg/mL fibronectin (Sigma) for 1 h. Bacteria were harvested and incubated for 1 h at room temperature in 200 μL of a 1:100 dilution of mouse anti–human fibronectin monoclonal antibody (Sigma). Bacteria were washed, incubated for 30 min in 200 μL of a 1:100 dilution of Alexa 488–conjugated goat anti–mouse antibody (Invitrogen), transferred to 2 mL of PBS containing 1% paraformaldehyde, and analyzed on a FACScan cytometer using CellQuest software (version 4.2; BD Biosciences). Anti–human fibronectin antibody and Alexa 488–conjugated anti–mouse antibody were added separately as negative controls.

The ability of *M. catarrhalis* to bind to salivary and colostrum IgA (Sigma) was analyzed as described elsewhere [17].

***Statistical analysis.*** Data were expressed as mean ± 1 standard deviation (SD). Differences between groups were analyzed by a 2-tailed *t* test and 1- or 2-way analysis of variance (ANOVA) with a Bonferroni posttest using Prism software (version 5.01; GraphPad). $P < .05$ was defined as statistically significant.

## RESULTS

***Increases in adherence of* M. catarrhalis *to epithelial cells of the upper respiratory tract due to cold shock.*** Because cold shock induces expression of UspA1, we investigated whether it affects the attachment of *M. catarrhalis* strain O35E to human pharyngeal and laryngeal epithelial cells. As shown in Figure 1, cold shock significantly enhanced adherence to pharyngeal (2.5-fold; Figure 1*A*) and laryngeal (3-fold; Figure 1*B*) cells, in comparison with exposure to 37°C. Furthermore, cold shock increased adherence of other *M. catarrhalis* clinical isolates to pharyngeal cells (Figure 1*C*), indicating that this effect is a general characteristic of *M. catarrhalis* that express UspA1. To evaluate whether increased adherence at 26°C is attributable to UspA1 only, the isogenic mutant O35E.*uspA1* was tested. The

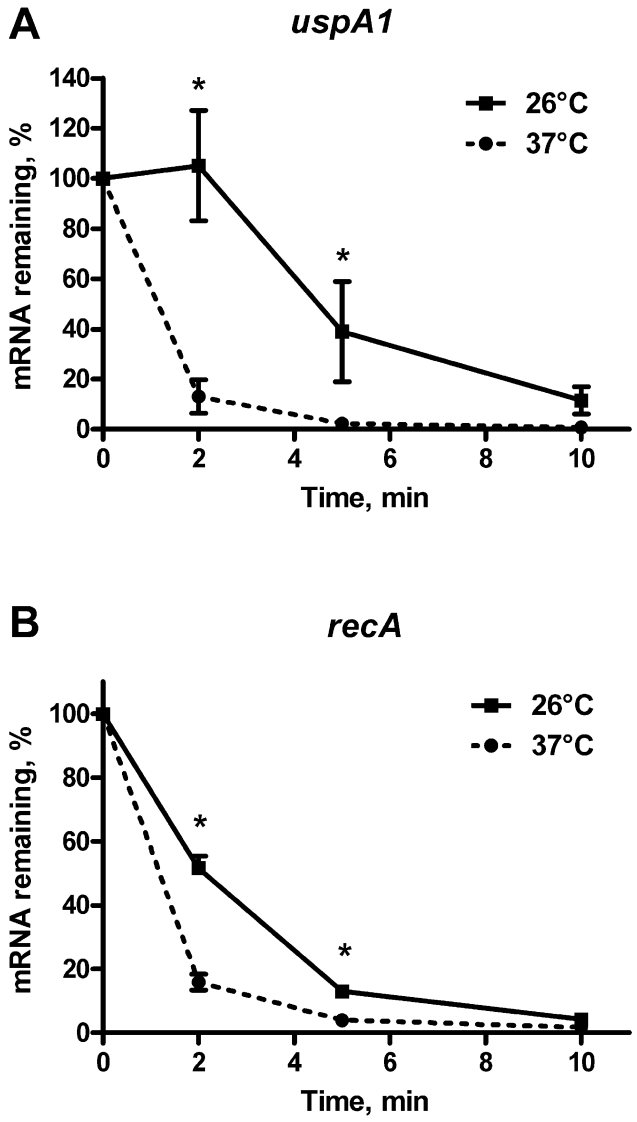


**Figure 2.** Increased levels of *Moraxella catarrhalis uspA1* and *recA* mRNA stability due to cold shock. *M. catarrhalis* strain O35E, grown to midlogarithmic phase (optical density read at 600 nm of 0.3), was exposed for 1 h to 26°C or 37°C. RNA isolated at different time points after the addition of rifampicin was analyzed by quantitative real-time reverse-transcription polymerase chain reaction to determine the amount of *uspA1* (*A*) and *recA* (*B*) transcripts. The percentage of mRNA remaining at each time point was determined. Results are expressed as means ± 1 standard deviation for 2 or 3 separate experiments performed in triplicate. **P* < .05 for 26°C versus 37°C (2-way analysis of variance).

absence of UspA1 substantially reduced adherence of *M. catarrhalis* to both cell lines (Figures 1*A* and 1*B*), demonstrating that UspA1 is a key adhesin; however, a cold shock effect was still observed in the mutant, indicating that other adhesins may also be involved. This concept is supported by comparative analysis of OMPs visualized by Coomassie blue staining, demonstrating some differences in the OMP profile of strain O35E exposed to the different temperatures (Figure 1*D*).

