LETTERS TO THE JOURNAL

Sir,

Rational Drug Targeting in *Acanthamoeba* Keratitis: Implications of Host Cell–Protozoan Interaction

It is now accepted that successful medical outcome of *Acanthamoeba* keratitis is very much dependent upon an early diagnosis,[1] followed by intervention with an effective drug regimen. Many such treatments have been suggested[2] and different institutes continue to suggest that theirs is the most appropriate and effective strategy. Recent examples of this are: topical propamidine and neosporin;[3] neosporin as monotherapy;[4] the combination of propamidine, neomycin and clotrimazole;[5] polyhexamethylene biguanide (PHMB);[6,7] and, more recently, the combination of chlorhexidine and propamidine.[8] Further, use of topical corticosteroids in treatment of *Acanthamoeba* keratitis also remains the subject of debate.[5]

Host–Protozoan Interaction

In order to rationalise the pharmacology of drug treatment of *Acanthamoeba* keratitis, it is first necessary to establish the mechanism of intra-corneal penetration of the amoebae, followed by the nature of the host–protozoan interaction. A further complication is that *Acanthamoeba* species are free-living protozoa. Although pathogens of the cornea (and elsewhere), they are not true parasitic protozoa, but merely accidental 'opportunists'.[9]

In the hamster model, there is a requirement for corneal abrasion as a prelude to *Acanthamoeba* keratitis.[10] After adhesion,[11] the amoebae penetrate the corneal epithelial layer by a process which remains poorly understood. The histopathological reaction(s) occurring in response to the presence of *Acanthamoeba* in the human cornea have been described.[12,13] There is an inflammatory reaction, which varies depending upon the location and extent of infection, but which comprises mainly macrophages and polymorphonuclear leucocytes; eosinophils and multinucleate giant cells may also be observed. In an animal model, the macrophage was a first line of defence in the infection, eliminating significant numbers of trophozoites.[14] *Acanthamoeba* can exert a cytopathic effect on corneal epithelial cells.[15] Cytological studies have shown that in *Acanthamoeba* infection of the cornea, the epithelial cells may be highly reactive, exhibiting an enlarged nucleus and prominent nucleolus.[16] Trophozoites may actively ingest these cells[17,18] (as well as keratocytes in the stroma).

It is uncertain, however, whether corneal epithelial cells might endocytose *Acanthamoeba* and/or inflammatory cells containing amoebal components, in a manner analogous to that suggested to occur in recurrent erosion syndrome,[19] or whether some amoebae may be voided from the cornea due to replicative turnover of the epithelium,[20] or as a consequence of apoptosis,[21] where the protozoa are attached to the epithelial cells as they are removed from the cornea. Such information is of considerable importance if the optimal pharmacokinetics of candidate drugs for treatment of *Acanthamoeba* keratitis is to be established.

Implications for Drug Therapy

The morphological features of the corneal epithelium–amoebal interaction are often difficult to interpret using tissues taken from human cases of *Acanthamoeba* keratitis. Fig. 1 illustrates the ultrastructural appearance by transmission electron microscopy of a group of corneal epithelial cells from a patient who was culture-positive for *Acanthamoeba*. Superficially there is an appearance of intra-epithelial cell localisation of what appears to be a macrophage containing, possibly, amoebal-derived cellular debris. This is unlikely, however. The micrograph actually represents a transverse thin section through a plane in the tissue which gives to it an appearance of intracellular localisation of what are in fact extracellular elements (Fig. 2); in essence, this micrograph demonstrates the well-known phenomenon of 'section-induced' artefact. A similar situation has been described in a study concerned with the cytopathogenicity of another free-living amoeba, *Naegleria fowleri*, in cell culture.[22]

Thus, further studies using serial sections of optimally fixed corneal tissue with appropriate histometric analysis are required to determine whether *Acanthamoeba* (or other free-living amoebae which can induce keratitis[23]) are localised within corneal epithelial cells (which may be the case

