The Role of Contact Lenses, Trauma, and Langerhans Cells in a Chinese Hamster Model of Acanthamoeba Keratitis

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Purpose. To determine the role of contact lenses, corneal trauma, and Langerhans cells in the development of keratitis caused by Acanthamoeba organisms in Chinese hamsters.

Methods. Various methods were used to induce corneal infections in Chinese hamsters, including application of parasite-laden contact lenses. The role of corneal epithelial defects in promoting parasite binding was examined in vitro in a microscopic binding assay. The role of corneal abrasion in the development of Acanthamoeba keratitis was also examined in Chinese hamsters exposed to parasite-laden contact lenses. Other experiments evaluated the effect of infiltrating Langerhans cells on the incidence and severity of Acanthamoeba keratitis.

Results. Corneal epithelial defects promoted extensive parasite binding to abraded corneas compared to intact, nonabraded counterparts. Corneal abrasion was absolutely necessary for the induction of Acanthamoeba keratitis in hamsters infected with contaminated contact lenses. Infection was never detected unless the corneas were abraded before exposure to parasite-laden contact lenses. The presence of Langerhans cells in corneas prevented the development of Acanthamoeba keratitis.

Conclusions. The highest incidence of Acanthamoeba keratitis occurs in corneas expressing epithelial defects and exposed to parasite-laden contact lenses. The presence of Langerhans cells in corneas exposed to parasite-laden contact lenses prevents the development of Acanthamoeba keratitis. Invest Ophthalmol Vis Sci 1993;34:1937–1944.

Acanthamoeba keratitis is a serious sight-threatening disease caused by a ubiquitous, pathogenic, free-living amoeba. Although Acanthamoeba spp. are is widely distributed in the environment and can be isolated from swimming pools, soil, dust, reservoirs, under ice and from the nasopharyngeal mucosa from healthy humans,1 corneal infections are very rare and mainly restricted to people who wear contact lenses.2 It is generally accepted that the predominant risk factor for Acanthamoeba keratitis is contact lens wear.3 Considering the fact that more than 24,000,000 persons in the United States wear contact lenses and the ubiquitous distribution of the Acanthamoeba, one would expect a high incidence of Acanthamoeba keratitis. However, the occurrence of the disease is very low. Therefore, it seems almost certain that other factors exist in the development of corneal infection. Such additional risk factors could be identified and examined in an appropriate animal model. Moreover, an animal model...
would also make it possible to explore the pathophysiology, cell biology, immunology, genetics, and therapy of this disease.

An animal model must conform to several basic criteria to be a useful tool in long-term studies. The most important one is that the model conform to Koch's postulates. The disease should be produced by live, infectious parasites, and viable parasites must be isolated from diseased tissue and grown in culture.

We have been unable to establish corneal infections in immunosuppressed mice and two species of immunocompetent rats. It was therefore concluded that some mammalian species were more susceptible to infection than others. Accordingly, we evaluated the parasite's ability to bind and penetrate full-thickness corneal buttons in vitro. The results indicated that of the eleven vertebrate species tested, only the corneas from human, pig, and the Chinese hamster were susceptible to in vitro infection with *Acanthamoeba castellanii*.4

As predicted from the in vitro studies, pigs were found to be susceptible to in vivo corneal infection and could serve as a valuable model for investigating the pathogenesis of *Acanthamoeba* keratitis.5 Based on in vitro studies,4 we predicted that the Chinese hamster would also be susceptible to in vivo infection.

The present study shows that *Acanthamoeba keratitis* develops in Chinese hamsters exposed to contaminated contact lenses and that this host can be used as a promising animal model of *Acanthamoeba* keratitis. Using this model, the role of contact lenses and trauma in promoting the infection was studied.

The involvement and the role of Langerhans cells in the pathogenesis of *Acanthamoeba* keratitis was studied for two reasons. First, Langerhans cells may contribute to corneal inflammation as has been suggested in herpes simplex stromal keratitis.6,7 Second, Langerhans cells are highly efficient antigen-presenting cells and might accelerate the development of protective immunity and thereby mitigate corneal disease. These two propositions were explored in the present study.

