Absence of an Antibiotic Effect of *Mycobacterium ulcerans*[1,2]

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Infection of man with *Mycobacterium ulcerans* causes extensive ulceration of the skin and is a serious health problem in some tropical countries, especially Uganda and Zaire. The infection is characterized by a spreading contiguous necrosis involving the full thickness of the skin. The infection and necrosis may extend to involve an entire limb (Fig. 1). Although these ulcers expose broad areas of subcutaneous tissue, secondary bacterial invaders, both gram-positive cocci and bacilli, are minimal and limited to a narrow zone at the margin of the ulcer in the most superficial necrotic tissues ([2]). The lack of significant secondary bacterial invasion is even more surprising considering the environments and native treatments to which these patients are exposed. Most patients, including the patient whose leg is shown in Figure 1, experience no pain, tenderness, lymphadenopathy, fever, malaise ([1, 2, 4, 5]) nor other manifestations of secondary bacterial invasion. Because of this, we

FIG. 1. Leg of a seven year old boy with *M. ulcerans* infection. The ulcer extended from midthigh to the toes and had been present for six months. AFIP Neg. 65-2976-1.

TABLE 1. *Cultures tested for sensitivity to culture filtrate and sonicated cells of* Mycobacterium ulcerans.

Aerobes

1. *Arizona* sp.
2. *Bacillus subtilis*
3. *Candida albicans*
4. *Citrobacter diversus*
5. *Citrobacter freundii*
6. *Citrobacter* sp.
7. *Corynebacterium equi*
8. *Enterobacter agglomerans*
9. *Enterobacter cloacae* (A)
10. *Enterobacter cloacae* (B)
11. *Escherichia coli*
12. *Klebsiella pneumoniae* (A)
13. *Klebsiella pneumoniae* (B)
14. *Klebsiella pneumoniae* (C)
15. *Klebsiella rhinoscleromatis*
16. *Listeria monocytogenes*
17. *Proteus mirabilis*
18. *Pseudomonas aeruginosa*
19. *Salmonella paratyphi* A
20. *Salmonella paratyphi* B
21. *Salmonella typhi*
22. *Sarcina lutea*
23. *Serratia marcescens*
24. *Shigella flexneri*
25. *Staphylococcus aureus*
26. *Staphylococcus epidermidis*
27. *Streptococcus agalactiae*
28. *Streptococcus faecalis* (enterococcus)
29. *Streptococcus faecalis* (nonenterococcus)
30. *Streptococcus pyogenes*
31. *Streptococcus pyogenes* (mucoid)
32. *Streptococcus salivarius*
33. *Streptococcus viridans*

Anaerobes

1. *Bacteroides melaninogenicus*
2. *Bacteroides* sp.
3. *Clostridium cochlearium*
4. *Clostridium perfringens*
5. *Clostridium sphenoides*
6. *Veillonella* sp.

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wondered if *M. ulcerans* elaborates a potent antibiotic that inhibits the growth of other microorganisms.

MATERIALS AND METHODS

The ability of *M. ulcerans* to produce an antibiotic substance was tested by determining the effect of various concentrations of culture filtrate (3.2, 9.0, 160.0, and 280 mg protein/ml) and sonicated *M. ulcerans* cells ([3]) (0.4 and 2.9 mg protein/ml) on the growth of 39 strains of bacteria (Table 1). Filter discs (7 mm in diameter) soaked in fractions were placed on inoculated brain-heart infusion (BHI) agar plates. Each plate (three per organism) contained three sample discs and one control (Dubos broth). Each of the aerobic cultures was also inoculated into 5 ml culture filtrate (effective concentration of 6.4 mg protein/ml). The fractions were concentrated by ultrafiltration on a UMO5 filter (Amicon) and the filter sterilized before testing.

RESULTS AND DISCUSSION

There was no detectable inhibition of growth of any of the organisms tested. We conclude, therefore, that the lack of secondary invaders is not caused by an antibiotic elaborated by *M. ulcerans*. Systemic or other local factors may play a role in inhibiting secondary bacterial invaders, and studies on some of these are planned.

SUMMARY

Fractions of *Mycobacterium ulcerans* were tested for the ability to inhibit growth of 39 bacterial strains. At protein concentrations of up to 280 mg/ml, there was no detectable effect on the growth of any of these bacterial strains.

RESUMEN

Se probaron fracciones de *Mycobacterium ulcerans* con respecto a su capacidad para inhibir el crecimiento de 39 cepas bacterianas. A concentraciones de proteína de hasta 280 mg/ml, no se observó un efecto detectable sobre el crecimiento de ninguna de estas cepas bacterianas.

RÉSUMÉ

Des fractions de *Mycobacterium ulcerans* ont été étudiés dans le but de juger de leur capacité à inhiber la croissance de 39 souches bactériennes. A des concentrations de protéines s'élevant jusqu'à 280 mg/ml, aucun effet n'a pu être détecté sur la croissance d'aucune de ces souches bactériennes.

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REFERENCES

1. Clancey, J., Dodge, R. and Lunn, H. F. Study of a mycobacterium causing skin ulceration in Uganda. Ann. Soc. Belg. Med. Trop. **4** (1962) 585-590.
2. Connor, D. H. and Lunn, H. F. Buruli ulceration. A clinico-pathologic study of 38 Ugandans with *Mycobacterium ulcerans* ulceration. Arch. Pathol. **81** (1966) 183-189.
3. Krieg, R. E., Hockmeyer, W. T. and Connor, D. H. Toxin of *Mycobacterium ulcerans*. Production and effects in guinea pig skin. Arch. Dermatol. **110** (1974) 783-788.
4. Lunn, H. F., Conner D. H., Wilks, N. E., Barnley, G. R., Kamunvi, F., Bee, J. D. A. and Clancey, J. K. Buruli (mycobacterial) ulceration in Uganda (a new focus of Buruli ulcer in Madi District, Uganda). Report of a field study. East Afr. Med. J. **42** (1965) 275-288.
5. Revill, W. D. L., Hutt, M. S. R. and Kiryabwire, J. W. M. Clinical features and treatment of pre-ulcerative Buruli lesions (*Mycobacterium ulcerans* infection: Report 11 of the Uganda Buruli Group). Br. Med. J. **2** (1970) 390-393.