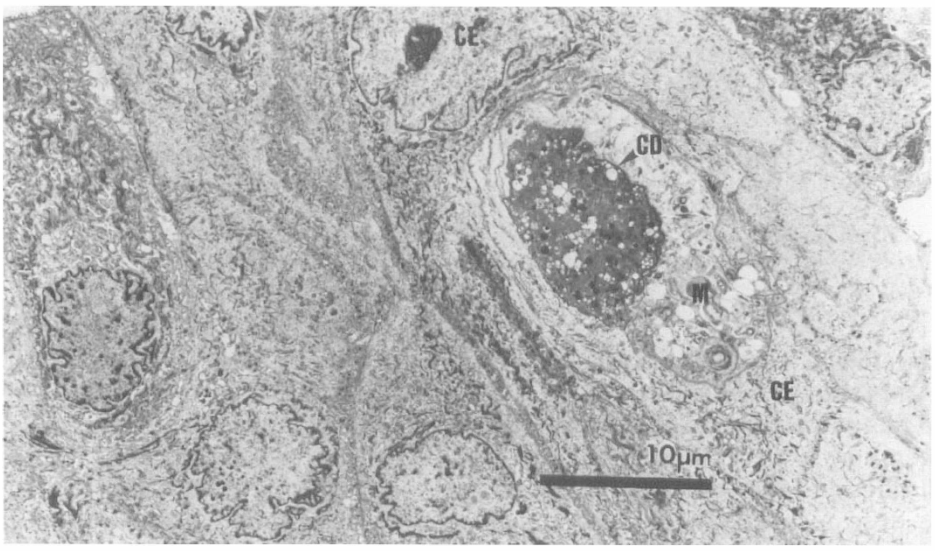

Fig. 1. *Transmission electron micrograph of corneal epithelial cells from a patient with culture-proven* Acanthamoeba *keratitis. Cursory examination reveals a macrophage (M), containing cellular debris (CD), possibly amoebal in origin and containing many electron-dense lipophilic structures, enveloped within a corneal epithelial cell (CE).*

for other phagocytic cells in the corneal erosion syndrome[19]) or whether the trophozoites gain access to the stroma by intercellular migration. Such findings have considerable implications for rationalisation of chemotherapeutic strategies to be used in the treatment of *Acanthamoeba* keratitis.

Since chlorhexidine is now being extensively used for treatment of *Acanthamoeba* keratitis,[8] such data would be invaluable. Chlorhexidine is delivered to the cornea at concentrations which are non-toxic to the native cells; thus if the amoebae were truly intracellular, they would not be expected to be

