Intracorneal Instillation of Latex Beads Induces Macrophage-Dependent Protection against Acanthamoeba Keratitis

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PURPOSE. Instillation of sterile 1.0 μM latex beads into the central corneal epithelium renders Chinese hamsters resistant to corneal infection with Acanthamoeba castellanii. By contrast, activation of the adaptive immune response by subcutaneous immunization with A. castellanii antigens fails to protect against Acanthamoeba keratitis. This study was undertaken to examine the mechanisms that mediate latex bead-induced resistance to Acanthamoeba keratitis.

METHODS. In vitro experiments examined the effect of latex bead treatment on the capacity of A. castellanii trophozoites to adhere to and kill corneal epithelial cells. In vivo administration of antineutrophil antiserum was used to evaluate the role of neutrophils in latex-bead-induced protection against Acanthamoeba keratitis. Liposomes containing the macrophagicidal drug clodronate were used to deplete conjunctival macrophages and determine the role of macrophages in the latex-bead-induced resistance.

RESULTS. Latex bead treatment did not affect adherence of trophozoites to the corneal epithelium or protect corneal epithelial or stromal cells from trophozoite-mediated cytolysis in vitro. Neutrophil depletion did not abrogate the latex beads’ protective effect. Latex bead treatment induced a significant infiltration of macrophages into the corneas that peaked at day 4 of infection. Moreover, depletion of conjunctival macrophages with the macrophagicidal drug clodronate eliminated the latex beads’ protective effect.

CONCLUSIONS. The results indicate that intracorneal injection of latex beads induces a remarkable resistance to Acanthamoeba keratitis that is largely, if not entirely, mediated by macrophages. These results underscore the importance of the innate immune apparatus in the resistance to Acanthamoeba keratitis. (Invest Ophthalmol Vis Sci. 2006;47:4917–4925) DOI:10.1167/iovs.06-0266

The Acanthamoeba spp. is a ubiquitous, free-living protozoan that has been isolated from a variety of habitats including public water supplies, soil, and air.1,2 Although Acanthamoeba spp. can cause granulomatous amebic meningoencephalitis (GAE) and cutaneous acanthamebiasis, they most commonly infect the eye to produce Acanthamoeba keratitis. Acanthamoeba keratitis is caused by at least seven species of pathogenic amoebae and is a potentially blinding corneal infection if left untreated.3,4 However, prompt antimicrobial treatment often controls infection and preserves vision. The pathogenic cascade of Acanthamoeba keratitis begins with trophozoite adherence to mannose glycoproteins on the corneal epithelium via a 136-kDa mannose-binding protein (MBP) on the trophozoite membrane.4 Exposure to mannose induces Acanthamoeba trophozoites to release a 133-kDa protein, termed mannose-induced protein (MIP-133), that is highly cytolytic to corneal epithelial cells in vitro.5 After the pathogen binds to the corneal epithelium, the pathogenesis of Acanthamoeba keratitis proceeds, with trophozoite-mediated destruction of the corneal epithelium and penetration of the underlying Bowman’s membrane. Subsequently, Acanthamoeba trophozoites elaborate a variety of proteases that facilitate destruction of the corneal stroma.6 The pathogenesis of Acanthamoeba keratitis abruptly stops before invasion of the corneal endothelium and entry into the anterior chamber of the eye, as Acanthamoeba spp. rarely progresses beyond the corneal endothelium to produce intraocular infections.7

Environmental exposure to Acanthamoeba spp. is common, as viable Acanthamoeba trophozoites have been isolated from nasopharyngeal and oral specimens collected from asymptomatic individuals.8 Moreover, peripheral blood lymphocytes from 50% of healthy individuals demonstrated T-cell proliferative responses to Acanthamoeba antigens, suggesting that environmental exposure to this protozoan stimulates the adaptive immune apparatus.9 At least one component of the adaptive immune apparatus, anti-Acanthamoeba secretory IgA antibody, protects against disease by preventing trophozoite adherence to the corneal epithelium by augmenting neutrophil-mediated lysis of trophozoites, and by inhibiting MIP-133-mediated lysis of the corneal epithelium and stroma.10 Mucosal IgA antibodies to Acanthamoeba antigens and MIP-133 are the only effective component of the adaptive immune response that seem to be capable of controlling Acanthamoeba keratitis and are only effective if induced before corneal infections are initiated in experimental animals.10–18 The adaptive immune response also appears to be ineffective in controlling Acanthamoeba keratitis in humans, as acute infections occur in the presence of IgG serum antibodies specific for Acanthamoeba antigens in patients with Acanthamoeba keratitis, including those with recrudescent infections.10 However, the adaptive recrudescence can occur in hosts not possessing anti-Acanthamoeba IgA antibodies, indicating that the adaptive immune apparatus is not effective at preventing reinfection.