***Increases*** **in uspA1** ***mRNA stability due to cold shock.*** A conceivable explanation for the greater abundance of *uspA1* mRNA molecules after cold shock [11] is greater stability at 26°C. To study this, mRNA stability experiments were per-

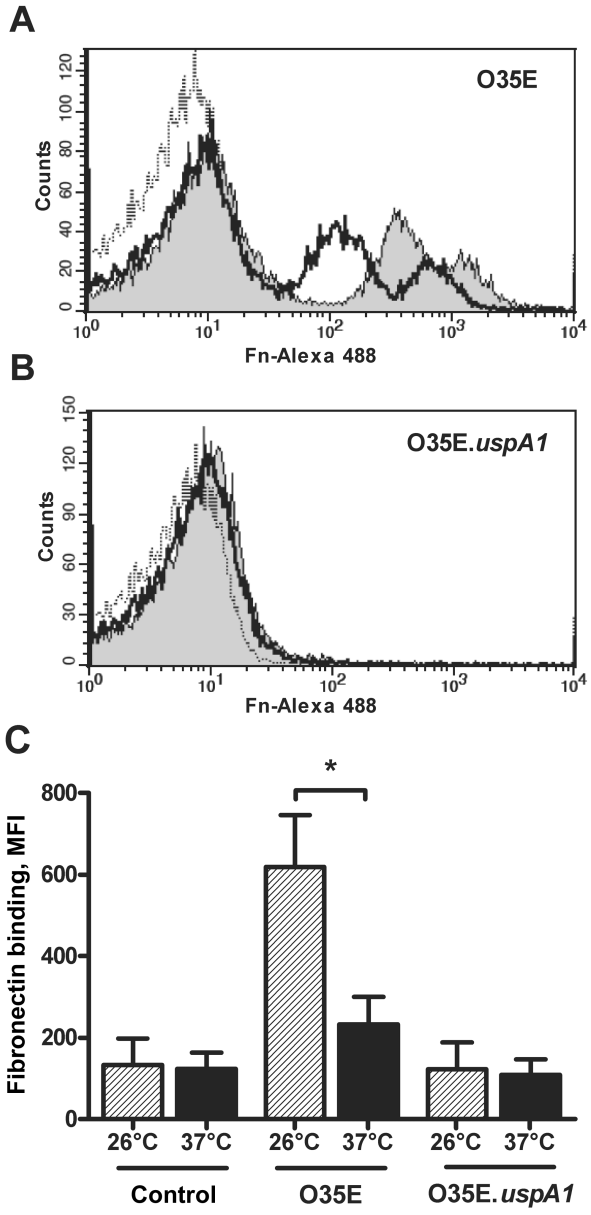


**Figure 3.** Binding of *Moraxella catarrhalis* to fibronectin. Strain O35E and its UspA1-deficient mutant (O35E.*uspA1*) were exposed to 26°C or 37°C for 3 h, harvested, and incubated with soluble fibronectin, followed by a mouse anti–human fibronectin antibody. Alexa 488–conjugated anti–mouse antibody was added, followed by flow cytometry analysis. Representative flow cytometry profiles of *M. catarrhalis* strain O35E (*A*) and O35E.*uspA1* (*B*) after exposure at 26°C (*gray*) or 37°C (*black*) show UspA1-dependent binding to soluble fibronectin. The dotted line represents the negative control (bacteria incubated with secondary antibodies only). The geometric mean fluorescence intensity ± 1 standard deviation for 2 experiments performed is shown in panel *C*. **P* < .05 for 26°C versus 37°C (1-way analysis of variance).

