Surfactant protein D contributes to ocular defense against Pseudomonas aeruginosa in a murine model of dry eye disease.
Surfactant Protein D Contributes to Ocular Defense against *Pseudomonas aeruginosa* in a Murine Model of Dry Eye Disease

Susan R. Heimer1,2, David J. Evans1,2, James J. Mun1, Michael E. Stern3, Suzanne M. J. Fleiszig1,2,4*

1 School of Optometry, University of California, Berkeley, California, United States of America, 2 College of Pharmacy, Touro University-California, Vallejo, California, United States of America, 3 Allergan Inc., Irvine, California, United States of America, 4 Graduate Groups in Vision Science, Microbiology, and Infectious Diseases and Immunity, University of California, Berkeley, California, United States of America

Abstract

Dry eye disease can cause ocular surface inflammation that disrupts the corneal epithelial barrier. While dry eye patients are known to have an increased risk of corneal infection, it is not known whether there is a direct causal relationship between these two conditions. Here, we tested the hypothesis that experimentally-induced dry eye (EDE) increases susceptibility to corneal infection using a mouse model. In doing so, we also examined the role of surfactant protein D (SP-D), which we have previously shown is involved in corneal defense against infection. Scopolamine injections and fan-driven air were used to cause EDE in C57BL/6 or Black Swiss mice (wild-type and SP-D gene-knockout). Controls received PBS injections and were housed normally. After 5 or 10 days, otherwise uninjured corneas were inoculated with 107 cfu of *Pseudomonas aeruginosa* strain PAO1. Anesthesia was maintained for 3 h post-inoculation. Viable bacteria were quantified in ocular surface washes and corneal homogenates 6 h post-inoculation. SP-D was measured by Western immunoblot, and corneal pathology assessed from 6 h to 4 days. EDE mice showed reduced tear volumes after 5 and 10 days (each by ~75%, p<0.001) and showed fluorescein staining (i.e. epithelial disruption). Surprisingly, there was no significant difference in corneal pathology between EDE mice and controls (~10–14% incidence). Before bacterial inoculation, EDE mice showed elevated SP-D in ocular washes. After inoculation, fewer bacteria were recovered from ocular washes of EDE mice (<2% of controls, p = 0.0004). Furthermore, SP-D knockout mice showed a significant increase in *P. aeruginosa* corneal colonization under EDE conditions. Taken together, these data suggest that SP-D contributes to corneal defense against *P. aeruginosa* colonization and infection in EDE despite the loss of barrier function to fluorescein.

Introduction

Bacterial keratitis is a severe, vision-threatening disease of the cornea associated with contact lens wear or ocular injury [1]. To this end, bacterial keratitis research has mostly focused on contact lens-wearing patient populations [2], or involved animal models of keratitis in which the cornea is either scratch-injured to allow infection or less commonly fitted with a contact lens [3–6]. These types of studies have helped identify numerous bacterial and host immune events that are important for disease pathogenesis, and have highlighted the resilience of the healthy ocular surface against infection. While other ocular surface diseases have also been associated with microbial keratitis, e.g. keratopathies [7] or dry eye diseases [8], little is known of the mechanisms involved.

The estimated prevalence of dry eye disease among microbial keratitis cases varies with study design, ranging from 7–15% in patients seeking treatment in a hospital or eye clinic setting [8–10], and up to 26% of patients dwelling in convalescent homes [11,12]. Causative agents are mostly well-recognized opportunistic ocular pathogens such as *coagulase-negative Staphylococcus* spp., *S. aureus*, *Corynebacterium* spp., *Streptococcus pneumoniae*, and *Pseudomonas aerugi- nosa* [11]. Specific changes in the tear film composition have been reported that suggest dry eye disease patients may be compromised in defenses against microbial colonization. For example, a hallmark of dry eye inflammation in Sjögren’s Syndrome is the depletion of conjunctival goblet cells which normally produce copious amounts of a gel-forming mucin MUC5A and MUC19 [13,14], which trap bacteria and facilitate their clearance [15]. Dry eye patient tear samples also have been reported to differ in...
the relative abundance of antimicrobial factors including lyso-
zyme, lactoferrin, lipocalin, MUC1, MUC4, MUC16, and beta-
defensins [16–21]. Proinflammatory cytokines, e.g. IL-1β, are
raised in patients with dry eye disease as are matrix metallocoproteinases such as MMP-9 [22]. Similar results have been
obtained in experimentally-induced dry eye (EDE) animal
models [23,24], and associated with changes in the structural integrity of the corneal epithelium [25,26]. More recently, the
proinflammatory cytokine IL-17 was shown to be important in the
pathogenesis of EDE [27,28]. Recent studies have also shown an
upregulation of secretory phospholipase A2 (sPLA2-IA), an
inflammatory disease biomarker and mediator, in patients with
dry eye disease and in EDE [29,30]. However, it is not yet known
if one or more of these tear and corneal epithelial changes
associated with dry eye disease or EDE predispose the cornea to
infection.

