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Modulation of *Pseudomonas aeruginosa* Adherence to the Corneal Surface by Mucus

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To gain access to the corneal epithelium and cause infectious keratitis, bacterial pathogens must first interact with ocular surface factors that could affect bacterial adherence. In this study, we demonstrated that the mucus layer, and, in particular, the mucin fraction of mucus, modulated adherence to intact corneal epithelium of *Pseudomonas aeruginosa* but not that of *Staphylococcus aureus* or *Streptococcus pyogenes*. Removal of endogenous mucus from rat or rabbit eyes increased the adherence of *P. aeruginosa* by 3- to 10-fold. Ocular mucus obtained from rat eyes, porcine stomach mucin, or bovine submaxillary gland mucin inhibited adherence of *P. aeruginosa* to uninjured corneal epithelium. The mucin fraction of ocular mucus, purified by ultracentrifugation, was found to contain the inhibitory activity, and inhibition was demonstrated at concentrations of mucin as low as 35 μg/ml. Ocular mucin was the only material tested that inhibited adherence of *P. aeruginosa* to an injured cornea. However, the binding of *P. aeruginosa* to immobilized substrates in vitro did not predict which fraction would possess antiadherence activity: bacteria bound well to whole ocular mucus, mucin, the nonmucin fraction of ocular mucus, and dilute human tears as well as to porcine stomach mucin and bovine submaxillary gland mucin. The effectiveness of the mucin fraction of ocular mucus at inhibiting the binding of *P. aeruginosa* to the cornea implies that this material is a barrier that protects the surface of the eye from *P. aeruginosa* adherence.

Studies of the pathogenesis of *Pseudomonas aeruginosa* infections of the mature cornea have focused on the initial adherence of bacteria to overtly injured corneas (21). However, not all corneal infections in adults are preceded by overt injury. For example, contact lens-related keratitis, which is the most common type of *P. aeruginosa* eye infection (1), likely follows more subtle disruptions to the ocular surface. In vivo, bacteria must pass through and interact with the tear film, the mucus layer, and the epithelial cell surface glycocalyx before they come into contact with corneal cells (7, 14). This aspect of the pathogenesis of infectious keratitis has received little attention.

We previously reported that mild acid treatment promotes bacterial adherence to the cornea without disrupting epithelial cell morphology (5). This observation suggested that there may be antiadherence factors at the corneal surface that are disrupted by mild acid. Other investigators have found that the surface mucus layer in the bladder inhibits bacterial adherence to the underlying healthy uroepithelial layer (15). Because the human tear film is composed substantially of mucus (16), we hypothesized that ocular mucus may modulate bacterial adherence to the healthy cornea. The results presented herein support this hypothesis. Thus, we propose that disruptions to mucus production, composition, or clearance may be an important factor in the enhanced susceptibility of contact lens wearers and patients with certain dry-eye conditions to infectious keratitis with *P. aeruginosa*.

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MATERIALS AND METHODS

Bacteria. *P. aeruginosa* 6294 (serogroup O6), isolated from a human corneal ulcer, was maintained in Trypticase soy broth at −70°C with 10% glycerol. This strain was nonmucoid and nonpiliated, similar to other human corneal isolates of *P. aeruginosa* (6, 22). Bacteria were inoculated onto a Trypticase soy agar plate covered with a dialysis membrane (molecular weight pore size, 12,000 to 14,000) (4) and were grown overnight at 37°C. The inoculum was prepared by resuspension of bacteria from the membrane into Hanks balanced salt solution (HBSS; Sigma Chemical Co., St. Louis, Mo.) until the appropriate optical density was achieved. In addition to strain 6294, nine other human corneal isolates of *P. aeruginosa* were used in microtiter well experiments to test bacterial interaction with mucin. These strains were 6489 and 6284 (serogroup O6), 6354 (serogroup O7), 6452 and 6436 (serogroup O10), and 6073, 6206, 6382, and 6389 (serogroup O11). Some experiments were also conducted with a strain of *Staphylococcus aureus* (capsular type 8) and a strain of *Streptococcus pyogenes* that was isolated from a human infection.

Animals. Male and female adult New Zealand White rabbits and Wistar rats were used in this study in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. Animals were sacrificed with an overdose of pentobarbital sodium (50 mg/ml), and their eyes were immediately removed.

