



Review

The Role of the Innate and Adaptive Immune Responses in *Acanthamoeba* Keratitis

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Abstract. Infections of the corneal surface are an important cause of blindness. Protozoal, viral, bacterial, and helminthic infections of the cornea account for up to 9 million cases of corneal blindness. Free-living amoebae of the genus *Acanthamoeba* produce a progressive infection of the cornea called *Acanthamoeba* keratitis. Disease is usually transmitted by *Acanthamoeba* trophozoites bound to soft contact lenses. Infection of the cornea is initiated when the parasite binds to the corneal epithelial surface. Recrudescence can occur and suggests that the adaptive immune response is not aroused by corneal *Acanthamoeba* infections. Systemic immunization with *Acanthamoeba* antigens elicits robust Th1 cell-mediated immunity and serum IgG antibody, yet fails to prevent the development of *Acanthamoeba* keratitis. However, immunization via mucosal surfaces induces anti-*Acanthamoeba* IgA antibodies in the tears and provides solid protection against the development of *Acanthamoeba* keratitis. Unlike other immune effector mechanisms that rely on cytolysis, inflammation, release of toxic molecules, or the induction of host cell death, the adaptive immune apparatus prevents *Acanthamoeba* infections of the cornea by simply preventing the attachment of the parasite to the epithelial surface. The beauty of this mechanism lies in its exquisite simplicity and efficacy.

Key words: cornea; *Acanthamoeba* keratitis; corneal blindness.

Background

Although the eye is only a few centimeters in diameter, it is composed of virtually every type of tissue found in the remainder of the body as well as cellular and noncellular elements found nowhere else³⁰. The neurological complexity of the eye is extraordinary. The million ganglion cells of the retina transmit 500 electrical signals per second, which in computer terms is equivalent to 1.5×10^9 bits of information³⁰. However, the remarkable complexity of the retina and its neurological communications with the brain are rendered meaningless if the transparency of the cornea is

compromised by trauma or infection. Indeed, infections of the cornea are a major cause of blindness. The nematode *Onchocerca volvulus* produces ocular onchocerciasis in over 17 million people in Africa, the eastern Mediterranean, and the Americas, and accounts for 270,000 cases of corneal blindness³⁷. Between 6 and 9 million individuals are blind from trachoma, a chlamydial infection of the cornea³⁷. Each year in the United States there are 20,000 new cases of ocular herpes simplex virus (HSV) infections²³. Interestingly, the blindness caused by each of these infectious diseases of the cornea is immune-mediated^{33, 37, 44}.

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Acanthamoeba spp. are free-living amoebae that are found in virtually every conceivable habitat, ranging from hot tubs to Antarctic soil^{5, 40}. *Acanthamoeba* spp. have been isolated from fresh water reservoirs, salt water, swimming pools, hot tubs, ventilation ducts, soil, bottled water, and even eyewash stations³. Although *Acanthamoeba* spp. exist as free-living amoebae subsisting on bacteria, they can infect the eye, brain and skin. In the eye, *Acanthamoeba* spp. produce a progressive, ulcerating, blinding infection of the cornea called *Acanthamoeba* keratitis. At least eight species of *Acanthamoeba* have been implicated in corneal infections: *A. castellanii*, *A. culbertsoni*, *A. polyphaga*, *A. hatchetti*, *A. rhyodes*, *A. lugdunensis*, *A. quina*, and *A. griffini*⁴¹. Environmental exposure to *Acanthamoebae* spp. is commonplace, as 50–100% of the normal population possesses serum antibodies to *Acanthamoeba* antigens^{1, 6, 8}. The leading risk factor for *Acanthamoeba* keratitis is contact lens wear, which is practiced by over 25 million individuals in the United States alone. Although >80% of the cases of *Acanthamoeba* keratitis occur in contact lens wearers³², less than one in 10,000 contact lens wearers will develop corneal *Acanthamoeba* infections⁴¹. The low incidence of *Acanthamoeba* keratitis is remarkable considering the ubiquity of the organism and the large population of contact lens wearers who are at risk for developing corneal infections. This may be due to a highly effective ocular immune apparatus. Although the eye is recognized for its immune-privileged nature, both innate and adaptive immune effector elements can and do function at the ocular surface and within the interior of the eye. In fact, a poorly regulated immune response to pathogens contributes to the pathogenesis of several major corneal infectious diseases.