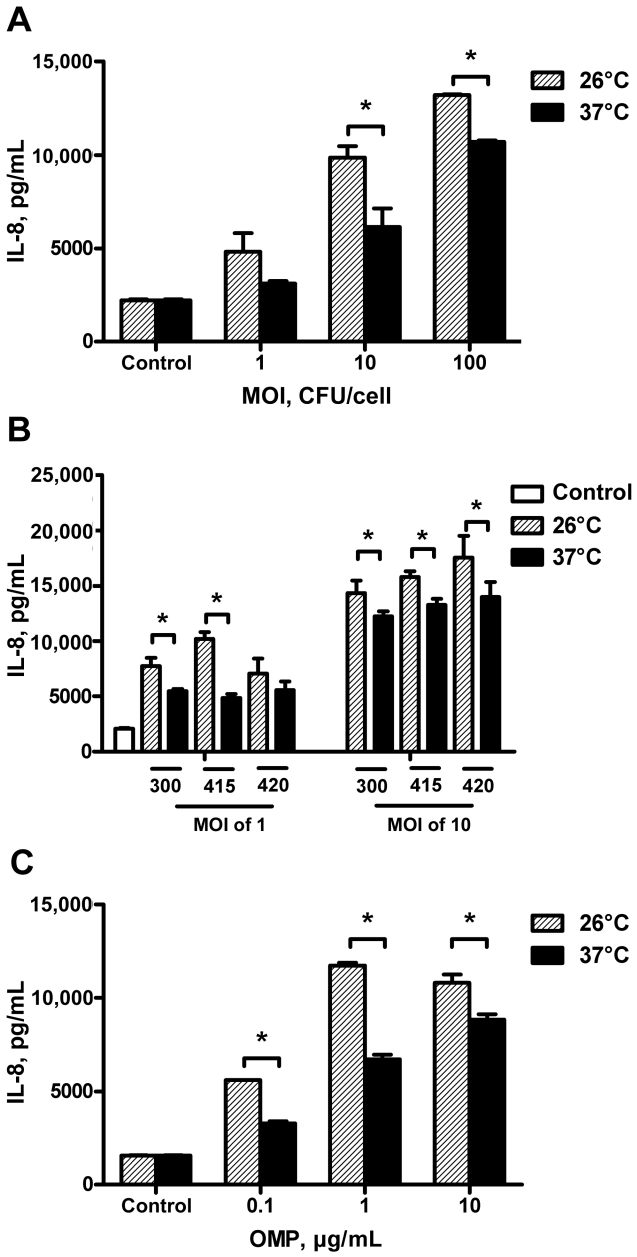


**Figure 4.** Increase in the outer membrane protein–mediated release of the proinflammatory cytokine interleukin 8 (IL-8) in Detroit 562 epithelial cells due to cold shock. Cells were either incubated for 16 h with increasing doses of heat-inactivated strain O35E (*A*) and isolates 300, 415, and 420 (*B*) exposed to 26°C or 37°C or stimulated with OMP isolated from *Moraxella catarrhalis* exposed to 26°C or 37°C *(C)*. IL-8 secretion in the supernatants was measured by an enzyme-linked immunosorbent assay. A representative experiment of 2 or 3 independent experiments is shown. Results are expressed as means ± 1 standard deviation of duplicate wells. **P* < .05 for 26°C versus 37°C (2-way analysis of variance).

formed using RNA isolated from strain O35E at different time points after addition of rifampicin to stop de novo RNA transcription. Quantitative real-time reverse-transcription PCR (RT-PCR) showed a significant difference between 26°C and 37°C with respect to the relative amount of *uspA1* mRNA remaining after the addition of rifampicin (Figure 2*A*). The calculated half-life of *uspA1* transcripts after incubation at 37°C was 1.8 min, whereas incubation at 26°C prolonged it to nearly 3.0 min. Similar differences in mRNA stability of *uspA1* were obtained with *M. catarrhalis* strain 420 (data not shown). Thus, cold shock at 26°C stabilizes *uspA1* transcripts. To assess whether prolonged *uspA1* mRNA half-life is specific to this particular transcript alone or reflects a more general phenomenon, we assessed mRNA stability of *recA*, another gene whose expression level is increased after cold shock [11]. Cold shock stabilized *recA* transcripts (although to a smaller extent than *uspA1*), with a calculated half-life of 1.8 and 2.3 min at 37°C and 26°C, respectively (Figure 2*B*).