Elements of the innate immune system are crucial in the resolution of Acanthamoeba keratitis in experimental animals.16,17 Neutrophils and activated macrophages are capable of killing trophozoites and cysts in vitro.14,20,22 Moreover, depletion of macrophages with the macrophagicidal drug clodronate results in a chronic, more severe form of Acanthamoeba keratitis.18 Similarly, depletion of neutrophils by in
vivo treatment with antineutrophil antiserum exacerbates disease in Chinese hamsters. Recent results suggest that neutrophils are also important in preventing *Acanthamoeba* keratitis from progressing to intraocular infection. Previous studies have shown that intracorneal instillation of sterile latex beads induces an increased number of Langerhans’ cells (LCs) in the central cornea. In other infectious diseases of the cornea, the induction of increased numbers of LCs into the central cornea exacerbates keratitis by promoting a more robust delayed-type hypersensitivity (DTH) response. However, induction of LC migration into the central cornea before infection as a means of promoting DTH responses to *Acanthamoeba* antigens did not exacerbate *Acanthamoeba* keratitis as anticipated, but instead resulted in a robust resistance to infection and a mitigation of corneal inflammation. The mechanism by which corneal latex bead treatment protects against *Acanthamoeba* keratitis remains unknown.

The adaptive immune apparatus is not usually effective against *Acanthamoeba* keratitis, as neither T-cell–mediated nor humoral immunity protects against disease. Moreover, the latex beads’ protective effect is observed within 4 days of exposure to *Acanthamoeba* trophozoites. Thus, if the immune apparatus is involved in the protective effect, it is likely to be the innate immune apparatus and not the adaptive immune apparatus that mediates protection.

This study explored the immune and nonimmune mechanisms that could be responsible for the latex beads’ protective effect. One hypothesis proposed that latex beads provide protection against infection by preventing the adhesion of trophozoites to the corneal epithelium. We further hypothesized that latex beads could protect against disease by inhibiting trophozoite-mediated cytolysis of corneal cells, including the corneal epithelium and the corneal stroma. A final hypothesis proposed that latex beads provide protection by recruiting and/or activating cells of the innate immune system, including neutrophils and macrophages, which can kill trophozoites.

**METHODS**

**Animals**

Chinese hamsters were purchased from Cytogen Research and Development, Inc. (West Roxbury, MA) and used at 4 to 6 weeks of age. All corneas were examined before experimentation to exclude animals with preexisting corneal defects. Animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Amoebae and Cell Lines**

*Acanthamoeba castellanii* (ATCC 30868), originally isolated from a human cornea, was obtained from the American Type Culture Collection (Manassas, VA). Trophozoites were grown as axenic cultures in peptone-yeast-glucose (PYG) medium at 35°C, as described previously. Chinese hamster corneal (HCORN) epithelial cells were immortalized with human papillomavirus E6 and E7 genes, as previously described and cultured in complete Eagle’s minimal essential medium (EMEM; BioWhittaker, Walkersville, MD) containing 10 mM HEPES buffer solution; 1% nonessential amino acid solution; 1% d-glutamine (BioWhittaker); 1% penicillin, streptomycin, and amphotericin B (Funigzone; BioWhittaker); 1% sodium pyruvate (BioWhittaker); and 10% fetal calf serum (FCS; HyClone Laboratories, Logan, UT). Normal human keratocytes (NHKs) were a generous gift from James Jester (University of California at Irvine). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; BioWhittaker) containing 10 mM HEPES buffer solution; 1% nonessential amino acid solution; 1% d-glutamine; 1% penicillin, streptomycin, and amphotericin B; 1% sodium pyruvate; and 10% fetal calf serum.