Several of our previous studies using P. aeruginosa have
highlighted the importance of tear fluid in protecting the cornea
from infection. These include direct effects of tear fluid on
bacteria, preventing invasion, cytotoxicity and epithelial traversal
[31,32], and indirect effects of tears by induction of corneal
epithelial antimicrobial and immunomodulatory factors, e.g.
RNase7 and ST-2 [33]. Our other previous studies have also
shown the importance of surfactant protein-D, found in tear fluid
and the corneal epithelium, in helping the ocular surface defend
against P. aeruginosa and its pathogenic mechanisms [34–36]. Here,
we tested the hypothesis that EDE would alter corneal suscepti-
bility to P. aeruginosa colonization and infection in vivo. Our results
showed that the murine cornea retained its resistance to P.
aeruginosa infection under EDE conditions, and part of that
resistance was associated with the increased expression of SP-D.

Materials and Methods

Ethics Statement

All procedures involving animals were carried out in accordance
with standards established by the Association for the Research in
Vision and Ophthalmology, and under a protocol approved by the
Animal Care and Use Committee, University of California,
Berkeley, an AAALAC accredited institution.

Experimentally-Induced Dry Eye (EDE) Murine Model

EDF was induced in female, 6–8 weeks old C57BL/6 mice
(Charles River Laboratories, Boston, MA), or in female or male 6–
8 weeks old SP-D gene knockout (sp-d−/−) Black Swiss mice (a
generous gift of Dr. Samuel Hawgood, University of California,
San Francisco) along with strain/age/sex-matched controls
(Taconic Farms, Cambridge, MA), or in female or male 6–
8 weeks old SP-D gene knockout (sp-d−/−) Black Swiss mice (a
generous gift of Dr. Samuel Hawgood, University of California,
San Francisco) along with strain/age/sex-matched controls
(Taconic Farms, Cambridge, MA). Mice were given subcuta-
nous injections of 1.5 mg ketamine, 0.17 mg xylazine, 21
mg acepro-
mazine per 20 g body weight. All mice (C57BL/6 or Black Swiss)
developed similar levels of EDE. At the conclusion of each
experiment, tissue samples were collected from euthanized
animals.

Fluorescein Staining

The corneas of anesthetized mice were topically infused with
5 µL of a sterile sodium fluorescein suspension (100 mL PBS rinse
of a Fluoret stick; Chavlin, Aubenas, FR) for 3 min. Excess
fluorescein was removed by washing with 1 mL of PBS. Corneal
staining was observed under 20x magnification with a dissecting
stereomicroscope (Zeiss, Jena, Germany) equipped with a blue
light illumination, and documented with a AxioCam MR (Zeiss,
Jena, Germany).

Bacterial inoculation and quantification

P. aeruginosa strain PAO1 (serogroup O5) was used for this study.
PAO1 is able to invade corneal epithelial cells and is virulent in a
scarified murine cornea [38]. Bacteria were grown on Trypticase
soy agar (TSA) at 37°C for 16 h and then resuspended in sterile
phosphate-buffered saline (PBS) to a concentration of 1011
cfu/mL. Bacterial concentrations were confirmed by quantitative
plating on TSA for viable counts. Following 5 or 10 day course of
EDE induction or control treatments, ocular surfaces of aneste-
tized mice were inoculated topically with 5 µL containing 109
cfu bacteria without introducing mechanical injury. Mice were
maintained under sedation for the initial phase of the challenge
~3 h. At various times after inoculation, viable bacteria in tear
fluids or corneal tissues were assessed using quantitative plating on
TSA [36].

