Acanthamoeba Keratitis: Synergy between Amebic and Bacterial Cocontaminants in Contact Lens Care Systems as a Prelude to Infection

EDWARD J. BOTTONE,* ROBERT M. MADAYAG, AND M. NASAR QURESHI

Clinical Microbiology Laboratories, The Mount Sinai Hospital, New York, New York 10029

Received 18 February 1992/Accepted 18 June 1992

We encountered a patient with *Acanthamoeba* keratitis whose contact lens care solution contained numerous trophozoites and cysts admixed with *Xanthomonas maltophilia* organisms, many of which were adherent to the trophozoite surface and internalized within endocytic vacuoles. Because of this finding, we investigated the role of bacterial cocontaminants in contact lens care systems as substrates for the growth of *Acanthamoeba* spp. Individual cocultivation of *Acanthamoeba castellanii* and *A. polyphaga* with *X. maltophilia*, *Flavobacterium breve*, and *Pseudomonas paucimobilis* showed better enhancement (1.5×) of ameba growth after 96 h than that obtained in the presence of *Staphylococcus aureus*, *S. epidermidis*, and *Escherichia coli*, the standard cocultivation species used for isolation of amebae from clinical specimens. Our data suggest that contamination of contact lens care systems with *Acanthamoeba* spp. and a bacterial species capable of supporting amebic growth may be the first step in the pathogenesis of ameba-induced keratitis by the provision of large inocula of amebae.

Free-living amebae belonging to the genera *Naegleria* and *Acanthamoeba* have been associated with human disease causing primary amebic meningoencephalitis and granulomatous amebic encephalitis, respectively (4, 8, 12). Recent interest in *Acanthamoeba* spp. has focused on their causative role in a painful, vision-threatening keratitis that occurs mainly in contact lens users (1). While the role of *Acanthamoeba* spp. in keratitis is undisputed (1), the exact pathogenesis of the infection remains unresolved. In non-contact lens wearers, it is generally accepted that eye infection ensues subsequent to minor corneal trauma with introduction of amebae from an environmental source (9). As most of these infections occur during warm weather and in warm climates (1), it is tacitly assumed (although not proven) that ameba trophozoites are the infecting stage. Inoculum densities leading to infection are equally unknown.

In contact lens wearers, the role of the contact lens itself is also unresolved, although it has been speculated that the contact lens may cause slight corneal abrasions and/or may become contaminated with ameba trophozoites and cysts and serve as a vehicle for eye entry of *Acanthamoeba* organisms. The latter assumption is supported by the fact that *Acanthamoeba* organisms have been shown to adhere to new and worn daily-wear, extended-wear, and disposable soft contact lenses (5, 7a). *Acanthamoeba* spp. can also, in vitro, adhere to and digest corneal epithelial cells (15), a process which appears to be both time and temperature dependent (11).

Despite these biologic revelations underscoring *Acanthamoeba* keratitis, a paucity of information exists concerning the role of contaminating microflora in the eye itself or in contact lens care systems as a prerequisite to eye infection. At The Mount Sinai Hospital, we recently encountered a patient with *Acanthamoeba* keratitis in which ameba trophozoites and cysts were seen in Giemsa-stained smears of corneal scrapings. Examination of the contact lens case

solution by phase-contrast microscopy revealed numerous trophozoites and cysts admixed with an impressive bacterial flora, which on culture proved to be *Xanthomonas maltophilia*. Strikingly, gram-stained smears of the lens care solution were remarkable for the presence of *Acanthamoeba* trophozoites encircled by a mantle of adherent gram-negative slender rods in a parallel arrangement. These findings raised several questions about the pathogenesis of *Acanthamoeba* keratitis. We therefore investigated the role of bacterial cocontaminants in contact lens care systems as substrates for the growth of *Acanthamoeba* organisms and compared these with the growth-supporting potential of various eye commensals and the traditionally used species *Escherichia coli* (6).