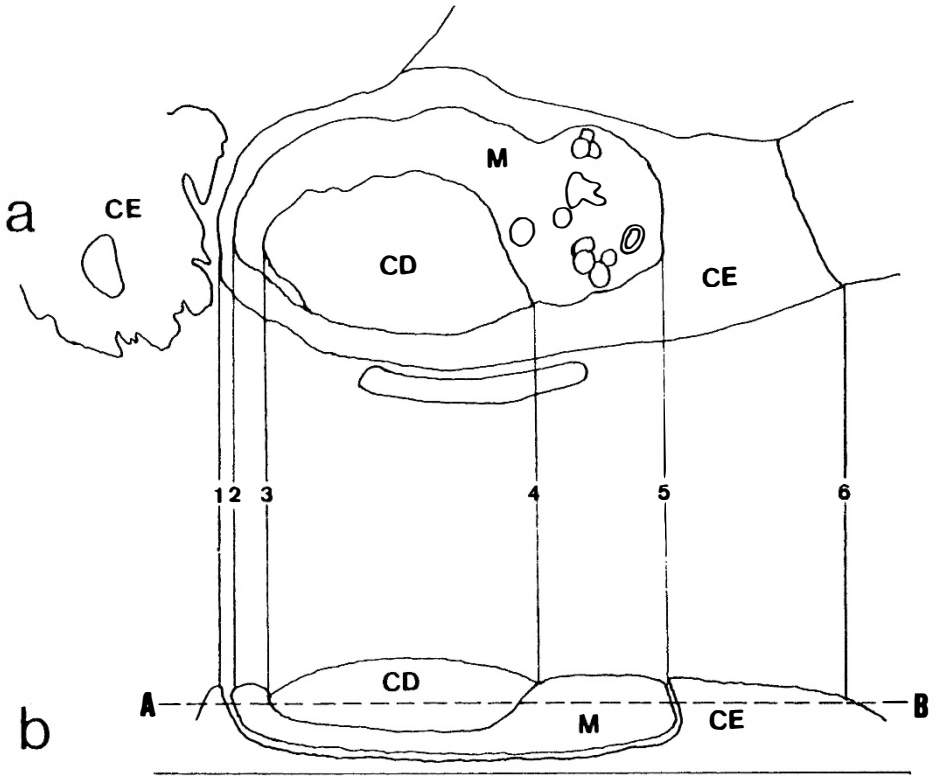

Fig. 2. *(a) Illustration of the salient features from Fig. 1. (b) Possible relationship at time of fixation between CE (1,6), M (2,5) and CD (3, 4). All entities are in a similar plane. A transverse thin section through plane A–B shows that the 'intracellular' appearance of M in CE is actually a section-induced artefact.*[22]

targeted by chlorhexidine. Chlorhexidine disrupts cell membranes prior to cytoplasmic coagulation.[24] For *Acanthamoeba* and the staphylococcal cell (MCC and MBC 2 μg/ml respectively) there is at least a thousand-fold difference in sensitivity relative to the mammalian cell for this action; the exception to this rule is the neuro-epithelial cell (such as the hair cell of the inner ear), which displays increased sensitivity.[8] Similarly, for treating chlamydial infection of the conjunctiva without toxicity,[25] chlorhexidine is considered to have activity against extracellular and not intracellular organisms, as has tetracycline, which cannot achieve sufficient intracellular concentration to be chlamydicidal within a host cell. If *Acanthamoeba* were located within corneal epithelial cells then the protozoan, not being exposed to active agent, would not respond to the cationic surfactants chlorhexidine and PHMB. These observations show the limitations, as far as pharmacological data is concerned, of two-dimensional morphological assessment and emphasise the absolute requirement for a detailed study of the temporal three-dimensional structure–function relationship between *Acanthamoeba* and its interaction with human corneal epithelium.