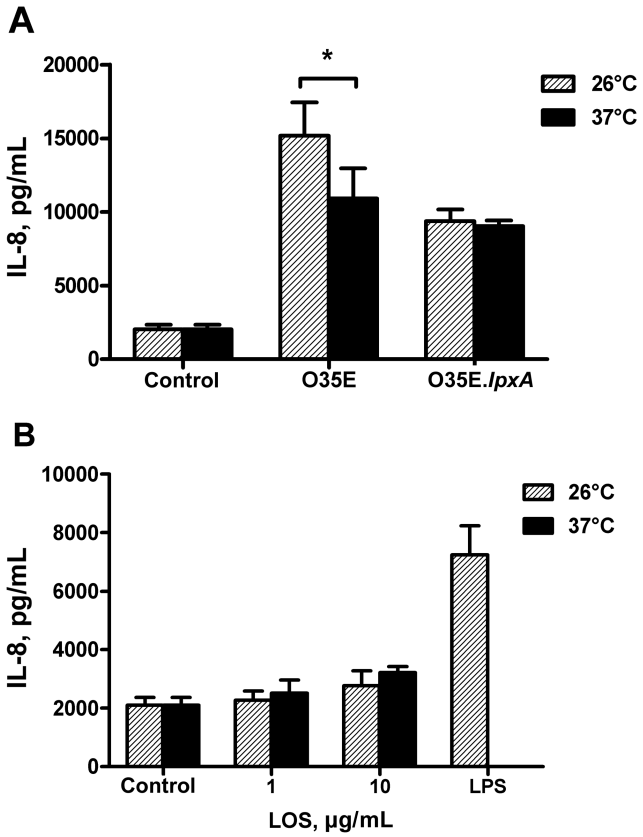


**Figure 5.** Lack of effect of cold shock on immunostimulatory properties of lipooligosaccharide (LOS) deficiency. *A*, Detroit 562 epithelial cells were infected (multiplicity of infection, 10) for 16 h with the heat-inactivated *M. catarrhalis* strain O35E or the LOS-deficient mutant O35E.*lpxA*, which were exposed to either 26°C or 37°C. *B*, Interleukin 8 (IL-8) secretion by LOS-stimulated Detroit 562 cells. Cells were stimulated for 16 h with LOS isolated from strain O35E, which was exposed to either 26°C or 37°C at the indicated concentrations. Lipopolysaccharide (LPS) (10 µg/mL) isolated from *Salmonella enterica* was used as a positive control. IL-8 secretion in the supernatant was measured by an enzyme-linked immunosorbent assay. Results are expressed as means ± 1 standard deviation for 2 separate experiments performed in duplicate. **P* < .05 for 26°C versus 37°C (2-way analysis of variance).

***Enhanced binding of fibronectin due to cold shock.*** UspA1 and, in some strains, UspA2 mediate adherence to epithelial cells by binding to cell-associated fibronectin [22]. In strain O35E, binding to fibronectin is exclusively dependent on UspA1 [22, 28]. Because a temperature drop from 37°C to 26°C induces an increase in surface expression of UspA1, we investigated whether it also affects binding to fibronectin. Strain O35E and its UspA1-deficient mutant were grown to midlogarithmic phase, exposed to 26°C or 37°C, and incubated with soluble fibronectin. Binding to fibronectin was significantly increased (63%) when bacteria were exposed to 26°C ($P < .05$) (Figures 3*A* and 3*C*). In contrast, the UspA1-deficient mutant did not bind to fibronectin (Figures 3*B* and 3*C*). These results indicate that the ability to bind to fibronectin is strongly enhanced by cold shock–induced UspA1 expression.

***Inducement of IL-8 release in pharyngeal epithelial cells due to cold shock.*** To study the contribution of cold shock to the proinflammatory response, we infected Detroit cells with bacteria grown at 37°C or after a temperature downshift to 26°C. Heat inactivation was performed before infection of cells to prevent bacterial replication during prolonged exposure at 37°C. Interaction between host cells and bacteria was investigated by comparing the release of IL-8 in cells after 16 h of incubation. Dose-specific IL-8 responses are shown in Figures 4*A* (O35E) and 4*B* (clinical isolates 300, 415, and 420). Cold-shocked bacteria induced a significantly greater release of IL-8 ($P < .05$). Similar (although not statistically significant) results for IL-8 secretion were obtained when pharyngeal cells were infected with the isogenic mutant O35E.*uspA1* (data not shown). Thus, cold-shocked *M. catarrhalis* whole cells induce a greater proinflammatory IL-8 response in pharyngeal epithelial cells than do control bacteria incubated at 37°C.

***Enhancement of IL-8 release due to OMVs isolated from cold-shocked bacteria.*** During infections, *M. catarrhalis* releases OMVs that contain OMPs and LOS [29]. OMVs are known to induce the secretion of various proinflammatory mediators, including IL-8, which contributes to bacterial pathogenesis [15]. To address the question of whether cold shock affects the immunostimulatory properties of the OM, we stimulated Detroit cells for 16 h with OMVs isolated from strain O35E exposed to either 26°C or 37°C and assessed IL-8 secretion. As shown in Figure 4*C*, OMVs isolated from *M. catarrhalis* grown at 26°C induced greater IL-8 release than did OMVs isolated from bacteria grown at 37°C. Similar results for IL-8 secretion were obtained when pharyngeal cells were stimulated with OMVs isolated from the isogenic UspA1-negative mutant (data not shown).

***Immunostimulatory properties of LOS not affected by cold shock.*** To study the contribution of LOS to the cold shock–induced inflammatory response, we assessed IL-8 secretion using the isogenic *lpxA* mutant of strain O35E [19, 30]. Detroit pharyngeal cells were infected for 16 h with heat-inactivated *M. catarrhalis* wild-type strain O35E and the LOS-deficient mutant O35E.*lpxA*, both exposed to either 26°C or 37°C. As shown in Figure 5*A*, the LOS-deficient mutant exposed at both temperatures elicited levels of IL-8 similar to the wild-type strain incubated at 37°C. To investigate whether cold shock affects the immunostimulatory properties of purified LOS, we stimulated pharyngeal cells for 16 h with LOS (1–10 $\mu$g/mL) isolated from strain O35E exposed to both temperatures. Again, there was no difference in IL-8 secretion (Figure 5*B*). Furthermore, the LOS-stimulated pharyngeal cells produced significantly lower levels of IL-8 than did the cells treated with whole bacteria or OMVs.