Ocular Surface Washes, Corneal Homogenates and
Determination of Ocular Pathology

Murine tear fluids were harvested by washing the ocular surface
of anesthetized mice with 3 µL of sterile PBS and collecting the
washes with sterile, glass microcapillary tubes (10 µL; Drummond
Scientific Inc, Broomall, PA) placed in the lateral canthus. These
corneal surface washes (2 µL) were serially diluted and plated for
viable bacteria. To prepare corneal homogenates, eyes were
collected from euthanized animals, corneal tissues were harvested
ex situ and washed extensively with PBS (10 µL). The corneas
were homogenized in 100 µL PBS containing 0.25% Triton X-
100 with sterile Kontes microtube pellet pestle (Daeiger, Vernon
Hills, IL) and sampled for viable bacteria. Corneal pathology was
assessed at various times pre- and post-inoculation, and docu-
mented with a digital CCD camera (Optronics, Goleta, CA)
stereomicroscope system (Stemi 2000-C; Carl Zeiss, Thornwood,
NY). Pathology was scored on 5-point grading system (0–4) based
on the amount of surface area involved, the density of an opacity,
and the overall surface regularity similar to that previously
described [38]. Scores ranged from 0 (no infection) to a maximum
of 12 (severe infection).

SP-D Detection

SP-D in murine tear fluids was detected by Western Immuno-
blot as described previously [35]. Tear fluids were collected by
washing the ocular surface with PBS as described above and
pooling samples from 10 mice per group. Total protein
concentration of pooled ocular surface washes was determined
with a BCA assay (Pierce, Rockford, IL), and equivalent amounts
of protein resolved by SDS-PAGE (Tris-HCl Ready Gel 10%,
BioRad, Hercules, CA) under denaturing conditions. Proteins
were transferred to nitrocellulose by electroblotting (180 mAmps
for 2 h) in transfer buffer (25 mM Tris, 190 mM glycine, and 10%
(v/v) methanol). Membranes were blocked with a solution of 10%
Dry Eye Disease and Defense against *P. aeruginosa*

**Results**

**EDE Mice Show Similar *P. aeruginosa* Tissue Colonization and Pathology to Controls**

EDE was induced in C57BL/6 mice for 5–10 days as described previously [26,37]. This model is known to induce damage in the lacrimal gland, cornea, and conjunctiva without grossly affecting other mucosal and non-mucosal tissues [39,40]. As previously reported [37], we observed a significant decrease in tear volume (~70%) compared to control mice within the first 2 days of administering scopolamine and evaporative air drafts, and this decrease was maintained for 10 days (Fig. 1A). EDE mice also showed fluorescein penetration of the cornea after 5 days, which intensified up to 10 days with continued scopolamine and air draft exposure (Fig. 1B, lower panels). Control mice did not show significant fluorescein staining (Fig. 1B, upper panels). After 10 days of EDE, mice were topically challenged with *P. aeruginosa* strain PAO1 (~10^9 cfu) without other prior injury to the cornea. Dry eye conditions were maintained and ocular pathology observed for up to 4 days (96 h) post-inoculation (pi). After 4 days, there were relatively few instances of ocular pathology. Less than 15% of challenged corneas displayed pathology, and there was no significant difference in disease incidence between EDE and control mice [EDE 14% versus control 10%; p-value (Chi square) = 0.75] (Table 1). When present, corneal pathology manifested as focal or punctate opacities observed within 24 h of challenge and varied from mild to moderate severity with disease scores of 4, 5, and 6, Table 1) after 4 days.

**Enhanced *P. aeruginosa* Clearance From Ocular Surface Washes of EDE Mice**

The initial clearance of *P. aeruginosa* from the ocular surface was assessed by measuring viable bacteria in corneal homogenates and ocular surface washes of EDE mice compared to normal controls at 6 h post-inoculation. EDE was induced for 5 days before bacterial inoculation. The majority of the *P. aeruginosa* inoculum (>99.9%) was rapidly cleared from the ocular surface (corneal homogenates and ocular surface washes) of both EDE and control mice after 6 h (Fig. 2). This was consistent with our previous studies using a similar “null infection” model [36,41]. EDE and control mice were not significantly different with respect to bacterial numbers in corneal homogenates after 6 h (Fig. 2A). However, there was a significant reduction (~50-fold) in the number of viable bacteria recovered from ocular surface washes of EDE mice compared to controls after 6 h (Fig. 2B, p = 0.049, Mann-Whitney test) showing that EDE enhanced the ocular clearance of *P. aeruginosa*.