**MATERIALS AND METHODS**

**Animals**

Chinese hamsters were purchased from Cytogen Research and Development, Inc. (West Roxbury, MA). Animals were used when 4–6 wk old and all corneas were examined before initiating the experiments to exclude animals with preexisting lesions or anomalies. All procedures were performed on the right eye. The left eyes were not manipulated. All animals were handled in accordance with the ARVO Resolution on the Use of Animals in Research and adhered to the tenets of the Declaration of Helsinki.

**Parasite Cultivation**

*Acanthamoeba castellanii*, originally isolated from a diseased human cornea, was obtained from American Type Culture Collection (ATCC #30868; Rockville, MD) and grown axenically in PYG (peptone-yeast-glucose) medium as described elsewhere.8

**Modes of Corneal Exposure to Parasites**

Several methods were used in an attempt to establish corneal infections with *A. castellanii*. These included: (A) intrastromal injection; (B) subconjunctival injection; (C) irrigation of abraded cornea with parasite-rich inocula; (D) deposition of parasite suspensions into the conjunctival cul-de-sac after tarsorrhaphy; and (E) application of parasite-laden “contact lens” onto intact or abraded corneal surfaces. Intrastromal injections were performed by injecting approximately 1.0 μl of a suspension of 4 × 10⁶ parasites/ml using a very fine 5 μl glass micropipette. Injections were aided by an automatic dispenser (Hamilton Co., Inc., Whittier, CA) while viewing the cornea under an operating microscope as described elsewhere.9,10 Subconjunctival injections were performed using 30-gauge needles mounted on tuberculin syringes. Parasites were deposited into the conjunctival cul-de-sac also with a 1 ml tuberculin syringe and 30-gauge needle without perforating the conjunctiva. In each case, a PYG suspension (3 × 10⁶ parasites/ml) was used. Parasite-laden contact lenses were prepared as described later.

**Contact Lens Preparation**

Contact lenses were prepared from Spectapor dialysis membrane tubing (Spectrum Medical Industries, Los Angeles, CA) using a 3 mm trephine. Sterile dialysis tubing lenses (DTL) were incubated at 35°C for 24 hr with *A. castellanii* in 200 μl PYG medium at a concentration of 3 × 10⁶ organisms/ml (50% trophozoites, 50% cysts) in sterile 96-well microtiter plates (Costar, Corp., Cambridge, MA). Attachment of parasites to the DTL was verified microscopically in a “hanging drop” technique.4

**In Vivo Corneal Infection**

Chinese hamsters were anesthetized with sodium pentobarbital (80 mg/kg; Abbott Laboratories, North Chicago, IL) injected intraperitoneally. Topical anesthesia consisted of proparacaine (Alcon Laboratories, Inc., Ft. Worth, TX). Twenty-five percent of the corneal surface was gently abraded under an operating microscope with a sterile cotton applicator before application of the parasite-laden DTL. After application of the DTL, the eyelids were closed by tarsorrhaphy with 10-0 Mersilene® sutures (Ethicon, Inc., Somerville, NJ). Control animals were treated the same way but without abrasion of the cornea. An additional
Hamster Model of Keratitis Caused by *Acanthamoeba* Organisms

The group was exposed to sterile DTL to examine possible bacterial infections or nonspecific corneal damage induced by the DTL. No topical or systemic antibiotics were used.

**Clinical Observations**

Sutures were removed 7 days after exposure to parasites and the DTL were removed. The experimental and the contralateral eyes were observed under a dissecting microscope. Animals were observed 3–4 times each week throughout the course of the study, and the eyes were scored according to the criteria given in Table 1.