***Increases in sIgA binding on the surface of* M. catarrhalis *due to cold shock.*** Salivary and sputum IgA antibodies are known to react with OMPs and LOS [17, 18]. Given that cold shock induces UspA1, we hypothesized that a temperature downshift might increase surface binding of sIgA. We preincubated saliva samples from healthy adults with *M. catarrhalis* grown at 37°C or 26°C and determined sIgA binding by flowed cytometry. Figures 6*A* and 6*C* demonstrate significantly increased binding of salivary IgA on the surface of cold shock–induced *M. catarrhalis* ($P < .05$). The absence of UspA1 significantly decreased binding of IgA (Figures 6*B* and 6*C*), and cold shock had no significant effect. The absence of expression of other major OMPs, such as UspA2 and Hag, had little influence on IgA binding to *M. catarrhalis* (data not shown). Similar cold shock–dependent IgA binding was found using IgA isolated from human colostrum (data not shown). Thus, UspA1 is required for the maximal binding of salivary IgA on the surface of cold shock–induced *M. catarrhalis*.

***Impairment of the adherence to pharyngeal cells due to binding of sIgA to* M. catarrhalis.** Because sIgA binds to UspA1, we investigated whether it also affects bacterial adherence to Detroit cells. Bacteria exposed to either 26°C or 37°C were incubated with or without sIgA and layered on confluent Detroit cells; adherence was then determined. Precoating of *M. catarrhalis* with sIgA significantly impaired the adherence to pharyngeal epithelial cells (Figure 6*D*). While binding of sIgA similarly inhibited (~3-fold) the adherence of *M. catarrhalis* exposed to both temperatures, the adherence of IgA-coated *M. catarrhalis* after cold shock exceeded the adherence of IgA-coated bacteria grown at 37°C.

## DISCUSSION

We have demonstrated in vitro that cold shock imitating physiologic downshifts in human nasopharyngeal temperature increases *M. catarrhalis* adherence to pharyngeal and laryngeal epithelial cells (Figure 1). This effect is mediated by increased expression and/or function of several surface adhesins. Foremost, rapid downshift of temperature from 37°C to 26°C in-

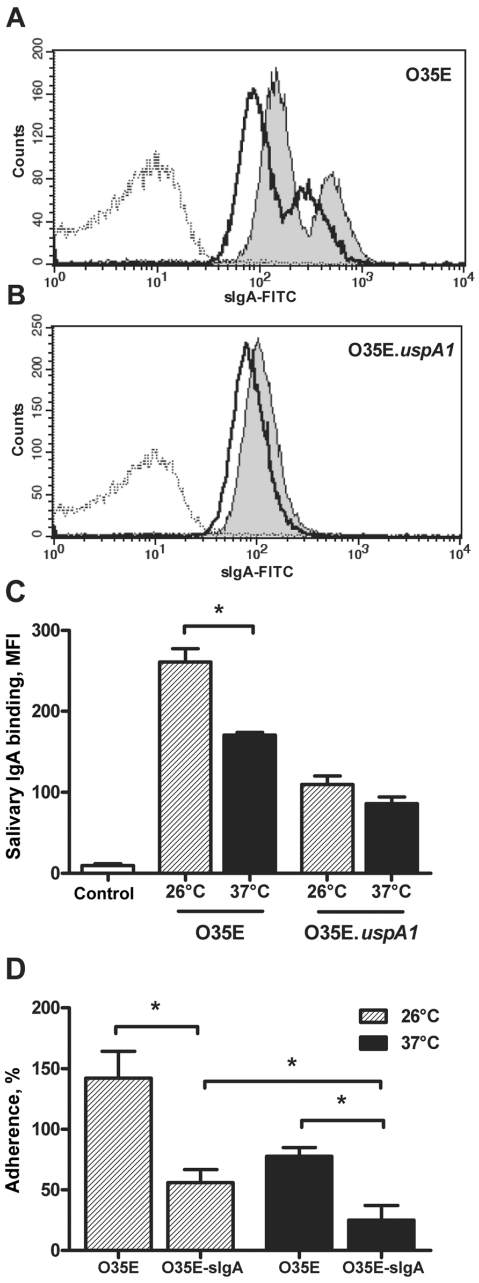


**Figure 6.** Increase in the binding of salivary immunoglobulin A (IgA) on the surface of *Moraxella catarrhalis* due to cold shock. Strain O35E and its isogenic mutant O35E.*uspA1* exposed to 26°C or 37°C for 3 h were preincubated with saliva samples (1:20 dilution) from healthy adults. Fluorescein isothiocyanate (FITC)–conjugated goat anti–human IgA antibody was added, and flow cytometry analysis was performed. Shown are representative flow cytometry profiles of strain O35E *(A)* and O35E.*uspA1 (B)* after exposure at 26°C (*gray*) or at 37°C (*black*), which demonstrate that UspA1 is required for effective secretory IgA (sIgA) binding on the surface of cold shock–induced *Moraxella catarrhalis*. The dotted line represents the negative control (bacteria incubated with secondary antibodies only). *C*, Salivary IgA binding. The mean fluorescence intensity (MFI) ± 1 standard deviation (SD) for 2 experiments performed is shown. *D*, Adherence assay demonstrating that binding of sIgA to *M. catarrhalis* impairs the adherence to pharyngeal epithelial cells. Wild-type strain O35E exposed to 26°C or 37°C was incubated with sIgA (100 μg/mL) isolated from human colostrums for 30 min and layered on confluent Detroit 562 epithelial cells; the number of adherent bacteria was determined after a 30-min incubation period. The level of adherence is expressed as the percentage of bacteria attached to human cells relative to that for the original inoculum added to the well. Means ± 1 SD for 3 independent experiments performed in triplicate are shown. *$P < .05$ for 26°C versus 37°C (1-way analysis of variance).