**Increased Expression of SP-D in Ocular Surface Washes of EDE Mice**

We have previously shown that SP-D, a member of the collectin family of innate defense molecules, is present in tear fluid and the corneal epithelium and plays a role in ocular defense against *P. aeruginosa* [34,36]. Thus, SP-D levels were assessed in EDE mice and controls after 5 days of EDE induction, and before and after (6 h) inoculation with 10^7 cfu PAO1. To account for differences in tear volume, equivalent amounts of total protein from each sample...
were used for analyses. EDE mice showed increased expression of SP-D in ocular surface washes compared to normal controls prior to bacterial inoculation (Fig. 3). This difference was not seen the post-inoculation ocular surface washes, although the latter samples did contain an additional form of SP-D which we have also observed in a previous study [34]. The antibody against SP-D did not react with bacteria alone. These data show that EDE also increases expression of SP-D at the murine ocular surface.

**EDE Increases *P. aeruginosa* Corneal Colonization in SP-D Deficient Mice**

Although EDE enhanced SP-D expression in ocular washes of normal mice prior to bacterial challenge, normal mice had previously shown no difference in ocular colonization between normal and EDE conditions. Thus, SP-D deficient (*sp-d\(^{-/-}\)*) mice were tested for *P. aeruginosa* corneal colonization under EDE conditions. Since *sp-d* gene knockout mice were available in a Black Swiss background, a control colonization experiment was done using wild-type Black Swiss mice. EDE did not affect corneal colonization by *P. aeruginosa* in wild-type Black Swiss mice (Fig. 4A), consistent with our earlier results from wild-type C57BL/6 mice (Fig. 2A). SP-D deficient mice were also exposed to EDE or normal conditions (NC) for 5 days then challenged with 10⁹ cfu of *P. aeruginosa* strain PAO1. A significant increase in bacterial corneal colonization (~5-fold) was observed in SP-D deficient mice under EDE compared to normal conditions at 6 h (Fig. 4B). Thus, without SP-D, EDE is associated with increased *P. aeruginosa* corneal colonization.

**Discussion**

Dry Eye Disease is denoted by low tear volumes and inflammatory damage to the conjunctiva and/or cornea [42]. As such, dry eye disease has the potential to increase susceptibility to infection. The results of the present study, however, show that induction of dry eye disease in a murine experimental model (EDE) did not increase corneal susceptibility to *P. aeruginosa* infection with minimal pathology observed in both normal and dry eye mice. The data also showed that EDE resulted in an increase in surfactant protein-D expression at the ocular surface (ocular surface washes) before bacterial inoculation, and this correlated with increased bacterial clearance from the tears (ocular surface

| Table 1. Incidence and severity of *P. aeruginosa* infections in EDE mice. |
|-------------------------------|-------------------|-----------------|-----------------|
| Treatment Group | Incidence of Pathology | Pathology Scores | Chi-square Value |
| Control | 1 (10 mice) | 6 | 0.75 |
| EDE | 2 (14 mice) | 4, 5 | |

C57BL/6 mice were exposed to EDE or control conditions for 10 d prior to topical challenge with 10⁹ cfu of *P. aeruginosa* strain PAO1. Mice were monitored for corneal infiltrates, opacities, and changes in epithelial surface regularity. Pathology was graded at 96 h post-inoculation (see Materials and Methods). Incidences of pathology in EDE and control groups were not significantly different (Chi-square analysis). Data is representative of two independent experiments. 
doi:10.1371/journal.pone.0065797.t001

![Figure 2. Ocular clearance of *P. aeruginosa* in EDE.](fig2) Levels of viable *P. aeruginosa* (cfu) in corneal homogenates (A) or ocular surface washes (B) of C57BL/6 EDE mice compared to normal controls (NC) at 6 h post-inoculation with 10⁹ cfu of *P. aeruginosa* strain PAO1 (*T* = 0). EDE was induced for 5 days prior to bacterial inoculation. Bacteria were rapidly cleared from the murine ocular surface of both groups of mice after 6 h. Similar bacterial levels were found in corneal homogenates (A), but fewer bacteria were recovered from the ocular surface washes of EDE mice compared to controls (*p* = 0.049, Mann-Whitney test) (B). Data are representative of three independent experiments (≥5 animals per group in each experiment). Data for each sample are shown as the median (black square) with upper and lower quartiles (boxed area), and range of the data (error bars). 
doi:10.1371/journal.pone.0065797.g002

