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Cornea and Ocular Surface Disease: Application of Cutting Edge Optometric Research

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Abstract

Clinician-scientists bridge the gap between basic research and patient care. At the 2012 Annual Meeting, a symposium highlighting the application of cutting edge optometric research within the anterior segment was held to present and discuss some of the recent basic scientific advances that will both shape and guide the development of future clinical care practices. This paper summarizes this work, bringing together four experts, all clinician-scientists in the field of cornea and ocular surface. Collectively, this work provides new insights to clinicians and researchers alike, as well as brings forth a greater appreciation of the impact of on-going optometric bench research in advancing clinical care.

Keywords

cornea; anterior segment; infection; diabetes; dry eye; endothelium; physiology

Advances in biomedical research and clinical care represent the culmination of two, often mutually exclusive arms of healthcare, the scientist and the clinician. Both working within their own scope of practice, too often lines are drawn, with each perceiving their own work as more relevant or fundamental to the problem at hand. Astute observations from clinicians drive the development of entire lines of research that are required to achieve landmark advances in clinical care. Defined as “bedside to bench” research, the identification of unmet clinical needs and the implementation of new techniques, therapeutic measures and medical devices often stimulate much needed basic research. In healthcare however, some may be less aware of the contributions that basic researchers make to the implementation of clinical care practices. These advances, termed “bench to bedside”, underlie some of our most significant developments yet frequently occur on a very different timeline.

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There is yet a third group or species that results from the intersection of the clinician and the scientist. Called the clinician-scientist, or scientist-clinician, with the latter defined as a researcher not involved in patient care, these individuals represent those whom have mastered both languages or scopes of practice in order to facilitate translation between them. In medicine, the clinician-scientist has been frequently referred to as an “endangered species”.[1,2] Within the optometry and vision science community however, the clinician-scientist is alive and well. At the 2012 American Academy of Optometry annual meeting in Phoenix, a symposium highlighting cutting edge optometric anterior segment research brought together four of our fellow clinician-scientists to address the impact of systemic disease, contact lenses, and the effects of commonly used topical medications on the cornea and ocular surface. As the moderator of this symposium, Tony Adams said at the time, “In optometry we have some superb examples of researchers who have both clinical and basic research credentials. In the area of cornea and anterior surface eye health we have some of our best examples of basic research, conducted by our clinically trained colleagues. That research is making a significant impact on advances in understanding mechanisms of health and disorders of the anterior eye with far reaching implications well beyond”.

Based on the Symposium presentations, in this paper our co-authors report on their own recent research findings and highlight the implications and applications of this work for the advancement of patient care. In the paragraphs that follow, they will guide us through their basic and translational research, beginning with new advances in our understanding of microbial infection stemming from contact lens wear and potential strategies for protection (Fleiszig), alterations in growth factor pathways that underlie the pathobiology of diabetic corneal and ocular surface disease (Robertson), the identification of critical autoimmune mechanisms leading to recalcitrant aqueous deficient dry eye (McNamara), and the impact of topical carbonic anhydrase inhibitors (CAIs) on corneal endothelial function (Bonanno). These brief illustrated expanded abstracts are accompanied by color figures and selected video clips. At the conclusion of each abstract, clinical commentary is provided by Optometry and Vision Science Clinical Editor Larry Alexander, to underscore the clinical significance and potential impact on clinical practice.

ERADICATING INFECTION (from the Research of Fleiszig and Colleagues)

Despite a plethora of new products, the incidence of contact lens-related infection has not changed since soft lenses were first introduced onto the market about 40 years ago. It is becoming increasingly clear that basic research to understand the mechanisms cannot be circumvented if the problem is ever to be solved. In recent years, our laboratory has capitalized on new technologies and an explosion of information in the field of molecular and cellular biology to develop various new models and methods. These are now providing answers to long standing questions about how the healthy cornea resists infection, how contact lens wear compromises that resistance, and how microbes take advantage of the contact lens-induced changes to cause disease. Data arising from these experiments are informing us about how lens wear could be made safer, and suggest exciting and novel strategies for managing infection whatever the cause.

How the Healthy Eye Rapidly Clears Bacteria

Knowing how lens wear predisposes to infection requires that we understand why the cornea is otherwise so resistant. Some microbes we commonly encounter, e.g. *Pseudomonas aeruginosa*, are armed with virulence factors that enable them to kill or invade corneal epithelial cells when they are grown in culture[3-6]. Surprisingly, little is known about how the healthy cornea protects itself against microbes *in vivo*. In contrast, much has been published about immune responses and inflammation initiated when an infection is underway. The lack of attention to protective mechanisms operating during health (homeostasis) is most likely a practical issue, in that it is easier to develop infection models to study the details of disease, than studying a healthy cornea to determine why an infection is not happening.

To assist us in our efforts to study health-related defenses of the cornea we recently developed a “null infection model”. This involves exposing healthy corneas of mice to highly virulent *P. aeruginosa* without any other manipulation of the cornea. As expected, there is no visible pathology. Importantly, our data show that even extremely large inocula are very quickly cleared from the ocular surface (Figure 1)[7]. Identifying the mechanisms involved in bacterial clearance and in corneal resistance to bacterial adhesion and subsequent penetration is critical in understanding homeostasis.

While tear fluid can protect corneal epithelial cells against *P. aeruginosa* (Figure 2)[8], we have found that *P. aeruginosa* can grow efficiently in human tear fluid removed from the eye[8]. This suggests that the rapid bacterial clearance from the ocular surface is not simply due to tear fluid antimicrobial activity. Rather, our data show that tear fluid acts upon the corneal cells to trigger their defenses against bacterial virulence strategies[9]. Indeed, human tear fluid protects mouse eyes against *P. aeruginosa* colonization.[10]

The protective activity of tear fluid is associated with changes in gene expression in corneal epithelial cells, and subsequently increased antimicrobial activity within them [9]. Figure 3 shows all the genes impacted upon exposure of corneal epithelial cells to tear fluid. Some (RNAse7 and ST2) were found directly responsible for this protective activity.[9]

Our studies of tear fluid effects on corneal cell antimicrobial activity have also led to the discovery of a novel class of antimicrobial that consist of small fragments of keratin[11]. These keratin derived antimicrobial peptides (KDAMPs) are smaller than other known host-expressed antimicrobials, and they are also more stable. Thus, they are easy to make and they are active in a wide variety of situations.

Figures 4, 5 and supplementary videos 1, 2 (all from [11]) show the quick killing activity of a highly cytotoxic strain of *P. aeruginosa* (6206) by a KDAMP, and its activity in normal salt conditions respectively.

Importantly, we have found that knocking down expression of the keratin from which they are derived compromises the capacity of the otherwise healthy mouse cornea to resist bacterial adhesion (Figure 6), showing that they are directly involved in defense against infection. The discovery that keratins can give rise to antimicrobial fragments and that they

are protective against bacteria, is likely to be of significance beyond the eye. It could also lead to the development of new approaches to preventing infection (see commentary[12]).

Contact Lens Wear Predisposes the Healthy Eye to Infection

If the cornea normally resists infection even when very large inocula of bacteria are added, why does contact lens wear predispose to infection? Our current working hypothesis, based on our research findings over more than 20 years, is that two events conspire to increase the probability of infection when a lens is worn; 1) suppressed production of defense molecules[13], and 2) bacterial adaptation to host defenses[14]. This would explain why extended wear is a risk factor for both people and rats (Figure 7). It would also explain why *P. aeruginosa*, which is particularly proficient at adaptation to its environment - including corneal epithelial defenses against bacterial penetration (unpublished data), is the most common cause of lens-related infections.[15]

Our understanding of health-related defenses, the impact of lens wear, and how bacteria adapt to host exposure is deepening. Given the vast body of new genomic and proteomic information, and the plethora of new research tools available in our labs, there is good reason to believe that we will soon be able to eradicate contact lens infections by either protecting/restoring normal corneal defenses or preventing bacterial adaptation.