creases the expression of UspA1, a trimeric autotransporter adhesin that mediates binding to the host cell surface via cell-associated fibronectin [22]. Our data indicate that fibronectin binding was significantly increased when *M. catarrhalis* was exposed to 26°C ($P < .05$) (Figures 3*A* and 3*C*). Clinical experiments have revealed that *Staphylococcus aureus* isolates that exhibited greater adhesion to fibronectin and endothelial cells led to persistent bacteremia [31]. Therefore, the finding that cold shock increases the adherence of *M. catarrhalis* to upper respiratory tract cells and enhances binding to fibronectin could be clinically relevant during the cold season by temporarily increasing the organism's virulence.

Our results indicate that UspA1 is not the only cold shock–induced adhesin. Enhanced adherence was also observed in the UspA1-deficient mutant, indicating that other adhesins may also be involved. This observation warrants additional investigation because *M. catarrhalis* expresses several adhesins that interact with different host cell receptors, whose levels of expression appear to be specific to cell type [22, 32–34]. This strategy could enable the organism to colonize different regions of the respiratory tract.

The cold shock response in bacteria is organized as a complex stimulon in which posttranscriptional events play an important role [10]. It was recently demonstrated that cold shock–dependent alterations in transcript abundances in *S. aureus* can be attributed mainly to alterations in mRNA stability [26]. Thus, we analyzed whether increased expression of UspA1 is a direct result of cold-induced stabilization of mRNA molecules and found that 26°C stabilizes *uspA1* transcripts (Figure 2*A*).

The proinflammatory cytokine IL-8 plays a pivotal role in mucosal inflammation during respiratory tract infections [35]. We demonstrate that cold-shocked *M. catarrhalis* enhances the release of IL-8 in pharyngeal epithelial cells in comparison with bacteria incubated at 37°C (Figures 4*A* and 4*B*). An enhanced proinflammatory response was also observed when epithelial cells were stimulated with OMVs isolated from bacteria exposed to 26°C (Figure 4*C*), thus indicating that cold-shock conditions affect the immunostimulatory properties of OMPs. Greater local concentrations of IL-8 may promote the recruitment of inflammatory cells, which cause respiratory tract symptoms (eg, purulent nasal discharge, swelling and edema of the Eustachian tube, and purulent otitis media). Pharyngeal epithelial cells infected with the *M. catarrhalis* mutant that lacked expression

of UspA1 showed a similar degree of IL-8 release as did cells infected with the wild-type strain, suggesting that *M. catarrhalis*–induced IL-8 release is not dependent on UspA1 (or at least is so to a lesser degree).

LOS is an important virulence factor inducing proinflammatory responses in related bacteria, such as *Haemophilus influenzae* [36], *Neisseria meningitidis* [37], and *Neisseria gonorrhoeae* [38]. Our data, however, demonstrate that *M. catarrhalis* LOS appears to be a minor contributor to the stimulation of IL-8 by epithelial cells and that its inflammatory properties are not dependent on cold shock.

Respiratory tract infections typically are mucosal, and protection against them is at least in part mediated by mucosal immune responses. Asymptomatic colonization with *M. catarrhalis* is associated with a greater frequency of sputum IgA than COPD exacerbation, indicating that IgA may protect against infection [39]. Here, we demonstrate that cold shock increases UspA1-mediated binding of sIgA to the surface of *M. catarrhalis.* This emphasizes its role as an important target of protective immune responses. Consequently, children who lack UspA1-specific sIgA may be more susceptible to *M. catarrhalis* infections, particularly after exposure to cold air. This concept is supported by the fact that the presence of sIgA against the pneumococcal surface protein PspA in early childhood was significantly associated with a lower risk of pneumococcal acute otitis media [40].

We also found that precoating bacteria with sIgA from human colostrum inhibited bacterial adherence to pharyngeal epithelial cells. In contrast, the presence of sIgA increases pneumococcal adherence to pharyngeal epithelial cells [41]. UspA1 is a major adhesin of *M. catarrhalis*, and anti-UspA1 IgA antibodies may have the potential to block the attachment of *M. catarrhalis* to epithelial cells by inhibiting binding to receptors on the host cell surface. Adherence of sIgA-coated *M. catarrhalis* after cold shock was greater than that of sIgA-coated bacteria incubated at 37°C, indicating that cold-shocked bacteria may require more sIgA to be prevented from attaching to epithelial cells than bacteria exposed to 37°C.

Interestingly, during infection, *M. catarrhalis* releases OMVs carrying UspA1 that bind to human C3 and protect *H. influenzae* from complement-mediated killing [29]. Hence, increased presence of UspA1 in *M. catarrhalis* OMVs after cold shock may have a collateral effect by promoting the survival of *H. influenzae* during coinfection.

