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Inhibition of *Acanthamoeba* Species by *Pseudomonas aeruginosa*: Rationale for Their Selective Exclusion in Corneal Ulcers and Contact Lens Care Systems

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Cocultivation of *Acanthamoeba castellanii* and *Acanthamoeba polyphaga* with live *Pseudomonas aeruginosa* and with broth filtrates of *P. aeruginosa* proved equally lethal to the *Acanthamoeba* spp. The *P. aeruginosa*-induced amebicidal activity is apparently toxin mediated and has two operative modes: it can function through binding of *P. aeruginosa* to the ameba membrane and in the presence of one or more *P. aeruginosa* exoproducts.

Free-living amebae of the genus *Acanthamoeba* are well-established causative agents of corneal ulcers (keratitis) (1). The role of various bacterial species in promoting *Acanthamoeba* growth, whether in their natural habitat (12) or in a contact lens care system, has been well documented (2, 8).

In the course of earlier studies (2), we found that in addition to *Escherichia coli*, several gram-negative, water-derived bacterial species, such as *Xanthomonas maltophilia*, *Flavobacterium breve*, and *Pseudomonas paucimobilis*, supported *Acanthamoeba* growth, while *Pseudomonas aeruginosa* proved lethal to *Acanthamoeba* spp. (10). Because the latter finding was unexpected, an attempt was made to define those factors (e.g., contact-dependent inhibition and toxin [exoenzyme] production) that could account for *P. aeruginosa*-induced ameba death and relate this finding to the clinical observation that *Acanthamoeba* spp. and *P. aeruginosa*, while equally eye destructive, may be selectively exclusive as eye pathogens. The results of these studies serve as the basis for this preliminary report.

Acanthamoeba castellanii (ATCC 30010) and *Acanthamoeba polyphaga* (ATCC 30461) maintained in axenic culture in Proteose Peptone yeast extract glucose medium (7) were individually cocultivated with clinical (blood) isolates of *P. aeruginosa* and *X. maltophilia*, were assessed for loss of cyst and trophozoite viability by methylene blue uptake (2), and were quantified by hemocytometer count. The percent nonviable cells was determined as follows: nonviable *Acanthamoeba* cells/(viable cysts + trophozoites + nonviable units) × 100.

Direct cocultivation of log-phase cultures containing approximately 10^5 cells of *A. castellanii* or *A. polyphaga* (~90% trophozoites) per ml in sterile physiologic saline with 0.5 ml of a saline suspension (~10^5 cells per ml) of *P. aeruginosa* resulted in marked amebicidal activity which approached 92.5% after 48 h (Fig. 1), an increase of approximately 64% over that observed in saline alone without a bacterial food source (28%). In contrast, in the presence of *X. maltophilia*, there was only a 10 to 15% loss of *Acanthamoeba* viability, with a concomitant increase in the total number of viable cysts and trophozoites. Results were reproducible in triplicate experiments.

Examination of ameba-bacterium interaction by phase-contrast microscopy and Gram-stained smears showed that both *P. aeruginosa* and *X. maltophilia* adhered to ameba trophozoites and cysts almost immediately (Fig. 2). Interestingly, however, *X. maltophilia* was internalized within 8 h of cocultivation, whereas *P. aeruginosa* remained membrane-bound to the *Acanthamoeba* surface and was not internalized even after 48 h of cocultivation (3).

Broth filtrates of *P. aeruginosa* were as amebicidal as the live organisms were in cocultivation. As noted in Fig. 3, the number of viable *A. castellanii* cells was significantly lower ($P < 0.01$) in the presence of *P. aeruginosa* filtrates than in the presence of *X. maltophilia* filtrates during the 48-h assay period. Similar results were obtained for *A. polyphaga*.

The amebicidal principle in cell-free broth filtrates of *P. aeruginosa* was as toxic to *A. castellanii* and *A. polyphaga* as cocultivation with live organisms, suggesting that the process was mediated by one or more *P. aeruginosa* exoenzymes.

In their ecosystems, *Acanthamoeba* spp. have been shown to display a differential feeding pattern toward bacterial food sources (12). Singh (12), through elegantly conceived but simple experiments, categorized bacterial substrates as utilizable, marginal, ineffectual, or outright toxic to ameba. For the latter category, he described the inhibitory activity of various pigment-producing bacterial species such as "*Bacillus pyocyanea*" (*P. aeruginosa*) against soil ameba, attributing toxicity to pigment production (pyocyanin) (13). Our results confirm and extend these early observations.

The *P. aeruginosa*-induced amebicidal activity apparently occurs as a result of a toxin-mediated phenomenon and has two operative modes, functioning in the presence of live bacteria and in the presence of one or more of its exoproducts. Initially, ameba death may be contact dependent, occurring through the adherence of *P. aeruginosa* to the cell surface. Direct microscopic evidence supporting this contention may be derived from the observation that *P. aeruginosa* (as well as *X. maltophilia*) markedly adheres to both *Acanthamoeba* species within minutes of cocultivation. In contrast to *X. maltophilia*, which is growth producing and readily internalized in *Acanthamoeba* spp. (2), *P. aeruginosa*, despite enveloping the ameba surface (Fig. 2), is not internalized within ameba trophozoites even after 48 h of cocultivation (3). Since trophozoite death occurs as early as 1 h postcocultivation, one may assume that failure to phago-