The Role of the Innate Immune Apparatus in *Acanthamoeba* Keratitis

The immune system is classified into two components: the innate or natural immune response and the adaptive or acquired immune response. Innate immunity rapidly mobilizes antigen-nonspecific immune elements, which include macrophages, granulocytes, natural killer cells, ($\gamma\delta$ T cells, and humoral factors including the complement system, as well as a variety of anti-bacterial peptides, such as interleukin 12 (IL-12), IL-18, and tumor necrosis factor α (TNF- α). Components of the innate immune system are best recognized for their participation in acute inflammatory responses and their capacity to phagocytize bacterial pathogens.

However, it is becoming increasingly clear that the innate immune response exerts enormous influence in the subsequent generation of antigen-specific, T cell-dependent immune responses^{11, 12}.

Disarming the innate immune apparatus can affect the course of protozoal infections. In experimental toxoplasmosis, depletion of neutrophils by *in vivo* treatment with anti-neutrophil antiserum results in an 80% reduction in the production of interferon γ , an 80% reduction in the number of CD4⁺ and CD8⁺ T cells, a significant increase in the severity of toxoplasma lesions in multiple organisms, and increased burdens of tachyzoites in the brain, lung, liver and spleen⁴.

The innate immune system appears to play an important role in *Acanthamoeba* keratitis. Histological evaluation of *Acanthamoeba* keratitis lesions in both humans and experimental animals has revealed large numbers of neutrophils^{16, 17, 19, 26}. *In vitro* studies have shown that both human and rat neutrophils can kill *Acanthamoeba* trophozoites^{13, 43}. *In vivo*, neutrophils influence the course of *Acanthamoeba* keratitis. Inhibition of neutrophil migration into corneas infected with *A. castellanii* results in profound exacerbation of *Acanthamoeba* keratitis in Chinese hamsters¹⁷. *Acanthamoeba* keratitis resolves spontaneously within approximately 3–4 weeks in normal Chinese hamsters. Resolution of *Acanthamoeba* keratitis coincides with the cornea's production of macrophage inflammatory protein 2 (MIP-2), a powerful chemotactic factor for neutrophils¹⁷. Subconjunctival injection of anti-MIP-2 antibody inhibits neutrophil migration into corneas infected with *A. castellanii*. Moreover, anti-MIP-2 antibody-treated Chinese hamsters develop corneal lesions sooner than untreated animals and do not resolve their ocular infections¹⁷. By contrast, intracorneal injection of MIP-2 protein prior to corneal infection with *Acanthamoeba* trophozoites induces neutrophil infiltration and results in a remarkable amelioration of *Acanthamoeba* keratitis and an early resolution in the corneal lesions.

Macrophages represent another arm of the innate immune apparatus. The importance of activated macrophages in controlling intracellular pathogens is well known. Like neutrophils, activated macrophages are also capable of killing *Acanthamoeba* trophozoites *in vitro*^{13, 24, 42}. Macrophages also limit the pathogenic cascade of *Acanthamoeba* keratitis. Depletion of conjunctival macrophages with the macrophagicidal drug dichloromethylene diphosphonate (clodronate) exacerbates the severity, chronicity, and incidence of *Acanthamoeba* keratitis in Chinese hamsters⁴⁹. Although there is a paucity of macrophages in corneal biopsy

specimens and corneal buttons from *Acanthamoeba* keratitis patients who receive penetrating keratoplasty, such histopathologic specimens are typically collected only after the active infection has been brought under control with antimicrobial drugs^{18, 26}.

Only a handful of the humoral factors that comprise the innate immune system have been examined for their effect on *Acanthamoeba* trophozoites. Early studies by FERRANTE and ROWAN-KELLY¹⁴ showed that *Acanthamoeba* trophozoites activate the complement cascade via the alternative pathway and succumb to complement-mediated lysis, even in the absence of complement-fixing antibodies. By contrast, other studies have reported that pathogenic strains of *Acanthamoeba* are resistant to complement-mediated lysis by virtue of their expression of complement regulatory proteins on the trophozoite cell membrane⁴⁶. The contribution of complement in controlling corneal infections with *Acanthamoebae spp.* is nebulous. Complement is present in the cornea and the tears that bathe the corneal surface^{7, 31}, yet systemic immunization and the generation of complement-fixing anti-*Acanthamoeba* antibodies does not affect the incidence, severity, or resolution of *Acanthamoeba* keratitis in animal models^{2, 35, 48}. The effect of other humoral components of the innate immune system in controlling *Acanthamoeba* keratitis is unknown. Neither TNF- α , IL-1 α , nor IL-1 β has an effect on the viability of *Acanthamoeba* trophozoites *in vitro*²⁴. The weight of evidence to date suggests that none of the humoral factors of the innate immune system appreciably affects the course of *Acanthamoeba* keratitis.