Removal of endogenous ocular mucus from rat and rabbit eyes. Endogenous ocular mucus was removed from rat and rabbit eyes in vitro by treatment for 20 min with 5% *N*-acetylcysteine (Sigma) dissolved in phosphate-buffered saline (PBS) (23). In one series of experiments, mucus was removed from only one eye of each animal to determine the effect on bacterial adherence. The other eye was treated with PBS alone and served as a control. All *N*-acetylcysteine-treated and

control (PBS-treated) eyes were washed twice with PBS before incubation for 20 min at 37°C in 4 ml of solution containing 1.5×10^8 CFU of bacteria per ml. This inoculum was chosen after preliminary studies indicated that smaller inocula did not lead to significant bacterial adherence to uninjured cornea. Each eye was then washed six times in 40 ml of PBS to remove nonadherent microorganisms. Corneas were excised from eyes, washed twice in 40 ml of PBS, and homogenized in 1 ml of Trypticase soy broth. A viable cell count was performed in triplicate to calculate the number of CFU of bacteria that had been adherent to each cornea.

Preparation of ocular mucus. Ocular mucus was prepared by two different means. To collect the mucus covering the entire eye, whole rat eyes were suspended in beakers containing 20 ml of HBSS; about 20 globes per beaker and a total of 178 eyes were used. After incubation for 4 h at 4°C, the eyes were removed and the remaining solutions were pooled. To collect mucus from only the corneal surface, 198 fresh rat eyes were embedded in agar (Difco Laboratories, Detroit, Mich.) in 10 petri dishes such that only the corneal surface of each eye protruded from the agar. The surface of each agar plate was soaked with 10 ml of HBSS for 4 h at 4°C, and the eluate was collected and pooled. Ten control agar plates were prepared without embedded eyes to test the effect of eluted agar components on bacterial adherence. Each of the eluate solutions was passed through filters (pore size, 3 μm; Nuclepore Corp., Pleasanton, Calif.), centrifuged three times at 5,000 rpm for 20 min (to remove cell debris and other solid material), dialyzed, lyophilized, and stored at −80°C until required.

Purification of mucin. Mucus derived from the corneal surface was separated into a purified mucin-rich fraction and a nonmucin fraction by ultracentrifugation in the presence of cesium bromide as described previously for purification of respiratory mucin (27) but without gel filtration. The carbohydrate-rich fraction banding at a density of 1.40 to 1.42 g/ml was collected, extensively dialyzed, and then lyophilized. This material, which was considered to be the mucin fraction, was rich in reducing sugars and absorbed poorly at 280 and at 260 nm, indicating minimal protein or nucleic acid contamination. The remaining material, considered to be the nonmucin fraction, was poor in carbohydrate and absorbed strongly at 280 and 260 nm. Glycolipids tend to separate into the nonmucin fraction after this type of ultracentrifugation purification procedure because of their buoyant density (27). In this case, there was little glycolipid present even in the nonmucin fraction as indicated by the absence of galactose and reducing sugars.

Effect of exogenous mucus on bacterial adherence to intact rat cornea. Solutions of mucus or mucin were prepared from the following sources: crude porcine stomach mucin (PSM; Sigma); crude bovine submaxillary gland mucin (BSGM; Sigma); whole-eye mucus; mucus collected from the corneal surface; purified corneal mucin; and the nonmucin fraction of corneal mucus. To determine the effects of these preparations on bacterial adherence, the endogenous ocular mucus layer was first removed with 5% *N*-acetylcysteine as described above. Each eye was then placed, cornea upwards, onto sterile cotton soaked in HBSS in a petri dish. Test eyes were coated with mucus or mucin by the addition of two aliquots of 8 μl each at 15-min intervals (total, 16 μl). Control eyes were treated with HBSS alone. All eyes were incubated at 37°C for a total of 30 min. The preparations were added in two aliquots to prevent drying of the corneal surface. Each cornea was then inoculated twice, with a 15-min interval intervening, with 10^7 CFU of bacteria in 8 μl of HBSS. Some eyes received the bacteria suspended in HBSS only, while other eyes were inoculated with a combination of bacteria and mucus that was prepared 30 min before addition to the cornea. After a total of 30 min of incubation at 37°C, each eye was washed six times in 5 ml of PBS to remove nonadherent microorganisms. Corneas were excised from each eye, washed twice in 5 ml of PBS and once in 50 ml of PBS, and homogenized in 1 ml of Trypticase soy broth. A viable cell count was performed in duplicate to calculate the number of CFU adherent to each cornea.