**Adherence Assay**

The ability of *Acanthamoeba* trophozoites to adhere to latex-bead-treated corneas was examined using a modification of the assay in van Klink et al. Briefly, hamsters were killed 10 days after latex bead treatment, and the eyes were enucleated. The corneal epithelium was abraded immediately before enucleation in all groups. Normal, abraded, but otherwise untreated eyes served as the control. Eyes were sterilized in 10% iodine in water for 1 minute and rinsed with saline. Subsequently, the eyes were placed into 200-μL pipette tips (eye cups), cornea side up. The eyes became wedged in the eye cups such that the corneal surface served as the bottom of the cup. *Acanthamoeba castellanii* trophozoites were metabolically labeled, as described previously. Briefly, trophozoites (5 × 10⁶/mL PYG) were cultured with 100 μg of [¹⁴C]methionine (ICN Biomedicals, Inc., Irvine, CA) overnight at 35°C. Radiolabeled trophozoites were washed three times in PYG and added to each eye cup at 10⁵ trophozoites/eye. The control cultures of trophozoites were incubated in the presence of 100 mM mannose on untreated corneas. Adherence of trophozoites to corneas was determined after 2 hours of incubation at 35°C. The eye cups were washed three times with PYG to remove unbound amoebae. The eyes were then transferred to scintillation vials containing 2 mL of scintillation cocktail (Ready-Gel; BD Biosciences, San Diego, CA), and counts were measured in a liquid scintillation counter (model LS8010; Beckman, Fullerton, CA). The results are expressed as the percentage adherence, with 100% corresponding to the number of trophozoites that bound to untreated corneas.

**Assay for Cytopathic Effects**

The cytopathic effect (CPE) of trophozoites on corneal cells was determined with a previously described photometric assay. Briefly, latex beads were added to confluent monolayers of HCORN or NHK cells to obtain a concentration of 10 beads/cell. Latex beads were not added to control wells. Trophozoites (5 × 10⁵, 5 × 10⁴, or 5 × 10³) were then added to the confluent monolayers and incubated for 48 hours at 37°C. Cells were stained with a manual staining system (Hema 3; Fisher Scientific, Pittsburgh, PA) and the optical density (OD) was read at 590 nm in a microplate reader (Molecular Devices, Menlo Park, CA). The OD of trophozoites alone was subtracted from the OD of experimental wells to determine the OD of live cells. The results are expressed as the percentage of cells alive.
In Vivo Corneal Infections

Acanthamoeba keratitis was induced in Chinese hamsters as described previously. Briefly, approximately 25% of the cornea was abraded with a sterile cotton applicator, and then amoeba-laden contact lenses were placed on the center of the cornea. The contact lenses were removed 4 days after infection, and the corneas were visually inspected for severity of disease. Visual inspection results were recorded daily during the times indicated, and infections were scored based on the degree of corneal infiltration, corneal neovascularization, and corneal ulceration. Each pathologic criterion was scored 0 to 5 based on the degree of corneal involvement: 0, no disease; 1, <10% of the cornea involved; 2, 10% to 25% involved; 3, 25% to 50% involved; 4, 50% to 75% involved; and 5, 75% to 100% involved. The scores for all three criteria were lumped together, and the average combined score was determined for each time point and was expressed as the clinical severity of keratitis (%). In Chinese hamsters, Acanthamoeba keratitis resolves in approximately 3 weeks.

Production of Anti-Chinese Hamster Neutrophil Antiserum

Anti-Chinese hamster neutrophil antiserum was produced and absorbed as described previously. Antiserum was absorbed for 2 hours at 4°C with hamster spleen cells to remove antibodies against Chinese hamster histocompatibility and lymphoid antigens. The absorbed serum was then centrifuged at 1700g for 10 minutes, and the antiserum was removed and tested for cytotoxicity to neutrophils in vitro and in vivo. To test for cytotoxicity in vivo, Chinese hamsters were injected with 1 ml of anti-Chinese hamster antiserum or normal rabbit serum IP. Blood was collected from tail veins at 0, 2, and 4 hours after injection and streaked on a slide. Slides were stained with the manual staining system (Hema 3; Fisher Scientific) for histologic examination. Neutrophils and lymphocytes were counted in 10 random fields per slide. The results are expressed as mean number of cells per five high-power fields.

The anti-neutrophil antibody was also examined with an in vitro cytotoxicity assay, as described previously.

Chinese hamsters were injected intraperitoneally with 0.5 ml of absorbed serum twice daily for 7 days beginning at day 3 after infection.