**Culture of Parasites**

*A. castellanii* organisms were isolated from the removed DTL by cultivating on lawns of *Escherichia coli* (ATCC #25922) cultured on non-nutrient agar. *E. coli* cultures were observed for the development of characteristic “amoeba trails.” Corneas demonstrating clinical signs of infection were examined for the presence of *A. castellanii* organisms and bacteria. *A. castellanii* organisms were isolated by culturing corneal specimens on lawns of *E. coli* as described earlier. Bacteria were isolated by culturing corneal specimens on BBL Brucella agar (Becton Dickinson Microbiological Systems, Cockeysville, MD). Bacterial identification was made using bacterial identification test panels (Biolog Micro Station System, Hayward, CA). At different time points of infection, animals were killed and their corneas were cut into two equal pieces. One half of each cornea was homogenized in saline using a glass tissue grinder and the homogenate was layered onto a lawn of *E. coli* and cultured for *A. castellanii*. The other half of the cornea was processed for histology.

**Histology**

Corneal specimens were fixed in Carson’s formalin, progressively dehydrated to 95% ethanol and embedded in glycol methacrylate (Sorvall, Wilmington, DE). Three μm sections were cut and stained with hematoxylin and eosin.

**Induction of Langerhans Cell Migration into the Central Cornea**

Latex beads were deposited into shallow corneal epithelial incisions as a simple and reproducible method for inducing the migration of Langerhans cells into the center of the corneal epithelium. Briefly, animals were anesthetized with Metofane (Pitman-Moore Inc., Mundelein, IL), and sterile 1.0 μM polystyrene latex beads suspended in phosphate-buffered saline (pH = 7.2) were deposited into shallow incisions in the center of the corneal epithelium 10 days before infection. Animals were infected with DTL after abrasion of the cornea, as described earlier. For comparison, animals without latex bead treatment were similarly infected.

Langerhans cell migration was also induced by the intracorneal injection of interleukin 1 (IL-1), as described in detail previously. Approximately 1 μl (7–12 U) of recombinant mouse IL-1 (Genzyme, Cambridge, MA) was injected through a very fine glass micropipette of approximately 5 μl. Injections were performed with the aid of an automatic dispenser while viewing the cornea under an operating microscope. Animals were infected either 2 or 7 days after the injection of IL-1 as described before. Control animals were injected with phosphate-buffered saline.

**Adenosine Diphosphatase Staining**

Langerhans cells were characterized by adenosine diphosphatase (ADPase) staining. ADPase staining is a convenient and sensitive method for demonstrating the presence of Langerhans cells in skin and cornea. To count and verify migration of Langerhans cells into the central cornea, Chinese hamsters were sacrificed ten days after latex bead treatment, and 2 or 7 days after IL-1 treatment. Experimental and control eyes were enucleated and corneal epithelial sheets were prepared as previously described and stained for ADPase activity. Flat whole epithelial sheets were prepared for histology.

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**TABLE 1. Clinical Scoring Criteria for *Acanthamoeba keratitis* in Chinese Hamster**

<table>
<thead>
<tr>
<th>Epithelial Defects</th>
<th>Stromal Edema</th>
<th>Vascularity</th>
<th>Stromal Opacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 = no defects</td>
<td>0 = no edema</td>
<td>0 = no vessels</td>
<td>0 = stroma clear</td>
</tr>
<tr>
<td>1 = ulceration, defect, &lt; 25% of surface area</td>
<td>1 = edema &lt; 25%</td>
<td>1 = vessels on &lt; 25% of circumference of cornea</td>
<td>1 = opacity &lt; 25% of cornea area, pupil easy visible</td>
</tr>
<tr>
<td>2 = defect 25 &lt; 50% of surface area</td>
<td>2 = edema 25 &lt; 50%</td>
<td>2 = vessels 25 &lt; 50%</td>
<td>2 = opacity 25 &lt; 50%, pupil hardly visible</td>
</tr>
<tr>
<td>3 = defect 50 &lt; 75% of surface area</td>
<td>3 = edema 50 &lt; 75%</td>
<td>3 = vessels 50 &lt; 75%</td>
<td>3 = opacity 50 &lt; 75%, pupil not visible</td>
</tr>
<tr>
<td>4 = defect &gt; 75% of surface area</td>
<td>4 = edema &gt; 75%</td>
<td>4 = vessels &gt; 75%</td>
<td>4 = opacity &gt; 75%, pupil and iris not visible</td>
</tr>
</tbody>
</table>
mounted on glass slides with Gel Mount (Biomedica Corp., Foster City, CA), and ADPase-positive cells were counted in the limbus and in the central cornea by counting the whole field using 400 × magnification under a normal light microscope at six randomly chosen fields for each cornea. The mean value was recorded as number of Langerhans cells/mm².