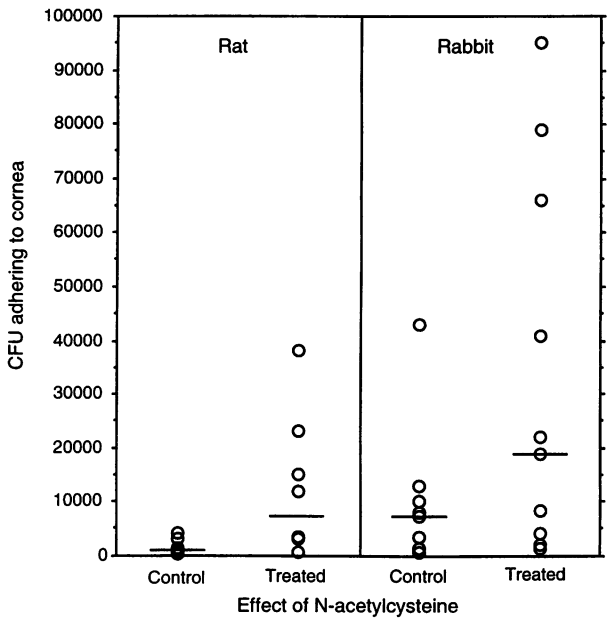

FIG. 1. Effect of removal of endogenous mucus on the adherence of *P. aeruginosa* 6294 to rat ($n = 16$) and rabbit ($n = 22$) corneas (P, 0.03 and 0.02, respectively). Mucus was removed by treatment of eyes with 5% *N*-acetylcysteine, and bacterial adherence in treated eyes was compared with that in untreated eyes. Bars represent the median of the measured values.

Two series of experiments assessed the effect of rat corneal mucus on bacterial adherence. One series was performed in which control eyes were treated with HBSS. In the other series, eyes coated with precorneal eluate were compared with eyes coated with the agar eluate.

To ascertain whether any effect on adherence was due to a viscosity barrier effect, mucus was prepared in two different concentrations. In some experiments, a concentration of 35 mg/ml was used to produce a viscous solution that might mimic the viscous mucus layer of the tear film. In other experiments, a concentration of 35 μg of mucin per ml was tested.

Exogenous mucus and bacterial adherence to injured cornea. Rat eyes were injured by first pressing a piece of filter paper onto the entire corneal surface, which produces a partial epithelial defect that leads to increased bacterial adherence (11), and then by producing a 2-mm full-thickness epithelial defect in the central cornea with a blunt spatula. The effect of various types of mucus on the adherence of *P. aeruginosa* to these corneas was examined as described above for intact corneas.

Bacterial interaction with mucus. To examine the mechanism for mucin inhibition of bacterial adherence to the cornea, the interaction of bacteria with the various types of mucin was studied as described previously (26). In brief, 96-well tissue culture plates (Linbro/Titertek; Flow Laboratories, Inc., McLean, Va.) were coated overnight with 100 μl of a solution containing 10 μg of mucin per ml of HBSS. After each well was

TABLE 1. Effect of exogenously applied mucus (35 mg/ml) on the adherence of *P. aeruginosa* to *N*-acetylcysteine-treated rat corneas

Type of mucus	No. of eyes	Median (range) CFU of adherent bacteria/cornea in control eyes[a]	Eyes with mucus added:						*P*
			Before inoculum		With inoculum		Before and with inoculum		
			Median (range) CFU of adherent bacteria/cornea	% Reduction[b]	Median (range) CFU of adherent bacteria/cornea	% Reduction	Median (range) CFU of adherent bacteria/cornea	% Reduction	
PSM	40	1,925 (640–3,200)[c]	950 (250–1,400)	51	575 (250–1,450)	70	250 (0–1,650)	87	0.0002
BSGM	24	5,825 (1,100–7,600)[c]	680 (300–1,750)	88	700 (150–1,750)	88	1,050 (400–2,250)	82	0.0045
Corneal mucus	15	1,400 (1,000–3,500)[c]	—[d]	—	—	—	200 (0–350)	86	0.001
Corneal mucus	24[e]	3,225 (485–7,000)[f]	—	—	—	—	670 (30–1,650)	79	0.008
Rat whole-eye mucus	24	4,175 (1,600–18,600)[c]	—	—	—	—	1,525 (250–5,400)	63	0.02