Clinical Commentary (Alexander): Keeping the Cornea Pristine

Contact lens-related corneal infection associated with contact lens wear remains a significant, on-going clinical problem. This presentation addresses the issue of corneal protection from microbial infection. The “null infection model” is presented to demonstrate what has been observed clinically for some time: a healthy cornea is very effective at blocking infection. Fleiszig demonstrated that tears protect the eye by triggering corneal cell defenses and “washing” the corneal epithelial surface. They also found that keratins, which are produced by the corneal cells, give rise to antimicrobial fragments that may lead to new the development of new therapeutic modalities. Fleiszig also offers a hypothesis regarding the predisposition toward infection that is inherent with contact lens wear and the impact on normal corneal defenses.

MECHANISMS OF CORNEAL EPITHELIAL MAINTENANCE: UNDERSTANDING THE EFFECTS OF SYSTEMIC DISEASE AND CONTACT LENS WEAR (from the Research of Robertson and Colleagues)

The corneal epithelium is a self-renewing stratified epithelial sheet that provides a barrier against invading pathogens and a smooth refracting surface essential for vision. A coordinated balance between proliferation, differentiation and apoptotic desquamation coupled with dynamic regulation of intercellular junction formation contributes to homeostatic tissue maintenance as cells divide, migrate and shed throughout the course of normal cellular turnover (Figure8). Using contemporary imaging, molecular and biochemical techniques, we are investigating fundamental gene regulatory mechanisms and protein-protein interactions that regulate normal corneal epithelial homeostatic renewal and are disrupted by injury or disease. To accomplish this, we have implemented two clinically

relevant models that perturb normal epithelial homeostatic renewal: contact lens wear, where mechanical and chemical effects from lenses and/or solutions alter both renewal and barrier function properties, predisposing the cornea to infection,[16, 17] and hyperglycemic-induced corneal epithelial changes as seen in diabetic disease.

Diabetes and the Effects of Hyperglycemia on Normal Ocular Surface Biology

Clinically, patients with diabetes can present with a spectrum of conditions including dry eye, superficial punctate keratitis, recurrent corneal erosions, persistent epithelial defects and severe neurotrophic keratopathy. These conditions are further complicated by impaired corneal wound healing mechanisms and a potential increased risk for microbial keratitis, during and in the absence of, contact lens wear. While the frequency of severe diabetic corneal complications has lessened due to earlier intervention and better glycemic monitoring and control; diabetic-induced corneal changes remain a significant understudied clinical problem.

Growth Factors and the Ocular Surface

It is well established that growth factors play a key role in the maintenance of the corneal epithelium. The classical pathways in which extracellular growth factors exert their effects involve interactions with surface membrane receptors, which initiate a series of events inside the cell that ultimately determine cell fate (such as growth or cell death, Figure 9). Unraveling the role of growth factors and how their effects are regulated at the ocular surface in the healthy and diseased eye is vital in both disease pathobiology and therapeutic management. Importantly, many, if not all, growth factors exert pleiotropic effects. This becomes clinically significant when using exogenous growth factors for therapeutic measures at the ocular surface. Similarly, changes in the presence of endogenous extracellular growth factors may negatively impact the corneal epithelium in disease states. Growth factors have been shown to be secreted from both the lacrimal gland and corneal epithelial cells, indicating both paracrine and autocrine regulatory pathways. At the cellular level, growth factors can mediate intracellular as well as extracellular effects. This means in the case of disease, problems can arise from the inside-out or the outside-in. Heterogeneous interactions between growth factors and their cognate receptors add yet another level of complexity.

The growth factor and receptor of interest in our laboratory are the insulin-like growth factor type 1 (IGF-1) and the IGF-1 receptor (IGF-1R, Figure 9). In the case of the IGF-1R, IGF-1R can interact with its fraternal twin, the highly homologous insulin receptor (INSR), to form a hybrid receptor (Hybrid-R). Our laboratory has been the first to identify the presence of the Hybrid-R in the corneal epithelium (Figure 10).[18] Importantly, studies using gene knockdown techniques have demonstrated preferential Hybrid-R formation compared to classical IGF-1R or INSR formation in corneal epithelial cells, underscoring the significance of this receptor in ocular surface biology.

The importance of the Hybrid-R in the corneal epithelium is two-fold. First, the Hybrid-R is present in the nucleus of corneal epithelial cells.[18] The presence of the Hybrid-R in the nucleus, where it interacts with DNA, means that it functions to regulate gene expression.

Using state-of-art chromatin immunoprecipitation-DNA sequencing technology (ChIP-seq), we have been able to identify potential genes from enriched genomic DNA sequences that are targeted by the IGF-1R/INSR Hybrid-R (Table 1). Of high clinical relevance to the pathobiology of corneal disease, these targets include cell survival factors, mediators of cellular adhesion and barrier function, and ocular surface defense.

Secondly, the Hybrid-R is important in regulating ligand specificity (ie: the ability of extracellular proteins to bind specific membrane receptors).[18] This is critical at the ocular surface, where both IGF-1 and insulin have been shown to be expressed in the preocular tear fluid.[19,20] In corneal epithelial cells, the Hybrid-R is stimulated by IGF-1 and not insulin (Figure 11). Therefore, the presence of the INSR in a Hybrid-R complex modulates the biological activity of tear insulin, which is an important growth and survival factor for corneal epithelial cells. In diabetes, hyperglycemic damage to the lacrimal gland can alter protein secretion into the tear fluid,[21,22] which may further impact the corneal epithelium through a reduction in bioavailable tear insulin.

Clinical proteomic tear studies in our laboratory have shown a significant increase in the IGF-binding protein 3, IGFBP3, in diabetic tears (Figure 12).[19] IGFBP3 can sequester IGF-1, thus the consequence of increased IGFBP3 in diabetic tear fluid is the inhibition of IGF-1 activity. While not yet investigated in the corneal epithelium but of high pathophysiological relevance, IGFBP3 has been shown to function independently of the IGF-1R to potentiate inflammatory and oxidative-mediated cell death pathways, which are critical pathways involved in mediating hyperglycemic damage.[23,24] While all patients in this early study were moderately well-controlled diabetics based upon HbA1c levels and the absence of any neuropathy or retinopathy, further studies are needed to investigate these changes in patients with moderate to severe disease.

Neuro-Epithelial Interactions: 3D Nerve Modeling

Diabetic keratopathy is a neuro-epithelial disease and corneal effects are mediated in part by alterations in the subbasal nerve plexus. Growth factors such as IGF-1 and insulin are neurotrophic mediators; however, the interactive effects of growth factors such as IGF-1 on the corneal epithelium and corneal nerve structure and function remain undefined (Figure 13). Our laboratory has developed and optimized new quantitative 3-dimensional methodology for evaluating the effects of diabetes on corneal nerve morphology *in situ*. Using laser scanning confocal microscopy we are able to generate full thickness, volumetric reconstructed images of the subbasal epithelial nerve plexus and associated epithelial nerve fibers (Figure 14). Volumetric reconstruction of the terminal epithelial nerves allows for quantitative assessment of corneal nerve parameters such as length and branching patterns within the corneal epithelium and provides a highly sensitive outcome measure for monitoring early and late diabetic changes. Using this methodology, we have been able to dissect out differential age and disease-related changes in the corneal nerves (unpublished data). 3-D images of the subbasal nerve plexus (SBNP) and epithelial nerves (EN) with animated rotation allow for enhanced visualization and are shown in the supplementary video files, SBNP 1, EN1, EN2.

