

Introduction

Numerous types of fungi are able to infect the eye, including *Fusarium*, *Aspergillus*, *Curvularia*, and *Candida* (4). The fungus responsible for the recent outbreaks of keratitis (infection of the cornea) due to ReNu MoistureLoc use is thought to be *Fusarium species*. There are multiple species within the genus *Fusarium*, with *Fusarium solani* being known as the most prevalent and dangerous variety. *Fusarium* is commonly found on plants or in the soil in warm, humid regions around the world.

Fusarium is a type of filamentous fungus, which means that it contains hyphae. A hypha is a long, branching, tube-like filament which may compose the majority of tissue in a fungus(1). When hyphae bundle together, a mycelium is formed. The mycelium functions to feed, grow, and eventually produce a reproductive structure (e.g., mushroom). A fungus starts out as a spore, and as it divides, cells making up hyphae are generated and grow outward in a thread-like fashion. Unlike normal cell division, hyphae grow by lengthening the tips on the ends, and when the proper length is reached, a new division known as a septum is formed between the old cell and the new cell. The same method can be used to make branches. Although there are walls dividing the boundaries of the cells, cytoplasm is shared between the cells. The movement of cytoplasm throughout the hyphae is known as cytoplasmic streaming (10). This allows nutrients to be shared along the entire fungus. Hyphae are initially composed of haploid cells, but if the hyphae of one fungus joins the hyphae from another fungus of the same species, they may fuse and form multinucleate cells.

In context of fusarium keratitis, the hyphae from the fungus may protrude through lesions in the cornea to gain access to the inside of the cornea. If it can get past the primary defenses, such as the skin or other mucosal associated lymphatic tissue (SALT and MALT respectively) tissue, then it can cause an infection. However the SALT and MALT tissues are reasonably effective at keeping *Fusarium* out of the body. Lesions in the cornea could be caused by physical damage (e.g., small particles in the air, plant matter) or by improper care for contact lenses. Treatments for fungi are limited because they share very similar DNA, RNA, and protein synthesis pathways to humans (4). Once the fungus gains access inside the cornea, it may grow and infiltrate other areas of the

eye.

Immune Response

The cornea has two main defenses against pathogens: 1) Langerhans cells and 2) immunoglobulins (1). Langerhans cells are mainly found on the outer regions of the cornea. The Langerhans cells are used to phagocytose recognized pathogens, and then present the digested fragments to the stroma of the cornea. They may also recognize antibodies or complement factors which have opsonized an antigen, flagging it for phagocytosis. In the case of fungal infections, complement factors will not work because the cell wall protects the cell-membrane of the fungus (1). After digestion, the expressed epitopes can be recognized by B-cells or T-cells with complementary receptors. This could trigger an immune response which culminates in inflammation of the cornea. Inflammation triggers the recruitment of natural killer cells (NK cells), leukocytes, and other lymphocytes to the site of infection. When the Langerhans cells phagocytose an antigen, they mature into dendritic cells by traveling to nearby lymph nodes. They then migrate to the corneal stroma where they can release cytokines or other inflammatory mediators. Cytokines released include IL-1, IL-6, IL-12, and TNF- α (textbook). IL-1, IL-6, and TNF- α are all synergistic with each other. They cause the release of acute phase proteins from the liver, and activate T-cells, B-cells, or inflammatory cells. IL-12 functions to activate NK cells and also causes the proliferation of T_H1 -cells. This will lead to the eventual increased production of IFN- γ . Taken together, a cell-mediated response seems to be highly favored.

The second defense consists of the immunoglobulins concentrated in the corneal stroma which have been secreted by plasma B-cells. On the surface of the cornea is a layer of mucous glycoprotein in association with glycocalyx, a network of polysaccharides (1). Immunoglobulin A (IgA), a major class of antibodies associated with serum and bodily secretions, is linked with these layers of mucous glycoproteins. IgA is essential as part of the defense against specific fungus antigens. Immunoglobulins not only attach to the surfaces of fungi marking them for extermination, but also neutralize dangerous mycotoxins released by the fungi.