[a] *N*-Acetylcysteine treated; no mucus added.
[b] Percent reduction in median bacterial adherence.
[c] Control eyes treated with HBSS.
[d] —, not done.
[e] Involved paired right and left eyes from the same animal.
[f] Control eyes treated with agar eluate.

washed four times to remove nonadherent mucin, a 30-μl volume of bacterial suspension (10^8 CFU/ml of HBSS) was added, and the preparation was incubated for 30 min at 37°C. Each well was washed 20 times in PBS to remove nonadherent bacteria and then filled with 300 μl of 0.5% Triton X-100 for 15 min to dislodge adherent bacteria and mucin. A viable cell count was performed with this solution to determine the number of bacteria that had been adherent to mucin in the well. At least six wells were used for each experiment. Adherence to six wells that were not coated with mucin was assessed as a control for each experiment. To determine whether human tears contain factors that bind *P. aeruginosa*, tears collected by irrigation of the surface of both corneas of 10 human volunteers with sterile saline as described previously (4) were collected into a total of 80 ml of saline. For each experiment, six wells were coated overnight with 100 μl of this solution. The remainder of the assay measuring binding of bacteria to tear factors was performed as described above.

Statistical analysis. In some experiments, eyes were paired such that the contralateral eye from each animal was used as a control for the treated eye. In these instances, the Wilcoxon signed-rank test was used to evaluate the significance of differences. For other adherence data, the Mann-Whitney U test (for the comparison of two groups) and the Kruskal-Wallis test (for the comparison of more than two groups) were used for statistical analyses. These analyses were performed on a Macintosh computer with Statview SE + Graphics software. Since nonparametric statistics were used, the results are presented as the median and range of the measured values.

RESULTS

Adherence of *P. aeruginosa* after removal of mucus from the cornea. Treatment with *N*-acetylcysteine to remove ocular mucus enhanced the adherence of *P. aeruginosa* to both rat and rabbit corneas (P =, 0.036 and 0.021, respectively; Fig. 1). *S. aureus* (median, 698 CFU; range, 10 to 2,050 CFU) and *S. pyogenes* (median, 28 CFU; range, 20 to 65 CFU) were found to adhere to rat corneas less efficiently than *P. aeruginosa*. The removal of ocular mucus with *N*-acetylcysteine did not significantly alter the adherence of *S. aureus* (median, 83 CFU; range, 50 to 1,215 CFU; P, 0.17) or *S. pyogenes* (median, 13 CFU; range, 0 to 55 CFU; P, 0.29) to rat corneas.

Effect of exogenous mucus on *P. aeruginosa* adherence to intact corneas. Initial experiments were undertaken to determine how different methods employed for adding mucin to the *N*-acetylcysteine-treated eye affected bacterial adherence. These experiments were performed with the two types of nonocular mucus (PSM and BSGM) and three different methods of treating eyes with mucus. One method involved the pretreatment of the cornea with mucus, the second involved the mixture of the bacterial inoculum with mucus, and the third involved the addition of mucus to both the cornea and the bacteria. In general, it was found that mucus treatment with either PSM or BSGM significantly reduced the adherence of *P. aeruginosa* to *N*-acetylcysteine-treated corneas (P, 0.0002 and 0.0045, respectively; (Table 1). When the results of the three methods of treatment were compared with those of untreated control eyes, all three methods were equally effective at

TABLE 2. Effect of low concentrations of exogenously applied mucus (35 μg/ml) on the adherence of *P. aeruginosa* to uninjured *N*-acetylcysteine-treated rat corneas

Type of mucus[a]	Median (range) CFU of adherent bacteria/cornea in control eyes[b,c]	Eyes with mucus added[b]		*P*
		Median (range) CFU of adherent bacteria/cornea	% Reduction in median adherence	
PSM	2,070 (990–5,150)	703 (315–1,390)	66	0.02
Purified rat corneal mucin	3,850 (2,300–6,100)	585 (110–1,870)	85	0.02
Nonmucin fraction of rat corneal mucus	1,395 (880–4,050)	1,255 (180–2,550)	10	NS[d]

[a] Each mucus group included 12 eyes.
[b] All paired left and right eyes.
[c] *N*-Acetylcysteine treated; no mucus added.
[d] Reduction not statistically significant.