Preparation of Clodronate Liposomes

Multilamellar liposomes were prepared as described previously. Clodronate liposomes were tested for in vitro toxicity against macrophages before use. Clodronate liposomes selectively deplete macrophages, and are not toxic to other phagocytic cells.

Clodronate Liposome Treatment

Clodronate-containing liposomes (50 μl) were administered via subconjunctival injection on days 9, 7, 5, and 3 before infection with Acanthamoeba-laden lenses. Clodronate liposomes were injected into four quadrants of the eye, encircling the entire conjunctiva. Van Klink et al. showed that injection of PBS-containing liposomes does not deplete resident macrophages or alter the severity of disease.

Quantification of NO Synthesis

Macrophage activation was quantified by measuring nitric oxide (NO) production. RAW cells were plated at 5 × 10^4 cells/well in a 24-well plate and allowed to confluence. As a positive control for activation, the indicated wells were stimulated with 2 μg/ml lipopolysaccharide (LPS; Sigma-Aldrich) and 100 ng/ml IFN-γ (BD-PharMingen, San Diego, CA), as described previously. To determine whether latex beads induce macrophages to produce NO, 1.0-μm latex beads were added to the indicated wells to obtain concentrations of 100, 1000, or 5000 beads/cell. After 24 and 48 hours of incubation at 57°C, 100 μL of supernatant was removed and assayed for nitrate, a stable reaction product of NO. Supernatants were then incubated with 100 μL of Griess reagent (Sigma-Aldrich) in a 96-well flat-bottomed plate for 10 minutes, and the OD was read at 590 nm in a microplate reader as described previously. To determine NO concentration, a standard curve of NO production was generated by incubating serial dilutions of sodium nitrite (Sigma-Aldrich) with Griess reagent. Results are expressed in micromol of NO.

To determine whether latex beads induce corneal epithelial cells to produce a factor that activates macrophages, BALB/c corneal epithelial cells were grown to confluence in a 24-well plate. Latex beads (1.0 μm) were added to the indicated wells to obtain concentrations of 10, 100, and 1000 beads/cell. After 24 hours of incubation at 37°C, the supernatant was collected and added to RAW cells in a 24-well plate. As a positive control for activation, the indicated wells of RAW cells were stimulated with 2 μg/ml LPS (Sigma-Aldrich) and 100 ng/ml IFN-γ (BD-PharMingen). After 24 and 48 hours, supernatant was removed and assayed for NO activity as just described. The same method was used to determine whether latex bead treatment induces BALB/c corneal keratocytes to produce a factor that activates macrophages.

Acid Phosphatase Staining

To determine whether there were more macrophages present in latex-bead-treated, Acanthamoeba-infected corneas than in untreated, infected corneas, eyes were processed and stained for macrophage-endogenous acid phosphatase activity, as described previously. Of importance, neutrophils and other leukocytes that may be present in the cornea do not stain positively with acid phosphatase. Thus, the acid phosphatase stain can be used to specifically stain macrophages in the cornea. Briefly, Chinese hamster corneas were infected with A. castellani, with or without prior latex bead treatment, as described earlier. Infected eyes of bead-treated and untreated Chinese hamsters (n = 5/group) were enucleated 3, 4, and 5 days after infection, sectioned, and processed for acid phosphatase staining. Frozen spleen sections were used as a positive control for acid phosphatase staining. Acid- phosphatase–positive cells were counted via light microscopy by three masked observers.

Statistics

Statistical analyses of all data except clinical scores were performed by using unpaired Student’s t-tests. Clinical severity scores were analyzed by the Mann-Whitney test.

Results

Effect of Latex Beads on Trophozoite Adherence to the Corneal Epithelium

Previous studies have shown that trophozoite adherence to mannose glycoproteins on the corneal epithelium is a crucial prerequisite for the establishment of infection. The extent of trophozoite binding to the corneal epithelium of various mammalian species correlates closely with the susceptibility of each mammalian species to corneal infection in vivo. Moreover, reports have shown that free-mannose, but not other sugars, inhibits trophozoite adherence to corneal epithelial cells in vitro. To ascertain the mechanism of the latex beads’ protective effect, it was first necessary to determine whether corneal latex bead treatment inhibits the binding of trophozoites to the corneal epithelium, thereby preventing infection. Accordingly, the ability of trophozoites to adhere to latex-bead–treated corneas was examined in an organ culture system. Corneal latex bead treatment 10 days before the adherence assay did not alter the adherence of A. castellani trophozoites to the corneal epithelium (Fig. 1). By contrast, binding of trophozoites to hamster corneas in the presence of 100 mM mannose was reduced by 85% compared with the untreated control.
Effect of Latex Bead Treatment on the Cytolysis of Corneal Epithelial and Corneal Stromal Cells