The Role of the Adaptive Immune Apparatus *Acanthamoeba* Keratitis

A large body of data indicates that *Acanthamoeba* antigens can stimulate the adaptive immune system³⁴. Environmental exposure to *Acanthamoeba* antigens is commonplace, as 50–100% of the normal adult population possesses anti-*Acanthamoeba* antibodies^{1, 6, 8} and 50% of the normal asymptomatic individuals demonstrate T cell proliferative responses to *Acanthamoeba* antigens⁴⁵. The anti-*Acanthamoeba* immune responses in the aforementioned studies were presumably the result of environmental exposure to *Acanthamoeba* antigens, as none of the individuals had a history of *Acanthamoeba* keratitis. This is a reasonable conclusion considering the ubiquitous distribution of *Acanthamoebae spp.* and the observation that *Acanthamoeba* trophozoites have been isolated from nasopharyngeal

washes and even the contact lens cases of asymptomatic individuals^{9, 38, 39}. The high incidence of anti-*Acanthamoeba* antibodies in the general population suggests that environmental exposure to *Acanthamoeba* antigens leads to “herd immunity” and that those individuals who contract *Acanthamoeba* keratitis represent a unique, infinitesimally small population that has an “immunologic blindspot” for *Acanthamoeba* antigens. A recent serological survey indicates that not only do *Acanthamoeba* patients possess anti-*Acanthamoeba* antibodies in their serum, but their overall antibody titers are significantly higher than the normal asymptomatic population¹. These findings beg two obvious questions. First, do corneal infections with *Acanthamoeba* trophozoites arouse the adaptive immune system? Second, does the adaptive immune response influence the establishment and pathological sequelae of *Acanthamoeba* keratitis? These questions can be best answered in prospective studies using appropriate animal models. With this in mind, we examined both T cell and B cell responses to ocular infections with *A. castellanii*. In both the pig and Chinese hamster models of *Acanthamoeba* keratitis, corneal infections fail to provoke either delayed type hypersensitivity (DTH) or IgG antibody responses^{2, 35, 48}. The failure to induce either DTH or IgG antibody responses suggests that ocular infection promotes the development of anergy or tolerance. However, animals experiencing *Acanthamoeba* keratitis are not tolerized, as subsequent intramuscular immunization with *Acanthamoeba* antigens elicits robust DTH and IgG antibody responses⁴⁸. The inability of corneal infections to activate the adaptive immune response is consistent with the immune-privileged nature of the eye.

The immune privilege of the cornea is due in part to the remarkable absence of resident antigen-presenting cells in the central portions of the corneal epithelium. However, peripheral antigen-presenting cells, namely Langerhans cells (LC), can be induced to migrate into the central cornea. The presence of LC in the cornea prior to infection with *Acanthamoeba* trophozoites robs the cornea of its immune privilege and promotes the development of DTH and IgG responses⁴⁸.

Although corneal infection with *Acanthamoeba* trophozoites does not normally activate the adaptive immune response, the question remains as to whether or not adaptive immunity can either prevent or mitigate *Acanthamoeba* keratitis. Histopathological evaluation of *Acanthamoeba* keratitis biopsies from patients and experimental animals reveals the presence of an inflammatory infiltrate that is comprised of macrophages and neutrophils, but a conspicuous absence of lympho-

cytes^{19, 26}. Induction of T cell-dependent immunity by systemic immunization with *Acanthamoeba* antigens induces DTH and IgG responses, but fails to protect either pigs or Chinese hamsters against corneal infection with *A. castellanii*^{2, 35}. Thus, classical Th1 immune mechanisms fail to influence the establishment and progression of *Acanthamoeba* keratitis.

Mucosal Immunity and Resistance to *Acanthamoeba* Keratitis

The pathogenesis of *Acanthamoeba* keratitis is a multifaceted cascade that begins with the adhesion of the trophozoite to the corneal epithelium. The ability of the parasite to bind to the corneal epithelium is believed to be a prerequisite for the establishment of *Acanthamoeba* keratitis. For example, there is considerable variability in the capacity of *Acanthamoeba* trophozoites to bind to the corneal epithelium of various mammalian species. *Acanthamoeba* trophozoites bind poorly to corneal buttons from the rat, cotton rat, horse, cow, dog, rabbit, mouse, and guinea pig³⁶. Attempts to produce *Acanthamoeba* keratitis through the application of parasite-laden contact lenses in rats, cotton rats, mice, and rabbits have been unsuccessful³⁶. By contrast, *Acanthamoeba* trophozoites bind extensively to corneal buttons from humans, Chinese hamsters, and pigs. Further studies have demonstrated that application of parasite-laden contact lenses to the eyes of either Chinese hamsters or pigs induces a form of *Acanthamoeba* keratitis that resembles the human counterpart, both histopathologically and clinically⁴⁷. Thus, binding to the corneal epithelium is critical for the establishment of *Acanthamoeba* infections of the cornea and for the development of *Acanthamoeba* keratitis.