PLOS ONE | www.plosone.org 4 June 2013 | Volume 8 | Issue 6 | e65797
While corneal colonization was unaffected by dry eye disease in wild-type mice, our data showed that sp-d gene knockout mice showed increased corneal colonization under EDE conditions. Together these data show that dry eye disease does not compromise ocular defenses against \textit{P. aeruginosa} infection, and suggest that SP-D contributes to ocular defense against infection under EDE conditions.

Upregulation of SP-D in ocular surface washes in response to dry eye conditions may reflect a compensatory innate defense response. This would be consistent with previous studies which have suggested that other ocular innate defenses are upregulated in patients with dry eye disease including membrane-associated mucins (e.g. MUC1) [21,43] and human beta-defensins [18,19]. SP-D has antimicrobial, aggregative and opsonizing properties against \textit{P. aeruginosa}; it is present in tear fluid, inhibits \textit{P. aeruginosa} internalization by corneal epithelial cells, and it promotes ocular clearance of \textit{P. aeruginosa} in vivo [34,36,44–47]. Each of these protective effects could help provide enhanced defense of the ocular surface from infection during dry eye disease, more so if combined with antimicrobial and anti-adhesive actions of other innate defenses, e.g. defensins and mucins respectively [41,48,49].

In our study, enhanced SP-D expression in ocular surface washes of dry eye mice correlated with reduced numbers of viable bacteria in those washes. However, further studies will be needed to determine the relative role(s) of SP-D, and other ocular surface antimicrobial defenses that are likely to be upregulated, in removing \textit{P. aeruginosa} from the ocular surface under the dry eye conditions in this model.

The mechanism for SP-D upregulation in ocular washes of EDE mice is not yet known. We have previously shown that \textit{P. aeruginosa} flagellin and LPS antigens can each upregulate SP-D production and secretion in corneal epithelial cells, the latter through a mechanism involving JNK [35]. However, upregulation in dry eye mice occurred before bacterial inoculation. Thus, other facets of dry eye disease must trigger increased SP-D expression at the ocular surface. Mechanisms of SP-D expression and upregulation in various mammalian cells are complex and incompletely understood [35,50,51]. However, dry eye disease in murine models or humans is known to involve increased expression of

![Figure 3. SP-D expression in EDE before and after \textit{P. aeruginosa} challenge.](image1)

Western immunoblot analysis of SP-D expression in pooled ocular surface washes from EDE and control mice (10 mice per group) after 5 days EDE induction, and before and 6 h after inoculation with \textit{P. aeruginosa} strain PAO1 (10⁹ cfu). To normalize for differences in tear volume, equivalent amounts of protein (2 µg) were used in the analysis (BCA protein assay). Purified recombinant SP-D (rSP-D, ∼43 kDa monomer), and a relevant number of bacteria suspended in PBS (5 × 10⁴ cfu, see Fig. 2B), were included as positive and negative controls, respectively. SP-D expression in ocular surface washes was increased under EDE conditions before bacterial inoculation. The experiment was repeated once.

doi:10.1371/journal.pone.0065797.g003

![Figure 4. Effect of EDE on \textit{P. aeruginosa} corneal colonization in SP-D knockout mice.](image2)

Corneal colonization by \textit{P. aeruginosa} in normal Black Swiss mice (A) or SP-D deficient age/sex-matched Black Swiss mice (B) under normal (NC) and experimental dry eye (EDE) conditions. After 5 days EDE induction, otherwise uninjured corneas were challenged with 10⁹ cfu of \textit{P. aeruginosa} strain PAO1 (T = 0). EDE did not affect bacterial colonization in wild-type mice after 6 h. However, EDE in SP-D knockout mice (sp-d -/-) resulted in a ∼5–fold increase in corneal colonization after 6 h. Data shown is representative of two independent experiments with SP-D-deficient Black Swiss mice (n=5 animals per group). P values were obtained using the Mann-Whitney Test. Data for each sample are shown as the median (black square) with upper and lower quartiles (boxed area), and range of the data (error bars).