In summary, our long term goals are to explain the pathobiological changes that disrupt epithelial homeostasis in the diseased cornea and during contact lens wear. This includes the identification of extracellular tear proteins as biomarkers for disease, the elucidation of how posttranslational modifications to existing proteins may modulate protein function and cellular processes, and investigations into the role of neuro-epithelial interactions in the maintenance of corneal epithelium.

Clinical Commentary (Alexander): The Effects of Hyperglycemia on Corneal Epithelial Cell Biology

The proper metabolism and thus health of the corneal epithelium is a very complicated balancing act. This presentation addresses the impact of hyperglycemia, as occurs in diabetes, on normal ocular surface biology and integrity. Diabetes is often associated with corneal complications including delayed healing and impacts a significant number of patients worldwide. Robertson reports on the impact of the insulin-like growth factor (IGF-1) and the receptor IGF-1R, and how this system may be altered by hyperglycemia. Robertson also reports on the interaction of IGF-1R with its twin INSR to form a hybrid receptor, Hybrid-R. The Hybrid-R is present in the nucleus of the corneal epithelial cell where it interacts with DNA and is posited to regulate gene expression. The Hybrid-R also is involved in regulating ligand specificity. The presence of the INSR in a Hybrid-R complex modulates the biological activity of tear insulin, which is important for growth and survival of corneal epithelium. The work presented in this study is critical in both the overall understanding of corneal biology in the non-diabetic and diabetic eye and may lead to the development of novel biomarkers or therapeutic strategies to mitigate diabetic corneal and ocular surface disease.

TACKLING THE CLINICALLY RECALCITRANT DRY EYE IN AUTOIMMUNE DISEASE (from the Research of McNamara and Colleagues)

One of the most debilitating forms of keratoconjunctivitis sicca (KCS) results from autoimmune-mediated destruction of the lacrimal gland. Despite powerful immunosuppressive and immune-modulatory therapy, KCS in autoimmune diseases like Sjögren's syndrome can lead to corneal opacification and vision loss through a process known as squamous metaplasia (Figure 15). Little is known about the pathogenesis of squamous metaplasia (SQM) and there is no cure. The goal of our research is to decipher how autoimmune-mediated inflammation provokes KCS and vision-threatening squamous metaplasia. Using three model systems, (i) human patients with Sjögren's syndrome; (ii) a validated mouse model deficient in the autoimmune regulator gene (Aire) that mimics the clinical characteristics of Sjögren's syndrome; and (iii) *in vitro* studies of cultured corneal epithelial cells, my lab has demonstrated an essential role for autoreactive CD4+ T cells and their interplay with the pro-inflammatory cytokine interleukin (IL)-1 in the pathogenesis of autoimmune KCS (Figure 16).[27-31]

The Role of IL-1 in Autoimmune Dry Eye

Knockdown of IL-1R (receptor) 1 in Aire-deficient mice improves ocular surface integrity and decreases SQM, providing evidence for a functional link between local signaling via

IL-1R1 in the pathogenesis of autoimmune KCS/SQM (Figure 17A). Accordingly, in clinical studies we have shown IL-1 beta protein is elevated in the tears of human patients with Sjögren's syndrome and its ocular surface expression is a significant predictor of ocular disease (Figure 17B).[28]

The development of immunoassays to identify novel biomarkers from small volume tear samples is underway, as well as the identification of novel drug targets to treat autoimmune dry eye and prevent the devastating consequences of squamous metaplasia. Inhibition of signaling via IL-1R1 represents one such drug target where topical application of the IL-1R1 antagonist, Anakinra, reduced ocular surface staining in Aire KO mice (Figure 18).[32]

To summarize, we have investigated local changes in the inflammatory response in human patients with Sjögren's syndrome and used this information to guide the development of targeted therapies for the treatment of autoimmune dry eye. To date, we have noted increased levels of IL-1 cytokines in the tears of Sjögren's syndrome patients and increased expression of IL-1 is highly correlated to the development of KCS/SQM in human patients. Using the Aire-deficient mouse, we have been able to provide a functional link between signaling via the IL-1/IL-1R1 pathway and autoimmune dry eye.[28,30] Accordingly, topical antagonism of IL-1R1 prevents local tissue damage and improves ocular surface phenotype. These data provide one example of a novel intervention that should be considered as a stand-alone or adjunct therapy in the treatment of dry eye. Alternative therapeutic targets are currently under investigation and may include a shift in therapeutic approach from anti-inflammatory agents to those with pro-secretory and mitogenic function.

Clinical Commentary (Alexander): IL-1 Expression in Sjogren's Patients Drives Ocular Surface Damage

The recognition of one's body as “non-self” results in autoimmune disease and forms the basis for the majority of tissue and organ specific destruction, particularly in women. The association of dry eye with various autoimmune diseases has been well established. This presentation developed and clarified the autoimmune dry eye hypothesis by establishing a relationship with increased levels of the cytokine IL-1 in the tears of Sjogren's patients. IL-1 expression was shown to directly correlate with the development of ocular surface pathology, including keratoconjuctivitis sicca and squamous metaplasia. The findings presented offer a targeted therapeutic approach to the disorder.

WHY ARE TOPICAL CAIS CONTRAINDICATED IN CORNEAS WITH LOW ENDOTHELIAL CELL COUNT? (from the Research of Bonanno and Colleagues)

The corneal endothelium is responsible for maintaining the hydration and transparency of the cornea (Figure 19A). Trauma, inflammation, or endothelial degeneration (e.g. Fuchs dystrophy) can reduce endothelial function leading to corneal edema and loss of vision (Figure 19B).

The endothelial “pump” requires the presence of bicarbonate, the activity of membrane bicarbonate transporters, Na-K ATPase, and carbonic anhydrase activity, which led to the hypothesis that the endothelial pump is a bicarbonate and carbonic anhydrase dependent ion secretory mechanism that creates osmotic gradients leading to water efflux that exactly counterbalances water influx driven by stromal glycosaminoglycans.[33] However, evidence for a bicarbonate secretory mechanism has not been revealed.

The Endothelial Pump: An Alternative Hypothesis

We provide an alternate hypothesis that is rooted in corneal metabolism and incorporates carbonic anhydrase as a component. Corneal metabolism is very glycolytic as opposed to being oxidative. 85% of the glucose consumed by the cornea simply goes through glycolysis and is converted to two lactate molecules[34]. The other 15% is shuttled to mitochondria where ATP is made and oxygen is consumed. Oxidative metabolism is relatively low because corneal epithelial cells and stromal keratocytes have very few mitochondria. This keeps light scatter low as well. As a result the cornea makes a lot of lactic acid, which is the end product of glycolysis. Because each glucose used is split into two lactate molecules, this represents a gain of osmotic particles. As such, these particles need to be removed or the cornea will become edematous. We know that this can happen because it has been shown that contact lens induced corneal hypoxia increases corneal lactate concentration and causes corneal edema[35]. Given the ‘”pump's” sensitivity to carbonic anhydrase inhibitors and the requirement for bicarbonate[36-38], we hypothesize that the endothelium is set up to facilitate the removal of lactic acid, as shown in Figure 20.

In this model glucose taken up from the anterior chamber diffuses to the stroma and epithelium where it is consumed and 85% of it is converted to 2 lactate molecules. Lactate leaves the epithelium through a lactate H^+ cotransporter. This diffuses back to the endothelium where lactate H^+ cotransporters move it across the cells. This flux of lactate across the cells is coupled to water flux (via Aquaporin channels, AQP1) by osmosis. The presence of bicarbonate and carbonic anhydrase activity helps to buffer the protons of lactic acid and thereby facilitates lactate flux.