**Attachment of Acanthamoeba castellanii to Chinese Hamster Corneas In Vitro**

Animals were killed 10 days after latex bead treatment of the cornea or immediately after abrasion of the corneal epithelium and both eyes were enucleated. The left, untreated eyes were used as controls. Eyes were sterilized in an iodine solution for 45 seconds and rinsed thoroughly with saline. The eyes were placed into tapered tubes 12 mm in length made from the upper part of 200 μl pipette-tips. Using this method, the eyes became stuck and a “well” with the corneal surface of the eye as the bottom was created. A 96-well microtiter plate was used as a holder for the separate “wells.” Parasites were labeled by a modified technique of Weisman and Korn.14 Fluorescent latex bead-labeled Acanthamoeba (1 × 10⁶ parasites/ml), were added to each well. After incubating at 35°C for 24 hr, the medium was removed, and each well rinsed twice with fresh PYG medium. Eyes were gently removed from the tube and placed into deep chamber slides with the epithelial side up. The corneas were then covered with a coverslip so that the center of the cornea was flattened and the number of Acanthamoeba attached to the cornea counted under a microscope (200 × magnification). Using an ocular grid, four randomly chosen fields were counted for each corneal specimen and the results were expressed as the number of parasites/mm².

**Statistical Analysis**

An unpaired, one-tailed Student’s t test and the Wilcoxon log rank test were used for statistical analysis. A probability value < 0.05 was considered statistically significant.

**RESULTS**

**Chinese Hamster Model**

As predicted by previous in vitro studies,⁴ Chinese hamsters were susceptible to in vivo corneal infection with A. castellanii. Of the various techniques for exposing corneas to viable parasites, exposure via parasite-laden contact lenses combined with abrasion of the corneal epithelium was the only method that consistently produced corneal infection in a large number of the experimental hosts. We were not able to induce keratitis by intrastromal injections, subconjunctival injections, irrigation of abraded corneas with parasite-rich inocula, or by deposition of parasites into the conjunctival cul-de-sac (Table 2).

Using parasite-laden DTL, the specific role of corneal trauma was examined. Eyes from 18 Chinese hamsters were abraded with cotton swabs and then infected with parasite-laden DTL. For comparison, 12 additional animals were also infected with parasite-laden DTL, but without abrasion of the cornea. At the removal of the contact lens on day 7 after infection, the majority of animals (66%) with abraded corneas developed keratitis as described later. By contrast, no sign of keratitis developed in any of the animals with nonabraded corneas. The highest incidence of infection occurred when the parasite-laden DTL remained in the eye for at least 7 days (Table 2).

Acute signs of infection were visible 7 days after exposure to parasite-laden DTL. Eyelids were inflamed and swollen, and the conjunctivae were in-

**TABLE 2. Modes of Corneal Exposure to Acanthamoeba**

<table>
<thead>
<tr>
<th>Exposure to Parasites*</th>
<th>Mean Clinical Score†</th>
<th>Histopathology</th>
<th>Isolation of Acanthamoeba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrastromal injection</td>
<td>No signs (8)</td>
<td>No inflammation</td>
<td>Negative</td>
</tr>
<tr>
<td>Subconjunctival injection</td>
<td>No signs (8)</td>
<td>No inflammation</td>
<td>Negative</td>
</tr>
<tr>
<td>Deposition in cul-de-sac</td>
<td>No signs (8)</td>
<td>No inflammation</td>
<td>Negative</td>
</tr>
<tr>
<td>Inoculation after abrasion of the cornea</td>
<td>No signs (8)</td>
<td>No inflammation</td>
<td>Negative</td>
</tr>
<tr>
<td>Contact lens (sterile)</td>
<td>No signs (12)</td>
<td>No inflammation</td>
<td>Negative</td>
</tr>
<tr>
<td>Contact lens (no abrasion)</td>
<td>No signs (12)</td>
<td>No inflammation</td>
<td>Negative</td>
</tr>
<tr>
<td>Contact lens (with abrasion)</td>
<td>3 days exposure</td>
<td>4+ (1/8)</td>
<td>PMN infiltrate</td>
</tr>
<tr>
<td></td>
<td>5 days exposure</td>
<td>4+ (4/9)</td>
<td>PMN infiltrate</td>
</tr>
<tr>
<td></td>
<td>7 days exposure</td>
<td>4+ (12/18)</td>
<td>PMN infiltrate</td>
</tr>
<tr>
<td></td>
<td>10 days exposure</td>
<td>4+ (6/9)</td>
<td>PMN infiltrate</td>
</tr>
</tbody>
</table>