Thus, "catching a cold"—unquestionably a viral infection in most instances—may also induce adaptive events in the residential upper respiratory tract flora, whose clinical implications (based on our study results) deserve to be the focus of future studies addressing this particular aspect of the transition from asymptomatic colonization to bacterial secondary infection. This study demonstrates that a 26°C cold shock up-regulates OM adhesin expression of *M. catarrhalis* by prolonging the mRNA half-life, promotes bacterial adherence to host cells via enhanced binding to fibronectin, increases the OMP-mediated proinflammatory activity of pharyngeal epithelial cells, and enhances sIgA binding on the bacterial surface. These findings indicate that cold air in the human upper respiratory tract may affect the nasopharyngeal host-pathogen interaction.

## References


1. Faden H, Duffy R, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. J Infect Dis **1997**; 175:1440–5.
2. Palmu A, Herva E, Savolainen H, Karma P, Mäkela PH, Kilpi T. Association of clinical signs and symptoms with bacterial findings in acute otitis media. Clin Infect Dis **2004**; 38:234–42.
3. Van Hare GF, Shurin PA. The increasing importance of *Branhamella catarrhalis* in respiratory infections. Pediatr Infect Dis J **1987**; 6:92–4.
4. Mbaki N, Rikitomi N, Nagatake T, Matsumoto K. Correlation between *Branhamella catarrhalis* adherence to oropharyngeal cells and seasonal incidence of lower respiratory tract infections. Tohoku J Exp Med **1987**; 153:111–21.
5. Sarubbi FA, Myers JW, Williams JJ, Shell CG. Respiratory infections caused by *Branhamella catarrhalis*: selected epidemiologic features. Am J Med **1990**; 88:9–14.
6. Hendley JO, Hayden FG, Winther B. Weekly point prevalence of *Streptococcus pneumoniae*, *Hemophilus influenzae* and *Moraxella catarrhalis* in the upper airways of normal young children: effect of respiratory illness and season. APMIS **2005**; 113:213–20.
7. Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. Nat Med **2008**; 14:558–64.
8. Griffiths NJ, Bradley CJ, Heyderman RS, Virji M. IFN-gamma amplifies NFkappaB-dependent *Neisseria meningitidis* invasion of epithelial cells via specific upregulation of CEA-related cell adhesion molecule 1. Cell Microbiol **2007**; 9:2968–83.
9. Rouadi P, Baroody FM, Abbott D, Naureckas E, Solway J, Naclerio RM. A technique to measure the ability of the human nose to warm and humidify air. J Appl Physiol **1999**; 87:400–6.
10 Weber MH, Marahiel MA. Bacterial cold shock responses. Sci Prog **2003**; 86:9–75.
11. Heiniger N, Troller R, Meier PS, Aebi C. Cold shock response of the UspA1 outer membrane adhesin of *Moraxella catarrhalis.* Infect Immun **2005**; 73:8247–55.
12. Kristo A, Uhari M, Kontiokari T, et al. Nasal middle meatal specimen bacteriology as a predictor of the course of acute respiratory infection in children. Pediatr Infect Dis J **2006**; 25:108–12.
13. Smith-Vaughan H, Byun R, Nadkarni M, et al. Measuring nasal bacterial load and its association with otitis media. BMC Ear Nose Throat Disord **2006**; 6:10.
14. Schwarz-Linek U, Hook M, Potts JR. The molecular basis of fibronectin-mediated bacterial adherence to host cells. Mol Microbiol **2004**; 52:631–41.
15. Fink J, Mathaba LT, Stewart GA, et al.*Moraxella catarrhalis* stimulates the release of proinflammatory cytokines and prostaglandin E from human respiratory epithelial cells and monocyte-derived macrophages. FEMS Immunol Med Microbiol **2006**; 46:198–208.
16. Xie H, Gu XX. *Moraxella catarrhalis* lipooligosaccharide selectively upregulates ICAM-1 expression on human monocytes and stimulates adjacent naive monocytes to produce TNF-alpha through cellular crosstalk. Cell Microbiol **2008**; 10:1453–67.
17. Stutzmann Meier P, Heiniger N, Troller R, Aebi C. Salivary antibodies directed against outer membrane proteins of *Moraxella catarrhalis* in healthy adults. Infect Immun **2003**; 71:6793–8.
18. Murphy TF, Brauer AL, Aebi C, Sethi S. Antigenic specificity of the