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References

1. Bacon AS, Dart JKG, Ficker LA, Matheson MH, Wright P. *Acanthamoeba* keratitis: the value of early diagnosis. Ophthalmology 1993;100:1238–43
2. Hay J, Kirkness CM, Seal DV, Wright P. Drug resistance and *Acanthamoeba* keratitis: the quest for alternative anti-protozoal chemotherapy. Eye 1994;8: 555–63.
3. Tay-Kearney M-L, McGhee CNJ, Crawford GJ, Trown K. *Acanthamoeba* keratitis: a masquerade of presentation in six cases. Aust NZ J Ophthalmol 1993; 21:237–45.
4. Sharma S, Srinivasan M, George C. *Acanthamoeba* keratitis in non-contact lens wearers. Arch Ophthalmol 1990;108:676–8.
5. D'Aversa G, Stern GA, Driebe WT Jr. Diagnosis and successful medical treatment of *Acanthamoeba* keratitis. Arch Ophthalmol 1995;113:1120–3.
6. Bacon AS, Frazer DG, Dart JKG, Matheson M, Ficker LA, Wright P. A review of 72 consecutive cases of *Acanthamoeba* keratitis. Eye 1993;7:719–25.
7. Gray TB, Gross KA, Cursons RTM, Shewan JF. *Acanthamoeba* keratitis: a sobering case and a promising new treatment. Aust NZ J Ophthalmol 1994;22:73–6.
8. Seal DV, Hay J, Kirkness CM, Morrell A, Booth A, Tullo A, Ridgway A, Armstrong M. Successful medical therapy of *Acanthamoeba* keratitis with topical chlorhexidine and propamidine. Eye 1996;10:413–21.
9. Jones DB. *Acanthamoeba* – the ultimate opportunist? Am J Ophthalmol 1986;102:527–30.
10. Klink van F, Alizadeh H, Ye YG. A Chinese hamster model of *Acanthamoeba* keratitis: role of contact lenses, trauma and Langerhans cells. Invest Ophthalmol Vis Sci 1993;34:1937–44.
11. Panjwani N, Zhao Z, Baum J, Pereira M, Zaidi T. Acanthamoebae bind to glycolipids of rabbit corneal epithelium. Infect Immun 1992;60:3460–3.
12. Garner A. Pathogenesis of acanthamoebic keratitis: hypothesis based on a histological analysis of 30 cases. Br J Ophthalmol 1993;77:366–70.
13. Mathers W, Stevens GS Jr, Rodrigues M, Chan CC, Gold J, Visvesvara GS, Lemp MA, Zimmerman LE. Immunopathology and electron microscopy of *Acanthamoeba* keratitis. Am J Ophthalmol 1987;103: 626–35.
14. Klink van F, Taylor WM, Alizadeh H, Jager MJ, Rooyen van N, Niederkorn JY. The role of macrophages in *Acanthamoeba* keratitis. Invest Ophthalmol Vis Sci 1996;37:1271–81.
15. Badenoch PR, Adams M, Coster DJ. Corneal virulence, cytopathic effect on human keratocytes and genetic characterisation of *Acanthamoeba*. Int J Parasitol 1995;25:229–39.
16. Rivasi F, Langanesi L, Casobari C, Croppo GP, Pierini G, Zunarelli E, Visvesvara GS. Cytologic diagnosis of *Acanthamoeba* keratitis: report of a case with correlative study with indirect immunofluorescence and scanning electron microscopy. Acta Cytol 1995;39:821–6.
17. Moore MB, Ubelaker JE, Martin JH, Silvany R, Dougherty JM, Meyer DR, McCulley JP. *In vitro* penetration of human corneal epithelium by *Acanthamoeba castellanii*: a scanning and transmission microscopy study. Cornea 1991;10:291–8.
18. Stopak SS, Roat MI, Nauheim RC, Turgeon PW, Sossi G, Kowalski RP, Thoft RA. Growth of *Acanthamoeba* on human corneal epithelial cells and keratocytes *in vitro*. Invest Ophthalmol Vis Sci 1991;32:354–9.
19. Aitken DA, Beirouty A, Lee WR. Ultrastructural study of the corneal epithelium in the recurrent erosion syndrome. Br J Ophthalmol 1995;79:282–9.
20. Dua HS, Watson NJ, Mathur RM, Forrester JV. Corneal epithelial cell migration in humans: 'hurricane and blizzard keratopathy'. Eye 1993;7:53–8.
21. Ren H, Wilson G. Apoptosis in the corneal epithelium. Invest Ophthalmol Vis Sci 1996;37:1017–25.
22. Brown T. Observations by immunofluorescence morphology on the cytopathogenicity of *Naegleria fowleri* in mouse embryo-cell cultures. J Med Microbiol 1979;12:363–71.
23. Aitken D, Hay J, Kinnear FB, Kirkness CM, Lee WR, Seal DV. Amebic keratitis in a wearer of disposable contact lenses due to a mixed *Vahlkampfia* and *Hartmanella* infection. Ophthalmology 1996;103: 485–94.

24. Russell AD. Chlorhexidine: microbicidal action and bacterial resistance. Infection 1986;14:212–5.
25. Nisbet IT, Graham DM, Spicer PE, Tibbs GJ. Chlorhexidine is an effective agent against *Chlamydia trachomatis in vitro* and *in vivo*. Antimicrob Agents Chemother 1979;16:855–7.

Sir,

Fungal Keratitis Caused by *Curvularia lunata*, with Successful Medical Treatment

Fungal keratitis is exacerbated by topical steroid use.[1] We present a case of a non-resolving corneal ulcer, treated with topical antibiotics and betamethasone. The ulcer was found to be infected by *Curvularia lunata*. This has not previously been described in this country although it is a recognised cause of keratomycosis in tropical and subtropical areas.

Case Report

A 53-year-old man was referred in December 1995 with a month's history of a non-resolving right corneal ulcer. His symptoms had developed in Israel while swimming in the Dead Sea, when he developed a painful and red right eye. He was otherwise well. In 1993 he had undergone surgery for right congenital ptosis. An ophthalmologist in Israel diagnosed a corneal ulcer and started treatment with topical antibiotics.

On his return to England a week later he consulted an ophthalmologist again. The corneal ulcer was treated with hourly topical ciprofloxacin 0.3% and gentamicin 1.5%, and betamethasone 0.1% four times a day. Corneal scrapes were negative for bacterial culture. After 3 weeks without clinical improvement, he was referred to the corneal clinic at the Croydon Eye Unit for further management.