The Effects of CAIs on the Corneal Endothelium

Our hypothesis would predict that inhibiting active transport, bicarbonate transporters or carbonic anhydrase activity will cause corneal swelling and an increase in lactate concentration. Figure 21A shows that topical Azopt or intracameral ouabain (active transport inhibitor) increases stromal lactate concentration and Figure 21B shows that as stromal lactate increases so does corneal swelling.[39,40]

Whereas this sensitivity to topical CAI in the rabbit cornea is dramatic, several studies in humans show that topical CAIs have no effect on corneal hydration.[41-44] However, there are several reports indicating that patients with low endothelial cell counts, guttatae, or Fuchs dystrophy are sensitive to topical CAIs[45-48]. Our explanation is that humans have more robust CA activity than rabbits, but that endothelial degeneration lowers the threshold for CAI sensitivity. The take home message is to not only measure corneal thickness, but also

take a good peek at the endothelium (or measure cell density if you have the instrumentation) before prescribing topical CAIs.

Clinical Commentary (Alexander): Topical CAI Treatment Is Contraindicated in Patients with Endothelial Compromise

This presentation by Bonanno exquisitely pointed to the importance of carbonic anhydrase in the maintenance of corneal endothelial integrity. It has been long known that carbonic anhydrase inhibitors may actually contribute to the degradation of corneal tissue in pre-existing disease such as Fuchs endothelial dystrophy. Bonanno posits that the endothelium is set up to facilitate the removal of lactic acid involving bicarbonate. The findings of the presentation open the door of understanding for potential methods of management of endothelial dystrophies.

SUMMARY

The translation, or bridging of the gap between basic researchers and clinicians, is an essential component in the advancement of evidence-based medicine. The primary goal of this symposium was to provide a forum for the presentation and discussion of on-going optometric research that either now or in the future, will play a role in guiding our clinical care practices. The end result however, was much greater. With this first of its kind publication, we hope to have provided new insights to both clinicians and researchers alike, as well as brought forth a greater appreciation of the significant impact of optometric bench research on the advancement of clinical care.

SUPPLEMENTAL DIGITAL CONTENT LEGENDS

SCD 1. Killing of *P. aeruginosa* by KDAMPS (KDAMP-19mer).

SCD 2. Control video showing *P. aeruginosa* activity in the absence of KDAMPS (KDAMP control – no peptide).

SCD 3. 3D reconstruction of the subbasal nerve plexus in the mouse central cornea (SBNP).

SCD 4. 3D reconstruction of the subbasal nerve plexus and associated terminal epithelial nerves (EN2).

SCD 5. 3D reconstruction of the terminal epithelial nerves branching off the subbasal nerve plexus and coursing throughout the corneal epithelium (EN1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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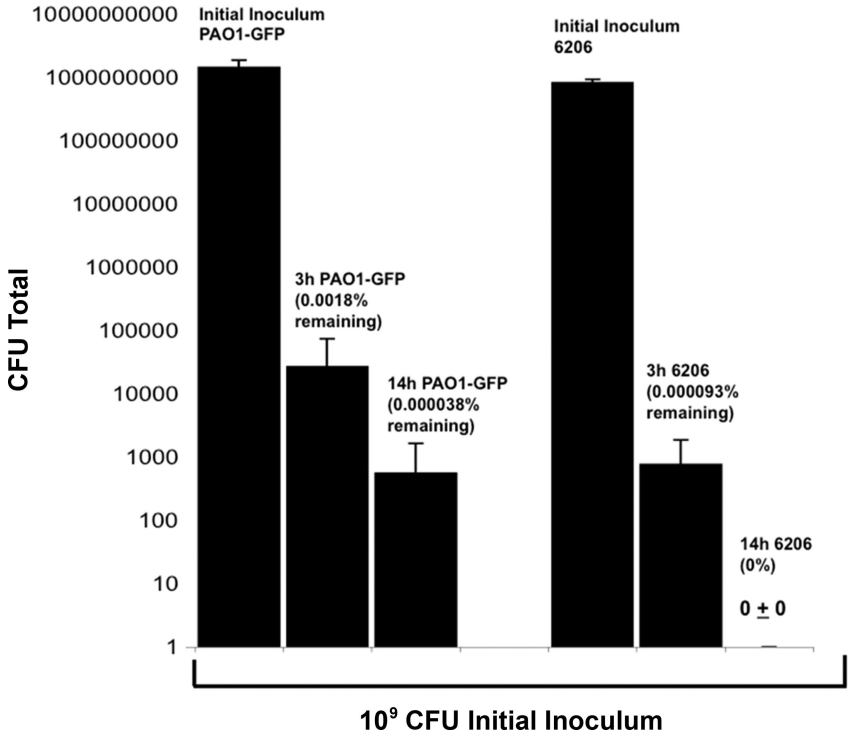

Figure 1.

The healthy eye rapidly clears bacteria. “Null mice” challenged with invasive and cytotoxic strains of *Pseudomonas aeruginosa* effectively clear bacteria from the corneal surface without any residual pathology.

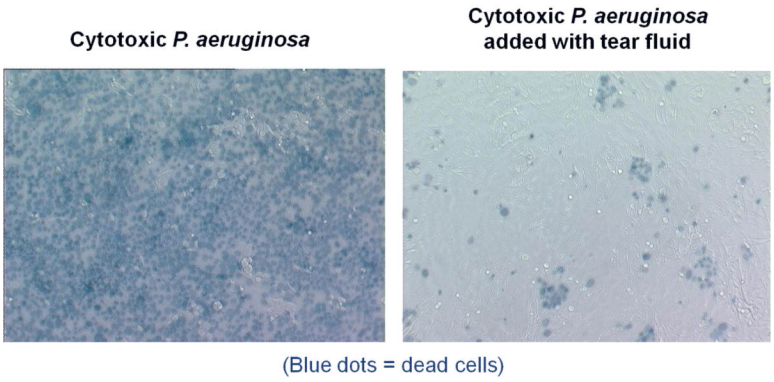

Figure 2.

Trypan blue staining of non-viable corneal epithelial cells exposed to cytotoxic P. aeruginosa. Corneal epithelial cells exposed to *P. aeruginosa* in the absence **(A)** and presence **(B)** of human tear fluid.

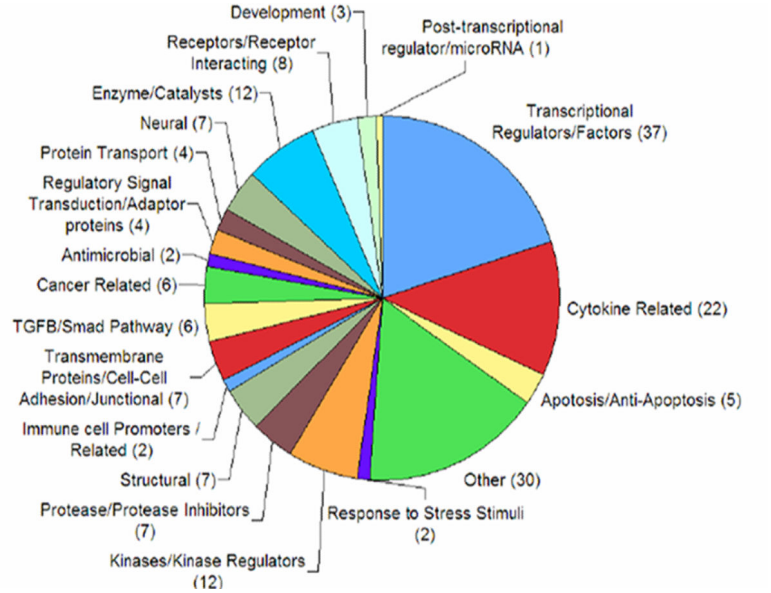

Figure 3.

Microarray analysis revealed the distribution of genes upregulated in corneal epithelial cells challenged with *P. aeruginosa* following pre-exposure to tear fluid.