mucosal antibody response to *Moraxella catarrhalis* in chronic obstructive pulmonary disease. Infect Immun **2005**; 73:8161–6.
19. Spaniol V, Heiniger N, Troller R, Aebi C. Outer membrane protein UspA1 and lipooligosaccharide are involved in invasion of human epithelial cells by *Moraxella catarrhalis.* Microbes Infect **2008**; 10:3–11.
20. Beveridge TJ. Structures of gram-negative cell walls and their derived membrane vesicles. J Bacteriol **1999**; 181:4725–33.
21. Murphy TF, Loeb MR. Isolation of the outer membrane of *Branhamella catarrhalis.* Microb Pathog **1989**; 6:159–74.
22. Tan TT, Nordstrom T, Forsgren A, Riesbeck K. The respiratory pathogen *Moraxella catarrhalis* adheres to epithelial cells by interacting with fibronectin through ubiquitous surface proteins A1 and A2. J Infect Dis **2005**; 192:1029–38.
23. Brooks MJ, Sedillo JL, Wagner N, et al. *Moraxella catarrhalis* binding to host cellular receptors is mediated by sequence-specific determinants not conserved among all UspA1 protein variants. Infect Immun **2008**; 76:5322–9.
24. Aebi C, Lafontaine ER, Cope LD, et al. Phenotypic effect of isogenic *uspA1* and *uspA2* mutations on *Moraxella catarrhalis* O35E. Infect Immun **1998**; 66:3113–9.
25. Attia AS, Hansen EJ. A conserved tetranucleotide repeat is necessary for wild-type expression of the *Moraxella catarrhalis* UspA2 protein. J Bacteriol **2006**; 188:7840–52.
26. Anderson KL, Roberts C, Disz T, et al. Characterization of the *Staphylococcus aureus* heat shock, cold shock, stringent, and SOS responses and their effects on log-phase mRNA turnover. J Bacteriol **2006**; 188: 6739–56.
27. Heiniger N, Spaniol V, Troller R, Vischer M, Aebi C. A reservoir of *Moraxella catarrhalis* in human pharyngeal lymphoid tissue. J Infect Dis **2007**; 196:1080–7.
28. McMichael JC, Fiske MJ, Fredenburg RA, et al. Isolation and characterization of two proteins from *Moraxella catarrhalis* that bear a common epitope. Infect Immun **1998**; 66:4374–81.
29. Tan TT, Morgelin M, Forsgren A, Riesbeck K. *Haemophilus influenzae* survival during complement-mediated attacks is promoted by *Moraxella catarrhalis* outer membrane vesicles. J Infect Dis **2007**; 195:1661–70.
30. Peng DX, Hong WZ, Choudhury BP, Carlson RW, Gu XX. *Moraxella catarrhalis* bacterium without endotoxin, a potential vaccine candidate. Infect Immun **2005**; 73:7569–77.
31. Xiong YQ, Fowler VG, Yeaman MR, Perdreau-Remington F, Kreiswirth BN, Bayer AS. Phenotypic and genotypic characteristics of persistent methicillin-resistant *Staphylococcus aureus* bacteremia in vitro and in an experimental endocarditis model. J Infect Dis **2009**; 199:201–8.
32. Hill DJ, Edwards AM, Rowe HA, Virji M. Carcinoembryonic antigen-related cell adhesion molecule (CEACAM)-binding recombinant polypeptide confers protection against infection by respiratory and urogenital pathogens. Mol Microbiol **2005**; 55:1515–27.
33. Bullard B, Lipski SL, Lafontaine ER. Hag directly mediates the adherence of *Moraxella catarrhalis* to human middle ear cells. Infect Immun **2005**; 73:5127–36.
34. Holm MM, Vanlerberg SL, Sledjeski DD, Lafontaine ER. The Hag protein of *Moraxella catarrhalis* strain O35E is associated with adherence to human lung and middle ear cells. Infect Immun **2003**; 71:4977–84.
35. Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol **2003**; 284:566–77.
36. Hong W, Mason K, Jurcisek J, Novotny L, Bakaletz LO, Swords WE. Phosphorylcholine decreases early inflammation and promotes the establishment of stable biofilm communities of nontypeable *Haemophilus influenzae* strain 86-028NP in a chinchilla model of otitis media. Infect Immun **2007**; 75:958–65.
37. Zarantonelli ML, Huerre M, Taha MK, Alonso JM. Differential role of lipooligosaccharide of *Neisseria meningitidis* in virulence and inflammatory response during respiratory infection in mice. Infect Immun **2006**; 74:5506–12.
38. Pridmore AC, Jarvis GA, John CM, Jack DL, Dower SK, Read RC. Activation of toll-like receptor 2 (TLR2) and TLR4/MD2 by *Neisseria* is independent of capsule and lipooligosaccharide (LOS) sialylation but varies widely among LOS from different strains. Infect Immun **2003**; 71:3901–8.
39. Murphy TF, Brauer AL, Grant BJ, Sethi S. *Moraxella catarrhalis* in chronic obstructive pulmonary disease: burden of disease and immune response. Am J Respir Crit Care Med **2005**; 172:195–9.
40. Simell B, Melin M, Lahdenkari M, et al. Antibodies to pneumococcal surface protein A families 1 and 2 in serum and saliva of children and the risk of pneumococcal acute otitis media. J Infect Dis **2007**; 196: 1528–36.
41. Quin LR, Onwubiko C, Moore QC, Mills MF, McDaniel LS, Carmicle S. Factor H binding to PspC of *Streptococcus pneumoniae* increases adherence to human cell lines in vitro and enhances invasion of mouse lungs in vivo. Infect Immun **2007**; 75:4082–7.