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Cytopathic Effect Mimicking Virus Culture Due to *Mycobacterium tuberculosis*

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***Mycobacterium tuberculosis* grew, from a bronchoalveolar lavage of a patient with AIDS, on a gentamicin-supplemented cell culture monolayer, causing a focal and slowly spreading cytopathic effect resembling that of a virological isolate. The same effect was observed after inoculation of two different inocula of *M. tuberculosis* onto the same cell culture.**

Detection of cytopathic effect (CPE) on cell cultures is the most common procedure to detect the presence of a particular virus in a clinical sample. Confirmation of a typical CPE may be done by using immunologic or genetic techniques that are usually performed in most clinical laboratories. However, some CPE can be produced by organisms other than viruses as well as by host cells and other substances (2, 3). We report herein a CPE mimicking virus infection due to *Mycobacterium tuberculosis* from a bronchoalveolar lavage (BAL) of an AIDS patient.

The BAL was obtained from a male AIDS patient with severe respiratory insufficiency. The clinical specimen was processed for bacterium, fungus, and virus isolation. For virological diagnosis, the BAL sample was first treated with gentamicin (80 mg/liter), vancomycin (50 mg/liter), and amphotericin B (2.5 mg/liter) for 1 h at 4°C. A heteroploid cell line of human oat cell pulmonary carcinoma (A-549), a heteroploid cell line of green monkey kidney (Vero), and human fetal diploid fibroblasts (HFD) were seeded with 200 μl of the decontaminated sample. The cell monolayers were cultured at 37°C in Eagle minimal essential medium supplemented with 2% fetal calf serum (EMEMS) containing 40 mg of gentamicin per liter and examined for up to 30 days.

After 15 days of incubation, a cell-associated virus-like CPE was exclusively observed on HFD monolayers (Fig. 1). The CPE grew slowly without generalization, but the cells did not react with monoclonal antibodies against respiratory syncytial virus, parainfluenza 3 virus, influenza A or B virus, adenovirus, or cytomegalovirus. Giemsa staining of the monolayer revealed many intracellular bacilli within the CPE foci. Ziehl-Neelsen staining was then performed, and the acid-fast nature of the bacilli was confirmed (Fig. 2). Conventional mycobacterial culture of the HFD altered monolayer as well as the mycobacterial cultures directly performed with the BAL sample yielded *M. tuberculosis* sensitive to 5 mg of gentamicin per liter.

In order to determine whether *M. tuberculosis* by itself was capable of producing the same CPE, the following experiment was carried out. From a Lowenstein-Jensen medium culture of *M. tuberculosis*, a bacterial suspension in buffered Hanks balanced salt solution, containing approximately 5×10^5 CFU/ml, was prepared. Two dilutions (10^{-2} and 10^{-4}) were prepared, and two aliquots from each one were managed in two different ways. One set of aliquots was treated by the same protocol used for the clinical sample; they were treated with gentamicin, vancomycin, and amphotericin B and then inoculated onto A-549, Vero, and HFD cell cultures in EMEMS containing gentamicin. The other set of samples was directly (not decontaminated) inoculated onto the same cell cultures in EMEMS without gentamicin, but this medium was replaced by a complete one (with gentamicin) 48 h later. No CPE was observed in those cell cultures inoculated with a *M. tuberculosis* inoculum, treated in the same way as the clinical sample after 25 days of observation. On the other hand, a CPE identical to that observed with the clinical sample was detected in HFD cells 14 to 18 days after inoculation with the nondecontaminated *M. tuberculosis* inoculum. Therefore, *M. tuberculosis* is capable of producing CPE in HFD cells if it is not inhibited by antibiotics. On the other hand, it seems that gentamicin, added to the cell culture 48 h after bacterial inoculation, is not able to inhibit *M. tuberculosis*; this could be related to the intracellular penetration of the organism, an inoculum