* Controls for intrastromal injection, subconjunctival injection, deposition into cul-de-sac, and inoculation onto abraded cornea consisted of hamsters exposed to parasite-free PYG medium in the same manner as the respective experimental group (e.g., intrastromal injection of sterile PYG medium). Controls for experiments involving contact lenses consisted of sterile contact lenses incubated in parasite-free PYG medium. Controls for experiments testing the effect of abrasion consisted of hamsters exposed to parasite-laden contact lenses placed onto intact corneas that were not abraded with cotton-tipped swabs.

† Clinical scores calculated as described in Table 1. Numbers in parentheses represent the number of hamsters in each group.
jected and hyperemic. Corneal stromas were moderately to severely opaque because of infiltration and edema. The epithelium was ulcerated in different degrees in most (90%) of the infected animals (Figure 1). Neovascularization was visible in the more severely infected animals. Histologic examination of acutely infected corneas revealed disruption of stromal lamellae, edema, neovascularization, and an inflammatory infiltrate consisting predominantly of neutrophils (Figure 2). Viable parasites were isolated from corneal smears, scrapings, and from the removed DTL. Corneas started to clear 3 days after removal of the DTL. Epithelial defects healed, stromal infiltration and edema resolved, and the keratitis was healed in 50% of the infected animals. By the fourteenth day after infection, all signs of keratitis had disappeared, although mild scarring of the cornea developed in a small number (10%) of hamsters.

Pathogenic bacteria such as *Pseudomonas* spp. were not detected at any time. Furthermore, sterile DTL applied in the same manner as the parasite-laden DTL did not produce any sign of disease or corneal damage after 7 days of exposure.

Role of Langerhans Cells in *Acanthamoeba* Keratitis

The role of Langerhans cells in the development of *Acanthamoeba* keratitis was evaluated by comparing the incidence and severity of keratitis in animals with normal corneas versus animals with Langerhans cells in the central cornea. Migration of Langerhans cells was induced by either the instillation of sterile latex beads or intracorneal injection of recombinant mouse IL-1. Parasite-laden DTL were applied to the corneas 10 days after latex-bead treatment and 2 and 7 days after IL-1 injection. The presence of infiltrating Langerhans cells in the central corneas of latex bead-treated and IL-1-treated corneas was confirmed by ADPase staining (N = 6 for all groups). Although the number of Langerhans cells was not significantly different in the limbus of the control corneas, latex bead-treated corneas, or IL-1-treated corneas, the number of Langerhans cells in the central of the corneas treated with either latex beads or IL-1 was significantly higher than controls (Figure 3). Histologic examination of the latex bead-treated and IL-1-treated corneas revealed that no inflammatory cells were present.

The presence of Langerhans cells in the central portions of the cornea exerted a profound effect on the development of *Acanthamoeba* keratitis. Clinical signs of *Acanthamoeba* keratitis developed in only 14% (1/7) of the latex bead-treated animals whereas 60% (6/10) of the untreated control animals had characteristic corneal lesions. Slightly different results occurred in corneas in which Langerhans cells were induced with IL-1. Here the time point of infection after the
injection of IL-1 seemed crucial. In the group infected 2 days after IL-1 treatment, the incidence of infection was the same as in the control group (50%), but the severity of infection was significantly reduced in the group treated with IL-1 2 days before exposure to parasite-laden DTL (Figure 4). In the group pretreated with IL-1 7 days before infection, only 28% (2/7) developed keratitis compared to 71% (5/7) in the control group.