FIG. 1. Enhanced growth of *A. castellanii* in the presence (cocultivation) of *X. maltophilia* (*X. malt.*), *P. paucimobilis* (*P. pauci.*), and *F. breve*, in contrast to that in the presence of *S. epidermidis* (*S. epi.*), *S. aureus*, and *E. coli*, after 96 h of incubation. Viable cysts and trophozoites were determined by methylene blue exclusion and enumerated by hemocytometer counting.

* Corresponding author.

FIG. 2. Enhanced growth of *A. polyphaga* in the presence (cocultivation) of *X. maltophilia* (*X. malt.*) and *F. breve* in contrast to that in the presence of the other species tested.

MATERIALS AND METHODS

Axenic cultures of *Acanthamoeba castellanii* ATCC 30010 and *A. polyphaga* ATCC 30461, maintained in a Difco Proteose Peptone yeast extract-glucose medium (6), were used to assess their growth characteristics by cocultivation with selected bacterial species isolated from a survey of 20

in-use contact lens care cases. These included *X. maltophilia*, *Flavobacterium breve*, *Pseudomonas paucimobilis*, *Staphylococcus aureus*, and *S. epidermidis*. Prior to cocultivation, amoeba suspensions were diluted 1:2 in 0.3% basic methylene blue and the numbers of viable cysts and trophozoites (methylene blue excluding) were determined by hemocytometer counting and confirmed by eosin staining (14). With either staining technique, nonviable *Acanthamoeba* cysts and trophozoites were stained within a few seconds, while viable cells remained unstained for 15 to 20 min. The amoeba counts were adjusted to approximately 4×10^5 cells per ml in normal saline. One milliliter of the *Acanthamoeba* suspension (containing approximately 70% cysts) in saline was transferred to individual tubes to which 1 ml of a saline suspension (1.0 McFarland unit) of *X. maltophilia*, *F. breve*, *S. aureus*, *S. epidermidis*, and *E. coli* was individually added. Final amoeba concentrations yielded approximately 2×10^5 amoebae per ml and a 0.5 McFarland unit suspension of bacteria. *Acanthamoeba* organisms in normal sterile saline served as an unsupplemented growth control. Cultures were maintained at 35°C, and amoeba counts were determined in triplicate by hemocytometer at 24-h intervals for 96 h.

For semiquantitative assessment of adherence and internalization of the selected bacterial species by *Acanthamoeba* trophozoites, *Acanthamoeba* organisms were cocultivated at 35°C with these species as described above, and at hourly

FIG. 3. Gram-stained smear of contact lens case solution showing an *Acanthamoeba* trophozoite encircled by adherent *X. maltophilia* organisms, several of which are contained within endocytic vacuoles. A similar presentation was observed by cocultivation of *Acanthamoeba* organisms with suitable bacterial substrates.

intervals for 8 h, a drop of the mixture was examined by phase-contrast microscopy and Gram staining.

RESULTS

Cocultivation of *A. castellanii* and *A. polyphaga* with bacterial species obtained from contact lens care systems was noteworthy because of the specificity of *Acanthamoeba* organisms for the bacterial species present and for the enhanced growth-promoting potential of environmental species.

In the presence of *X. maltophilia*, *F. breve*, and *P. paucimobilis*, there was better enhancement (1.5×) of growth of both *Acanthamoeba* species than with *E. coli*. By 96 h of cocultivation, counts of *A. castellanii* cysts and trophozoites in the sole presence of *X. maltophilia* and *P. paucimobilis* increased considerably compared with the unsupplemented control or *E. coli* (Fig. 1). For *A. polyphaga*, *F. breve* appeared to be a better growth-promoting bacterial substrate than *X. maltophilia* or *P. paucimobilis* (Fig. 2). It is interesting that *E. coli*, the recommended microbial species for cocultivation (6), was essentially equivalent to the staphylococcus species in growth-promoting potential. For neither *Acanthamoeba* species was *E. coli* equivalent to the environmental species as a native growth substrate.