In the pathogenic cascade of *Acanthamoeba* keratitis, adhesion to the corneal epithelium is followed by trophozoite-mediated destruction of the corneal epithelium and penetration of the underlying Bowman’s membrane. It is possible that latex bead treatment protects the corneal epithelium from trophozoite-mediated cytolysis. Therefore, we next examined the ability of trophozoites to kill HCORN cells that were pretreated with latex beads in vitro. The results show that latex beads (10/cell) did not protect HCORN cells from trophozoite-mediated killing (Fig. 2A). Trophozoites killed as many as 60% of latex-bead–treated and untreated HCORN cells after 48 hours. Similar results were obtained with HCORN cells that were pretreated with 100 and 1000 beads/cell (data not shown).

After desquamation of the corneal epithelium, the pathogenesis of *Acanthamoeba* keratitis continues, with penetration and dissolution of the underlying stroma. It is possible that latex bead treatment provides resistance against *Acanthamoeba* keratitis by protecting the corneal stroma from trophozoite-mediated cytolysis. However, latex beads did not protect normal human corneal stromal cells (NHK) cells from trophozoite-mediated killing (Fig. 2B). Similar results were obtained with NHK cells that were pretreated with 100 and 1000 beads/cell (data not shown).

Role of Neutrophils in the Latex Beads’ Protective Effect

Neutrophils are capable of killing *Acanthamoeba* trophozoites and cysts in vitro. Moreover, in vivo depletion of neutrophils by intraperitoneal injection of anti-Chinese hamster neutrophil antibody results in a more severe infection compared with untreated control cells. Therefore, the possibility that neutrophils are involved in the latex beads’ protective effect was explored. To determine the role of neutrophils in the protective effect, rabbit anti-Chinese hamster neutrophil antiserum was generated to deplete neutrophils in latex-bead–treated animals. First, the efficacy of the rabbit anti-Chinese hamster neutrophil antibody was examined in vivo. Antiserum injections resulted in approximately an 85% decrease in peripheral blood neutrophils after 2 hours and a 95% decrease after 4 hours (Fig. 3). Lymphocyte and monocyte counts were not significantly decreased. Moreover, anti-Chinese hamster neutrophil antibody (1:100) lysed 83% of neutrophils in the presence of complement without significantly depleting splenocytes in vitro (data not shown). As a control, normal rabbit serum was found to be nontoxic to neutrophils in vivo or in vitro (data not shown). Successful depletion of neutrophils with anti-neutrophil antiserum resulted in a more severe disease than appeared in the untreated control animals. As reported previously, latex bead treatment reduced the inci-

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FIGURE 1. Effect of latex bead treatment on binding of trophozoites to hamster corneas. Sterile latex beads suspended in PBS were deposited into shallow incisions in the center of the corneal epithelium of Chinese hamsters. Ten days later, the corneas were abraded immediately before enucleation. Adherence of radiolabeled trophozoites to hamster corneas, with or without prior latex bead treatment, was examined after 2 hours of incubation in an organ culture system. As a control, the adherence assay was also performed in the presence of 100 mM mannose. Data are the mean ± SD of results in five hamster corneas. The experiment was performed in triplicate. There was no significant difference between latex-bead–treated and normal corneas (P > 0.05). Attachment to corneas in the presence of 100 mM mannose was reduced by 85%. **P < 0.01.