doi:10.1371/journal.pone.0065797.g004
proinflammatory mediators such as IL-1β, IL-6, IL-8, and involve MAP kinase signaling proteins including JNK [22,52–54]. SP-D is also known as an immuno-modulator with sp-d knockout mice showing enhanced inflammatory-mediated tissue pathology in the cornea and other animal infection models [47,55]. It is possible, therefore, that increased SP-D expression in EDE occurs in response to ocular inflammation, and that it functions to modulate those responses and protect against bacterial challenge.

Two different mouse strains were used in this study (C57BL/6 and Black Swiss). We are unaware of any differences in SP-D expression between these and other mouse strains. Black Swiss mice show a bias towards Th2 responses, and a SP-D knockout mouse in that strain could show greater Th2 responsiveness, as shown in models of lung allergy [56]. It has been also shown that BALB/c mice (which also show a Th2 bias) produce lower levels of pro-inflammatory cytokines and display less severe changes in goblet cell density under EDE conditions compared to C57BL/6 mice which have a Th1 bias [57]. However, further studies will be needed to determine the relationship, if any, between different mouse strains, SP-D expression, and \( \text{P. aeruginosa} \) colonization under EDE conditions.

In conclusion, experimental dry eye mice were not inherently more susceptible to \( \text{P. aeruginosa} \) infections than controls. These animals were able to displace bacteria from the ocular surface and displayed relatively low incidence rates of mild to moderate pathology, comparable to normal controls. Although dry eye disease in this model can promote desquamation of the superficial corneal epithelial cells, decrease the relative number of intercellular tight junctions [25,26], it is recognized that other protective defenses can be upregulated in dry eye including pro-inflammatory mediators, defensins and mucins. Our data shows that SP-D is also upregulated in dry eye conditions, and may contribute to ocular resistance to infection during desiccating stress.

Acknowledgments

Thanks to Dr. Samuel Hawgood, University of California, San Francisco for generous provision of antibody to SP-D, recombinant SP-D, and SP-D knockout mice.

Author Contributions

Conceived and designed the experiments: SF DE MS SH. Performed the experiments: SH JM. Analyzed the data: SH DE SF. Wrote the paper: SH DE MS SF.