The ocular mucosal epithelium, including the cornea, is regularly exposed to a wide range of environmental agents, including pathogens. The corneal surface is continuously bathed in tears, which contain a variety of humoral factors that protect it from pathogenic insults. IgA antibodies are one of the most abundant antimicrobial components of tears. The production and accumulation of IgA antibodies in the tears can be elicited by immunization via mucosal surfaces, such as the gastrointestinal tract. The observation that immunization through one mucosal surface leads to the appearance of secretory IgA antibodies in multiple mucosal secretions is the basis for the concept of a common mucosal immune system. If trophozoite binding to the corneal epithelium is essential for the establishment of ocular *Acanthamoeba* infections, then mucosal im-

munization should lead to the appearance of anti-*Acanthamoeba* antibodies in the tears and resistance to *Acanthamoeba* keratitis. This hypothesis was confirmed in both the pig and Chinese hamster models of *Acanthamoeba* keratitis^{2, 35}. Oral immunization with *Acanthamoeba* antigens conjugated with the mucosal adjuvant, neutralized cholera toxin, produces solid protection against corneal infection with *A. castellanii*. The mucosally induced immunity correlates with the appearance of anti-*Acanthamoeba* antibodies in the tears of mucosal immunized animals²⁰. Moreover, mucosal IgA antibodies inhibit the binding of trophozoites to the corneal epithelium, but do not affect parasite viability. A similar level of protection against *Acanthamoeba* keratitis can be produced by passive transfer of a monoclonal anti-*Acanthamoeba* IgA antibody²². Animals receiving monoclonal anti-*Acanthamoeba* IgA antibody are protected against corneal infection, while animals treated with an irrelevant IgA antibody are just as susceptible as control animals. Mucosal immunity is effective only in preventing the establishment of corneal infection and is incapable of influencing the disease course once the parasites have entered the cornea²¹. Mucosal immunization must be initiated before the parasite-laden contact lenses are applied to the corneal surface; delaying mucosal immunization until after corneal infection has been established fails to mitigate keratitis²¹. Based on these animal studies, we considered the hypothesis that the extraordinarily low incidence of *Acanthamoeba* keratitis – 1.65 to 2.01 cases per million contact lens wearers⁴¹ – might be due to the protective effects of mucosal immunity that is naturally acquired through environmental exposure to ubiquitous *Acanthamoeba* spp. This hypothesis would also predict that, in general, those individuals who contract *Acanthamoeba* keratitis possess feeble or nonexistent mucosal immunity to *Acanthamoeba* antigens. A recent serological study supports this hypothesis and shows that *Acanthamoeba* keratitis patients display significantly lower anti-*Acanthamoeba* IgA antibody levels in their tears compared with asymptomatic, normal individuals¹. *Acanthamoeba* keratitis patients do not appear to suffer from any remarkable immune deficiency, other than possessing significantly lower levels of anti-*Acanthamoeba* IgA antibodies in their tears. Interestingly, the same patients have significantly higher titers of serum IgG antibodies specific for *Acanthamoeba* antigens. Thus, the *Acanthamoeba* keratitis patients' immunological "blind spot" for *Acanthamoeba* antigens may be restricted to the IgA antibody response. The importance of IgA antibodies in preventing the establishment of *Acanthamoeba* keratitis should come as no surprise.

IgA antibody is the most abundant immunoglobulin synthesized in the body and is produced in quantities greater than all of the other immunoglobulins combined²⁷.