FIGURE 2. Effect of latex bead treatment on trophozoite-mediated cytolysis of corneal cells. Latex beads were added to HCORN (A) or NHK (B) cells and incubated for 24 hours at 37°C. Trophozoites were then added to the indicated wells and incubated for 48 hours. CPE was assessed spectrophotometrically. Data are the mean ± SD of triplicate counts. CPE on latex-bead–treated cells was not significantly different from that on untreated cells. (P > 0.05). Trophs, trophozoites; HCORN, Chinese hamster corneal epithelial cells; NHK, normal human keratocytes.
The Role of Macrophages in the Latex Beads’ Protective Effect

The role of macrophages in resistance to *Acanthamoeba* keratitis is well-established. In vivo depletion of conjunctival macrophages with the macrophagicidal drug, dichloro-methylene diphosphonate (clodronate), results in a chronic, exacerbated form of *Acanthamoeba* keratitis. Moreover, macrophages can kill *Acanthamoeba* trophozoites and cysts in vitro. We hypothesized that corneal latex bead treatment induces the migration and/or activation of macrophages that mitigate disease. To ascertain the role of macrophages in the latex beads’ protective effect, clodronate was administered subconjunctivally to remove corneal macrophages in latex-bead–treated animals. The results of this experiment showed that clodronate treatment eliminated the protective effect of the beads, exacerbating disease similar to that in the clodronate alone group (Fig. 5). Furthermore, the latex beads/clodronate group was not significantly different from the clodronate-treatment–alone group.

We next proposed that latex bead treatment may induce the activation of macrophages, which could kill trophozoites. To determine whether latex bead treatment activates macrophages, the beads were added to the RAW macrophage cells in vitro. Macrophage activation was determined by measuring nitrite generation, a stable reaction product of NO. However, the results showed that latex beads did not increase the pro-

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**Figure 4.** The role of neutrophils in the protective effect of latex beads. Sterile latex beads suspended in PBS were instilled into shallow corneal epithelial incisions in Chinese hamster corneas 10 days before infection in the indicated groups. Hamsters were injected IP with 0.5 mL of anti-Chinese hamster neutrophil antiserum or naïve rabbit serum twice daily for 7 days beginning 3 days before corneal infection. Latex bead treatment reduced the severity of disease at days 4, 5, 6, and 14 after infection compared with that in the untreated (infection alone) group (\(P < 0.05\)). Anti-neutrophil antiserum treatment increased the severity of disease at all times examined in both latex-bead–treated and untreated hamsters (\(P < 0.05\)). The clodronate treatment alone group was not significantly different from the latex-bead–treated, clodronate-treated group (\(P > 0.05\)). The results are representative of three separate experiments (\(n = 7\) group).

**Figure 5.** The role of macrophages in the latex beads’ protective effect. Latex beads were administered to hamster corneas 10 days before infection in the indicated groups. Clodronate-containing liposomes were administered via subconjunctival injection 9, 7, 5, and 3 days before infection with *Acanthamoeba*-laden lenses. Corneas were observed for signs of clinical disease beginning at day 4 after infection. Latex bead treatment reduced the severity of disease at days 4 and 5 after infection compared with that in the untreated (infection alone) group (\(P < 0.05\)). Clodronate treatment increased the severity of disease at all times examined in both latex-bead–treated and untreated hamsters (\(P < 0.05\)). The clodronate treatment alone group was not significantly different from the latex-bead–treated, clodronate-treated group (\(P > 0.05\)). The results are representative of three separate experiments (\(n = 7\) group).
duction of NO at 24 or 48 hours (Fig. 6A). Treatment with IFN-γ and LPS significantly activated macrophages compared with the untreated control group. Alternatively, it is possible that latex beads induce corneal epithelial cells to produce a factor that activates macrophages. To test this, BALB/c corneal epithelial cells were incubated with latex beads for 24 hours. Subsequently, latex-bead–treated BALB/c epithelial cell supernatant was added to RAW macrophages. Latex-bead–treated corneal epithelial cell supernatant did not increase production of NO at 24 or 48 hours (Fig. 6B).

We next hypothesized that latex bead treatment before corneal infection may induce the migration of macrophages to the central cornea, which kill trophozoites and result in the mitigation of corneal disease. Previous work by Hazlett et al. demonstrated that intracorneal instillation of latex beads before corneal infection with *P. aeruginosa* induces the corneal migration of macrophages. Acid phosphatase staining of corneal macrophages in latex-bead–treated *Acanthamoeba*–infected corneas revealed that latex bead treatment induced a significant increase in the infiltration of macrophages at day 4 of corneal infection (Fig. 7).