Unique to infections involving *Fusarium* is the lack of most granulocytes, also known as polymorphonuclear leukocytes, during inflammation (1). This class of leukocytes includes the neutrophils, eosinophils, and basophils. These cells function to release enzymes or oxygen radicals to destroy foreign antigen. Basophils and eosinophils secrete Type II cytokines (IL-4 or IL-5 respectively) and tend to stimulate humoral responses within the body (textbook). Thus, it makes sense that there is an absence of humoral-mediated responses in the body. It has been suggested that these leukocytes are kept away from the eye as a defense mechanism to prevent hazardous inflammation which could damage the eye.

The primary method of dealing with fungal infections happens to be cell-mediated. Neutrophils and monocytes are very effective against the hyphae of *Fusarium* (1). These cells migrate to sites of infection, and use reactive oxygen species or enzymes to kill ingested organisms. They are attracted to the sites of infection by the Type I cytokines, IFN- γ and G-CSF, which are secreted by T_H1-cells or NK cells and fibroblasts or monocytes respectively (1). These two cytokines greatly enhance the functioning of the neutrophils and monocytes in the eye. Monocytes can also secrete IL-1 to stimulate T-cell activation and promote B-cell clonal expansion and maturation.

The important area needed for all of these responses can be found within the protective epithelium of the conjunctiva, the membrane covering the sclera and lining the inside of eyelids (12). The conjunctiva is likely to become inflamed during infection. Lymphoid tissue can be found in this area, and houses the colonies of B-cells and T-cells. B-cells here undergo maturation after being exposed to antigen presented by langerhans cells. The B-cells then migrate to nearby lymph nodes to proliferate into plasma cells. The mature cells then return to the conjunctiva or go to the blood marrow to produce antibodies. If in the eye, IgA will be secreted (4). T-cells are also stimulated by the antigen presented by langerhans cells or B-cells (via MHC II). Helper T-cells similarly migrate nearby lymph nodes to mature, and then return to the conjunctiva in order to release cytokines to activate leukocytes or provide co-stimulation for the functioning of other lymphocytes.

Progression of Disease

The majority of fungal keratitis occurs after trauma to the cornea. The early symptoms of fungal keratitis are blurred vision, a red and painful eye that does not improve when contact lenses are removed, increased sensitivity to light, and excessive tearing or discharge (11). Fungal keratitis is recognizable by the presence of a coarse granular infiltration of the corneal epithelium and the anterior stroma. The corneal defect usually becomes apparent within 24 to 36 h after the trauma. There is minimal to absent host cellular infiltration. The absence of inflammatory cells is likely a good prognostic finding, since products of polymorphonuclear leukocytes contribute to the destruction of the cornea. It has been contended that eyelid lesions are common in patients with eye infections.

The eye will appear white with filamentous projections from the cornea after about a week. *Fusarium* Keratitis may completely destroy an eye within a few weeks and malignant glaucoma can ensue. If not treated after 5 or 6 weeks, blindness may result. A systemic infection is unlikely. If the fungus does get past the eye's defense system and into other tissues, it will be exposed to all of the immune defenses of the body. This includes inflammatory macrophages, granulocytes, and humoral immunity. However, this can lead to inflammation of the tissue surrounding the eye. The eye will appear severely red and tearing will ensue to lower the pH of the eye and introduce lysozyme to the fungus.

Therapy

Fungal and human cells have similar mechanisms for DNA, RNA and protein synthesis. This greatly limits the number of potential antifungal drug targets because the vast majority of compounds that inhibit fungi also are toxic to human cells. Therefore, antifungal drugs tend to target the few features of fungal cells that differ from human cells. Most fungal cell membranes contain ergosterol, rather than cholesterol, which is the sterol found in human cell membranes. Amphotericin B directly binds to ergosterol, whereas the 'azoles' (e.g., itraconazole, fluconazole, voriconazole, miconazole,

ketoconazole) and terbinafine target ergosterol synthesis (4). However, the major distinguishing feature of fungal cells is a rigid cell wall containing chitin, mannans and glucans (the target of the echinocandin class of antifungal drugs).