References

1. Green M, Apel A, Stapleton F (2008) Risk factors and causative organisms in microbial keratitis. Cornea 27: 22–27.
2. Stapleton F, Curns N (2012) Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. Eye (Lond) 26: 183–193.
3. Hazlett LD (2007) Bacterial infections of the cornea (\( \text{Pseudomonas aeruginosa} \)). Chem Immunol Allergy 92: 185–194.
4. Pearlman E, Johnson A, Adhikary G, Sun Y, Chimney HR, et al. (2008) Toll-like receptors at the ocular surface. Ocul Surf 6: 108–116.
5. McDermott AM (2009) The role of antimicrobial peptides at the ocular surface. Ophthalmic Res 41: 60–75.
6. Tam G, Mun J, Evans DJ, Fleisch SM (2010) The impact of inoculation parameters on the pathogenesis of contact lens-related infectious keratitis. Invest Ophthalmol Vis Sci 51: 3100–3106.
7. Bourrier T, Thomas F, Borderie V, Chaumet C, Laroch L (2003) Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. Br J Ophthalmol 87: 834–838.
8. Schaefer F, Brunton O, Zografos L, Guex-Crosier Y (2001) Bacterial keratitis: a prospective clinical and microbiological study. Br J Ophthalmol 85: 842–847.
9. Wong T, Ormonde S, Gamble G, McGhee CN (2003) Severe infective keratitis leading to hospital admission in New Zealand. Br J Ophthalmol 87: 1103–1108.
10. Al-Yousuf N (2009) Microbial keratitis in kingdom of bahrain: clinical and microbiology review. Middle East Afr J Ophthalmol 16: 3–7.
11. Butler TK, Spencer NA, Chan CC, Singh Gilhotra J, McClellan K (2005) Infective keratitis in older patients: a 4 year review, 1998–2002. Br J Ophthalmol 90: 594–596.
12. Jianyi V, Constantinou M, Taylor HR, Vajpayee RB (2009) Microbiological and clinical profiles of patients with microbial keratitis residing in nursing homes. Br J Ophthalmol 93: 1639–1642.
13. Argueso P, Balaram M, Spurr-Michaud S, Keutmann HT, Dana MR, et al. (2002) Decreased levels of the goblet cell mucin MUC5AC in tears of patients with Sjogren syndrome. Invest Ophthalmol Vis Sci 43: 1004–1101.
14. Yu DF, Chen Y, Han JM, Zhang H, Chen XP, et al. (2008) MUC19 expression reduces apical corneal epithelial cell size-modulation by the metaboliteprotein inhibitor doxycycline. Cornea 27: 933–940.
15. Beardsley RM, Farley W, Luo L, Chen LZ, de Paiva CS, et al. (2005) Matrix metalloproteinase-9 knockdown confers resistance to corneal epithelial barrier disruption in experimental dry eye. Am J Pathol 166: 61–71.
16. De Paiva CS, Chotikanawich S, Pangelinan SB, Pitcher JD 3rd, Fang B, et al. (2009) \( \text{II}-\text{I7} \) disrupts corneal barrier following desiccating stress. Micromol Cell Biol 2: 235–245.
17. Chauhan SK, El Amann J, Eozoff T, Goyal S, Zhang Q, et al. (2009) Autoimmunity in dry eye is due to resistance of Th17 to Treg suppression. J Immunol 182: 1247–1252.
18. Wei Y, Epstein SP, Fukosaka S, Birmingham NP, Li XM, et al. (2011) \( \text{pPLA2-IIa} \) amplifies ocular surface inflammation in the experimental dry eye (DE) BALB/c mouse model. Invest Ophthalmol Vis Sci 52: 4780–4786.
19. Chen D, Wei Y, Li X, Epstein S, Wolosin JM, et al. (2009) \( \text{pPLA2-IIa} \) is an inflammatory mediator when the ocular surface is compromised. Exp Eye Res 88: 880–886.
20. Fleisch SM, Kwong MS, Evans DJ (2003) Modification of \( \text{Pseudomonas aeruginosa} \) interactions with corneal epithelial cells by human tear fluid. Infect Immun 71: 3866–3874.
21. Kwong MS, Evans DJ, Ni M, Cowell BA, Fleisch SM (2007) Human tear fluid protects against \( \text{Pseudomonas aeruginosa} \) keratitis in a murine experimental model. Infect Immun 75: 2325–2332.
22. Mun J, Tam C, Evans DJ, Fleisch SM (2011) Modulation of epithelial immunity by mucosal fluid. Sci Rep 1: 8.
23. Ni M, Evans DJ, Hawgood S, Anders EM, Sack RA, et al. (2005) Surfactant protein D is present in human tear fluid and the cornea and inhibits epithelial cell invasion by \( \text{Pseudomonas aeruginosa} \). Infect Immun 73: 2147–2156.
24. Ni M, Tam C, Verma A, Ramphal R, Hawgood S, et al. (2008) Expression of surfactant protein D in human corneal epithelial cells is upregulated by \( \text{Pseudomonas aeruginosa} \). FEMS Immunol Med Microbiol 54: 177–184.
25. Mun J, Tam C, Kowbel D, Hawgood S, Barnett MJ, et al. (2009) Clearance of \( \text{Pseudomonas aeruginosa} \) from a healthy ocular surface involves surfactant protein D
and is compromised by bacterial elastase in a murine null-infection model. Infect Immun 77: 2392–2398.

37. Dursun D, Wang M, Mourouy D, Li DQ, Lokeshwar BL, et al. (2002) A mouse model of keratoconjunctivitis sicca. Invest Ophthalmol Vis Sci 43: 632–638.

38. Lee EJ, Ecusse DJ, Fleiszig SM (2003) Role of Pseudomonas aeruginosa ExoA in penetration through corneal epithelium in a novel in vivo model. Invest Ophthalmol Vis Sci 44: 5220–5227.

39. Pitcher JD 3rd, De Paiva CS, Pelegino FS, McClellan AJ, Raince JK, et al. (2011) Pharmacological cholinergic blockade stimulates inflammatory cytokine production and lymphocytic infiltration in the mouse lacrimal gland. Infect Ophthalmol Vis Sci 52: 3221–3227.