At that time his visual acuity was 6/12 in the right eye and 6/9 in the left. The conjunctiva was injected. There was an area of peripheral corneal ulceration at 4 o'clock measuring 2.5 mm × 1.5 mm. This was associated with overlying hard plaque formation and surrounding infiltration and corneal melting (Fig. 1). There was minimal anterior chamber activity and the left eye was normal.

A Gram stain of corneal scrapings demonstrated some fungal elements, confirming the clinical picture of fungal keratitis. The day after stopping the patient's medication, further scrapings were taken for inoculation on blood, chocolate and two Sabouraud agar plates. Treatment of the ulcer was then initiated with topical natamycin 5% (Pimaricin) hourly; 2-hourly gentamicin 1.5% was continued. The blood and chocolate plates were inoculated in CO_2 at 37 °C overnight; one Sabouraud plate was incubated at 37 °C and the other at 30 °C. Dark brown velvety colonies grew on the chocolate and on the Sabouraud plates incubated at 37 °C after 2 days. These were identified as *Curvularia* species by the distinctive curved macronidia. Bristol Public Health Laboratory Services confirmed the fungus as *C. lunata* and found it to be sensitive to amphotericin B, itraconazole and clotrimazole (Pimaricin was not available for testing).

The corneal ulcer slowly resolved and the gentamicin was discontinued. After 1 week, tetracyline 250 mg orally q.i.d. was added for 2 weeks. The area of infiltration decreased in size over 3 weeks and topical medication gradually tapered off. Four weeks after the initiation of antifungal treatment the epithelial defect had healed completely. There was a residual area of stromal thinning with underlying vascularisation (Fig. 2). The patient's final right visual acuity was 6/12.

Discussion

Fungal keratitis is rare in the United Kingdom, accounting for 3% of suppurative keratitis presenting to Moorfields Eye Hospital in 1981.[2] The incidence is much higher in tropical or subtropical regions and is related to climatic conditions and agrarian popula-

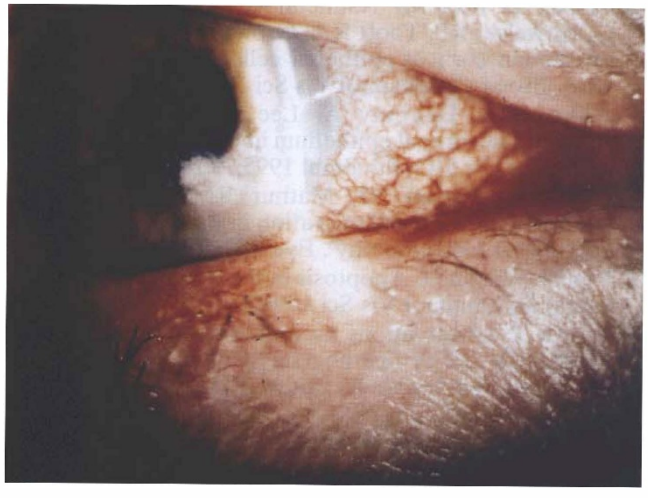

Fig. 1. *The right eye at presentation, showing corneal ulceration at 4 o'clock.*

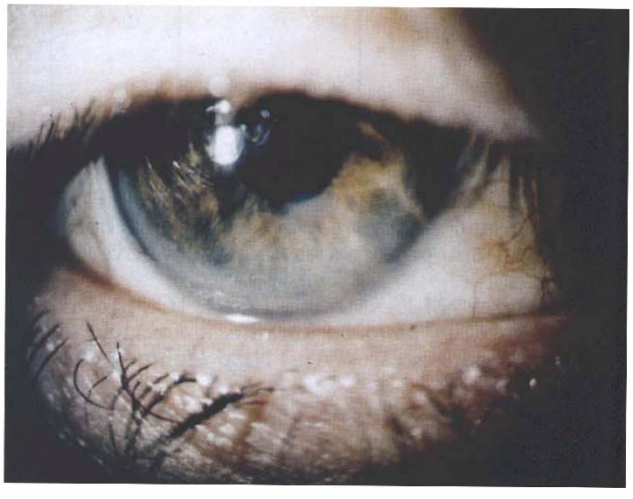

Fig. 2. *The right eye 4 weeks after initiation of antifungal treatment, showing complete healing of the epithelial defect.*