**PEARLMAN RESEARCH PROGRAM**

Dr. Pearlman’s research interest is in the pathogenesis of bacterial and fungal keratitis, and his current studies examine host responses in infected individuals, and uses animal models of microbial keratitis.


**Impact of Fungal Keratitis on Global Visual Health**

Fungal keratitis (Infection and inflammation of the cornea) is an important cause of blindness and visual impairment in the USA and other industrialized countries, where contact lens wear is the primary risk factor. This was illustrated by several hundred cases in the USA, Western Europe and Singapore during the 2005/2006 outbreak of contact lens associated *Fusarium* keratitis, and contact-lens and trauma associated fungal keratitis are continually reported in the USA. However, in developing countries, trauma to the ocular surface, most commonly in relation to agricultural work, is the major risk factor for fungal keratitis. On a global scale, fungal keratitis accounts for ~65% of all corneal ulcers. In India, it is estimated that 80,000 total cases and 10,000 corneal transplants are performed each year due to fungal corneal infections.

*Aspergillus* (*A. flavus, A. fumigatus*) and *Fusarium* (*F. solani, F. oxysporum*) species are the main etiologic agents of fungal keratitis. However, other species and genera of filamentous fungi that cause keratitis include: *Curvularia, Alternaria,* and *Penicillium*. Most infections with these organisms are initiated in an agricultural environment as a result of ocular surface trauma caused by plant material, insects or branches contaminated with fungal spores. In the corneal stroma, conidia germinate into hyphae, which then penetrate throughout the stroma and the basement membrane, where they also infect the anterior chamber, causing severe pain, photophobia and vision loss. Topical natamycin or voriconazole are effective if given very early, but fungal keratitis is notoriously difficult to treat, especially after the hyphae penetrate deeper stromal layers. Depending on the inoculum, the time until treatment, infected individuals will require corneal transplantation (10% of cases). Following transplantation, the rejection or reinfection of transplanted cornea tissue can occur, and in severe cases the only recourse is enucleation of the affected eye {Thomas, 2003 #64}. In milder cases, resolution of infection is accompanied by fibrosis, resulting in visual impairment. In contrast to trauma – induced fungal keratitis, contact lens associated fungal keratitis is likely due to the hyphal stage. Airborne conidia settle in a lens case, germinate and form a biofilm on the lenses and the case. Following contact with the ocular surface, hyphae penetrate into the cornea stroma through minor epithelial abrasions where they establish infection.

**2.1 Anti-Microbial Defenses at the Ocular Surface**

*Figure 1A* illustrates a cross-sectional diagram of the human eye and cornea. The transparent cornea provides most of the refractive index that is essential for the accurate transmission of light through the pupil and to the retina, where photoreceptor cells transmit images to the visual cortex of the brain. Infection or inflammation disrupts the role of the cornea, as inflammation is associated with edema and change in refractive index, resulting in impaired vision. The avascularity of the cornea is also important in maintaining corneal clarity.
The cornea and ocular surface are protected from trauma and infection by physical and molecular defenses. Perhaps the simplest and most effective defense involves eyelid closure and blinking, which protects the cornea from physical trauma, and removes microbes from the ocular surface. Figure 1B is a histological section of the normal human cornea, showing the main layers of cornea, which are the corneal epithelium, stroma, endothelium, in addition to the underlying anterior chamber. The major physical barrier against bacterial and fungal infection is the non-keratinized, stratified corneal epithelium, comprising three layers of epithelial cells with tight junctions that form a physical barrier preventing microbial access to the corneal stroma. Murine studies of fungal keratitis or *Pseudomonas aeruginosa* have shown that an intact corneal epithelium will restrict access of millions of live organisms to the corneal stroma even under stressful conditions such as long term contact lens wear. However, the main site of infection is the corneal stroma, which comprises 90% of the tissue, and is a dense, highly organized matrix, with anti-parallel layers of collagen separated by keratan sulfate proteoglycans that are essential for corneal transparency.

We developed a murine model of trauma induced *Fusarium* and *Aspergillus* keratitis in which conidia are injected directly into the corneal stroma [Leal, 2010 #29;Tarabishy, 2008 #63]. Following germination, hyphae spread through the corneal stroma, and neutrophils infiltrate, causing pronounced corneal opacification. Figure 1D shows an example of this model using RFP expressing *A.fumigatus* conidia and GFP expressing neutrophils. At 48h post-infection, corneal opacity and neutrophil infiltration increase further, while RFP expressing fungi decreases. Neutrophils are detected at higher magnification in the live cornea *(Fig 1D, central panels)*. Subsequent experiments found that systemic neutrophil depletion resulted in uncontrolled fungal growth, thereby implicating neutrophils as having an essential role in fungal killing.

### 3. Proposed sequence of events in fungal keratitis

Studies using infected human corneas and murine models of *Fusarium* and *Aspergillus* keratitis are consistent in implicating Dectin-1, TLR4, and neutrophils, in addition to inflammasomes and IL-1β in the pathogenesis of fungal keratitis. Results from these studies led us to propose the sequence of events illustrated in Figure 2. 1) Trauma to the eye caused by dirt or plant material with adherent conidiophores containing multiple conidia. 2) Once in the corneal stroma, conidia germination results in shedding the hydrophobin layer that coats resting conidia, and exposing cell wall β-glucan on the surface. β-glucan binds to Dectin-1 on resident corneal macrophages. 3) Dectin-1 signals through syk and CARD9 to activate NFκB and transcription of CXC chemokines and IL-1β, which is cleaved by inflammasomes and caspase 1, and activates neighboring cells to produce CXCL chemokines and upregulates ICAM-1 expression on vascular endothelial cells in the peripheral vessels (as the cornea is avascular). 4) Elevated ICAM-1 and CXC chemokines mediate recruitment of neutrophils to the corneal stroma, and 5) Cell surface β-glucan and mannosyl residues on hyphae in the cornea activate Dectin-1 and TLR4 on neutrophils, stimulating production of ROS and fungal killing, although corneal inflammation also results in corneal opacification and loss of vision.