Species specificity with regard to the preferred bacterial substrates could also be judged by the number of bacteria adhering to the surface of the amebae as a prerequisite to engulfment. As assessed by phase-contrast microscopy and evaluation by Gram-stained smears of bacterium-ameba cultures, the gram-negative environmental species, especially *X. maltophilia* and *F. breve*, were markedly adherent to cysts and trophozoites of both *Acanthamoeba* species (Fig. 3). Further, many phagocytized bacteria were seen as early as 2 h post cocultivation in the cytoplasm of trophozoites enclosed in endocytic vacuoles (Fig. 3). Although *E. coli* and staphylococci also adhered well to ameba cysts and trophozoites, the degree of adherence and trophozoite internalization of these species was more time dependent. Evaluation of 10 consecutive trophozoites after 2 h of cocultivation showed irregular adherence and random internalized bacteria, in contrast to *X. maltophilia* and *F. breve*. At 8 and 24 h post cocultivation, the environmental species saturated the ameba surface and were readily found intracellularly, whereas with *E. coli* and staphylococci, random internalization still prevailed.

DISCUSSION

It has long been recognized that free-living amebae display a differential feeding pattern on bacterial substrates they encounter in their natural environment (13). Transposition of these findings to ameba-bacterium interactions in other environmental niches, such as might exist in a contact lens care system, indicates that the nature of the bacterial cocontaminants either ensures or aborts ameba growth. This principle is germane to *Acanthamoeba*-induced keratitis.

Although we did not use a nonnutrient agar plate seeded with the test bacteria, our findings on the equivalence of *S. epidermidis* and *E. coli* as nutrient sources for *Acanthamoeba* growth parallel those of Larkin and Easty (7). These investigators inoculated 100 amebae on an agar lawn of the two bacterial species and compared the migration distance of the 50th trophozoite at the end of 6 days of incubation as an index of the growth suitability of the two test species. These researchers showed that *Acanthamoeba*

FIG. 4. Proposed hypothesis to account for the role of bacterial cocontaminants in contact lens care systems as a factor in *Acanthamoeba*-induced keratitis. A suitable bacterial substrate cocontaminates the contact lens care system with *Acanthamoeba* cysts from an environmental source. Excystment takes place, and trophozoites feed on bacteria, multiply, adhere to the contact lens (5), and invade the cornea subsequent to trauma, resulting in keratitis.

organisms migrated with equal speed on lawns of *E. coli* and *S. epidermidis*.

The results reported herein also confirm the early findings of Singh (13) on selective bacterial feeding by *Acanthamoeba* trophozoites, especially against species which cohabitate in aquatic and soil environments with amebae. This finding is not only reflective of the natural predatory activity of free-living amebae but is also of clinical importance, as the bacterial species tested, e.g., *X. maltophilia*, *F. breve*, and *P. paucimobilis*, are common contaminants of contact lens care systems (3, 7a). As noted herein, early internalization of these species may favor rapid ameba growth.

The initial stage in the development of *Acanthamoeba* keratitis is thought to be contamination of a contact lens care system with an *Acanthamoeba* cyst(s) introduced from the environment, in which they are widespread (9). By itself, this event does not pose a particular problem to the lens wearer, either because ophthalmic solutions used to clean contact lenses are not growth promoting for amebae (2) or because in the absence of a cocontaminant, excystment may not take place. However, in the concomitance of a bacterial contaminant (usually introduced through homemade saline) which is capable of supporting ameba growth, excystment does take place (14), liberating the amebic trophozoite. Continued feeding on the bacterial substrate results in ameba multiplication. Attachment of *Acanthamoeba* trophozoites to the contact lens surface (5, 10) may then appose numerous amebae on the corneal surface. Minor trauma to corneal conjunctival cells may then facilitate eye invasion by amebae (1, 5). These events are summarized in Fig. 4.