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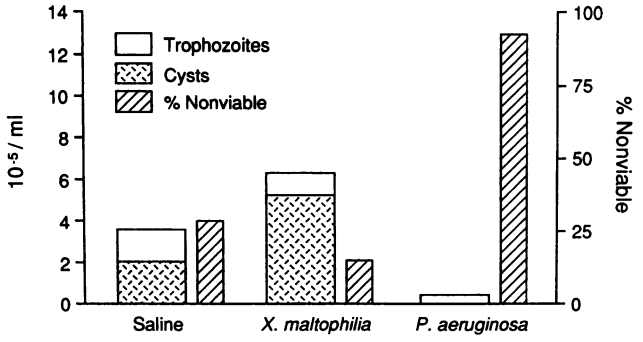

FIG. 1. Loss of viability (92.5%) of *A. castellanii* by cocultivation for 48 h with *P. aeruginosa* in contrast to promotion of growth by *X. maltophilia*. In a saline control without bacterial supplementation, loss of viability (28.6%) was greater than that seen with *X. maltophilia* (10%). Similar results were obtained for *A. polyphaga*.

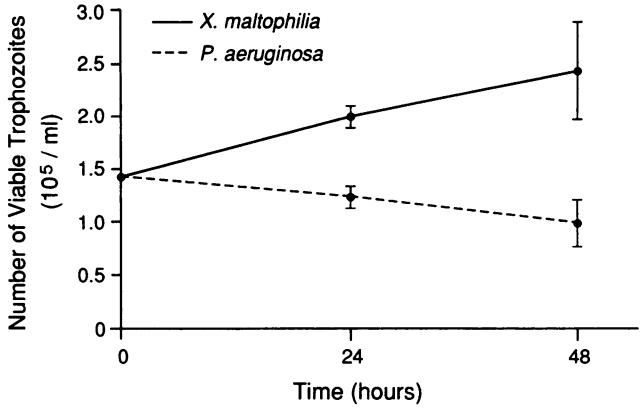

FIG. 3. Effect of bacterial filtrates on *A. castellanii*. The linear decrease in viability of *A. castellanii* in the presence of cell-free filtrate of *P. aeruginosa* is contrasted to the absence of growth inhibition in the presence of *X. maltophilia* filtrate. Similar results were obtained for *A. polyphaga*.

cytose adherent bacteria, an apparently energy-dependent phenomenon for *Acanthamoeba* spp. (15), is a consequence of amebicidal activity. Failure to internalize adherent pseudomonas cells also suggests that a pseudomonas exoenzyme, operative at the ameba membrane, may account for early ameba death.

Interaction with cell-free cultures (filtrates) of *P. aeruginosa* was equally toxic to the *Acanthamoeba* species studied. The *P. aeruginosa*-induced amebicidal principle appears to be exoenzyme mediated. *P. aeruginosa* produces several exoenzymes, such as phospholipase C (which has hemolytic activity), various proteases (including elastase) known to degrade protein in human epithelial cells, and the blue-green pigment pyocyanin, which inhibits ciliary function in human nasal epithelial cells (16). These pseudomonas exoproducts are all potential candidates for causing membrane lesions in *Acanthamoeba* spp. Future experiments using *P. aeruginosa* (PAO1) mutants deficient in exotoxin A (PAO1-3) and phospholipase C (9) should resolve which pseudomonal components may conspire to induce the amebicidal activity.

Acanthamoeba spp. and *P. aeruginosa* share many characteristics as eye pathogens. Both can adhere to, colonize, and invade injured corneal tissue (11, 14); both produce tissue-destructive enzymes (5, 6, 16); and both have been recovered individually as contaminants of contact lens care systems (4). Despite these overlapping attributes, these microbial species seem selectively exclusive as eye pathogens. Indeed, an extensive review of 35 cases of *Acanthamoeba* keratitis (1) has not revealed a single instance of the simultaneous recovery of an *Acanthamoeba* sp. with *P. aeruginosa*. The data reported herein provide an explanation for this observation. The clinical significance of these findings remains to be elucidated.

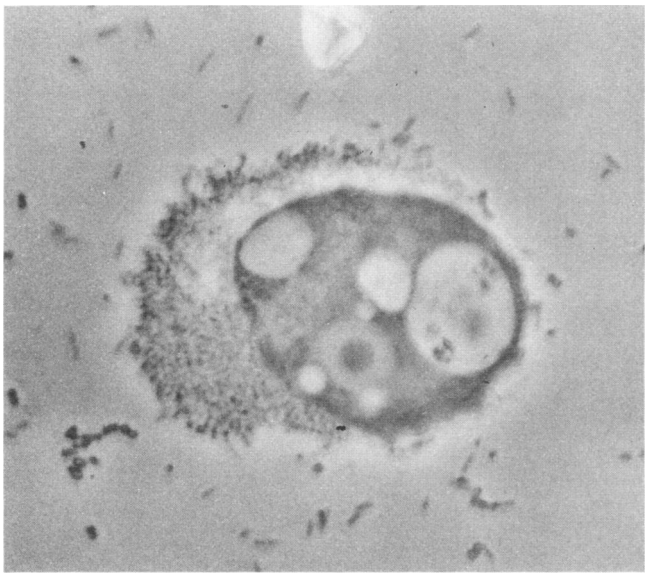

FIG. 2. Phase-contrast photomicrograph showing multilayered mantle of *P. aeruginosa* cells adherent to an *A. polyphaga* trophozoite. Adherence began within 2 min of interaction (cocultivation) of *A. polyphaga* and *P. aeruginosa*. Magnification, ×1,200.

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