**DISCUSSION**

The original rationale for instilling latex beads into the central cornea was based on the hypothesis that latex beads would stimulate the appearance of LCs in the central cornea, which in turn would enhance the development of DTH to *Acanthamoeba* antigens, as LCs are potent antigen-presenting cells and are known to enhance DTH responses to alloantigens on corneal transplants and infectious agents introduced into the central cornea. The pathophysiology of herpes simplex virus (HSV) keratitis and *Pseudomonas* keratitis is largely mediated by DTH responses to the ocular pathogens and is exacerbated in corneas containing resident LC at the time of ocular infection. However, as shown here and elsewhere, intracorneal instillation of sterile latex beads 10 days before ocular challenge with *A. castellanii* reduced, rather than exacerbated, the incidence, severity, and duration of *Acanthamoeba* keratitis. Although intracorneal instillation of sterile latex beads before corneal infection with *A. castellanii* mitigates disease, it also curiously promotes the development of parasite-specific DTH. This is in sharp contrast to the condition in HSV keratitis and *Pseudomonas* keratitis, in which latex bead treatment enhances the DTH responses to the ocular pathogens and thus contributes to the exacerbation of corneal inflammation.

The latex beads’ protective effect does not appear to be mediated by the adaptive immune response, as protection develops within 4 days of bead installation, a time well before the peak of the adaptive immune response. Moreover, previous studies in both pigs and Chinese hamster models of disease have shown that both corneal abrasion and contact lenses upregulate mannose glycoproteins on the corneal epithelium, which latex bead treatment enhances the DTH responses to the ocular pathogens and is exacerbated in corneas containing resident LC at the time of ocular infection. However, as shown here and elsewhere, the pathophysiology of herpes simplex virus (HSV) keratitis and *Pseudomonas* keratitis is largely mediated by DTH responses to the ocular pathogens and is exacerbated in corneas containing resident LC at the time of ocular infection. However, as shown here and elsewhere.

**FIGURE 6.** Effect of latex beads on macrophage activation. (A) RAW cells were treated with various concentrations of latex beads and incubated at 37°C. As a measure of macrophage activation, NO production was determined at 24 and 48 hours. IFN-γ and LPS treatment resulted in increased production of NO at 24 and 48 hours **P < 0.05.** Latex bead treatment did not increase NO production at 24 or 48 hours. (B) BALB/c corneal epithelial cells were treated with various concentrations of latex beads and incubated at 37°C for 24 hours. The supernatant was collected and added to RAW cells in a 24-well plate. NO production was determined at 24 and 48 hours. BALB/c corneal epithelial supernatant did not increase NO production at 24 or 48 hours. The results are representative of three experiments. SN, supernatant.
Effect of corneal latex bead treatment on the migration of macrophages. Chinese hamster corneas were infected with *A. castellanii*, with or without prior latex bead treatment. Infected eyes of bead-treated and untreated Chinese hamsters were enucleated 3, 4, and 5 days after infection and 8-μm cryostat sections were tested for endogenous acid phosphatase activity as an indicator of macrophage infiltration. Acid phosphatase–positive cells (*pinkish red*) were counted by three masked observers. (A) Control eyes not treated with latex beads and collected on day 4 after infection; (B) latex-bead–treated eyes collected on day 4 after infection; and (C) number of acid-phosphatase–positive macrophages in the corneas of infected mice. Numbers are representative of the three observers’ counts in untreated and latex-bead–treated corneas. Acid phosphatase–positive cells were counted in the entire cornea (conjunctiva to conjunctiva) in four corneal sections per eye in each of three separate experimental eyes per group. Mean ± SD; *p* < 0.05.
atritis in experimental animals. However, elements of the adaptive immune system can exacerbate the pathologic sequelae of HSV keratitis and Pseudomonas keratitis. Intraocular instillation of latex beads before ocular challenge with P. aeruginosa results in a corneal infiltration of macrophages and LCs and an increased expression of IFN-γ. These observations led Hazlett et al. to conclude that latex beads promote the generation of a Th1 immune response to Pseudomonas antigens, which results in more severe keratitis, especially in Th2-prone BALB/c mice. They further proposed that the excessive number of macrophages elicited by latex bead treatment contributes to corneal perforation in Pseudomonas keratitis. This conclusion is in sharp contrast to the present and previous findings suggesting that macrophages are crucial for the resolution of Acanthamoeba keratitis. Thus, we favor the hypothesis that latex bead treatment stimulates the centrifugal migration of both LC and macrophages into the infected cornea. The infiltrating macrophages appear to be the primary mediators of the latex bead-induced protection through their capacity to eliminate trophozoites from the ocular surface.

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References