40. Niederkorn JY, Stern ME, Pflugfelder SC, De Paiva CS, Corrales RM, et al. (2011) Pharmacological cholinergic blockade stimulates inflammatory cytokine production and lymphocytic infiltration in the mouse lacrimal gland. Infect Ophthalmol Vis Sci 52: 3221–3227.

41. Augustin DK, Heimer SR, Tam C, Li WY, Le Due JM, et al. (2011) Role of defensins in corneal epithelial barrier function against Pseudomonas aeruginosa traversal. Infect Immun 79: 595–605.

42. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC (2004) The role of the lacrimal functional unit in the pathophysiology of dry eye. Exp Eye Res 78: 409–416.

43. Gipson IK, Spurr-Michaud SJ, Senchyna M, Ritter R 3rd, Schaumberg D (2011) Comparison of mucin levels at the ocular surface of postmenopausal women with and without a history of dry eye. Cornea 30: 1346–1352.

44. Douda DN, Jackson R, Grasemann H, Palaniyar N (2011) Innate immune collectin surfactant protein D simultaneously binds both neutrophil extracellular traps and carbohydrate ligands and promotes bacterial trapping. J Immunol 187: 1856–1865.

45. Bufler P, Schmidt B, Schikor D, Bauernfeind A, Crouch EC, et al. (2003) Surfactant protein A and D differently regulate the immune response to nonmucoid Pseudomonas aeruginosa and its lipopolysaccharide. Am J Respir Cell Mol Biol 28: 249–256.

46. Wu H, Kazmenko A, Wao S, Schaffer L, Weiss A, et al. (2003) Surfactant proteins A and D inhibit the growth of Gram-negative bacteria by increasing membrane permeability. J Clin Invest 111: 1589–1602.

47. Giannoni E, Sasca T, Allen L, Wiener-Kronish J, Hawgood S (2006) Surfactant proteins A and D enhance pulmonary clearance of Pseudomonas aeruginosa. Am J Respir Cell Mol Biol 34: 704–710.

48. Fleiszig SM, Zaidi TS, Per GB (1994) Mucus and Pseudomonas aeruginosa adherence to the cornea. Adv Exp Med Biol 350: 359–362.

49. Redfern RL, Reins RG, Mcdermott AM (2011) Toll-like receptor activation modulates antimicrobial peptide expression by ocular surface cells. Exp Eye Res 92: 209–220.

50. He Y, Crouch EC, Rast K, Spaite E, Brody SL, (2000) Proximal promoter of the surfactant protein D gene: regulatory roles of AP-1, forkhead box, and GT box binding proteins. J Biol Chem 275: 31051–31060.

51. Whisett JA (2010) Review: The intersection of surfactant homeostasis and innate host defense of the lung: lessons from newborn infants. Innate Immun 16: 130–142.

52. De Paiva CS, Pangelinan SB, Chang E, Yoon KC, Farley WJ, et al. (2009) Essential role for c-Jun N-terminal kinase 2 in corneal epithelial response to desiccating stress. Arch Ophthalmol 127: 1625–1631.

53. Enriquez-de-Salamanca A, Castellanos E, Stern ME, Fernandez I, Carreno E, et al. (2010) Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. Mol Vis 16: 862–873.

54. Lam H, Bleiden L, de Paiva CS, Farley W, Stern ME, et al. (2009) Tear cytokine profiles in dysfunctional tear syndrome. Am J Ophthalmol 147: 190–203 e191.

55. McCormick CC, Hobden JA, Baliti CL, Reed JM, Caballero AR, et al. (2007) Surfactant protein D in Pseudomonas aeruginosa keratitis. Ocul Immunol Inflamm 15: 371–379.

56. Madan T, Redl KB, Singh M, Sarma PU, Kishore U (2005) Susceptibility of mice genetically deficient in the surfactant protein (SP)-A or SP-D gene to pulmonary hypersensitivity induced by antigens and allergens of Aspergillus fumigatus. J Immunol 174: 6941–6954.

57. Corrales RM, Villarreal A, Farley W, Stern ME, Li DQ, et al. (2007) Strain-related cytokine profiles on the murine ocular surface in response to desiccating stress. Cornea 26: 579–584.