

Pre-exposure of Corneal Epithelial Cells to Human Tear Fluid Induces Protective Activity Against *P. aeruginosa* Virulence Mechanisms

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Purpose: Our previous studies have shown that human tear fluid increases transepithelial resistance of corneal epithelia *in vitro* and protects them against *P. aeruginosa* invasion, cytotoxicity and translocation but only retards the growth of some *P. aeruginosa* strains. However, we have also found that if a murine eye is removed and suspended in bacteria without tear fluid, the corneal epithelium remains resistant to *P. aeruginosa* translocation. Thus, we tested the hypothesis that pre-exposure of corneal epithelial cells to tear fluid is sufficient to induce protective activity against *P. aeruginosa* invasion and cytotoxicity.

Methods: Tears were freshly collected from the lower conjunctival sac of healthy, non-contact lens wearing subjects. Human corneal epithelial cells (telomerase-immortalized) were exposed to human tear fluid or cell culture media without antibiotics for 16 h then inoculated with $\sim 10^4$ cfu of *P. aeruginosa* strain 6206, 19660, 6294 or PAO1 for 3 h. Invasion was quantified by a gentamicin survival assay and cytotoxicity assessed by trypan blue staining. Bacteriostatic activity of tears with and without pre-exposure to corneal epithelial cells was measured by viable counts. Pathway Reporter Arrays were used to determine luciferase expression controlled by pathway specific transcription factors in corneal epithelial cells exposed to bacteria with and without tear fluid.

Results: Pre-exposure to corneal epithelial cells for 16 h did not influence the bacteriostatic activity of human tears. However, cells pre-exposed to human tears showed reduced susceptibility to *P. aeruginosa* invasion and cytotoxicity. For strain 6294, tear fluid pre-exposure resulted in a (A) 5-fold reduction in invasion ($P = 0.007$, t-test) with 3.5- and 2.5-fold reductions observed for strains PAO1 ($P = 0.02$, t-test) and 19660 ($P = 0.048$, t-test), respectively. (B) Strain 6206 cytotoxicity was reduced by ~ 5 -fold with tear fluid pre-exposure. (C) Tear fluid magnified bacterial-induced increases in luciferase expression controlled by NFkB and AP-1 transcription factors.

Conclusions: These data suggest that the protective effects of tear fluid against *P. aeruginosa* invasion and cytotoxicity involve the modulation of corneal epithelial cell defenses, some of which are known to be regulated by NFkB and AP-1. Magnified increases in luciferase expression controlled by these transcription factors suggest that enhanced upregulation of innate defense molecules occurs in corneal epithelial cells in response to bacteria when cells are exposed to tear fluid.