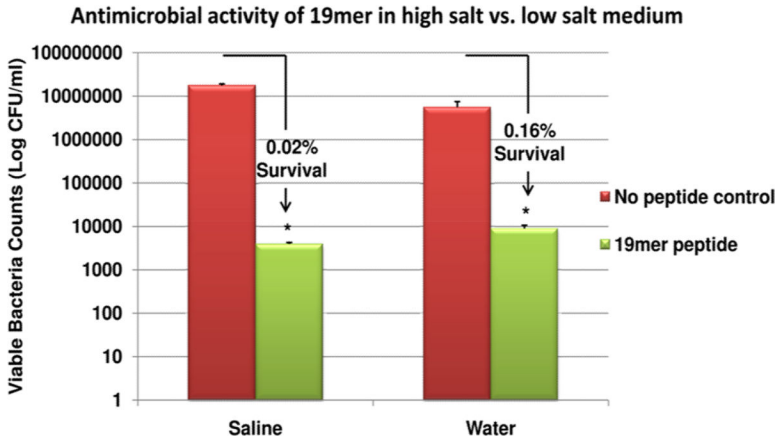

Figure 4.

A comparison of *P. aeruginosa* survival when exposed to KDAMPS in physiological saline (0.9% NaCl) compared to water showed that killing activity was independent of salt concentration.

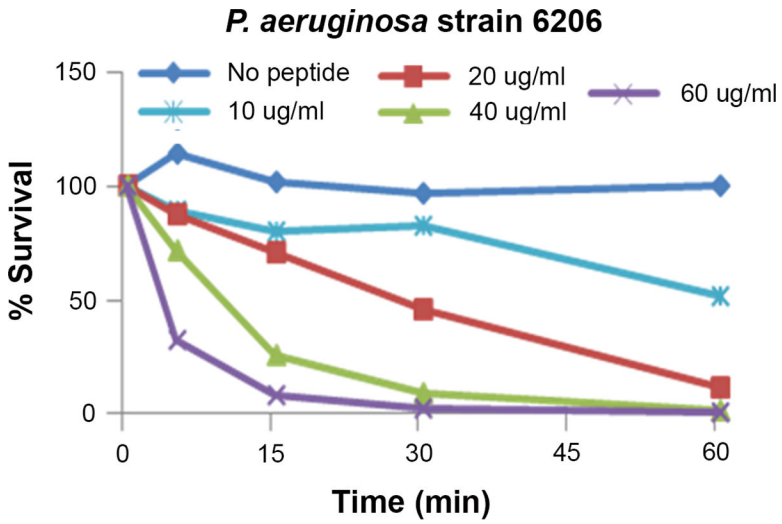

Figure 5.

Rapid killing of cytotoxic *P. aeruginosa* by KDAMPS was seen at all peptide concentrations tested.

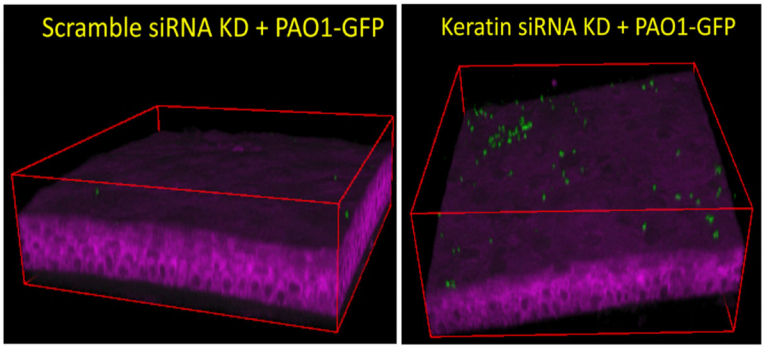

Figure 6.

Two-photon confocal imaging of the mouse cornea in situ. Gene knockdown targeting keratin K6A increased *P. aeruginosa* (green) adherence to the corneal epithelial surface (purple). In contrast, no bacterial adherence was detected in the control cornea (scrambled).

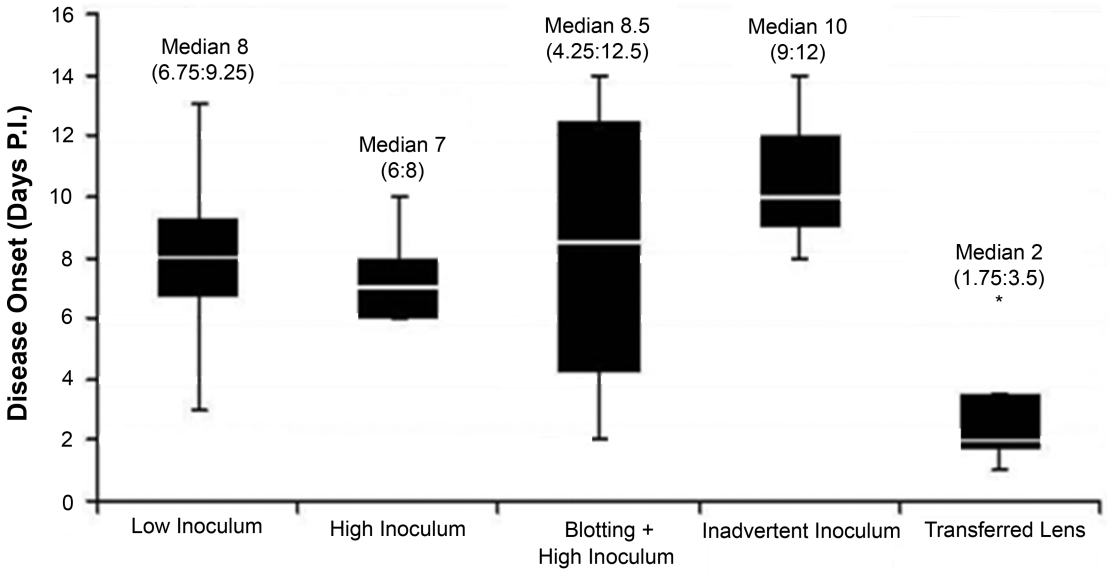

Figure 7.

"In the lens-wearing rats, the onset of infection can be reduced from ~8 days to 2 days if lenses harboring the infecting bacteria are transferred from the eyes of infected rats to naïve rats". Reprinted with permission from The Association in Research for Vision and Ophthalmology, ©ARVO.

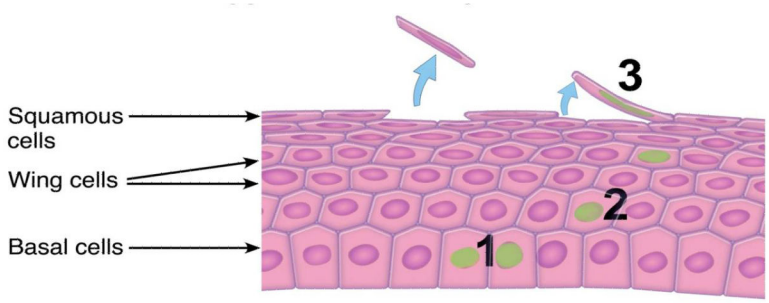

Figure 8.

Model of corneal epithelial homeostasis. Mitotic basal cells divide (1) and migrate vertically (2) towards the corneal surface, where surface cells undergo apoptotic-mediated desquamation (3).