A summary of the various factors influencing the development of *Acanthamoeba* keratitis is found in Table 3.

**Attachment of *Acanthamoeba castellanii* to Chinese Hamster Corneas In Vitro**

It was previously shown that the extent to which *Acanthamoeba* organisms bound to the corneal epithelium of various mammalian species correlated closely with the parasite's ability to produce corneal infections. The next experiments evaluated the effect of latex bead treatment and corneal abrasion on the binding of *Acanthamoeba* organisms to corneal epithelium. Abrasion of the cornea before exposure to parasites had a profound effect on parasite binding resulting in an 18 fold increase in the number of parasites adhering to either latex bead-treated corneas or normal corneas (Figure 5). By contrast, latex bead treatment did not influence the degree of binding of parasites to either nonabraded or abraded corneas.

**DISCUSSION**

The incidence of *Acanthamoeba* keratitis has increased sharply since its first description in 1973. However, the overall incidence is still low, especially considering the ubiquity of the parasite, and the large number of people wearing contact lenses. Although the latter is generally accepted as the major risk factor for *Acanthamoeba* keratitis, the observed rate of infection is only 0.01% of the contact lens wearers in the United States. This suggests that there must be other risk factors involved in the development of this corneal disease. An animal model of *Acanthamoeba* keratitis would be a useful tool to examine possible risk factors, pathophysiology, immunology, and therapy of this disease.

In the past there have been few attempts to establish an animal model of *Acanthamoeba* keratitis. Font et al reported that infections could be induced by intraocular inoculations in immunosuppressed rabbits. Badenoch et al described that intrastromal injections of *Acanthamoeba* organisms could only cause infections when coinfected with Corynebacteria organisms and not by injecting *Acanthamoeba* alone. By contrast, Larkin et al were able to induce keratitis by intrastromal injections in the rat by using *Acanthamoeba polyphaga*. In each of these studies, keratitis was induced by the direct inoculation of parasites into the corneal stroma. However, He et al recently described a model of *Acanthamoeba* keratitis in pigs infected by parasite-laden contact lenses.

The Chinese hamster model of *Acanthamoeba* keratitis has advantages over most previous models. The most important one is that the mode of infection is the same as what is believed to be the major source of
TABLE 3. Factors Influencing Acanthamoeba Keratitis in Chinese Hamsters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Incidence of Keratitis (%)</th>
<th>Nature of Keratitis</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method of exposure*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrastromal injection</td>
<td>0</td>
<td>None</td>
<td>Exposure via parasite-laden contact lenses and corneal abrasion is necessary to produce disease</td>
</tr>
<tr>
<td>Subconjunctival injection</td>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Deposition into cul-de-sac</td>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Inoculation onto abraded cornea</td>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Contact lens + abrasion</td>
<td>0</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td>Contact lens without abrasion</td>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Time of exposure to parasite-laden contact lens†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td>12</td>
<td>Severe</td>
<td>Exposure to parasite-laden contact lenses for 7 days or longer produces highest incidence of disease</td>
</tr>
<tr>
<td>5 days</td>
<td>44</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>66</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td>66</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td>10 days (normal contact lens)</td>
<td>66</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Effect of Langerhans’ cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC induced by IL-1</td>
<td>28</td>
<td>Moderate</td>
<td>Presence of Langerhans’ cells at the time of exposure to parasite-laden contact lenses reduces both incidence and severity of disease</td>
</tr>
<tr>
<td>LC induced by latex beads</td>
<td>14</td>
<td>Mild</td>
<td></td>
</tr>
<tr>
<td>Untreated controls</td>
<td>60</td>
<td>Severe</td>
<td></td>
</tr>
</tbody>
</table>

* Positive controls consisted of parasite-laden contact lenses placed onto abraded corneas for 7 days.
† Negative controls consisted of normal contact lenses incubated with PYG medium not containing parasites.