Fluconazole is an oral anti-fungal medication known to be effective in curing numerous types of fungal infections throughout the body. It functions to block the pathway for sterol synthesis in Fungi (3). Clinical trials have shown that it is able to fight fungal eye infections like *Fusarium* keratitis. The active ingredient is 2,4-difluoro- α , α 1-bis(1H-1,2,4-triazol-1-ylmethyl) benzyl alcohol. Fluconazole is sold under the name Diflucan, made by Pfizer. One dose is 100mg, and 30 pills costs \$434.98. Two pills are taken daily for two weeks, or until symptoms cease to exist.

Natamycin is highly effective at fighting fungal Keratitis. Natamycin is a tetraene polyene antibiotic derived from *Streptomyces natalensis*. The mechanism of action appears to be through binding of the molecule to the sterol moiety of the fungal cell membrane. The polyenesterol complex alters the permeability of the membrane to produce depletion of essential cellular constituents. The active ingredient is a stereoisomer of 22-[(3-amino-3, 6-dideoxy-(beta)-D- mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.0^{5,7}] octacos-8,14,16,18,20-pentaene-25-carboxylic acid (3). The manufacturer is Alcon, and sold under the name Natacyn. A 15mL bottle costs \$220. Natacyn is administered is through the use of eyedrops. The user usually takes one eyedrop every 4 to 6 hours, though more serious infections may require a higher dose.

Due to the economic restraints held by some of the sufferers of the *Fusarium* Keratitis infection, our outlook would be that an alternative more affordable means of therapy should also be looked at as a potential cure. We recommend the product *Melaleuca Alternifolia* or more commonly known as tea tree oil. It has already been proven through laboratory research to be an effective substitute from conventional medicine in treating fungal infections of the mouth and foot (8). The active ingredients involved in tea tree oil are Terpinen-4-ol and Cineole (8). It is mainly produced in

Australia and marketed in the United States by the company Desert Essence. They sell their product for the price of \$19.99 for every 2 fluid ounces (7). We propose that the initiative be taken to enhance the product into a form suitable for application to the eye (i.e. eye drops). As of now only a topical solution is available which is both used in the diluted form for gargling in the mouth, and as a direct applicant to the skin. Eye drop usage once every 4-6 hours as has been shown to be effective with Natacyn may be sufficient, but if results did not show then an increase in the frequency of application could be imposed.

Other Possible Causes

The ReNu MoisturLoc product may have been sterile when it left the plant in Greenville. However, the ingredients in MoisturLoc are different from the other formulas made by Bausch. It is important to note that only ReNU MoisturLoc is related to the greater chance of developing the *Fusarium Keratitis* infection. One reason this could be possible is because the MoisturLoc provides an environment where the *Fusarium* can thrive and reproduce in. The MoisturLoc may be a sort of “minimal essential media” for the fungus. It is already known that the *Fusarium* did not come from the factory. However, when the user puts the MoisturLoc in the contact cases, *Fusarium* is able to grow on the lens. The method by which *Fusarium* gets access to the solution is unknown, but since spores can be airborne it is possible for the fungus to randomly land in the solution. In this case, the probability of contaminating the solution depends on the user; if the user keeps the solution near outdoor areas or other places with spores, then the likelihood of contamination increases. After the user puts the infected contacts in their eyes, the fungus on the lens will come into contact with the eye. The fungus is then trapped between the eye and contact lens for the entire day which gives the *Fusarium* enough time to get through the cornea and reproduce in the eye, causing infection.

Another possible reason that the ReNu MoistureLoc may be causing infection is because a chemical in MoistureLoc may be causing the defense system of the eye to malfunction. An ingredient in the solution may be acting as an inhibitor of a defense

mechanism. Normally the eye may come into contact with *Fusarium* on a daily or weekly basis. The lymphoid tissue of the conjunctiva is usually able to fight off the infection under normal conditions. However, when a patient who uses contacts with MoisturLoc comes into contact with the eye, perhaps the conjunctiva lymphoid tissue is no longer able to fight off the disease. This may be because the MoisturLoc is inhibiting cytokines (e.g., IL-1, IL-6, IL-12, or TNF- α) that normally promote defensive cells to attack the fungus. In this way, a *Fusarium* Keratitis infection would proceed easier.

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