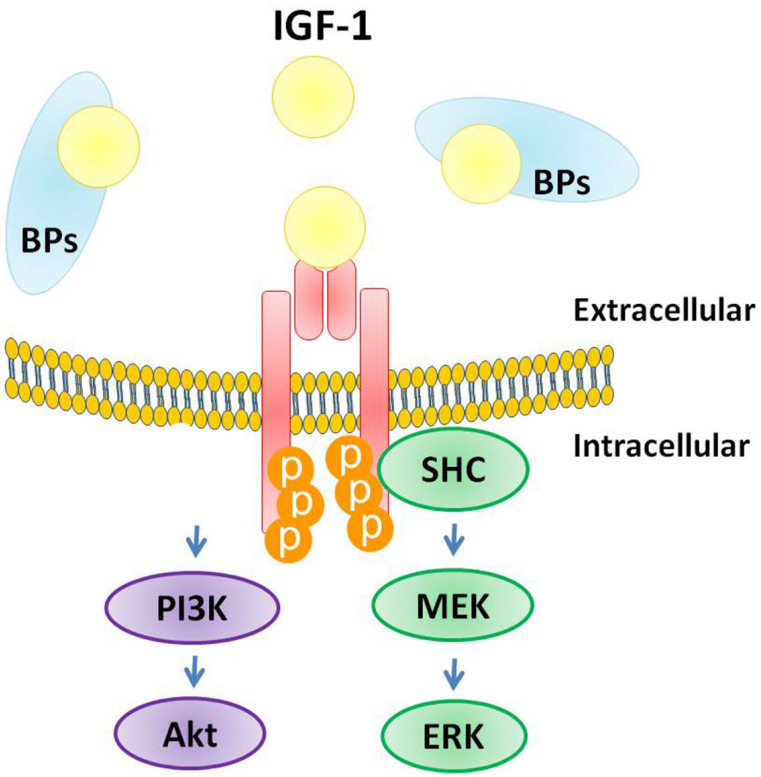

Figure 9.

Extracellular IGF-1 (yellow) binds to the IGF-1R receptor (red) to stimulate receptor phosphorylation. IGF-binding proteins (BPs, blue), can bind and sequester IGF-1, blocking IGF-1 activity. PM: plasma membrane; P: phosphate. Phosphorylation of the IGF-1R leads to activation of PI3K/Akt and MEK/ERK pathways.

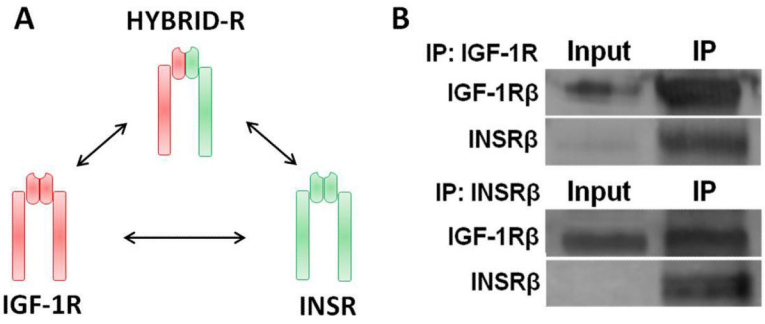

Figure 10.

Identification of the IGF-1R:INSR hybrid. **(A)** The IGF-1R (red) can dimerize with the INSR (green) to form the Hybrid-R. **(B)** Reciprocal co-immunoprecipitation and western blot assay demonstrating the presence of the Hybrid-R in corneal epithelial cells. Input: whole cell lysate; IP: immunoprecipitated lysate. Adapted from Wu et al.[18]

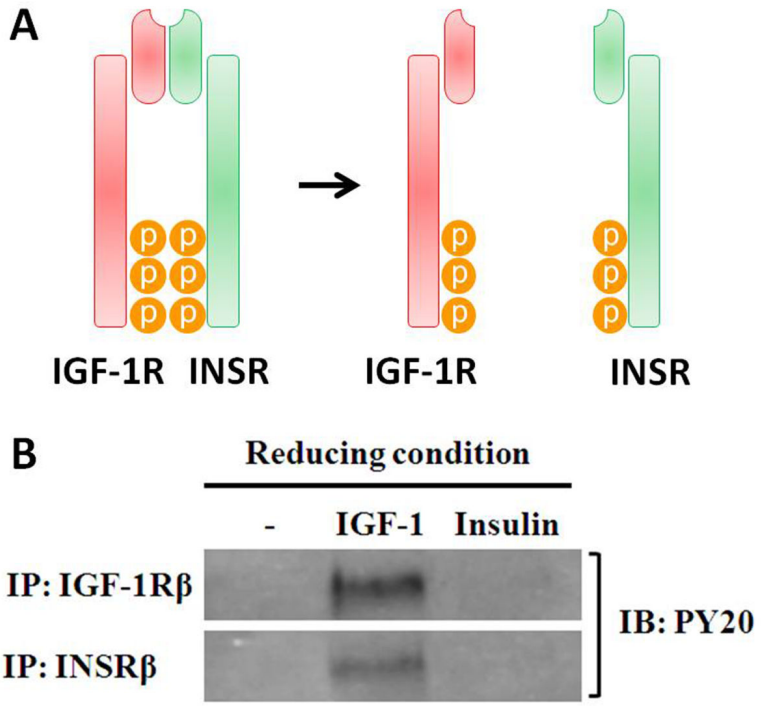

Figure 11.

IGF-1 stimulation of Hybrid-R. **(A)** Treatment with a reducing agent disrupts the disulfide bonds joining the IGF-1R/INSR subunits. **(B)** Reducing immunoprecipitation and western blot showing IGF-1 activation of both IGF-1R and INSR hybrid subunits. IGF-1 was unable to activate the homodimeric INSR (not shown). Adapted from Wu et al.[18] (-) non-stimulated; IP: immunoprecipitated lysate; IB: immunoblot; PY20: antibody recognizing a phosphorylated receptor.

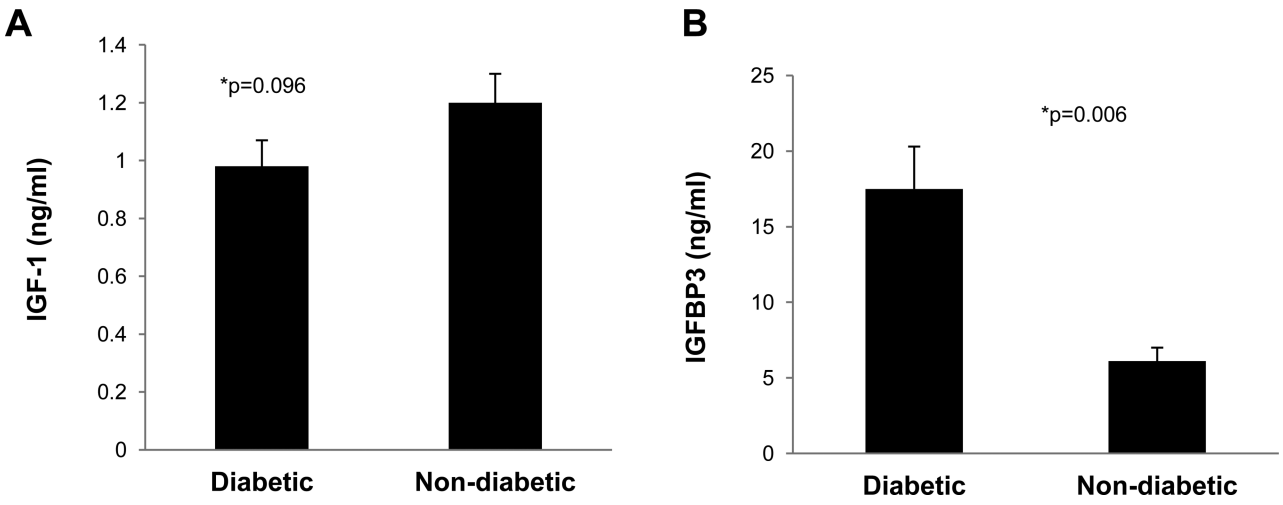

Figure 12.