human infection, namely by application of parasite-laden contact lenses. However, certain minimal requirements are necessary to establish keratitis in Chinese hamsters: parasite-laden contact lenses are necessary to induce keratitis, the parasite-laden contact lens must remain in the eye for a minimum of 7 days, and the cornea must be partially abraded before exposure to parasite-laden contact lenses. Removal of the contact lens before day 7 reduces the rate of keratitis. Using these criteria, the rate of keratitis (ie, 60–70%), was consistent and predictable. The reason that infection did not approach 100% might be attributable to immunologic considerations. It is possible that a significant percentage (30–40%) of the unmanipulated animals had been previously exposed to Acanthamoeba through contaminated bedding or through other environmental sources and, as a result, developed protective immunity. Clinically and histopathologically, the keratitis closely resembled the acute stages of the human counterpart including epithelial ulceration, corneal opacity, edema, neutrophilic infiltration, and neo-vascularization. Moreover, the ability to isolate and culture viable parasite from diseased corneas adds credibility to the Chinese hamster model. Another advantage of this model is that the Chinese hamster is inexpensive (compared to pigs), easy to work with in a laboratory setting, and inbred strains are readily available.

The results from the in vitro binding study demonstrated that abrasion of the corneal epithelium greatly increased the binding of the parasite to the cornea. Moreover, the in vivo results confirm that abrasion is necessary for the development of Acanthamoeba keratitis and implies that damage of the corneal epithelium is an additional risk-factor for Acanthamoeba keratitis. This might mean that infections in contact lens wearers could be partly due to poorly fitting contact lenses or minor trauma related to contact lens wear, thereby resulting in damage of the corneal epithelium. This would occur only in combination with contaminated contact lenses.

It is somewhat surprising that the presence of Langerhans cells in the central cornea actually protected the cornea against Acanthamoeba keratitis. These cells are known to be involved in the immune response and their presence in the central cornea might provide a barrier against the parasite. The finding suggests that the immune system plays a role in the development of the disease and that the immune response might be a contributing factor in the establishment of keratitis.

![FIGURE 5. Attachment of Acanthamoeba castellanii to normal Chinese hamster corneas and corneas 10 days after latex bead treatment, both with and without abrasion of the corneal epithelium. Each bar represents the mean and standard error. N = 10 for all groups. Attachment to the abraded corneas was significantly higher than the nonabraded corneas (P < 0.05). There was no significant difference between latex bead-treated and normal corneas (P > 0.05).](https://iovs.arvojournals.org/pdfsaccess.ashx?url=/data/journals/iovs/933399/ on 11/30/2018)
findings contrast with previous studies of herpes simplex keratitis, in which the presence of Langerhans cells greatly increased the incidence and severity of the keratitis. 6,7 The mechanism whereby Langerhans cells protect against Acanthamoeba keratitis might be an indirect immune-mediated process because Langerhans cells are highly efficient antigen-presenting cells. Alternatively, Langerhans cells might exert a direct effect by damaging the parasites or inducing their encystment. Both of these possibilities are currently under investigation. The results from the in vitro attachment study showed that neither the latex bead treatment nor the IL-1 treatment used to induce Langerhans cell migration altered the binding of parasites to the corneal epithelium. Thus, the protective effects of latex bead and IL-1 treatment appear to be mediated by Langerhans cells and are not attributable to inhibition of parasite binding to the corneal epithelium.

The Chinese hamster model provides exciting opportunities to explore the pathophysiology, cell biology, immunology, and therapy of Acanthamoeba keratitis. However, it has limitations, especially the fact that Acanthamoeba keratitis in Chinese hamsters is an acute, self-limiting infection. Therefore, this model might not be suitable for studying the progressive and chronic stages of Acanthamoeba keratitis. However, an important advantage is its facility for immunologic studies that are not feasible in the pig model. Another important feature of this model is that the mode of infection (ie, contact lenses) is the same as that believed to occur in human patients. Thus, the Chinese hamster is not only a model of disease, but it is also a model of infection. This in turn offers an opportunity for designing and testing new therapeutic methods for the prevention of Acanthamoeba keratitis in contact lens wearers.

**Key Words**

Acanthamoeba sp, hamster, contact lens, cornea, Langerhans' cells

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