TABLE 3. Effect of mucus on adherence of *P. aeruginosa* to injured rat corneas

Type of mucus[a]	Mucus concn	Median (range) CFU of adherent bacteria/cornea (10^3) in control eyes	Eyes with mucus added		P
			Median (range) CFU of adherent bacteria/cornea (10^3)	% Reduction in median adherence	
Rat whole-eye mucus	35 mg/ml	1,474 (500–2,005)	380 (240–1,300)	74	0.01
PSM	35 mg/ml	1,520 (490–1,780)	890 (560–1,630)	41	NS[b]
Purified rat corneal mucin	35 μg/ml	692 (475–1,155)	280 (175–430)	60	0.006
Nonmucin fraction of rat corneal mucus	35 μg/ml	683 (500–1,260)	488 (160–800)	29	NS

[a] Each mucus group included 12 eyes.
[b] NS, reduction not statistically significant (P ≥ 0.1).

reducing bacterial adherence; that is, there were no significant differences in the degree of inhibition of adherence (*P*, 0.1 for PSM and 0.4 for BSGM). In addition to the nonocular mucus, preparations of ocular mucus inhibited the adherence of *P. aeruginosa* to *N*-acetylcysteine-treated, uninjured rat corneas when added at a concentration of 35 mg of mucus per ml (Table 1). Experiments in which the agar eluate rather than HBSS was used as a buffer to coat control eyes demonstrated that inhibition of bacterial adherence by corneal mucus was due to ocular factors and not to factors derived from agar (*P*, 0.008).

Low concentrations of either PSM or purified mucin (35 μg of mucin per ml) also inhibited the adherence of *P. aeruginosa* to *N*-acetylcysteine-treated rat corneas (*P*, 0.02 in both cases; Table 2). Thus, the inhibition of adherence by mucin seemed not to be the result of a viscosity barrier effect. A minor reduction in the adherence of *P. aeruginosa* after the addition of the nonmucin fraction from rat corneas was not statistically significant (*P*, 0.17).

Both *S. aureus* (median, 1,230 CFU per cornea; range, 185 to 1,590 CFU) and *S. pyogenes* (median, 380 CFU per cornea; range, 120 to 680 CFU) adhered to *N*-acetylcysteine-treated corneas to some degree. In contrast to the results obtained with *P. aeruginosa*, purified ocular mucin (35 μg/ml) did not significantly inhibit the adherence of *S. aureus* (median, 850 CFU; range, 315 to 2,000 CFU) or *S. pyogenes* (median, 333 CFU; range, 135 to 630 CFU) to the cornea (*P*, 0.60 and 0.46, respectively).

Effect of exogenous mucus on *P. aeruginosa* adherence to injured corneas. Only ocular mucus and mucin inhibited the adherence of *P. aeruginosa* to the cornea after injury (Table 3). At a concentration of 35 mg/ml, whole-eye mucus inhibited adherence to injured corneas (*P*, 0.01); however, PSM did not (*P*, 0.15). Mucin purified from corneal mucus inhibited adherence to injured rat corneas (*P*, 0.006) at concentrations as low as 35 μg/ml. The small reduction in adherence associated with the nonmucin fraction of ocular mucus was not statistically significant (*P*, 0.1).

Bacterial binding to mucus and human tears. Of the 10 corneal isolates of *P. aeruginosa* examined, 9 bound to mucus at a level of at least 10^4 CFU per well. Only one strain, 6206, did not bind to mucus-coated wells. The results of one series of experiments investigating the adherence of *P. aeruginosa* 6294 to mucus materials are presented in Table 4. This strain did not bind to empty wells and always bound to mucus- or tear-coated wells.

P. aeruginosa 6294 bound more effectively to mucus and human tears than *S. aureus* and *S. pyogenes* (Table 4). The levels of binding of *S. aureus* to the mucin and nonmucin fractions of ocular mucus were only about 0.23 and 3%, respectively, of the levels observed for *P. aeruginosa* with comparable inocula. *S. pyogenes* did not bind in significant numbers to any of the mucus materials tested. Interestingly, in vitro adherence to the nonmucin fraction of corneal mucus was significantly greater than adherence to the mucin fraction for both *P. aeruginosa* (*P*, 0.03) and *S. aureus* (*P*, 0.005), even though only the mucin fraction inhibited binding of *P. aeruginosa* to intact and injured corneas.