There is perhaps a secondary role for bacterial cocontaminants of contact lens care systems in the pathogenesis of amebic keratitis. In our patient, the degree of bacterial contamination in the contact lens case system was prodigious, as evidenced by smears and cultures and by the mantle of gram-negative slender rods (*Xanthomonas* sp.) encircling *Acanthamoeba* trophozoites observed directly in smears of the contact lens care solution. These bacterial contaminants may incite small lesions in the conjunctiva, as a consequence of either their accumulated metabolic toxins or a minor infection (3). Once scarification of the cornea has ensued, the amebae may gain entrance and continue to feed on the adherent-ingested bacteria, thereby facilitating early establishment in the corneal stroma. Subsequent to invasion and exhaustion of the bacterial food source, amebae may then degrade corneal cells as a food substrate (15) and become firmly established in the corneal stroma. The consequence of these events is clinically apparent keratitis.

ACKNOWLEDGMENT

This research was partially supported by grant 5T35 DK07420 from the National Institutes of Health.

REFERENCES

1. Auran, J. D., M. B. Starr, and F. A. Jakobiec. 1987. *Acanthamoeba* keratitis. A review of the literature. *Cornea* 6:2-26.
2. Brandt, F. H., D. A. Ware, and G. S. Visvesvara. 1989. Viability of *Acanthamoeba* cysts in ophthalmic solutions. *Appl. Environ. Microbiol.* 55:1144-1146.
3. Donzis, P. B., B. J. Mondino, B. A. Weissman, and D. A. Bruckner. 1987. Microbial contamination of contact lens care systems. *Am. J. Ophthalmol.* 104:325-333.
4. Fowler, M., and R. F. Carter. 1965. Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. *Br. Med. J.* 2:740-742.
5. John, T., D. Desai, and D. Sahm. 1991. Adherence of *Acanthamoeba castellanii* cysts and trophozoites to extended wear soft contact lenses. *Rev. Infect. Dis.* 13(Suppl. 5):S419-S420.
6. Krogstad, D. J., G. S. Visvesvara, K. W. Walls, and J. W. Smith. 1991. Blood and tissue protozoa, p. 727-750. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
7. Larkin, D. F. P., and D. L. Easty. 1990. External eye flora as a nutrient source for *Acanthamoeba*. *Graef's Arch. Clin. Exp. Ophthalmol.* 228:458-460.
- 7a. Larkin, D. F. P., S. K. Livingston, and D. L. Easty. 1990. Contamination of contact lens storage cases by *Acanthamoeba* and bacteria. *Br. J. Ophthalmol.* 70:133-135.
8. Martinez, A. J. 1980. Is *Acanthamoeba* encephalitis an opportunistic infection? *Neurobiology* 30:567-574.
9. Mergeryan, H. 1991. The prevalence of *Acanthamoeba* in the human environment. *Rev. Infect. Dis.* 13(Suppl. 5):S390-S391.
10. Moore, M. B., J. Ubelaker, R. Siluany, J. Martin, and J. P. McCulley. 1991. Scanning electron microscopy of *Acanthamoeba castellanii*: adherence to surfaces of new and used contact lenses and to human corneal button epithelium. *Rev. Infect. Dis.* 13(Suppl. 5):S243.
11. Morton, L. D., G. L. McLaughlin, and H. E. Whitley. 1991. Adherence characteristics of three strains of *Acanthamoeba*. *Rev. Infect. Dis.* 13(Suppl. 5):S424.
12. Robert, V. B., and L. B. Rorke. 1973. Primary amebic encephalitis, probably from *Acanthamoeba*. *Ann. Intern. Med.* 79:174-179.
13. Singh, B. N. 1946. A method for estimating the number of soil protozoa, especially amoebae, based on their differential feeding on bacteria. *Ann. Appl. Biol.* 33:112-119.
14. Singh, B. N., V. Sayena, and S. S. Iyer. 1965. Production of viable sterile cysts of free-living amoebae and role of bacteria on excystment. *Indian J. Exp. Biol.* 3:110-112.
15. Stopak, S. S., M. I. Roat, R. C. Nauheim, P. W. Turgeon, G. Sossi, R. P. Kowalski, and R. A. Thaft. 1991. Growth of *Acanthamoeba* on human corneal epithelial cells and keratocytes in vitro. *Invest. Ophthalmol. Visual Sci.* 32:354-359.