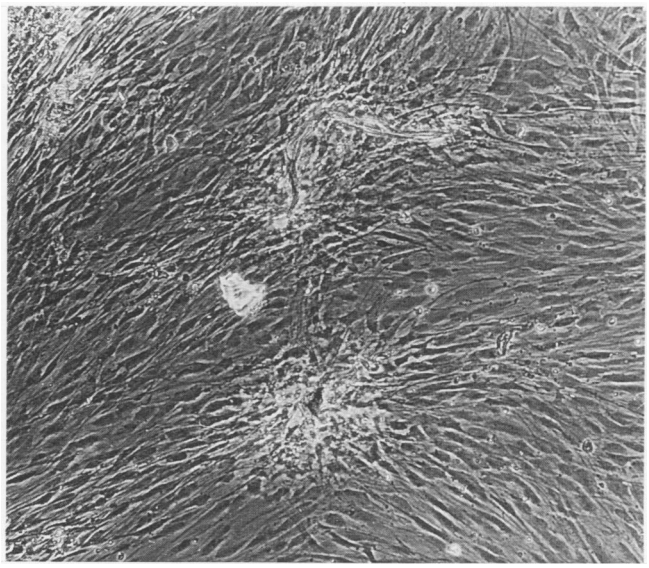

FIG. 1. CPE foci after 15 days of incubation of BAL on HFD. Phase-contrast micrograph. Magnification, ×100.

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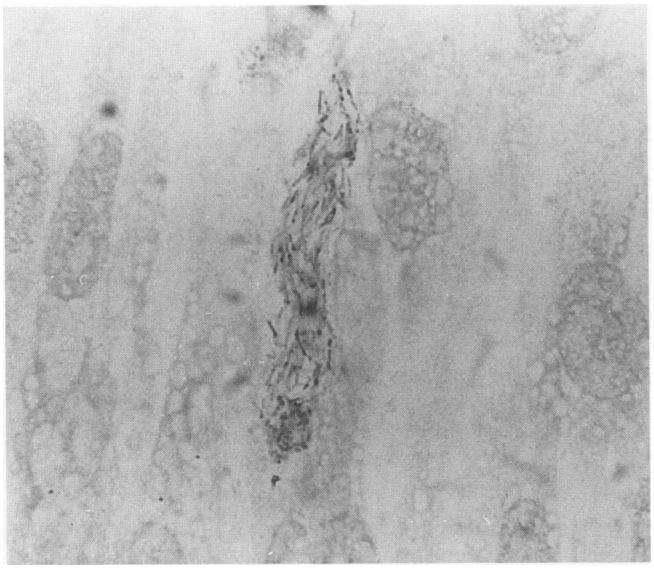

FIG. 2. Higher-power magnification of Ziehl-Neelsen-stained HFD seeded with BAL revealing a large number of intracellularly acid-fast bacilli.

effect, or both. Because the organisms recovered from the cell cultures remained fully sensitive to gentamicin, the growth of *M. tuberculosis* in the presence of such an antibiotic must be due to factors other than a possible selection of antibiotic-resistant mutants. Why *M. tuberculosis* present in the clinical sample was able to grow in HFD cells despite previous antibiotic decontamination and culture of HFD cells with added gentamicin is a matter for speculation. It is possible that some patients with tuberculosis and AIDS eliminate huge amounts of bacilli, making possible an inoculum effect-related phenotypic resistance to gentamicin. On the other hand, intracellular bacilli present in the clinical sample could infect cell cultures without being efficiently exposed to the antibiotic present in the cell culture medium.

AIDS patients frequently have multiple infections, and more than one organism may be involved. Although it is not possible to rule out the possibility of coinfection with a virus different from those screened in our clinical sample, the experimental data obtained by direct inoculation of *M. tuberculosis* onto cell cultures confirm that this organism is capable of producing CPE mimicking virus culture. *M. tuberculosis* and other mycobacteria have been grown on cell cultures mainly for investigative purposes (1, 4), but we have no knowledge of their isolation from clinical samples. Microbiologists must be aware of these possibilities and must confirm all CPE with the most advanced technological procedures. On the other hand, there is a real possibility of finding mycobacteria and viruses in a BAL from a patient with AIDS, but such a finding does not preclude the presence of other pathogenic organisms.

ADDENDUM

Since the first submission of the manuscript we have detected two additional cases of CPE mimicking virus culture due to *M. tuberculosis* from two BAL samples taken from patients with AIDS. One of them also produced CPE on A-549 and Vero cells, and screening for virus was negative in both cases.

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