DISCUSSION

The data presented in this study show that factors in the overlying mucus layer affect access of *P. aeruginosa* to the corneal epithelium. Injury to the cornea also affected *P. aeruginosa* adherence, consistent with results of previous studies (18). Removal of endogenous mucus from the corneal surface with *N*-acetylcysteine enhanced *P. aeruginosa* adherence 3- to 10-fold, whereas corneal injury increased *P. aeruginosa* adherence by approximately 1,000-fold. This increased adherence of *P. aeruginosa* to *N*-acetylcysteine-treated corneas could be reversed by the addition of various types of mucus to the eye. However, only ocular mucus and mucin purified from

TABLE 4. Bacterial binding to wells coated with various types of mucus[a]

Type of mucus	Median (range) CFU of bacteria binding/well (10^2)		
	P. aeruginosa	*S. aureus*	*S. pyogenes*
None	0 (0–0)	0.2 (0–0.4)	0 (0–0)
PSM	1,575 (410–3,300)	685 (400–1,100)	0.4 (0.2–1.3)
BSGM	2,150 (130–3,300)	288 (150–560)	0.09 (0–0.3)
Purified rat corneal mucin	275 (31–605)	0.3 (0–3)	0 (0–0.2)
Nonmucin fraction of rat corneal mucus	1,800 (275–2,900)	8 (3–24)	0 (0–0.08)
Dilute human tears[b]	310 (300–365)	2.3 (0.8–6.8)	1.6 (0.3–2.3)

[a] All mucins were added at a concentration of 10 μg/ml except for human tears, which consisted of one 5-s irrigation of each cornea per 4 ml (glycoprotein concentration not determined).
[b] Precorneal tears collected by irrigation of cornea.

ocular mucus inhibited *P. aeruginosa* adherence to injured corneas, and the effect of injury on adherence was only partially reversed by these materials. The nonmucin fraction of ocular mucus did not inhibit *P. aeruginosa* adherence to either intact or injured corneas. Adherence was inhibited with mucin concentrations as low as 35 μg/ml, indicating that the effect of mucin was likely a specific effect and not due to the viscosity of mucus solutions. These findings highlight the differences between mucins derived from disparate sources in regards to their ability to inhibit *P. aeruginosa* binding to the injured corneas.

Various studies have shown that bacterial interactions with mucins and the effects of mucins on bacterial adherence to surfaces depend upon the bacterial strain, the source of mucin, and the surface or cellular target. For example, it has been demonstrated that the adherence of *Escherichia coli* to polystyrene wells may be inhibited by colonic mucin (12). We and other investigators have found that *P. aeruginosa* is able to bind to different types of mucins when they are immobilized on tissue culture wells (26). Respiratory mucins inhibit adherence of *P. aeruginosa* to acid-injured tracheas (19), but neither respiratory mucin nor intestinal mucin affect *P. aeruginosa* adherence to buccal epithelial cells (20). Mucin in whole human saliva inhibits the adherence of *S. pyogenes* to buccal cells (2), whereas here we show that ocular mucin does not affect *S. pyogenes* binding to corneal cells. Our results further indicate that bacterial binding to epithelial cells in the presence of mucin is dependent upon the mucin source and type of target cell.

The ability of mucus layers to prevent bacterial access to the underlying epithelium has been attributed to various mechanisms. In the bladder, repulsion by negatively charged groups on glycosaminoglycans is thought to be responsible for the nonspecific antiadherence effect of bladder mucus (15). In contrast, respiratory mucins are believed to bind and entrap *P. aeruginosa* (26). In our study, all of the various mucins tested were found to bind *P. aeruginosa*, suggesting that mucins in general bind and entrap this bacterium when it is present on an intact mucosal surface. It was also noted that whole human tears collected by irrigation of the corneal surface contained a factor(s) that binds *P. aeruginosa*. Whether this factor(s) includes mucins is yet to be determined. Interestingly, purified ocular mucin was not able to bind or inhibit adherence of two different gram-positive bacteria, *S. aureus* and *S. pyogenes*, supporting the concept that inhibition of adherence by *P. aeruginosa* to the cornea is associated with bacterial binding to mucins.