The Immunology of the *Acanthamoeba* Cyst

Acanthamoeba is one of the few extracellular protozoal parasites that encysts in human tissue. The *Acanthamoeba* cyst is an extraordinary structure whose major constituent is cellulose and which can remain viable for up to 24 years^{15, 28}. One of the problematic features of *Acanthamoeba* keratitis is the encystment of trophozoites within the cornea and the persistence of cysts after clinical disease has been brought under control. Cysts can evade immune elimination and persist in the corneal stroma for months and are believed to be a source of recrudescence in *Acanthamoeba* keratitis patients^{10, 18, 20, 25}. The failure of the immune system to eliminate cysts within the corneal stroma could be due to either weak immunogenicity of the cysts or to the immune privilege of the cornea. However, animal studies have revealed that *Acanthamoeba* cysts are immunogenic when introduced into extraocular sites and will elicit DTH and IgG antibody responses (McCLELLAN et al., submitted for publication). Moreover, cyst antigens elicit inflammatory responses in the form of DTH lesions when injected into mice that have been immunized with *Acanthamoeba* trophozoites. Thus, *Acanthamoeba* cysts are both immunogenic and antigenic. The failure of cysts to elicit inflammatory responses and undergo immune elimination in the stroma following ocular infections may be the combined effect of an insufficient quantity of cyst antigen and the immune privilege of the cornea itself.

The presence of *Acanthamoeba* cysts in ocular tissues is a clinical dilemma for the ophthalmologist. Cysts can remain viable within ocular tissues for months and produce recrudescence, especially in patients whose vision is restored with a corneal transplant. Topical corticosteroids are the most widely used immunosuppressive drugs that are routinely used to prevent the immune rejection of corneal transplants. However, the use of corticosteroids might unwittingly activate dormant *Acanthamoeba* cysts that are known to persist in the corneal stroma. In addition to inhibiting the innate immune apparatus, corticosteroids have a profound effect on the behavior of *Acanthamoeba* cysts. *In vitro* treatment with dexamethasone induces a 4 to 6 fold increase in the rate of excystment and a greater than two fold increase in the proliferation of

trophozoites²⁹. In addition to stimulating excystment and proliferation, corticosteroids also stimulate trophozoites to produce more extensive cytolysis of corneal cells *in vitro*²⁹. Moreover, *in vivo* treatment with dexamethasone dramatically exacerbates the severity and persistence of *Acanthamoeba* keratitis in Chinese hamsters²⁹. Thus, the use of corticosteroids in the management of *Acanthamoeba* keratitis is a “double-edged sword”. On the one hand, corticosteroids are useful in alleviating the pain and inflammation that accompany *Acanthamoeba* keratitis. On the other hand, corticosteroids promote recrudescence and exacerbate infections by inducing excystment of dormant cysts and stimulating proliferation and activation of the emerging trophozoites. These effects are compounded by the fact that corticosteroids paralyze the innate immune apparatus that helps to restrict the progression of *Acanthamoeba* keratitis. This is consistent with previous findings indicating that depletion of either conjunctival macrophages or neutrophils exacerbates *Acanthamoeba* keratitis^{17, 49}.

Summary and Conclusions

Acanthamoebae spp. are remarkable protozoal organisms. They normally exist as free-living amoebae subsisting on soil bacteria, but on occasion they behave as opportunistic parasites and produce blinding infection and lethal meningoencephalitis. *Acanthamoeba* is the only major human extracellular protozoal parasite that encysts in human tissue and it is the tissue-bound cyst that presents the ophthalmologist with a therapeutic dilemma. Topically applied corticosteroids are highly effective in alleviating pain and dampening inflammation, yet they promote recrudescence by: a) inducing the excystment of dormant cysts, b) enhancing the cytopathogenicity of trophozoites, and c) paralyzing the innate immune apparatus.

The role of the adaptive immune response in protozoal infections is well recognized. Th1 immune mechanisms are crucial for resistance to many protozoal parasites such as *Plasmodium*, *Toxoplasma*, *Cryptosporidium*, and *Trypanosoma*. However, induction of Th1 immune responses to *Acanthamoeba* antigens has no demonstrable effect in either mitigating or preventing *Acanthamoeba* keratitis. Likewise, anti-*Acanthamoeba* IgG antibody does not appear to have any effect in this disease. The common mucosal immune system, however, has a profound effect in preventing *Acanthamoeba* keratitis. It does so by simply preventing the infectious trophozoites from binding to the corneal epi-

thelium. Thus, adhesion of the parasite to the corneal surface never occurs, and the shear forces of the blinking reflex along with the cleansing effects of the tears result in the expulsion of trophozoites from the corneal surface. Unlike other immune effector mechanisms that rely on cytolysis, inflammation, release of toxic molecules, or the induction of host cell death, the adaptive immune apparatus prevents *Acanthamoeba* infections of the cornea by the most innocuous of all means: by simply preventing the attachment of the parasite to the epithelial surface. The beauty of this mechanism lies in its exquisite simplicity and efficacy.

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