IGF-1 and IGFBP3 in human tears. **(A)** IGF-1 was not significantly reduced in diabetic tears; **(B)** IGFBP3 was increased 3 fold in diabetic tears compared to non-diabetic controls. Adapted from Wu et al.[19]

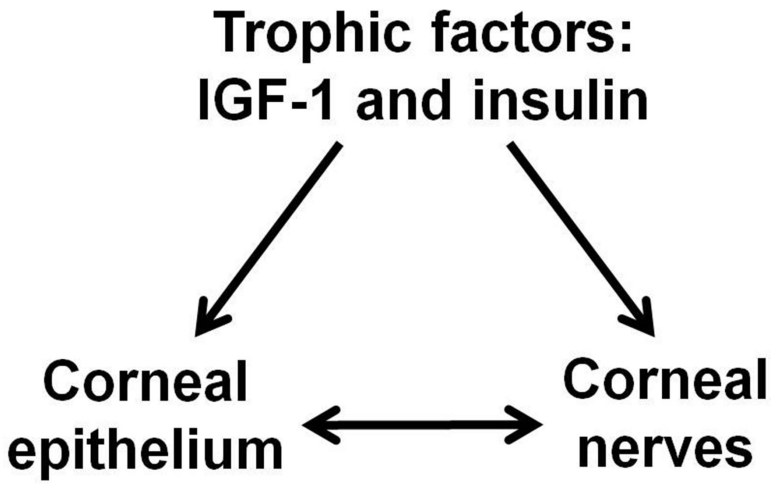

Figure 13.
Interactive effects between trophic factors, IGF-1 and insulin, on the corneal epithelium and corneal nerves.

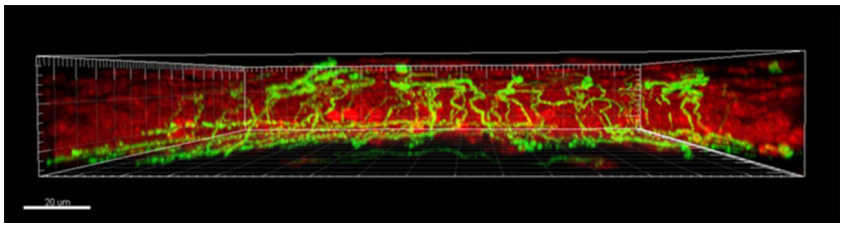

Figure 14.

Three-dimensional reconstruction of the subbasal nerve plexus and terminal epithelial nerves in the mouse cornea. Corneal nerves are labeled with neuronal tubulin (green) and nuclei are counterstained with propidium iodide (red). Scale: 20μm.

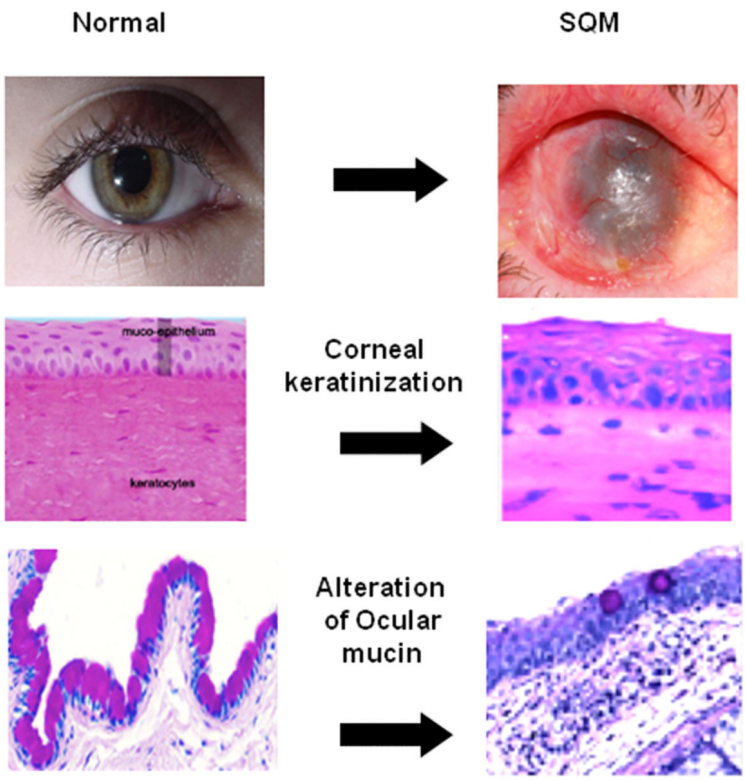

Figure 15.

The healthy ocular surface is comprised of a non-keratinized, squamous corneolimbal epithelium (middle left) and a conjunctival surface rich in GCs (bottom left, pink cells). In autoimmune dry eye, chronic inflammation sets off a process of altered differentiation, known as squamous metaplasia (SQM), where the transparent cornea becomes pathologically keratinized (middle right) and the conjunctiva is depleted of GCs (bottom right). An end-stage consequence of SQM is corneal opacification and vision loss.[25,26]

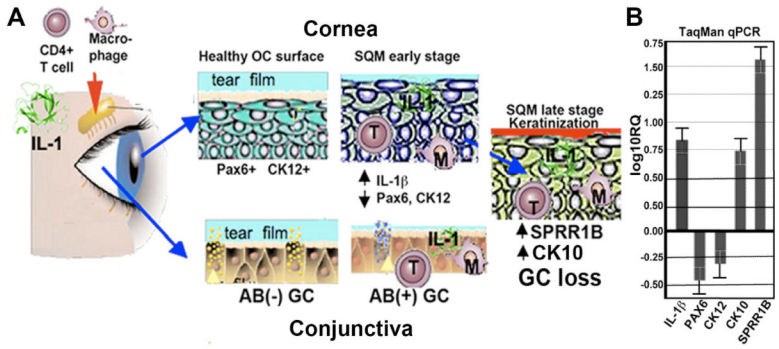

Figure 16.

(A) Schematic summary of SQM in autoimmune dry eye. Autoreactive CD4+ T cells and mononuclear cells infiltrate the lacrimal gland and ocular surface to set-off a process of IL-1-mediated altered differentiation. SQM is phenotypically characterized by downregulation of ocular hallmarks Pax6, CK12 & upregulation of epidermal hallmarks (CK10, SPRR1B). Conjunctival goblet cells (GCs) undergo aberrant glycosylation (Alcian Blue (AB)+) followed by GC loss. **(B)** qPCR profile of SQM in *Aire* KO ocular epithelial cells, expressed as relative quantification (RQ) in Log10, (*Aire* WT designated as baseline log10 = 0).

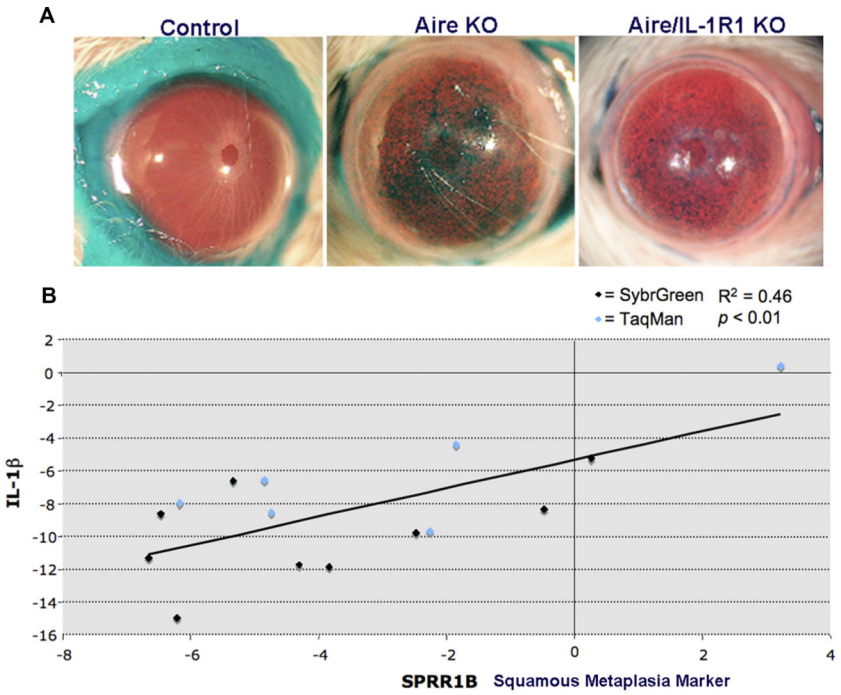

Figure 17.