Mucin binding of *P. aeruginosa* did not account for the inhibition of bacterial binding to injured corneas. All of the mucins, as well as the nonmucin fraction of ocular mucus, bound *P. aeruginosa* efficiently, yet only whole ocular mucus, and its purified mucin fraction, inhibited bacterial adherence to the injured cornea. Thus, it appears that bacterial binding to an interfacing molecule does not necessarily prevent bacterial binding to an underlying epithelial surface. These findings suggest that interactions among *P. aeruginosa*, the cornea, and interfacing molecules covering the cornea may be affected by the strength of the molecule-bacterium bond and/or the interaction between the molecule and the cornea. Alternatively, it may be that certain mucins and nonmucin fractions of mucus bind to different bacterial surface structures than those that are important for binding to cells. Our results suggest that ocular mucin, by virtue of its ability to inhibit *P. aeruginosa* binding to injured corneas, either binds to a different bacterial structure than do the other mucins or that bacteria attached to ocular mucins are more easily removed from the injured cornea than are bacteria bound to other mucins.

The mucus layer is the innermost layer of the tear film and is in intimate contact with the corneal surface. In the eye, mucus is secreted from conjunctival goblet cells and spreads over the surface of the cornea and conjunctiva; it is thought to be loosely bound to the glycocalyx of the epithelial microvilli (14). Thus, avid binding of bacteria to mucin would likely promote bacterial clearance since mucin is not bound to the ocular surface but rather appears to act as a debris removal system (7). However, interruption of the normal movement of mucin might produce the opposite effect, thereby promoting bacterial adherence to a surface. The importance of mucus in defense against ocular *P. aeruginosa* infection may, at least in part, explain why the intact infant mouse eye, which has not yet developed a mucus layer (8), is easily infected with *P. aeruginosa*, while adult mouse eyes with ocular mucus must be injured before *P. aeruginosa* can cause disease (9, 18). Some dry-eye conditions are associated with mucin deficiencies (10). Hypovitaminosis A xerophthalmia, a condition in which mucin production is severely impaired, predisposes to *P. aeruginosa* keratitis (3). Indeed, one study reported the isolation of *P. aeruginosa* from the corneas of 36% of patients with severe forms of this disease (24).

Contact lens wear, which can alter the quantity and composition of ocular mucin, is a well-described risk factor for *P. aeruginosa* keratitis (1). Significant decreases in sialic acid, *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose-*N*-acetylgalactosamine, and mannose in the mucus of contact lens wearers have been reported, and mucin-producing cells from the eyes of contact lens wearers contain sulfomucins that are not detectable in non-lens wearers (25). Monosaccharides are thought to be receptors for *P. aeruginosa* (17); therefore, a reduction in these could result in a loss of the protective effect of mucin. In addition, since sulfated mucin fractions do not appear to bind *P. aeruginosa* (17), the switch to sulfomucins may further reduce ocular protection. Thus, contact lens wear could interfere with the protective effect of mucin. It is also conceivable that, during contact lens wear, bacterial binding to mucin could actually promote infection. The stagnation of the preocular tear film under a contact lens may prevent the removal of mucin-bound bacteria from the ocular surface. It is interesting that the incidence of *P. aeruginosa* keratitis is highest among wearers of soft contact lenses (1), which, because of their ability to wrap closely over the cornea, are the most disruptive to the precorneal tear film (10).

Another manner by which mucins may be involved in the pathogenesis of contact-lens associated keratitis would be to promote bacterial binding to the contact lens, which could serve as an inoculum to initiate the infectious process. Mucins have been shown to enhance the binding of *P. aeruginosa* to soft contact lenses (13); in this situation, mucins most likely act as a glue to bind bacteria to the lens surface. In cystic fibrosis, a disease associated with a high risk of *P. aeruginosa* respiratory tract infection, there are similar alterations to respiratory mucin, including changes in mucin composition, alterations in the capacity of mucin to bind *P. aeruginosa*, and stagnation of mucin in the respiratory tract (17).

Since the intact cornea is highly resistant to infection, infectious keratitis among healthy individuals who do not wear contact lenses is almost always associated with injury to the ocular surface and is often due to gram-positive bacteria (1), possibly because of higher levels of gram-positive organisms on the skin and the conjunctiva. The lack of interaction of *S. aureus* and *S. pyogenes* with mucins suggests that these organisms are unable to take advantage of the altered and/or

stagnated mucus trapped beneath a contact lens to serve as a repository for infection.

Overall, our observations suggest that, under normal circumstances, *P. aeruginosa* binds to ocular mucin and protects the cornea from bacterial adherence. Conditions associated with an increased risk of *P. aeruginosa* infection may involve alterations to mucins that interfere with the effective removal of bacteria from the eye.

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