(A) IL-1R1 knockdown in Aire-deficient mice demonstrated the functional role of IL-1/IL-1R signaling in the pathogenesis of autoimmune dry eye.[28] **(B)** Accordingly, ocular surface disease in Sjögren's syndrome-KCS was correlated with increased levels of IL-1 beta in tears and on the ocular surface. Transcript levels of IL-1beta and SQM marker, SPRR1B, were examined by qPCR using mRNA extracted from impression cytology specimens and revealed a strong correlation between IL-1beta and SPRR1B (R2 = 0.46, p<0.01) in human patients. Reprinted with permission from: Chen et al. [28]

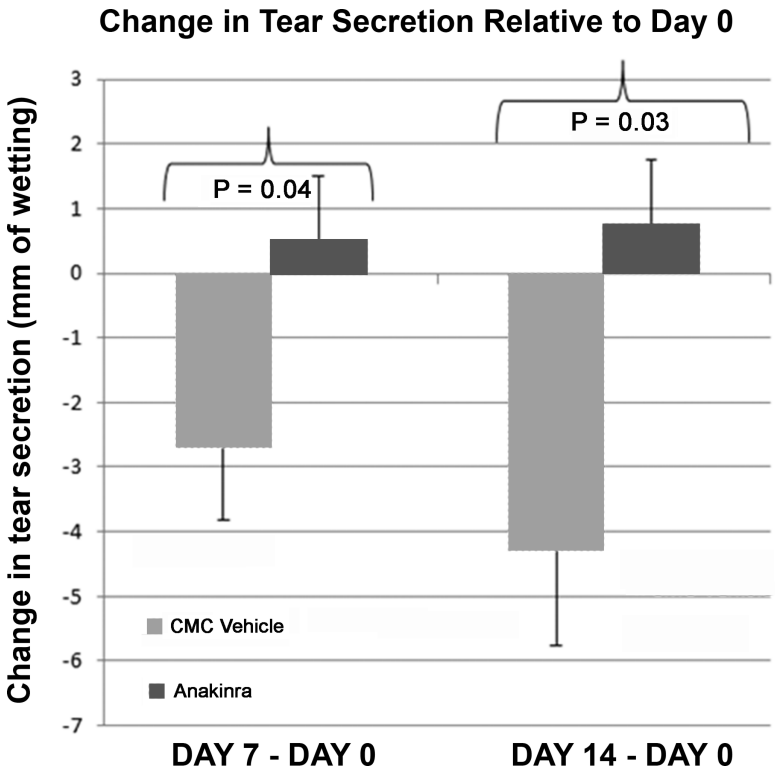

Figure 18.

Lissamine green (LG) dye was used to assess loss of corneal epithelial integrity in the eyes of Aire KO mice treated with IL-1R1 antagonist, Anakinra (AnkTx) or vehicle control (UnTx). A score of 1 represents < 25% of the ocular surface stained with LG; 2 represents 25-50% stained; 3 represents 50-75% stained and 4 represents > 75% of the ocular surface stained with LG. The difference in LG staining at Day 7 and Day 14 with respect to baseline at Day 0 is shown. Relative to the baseline, the Anakinra-treated group shows an appreciable reduction in LG staining score by Day 7 that progresses to a statistically significant decrease by Day 14.[30]

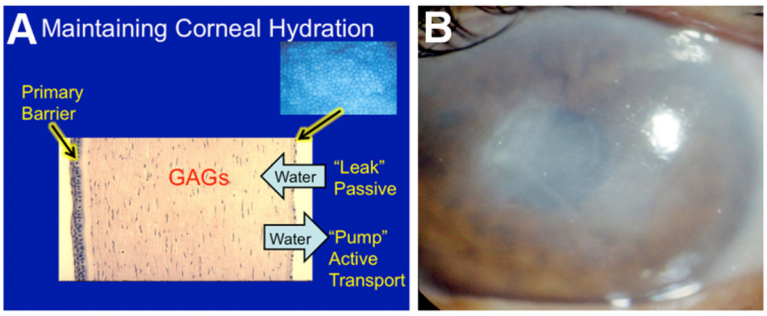

Figure 19.

(A) Stromal GAGs (glycosaminoglycans) suck water into the cornea (Leak). The endothelial pump removes water thereby maintaining corneal hydration and transparency. **(B)** When the pump fails, corneas become edematous and vision is poor. This is an example of Fuchs Dystrophy.

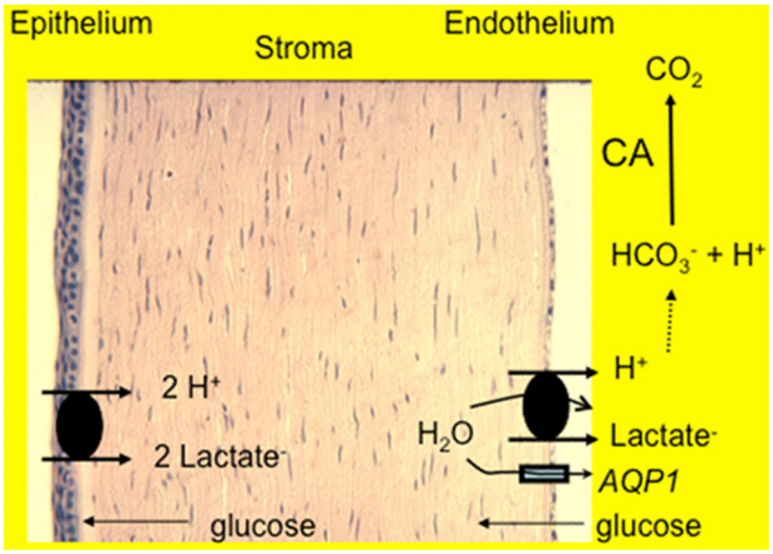

Figure 20.
Schematic diagram illustrating the removal of lactic acid by the corneal endothelium.

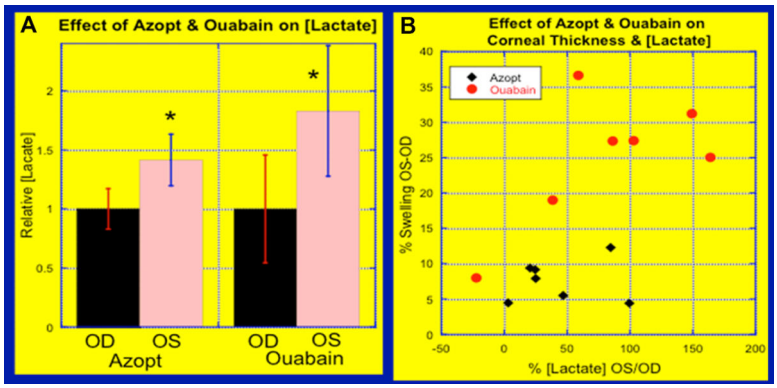

Figure 21.

(A) Rabbits received 2 drops of topical Azopt or injection of intracameral ouabain, OS. Several hours later corneal thickness was measured and they were euthanized. Stromal lactate was then determined. **(B)** Relationship between stromal lactate and corneal swelling.

Table 1

Adapted from Wu et al.[18]

	IGF-1R	INSR
Total Functional Genes Identified	**52**	**31**
Involved in regulation of:		
Cell proliferation/cell cycle progress	5	6
Cell death/apoptosis	5	1
Cell differentiation	6	1
Cell adhesion	6	3
Signal transduction	11	5
Cellular protein metabolic process	8	7
Cell communication	3	3