Isolation of *Trichomonas vaginalis* from Cycloheximide-Treated McCoy Cells

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Sir,

Inoculation of cycloheximide-treated McCoy cell monolayers for culture of *Chlamydia trachomatis* from urogenital specimens is a widely used technique in laboratories with cell-culture facilities. Unfortunately, up to 15 % of the cultures cannot be interpreted due to destruction of the monolayer by bacterial, viral, toxic or other factors (1). Filtration of specimens through a 450 nm membrane filter, as is usual before inoculation of a specimen for viral culture, is not possible in chlamydial cultures due to the large size of the infective elementary bodies of *Chlamydia trachomatis*. Addition of antimicrobial drugs to the culture medium minimizes bacterial overgrowth, but resistant bacteria may survive, multiply and destroy the monolayer completely. Cell destruction without visible bacterial growth may be due to the presence of bacterial toxin in the specimen – for instance in toxic shock syndrome staphylococcal toxin may be present in high quantities. Viral cytopathogenic effect (CPE) is mainly attributed to the presence of herpes simplex virus (HSV). Subculture after freeze-thawing and membrane filtration of a duplicate culture reveals the typical HSV-CPE on HSV-susceptible cell-lines.

We report here on a less well-recognised cause of cell-monolayer damage due to growth of *Trichomonas vaginalis* on McCoy cells. Pindak et al. (2) recently reported that *Trichomonas vaginalis* readily multiplies on different cell-lines, including McCoy cells. In a four years period 1797 cultures for *Chlamydia trachomatis* were performed with 88 (4.9 %) positive results; 96 (5.3 %) of the monolayers were lytic, in 16 instances (16.7 %) due to growth of *Trichomonas vaginalis* and in 12 instances (12.5 %) due to a HSV-CPE. *Trichomonas vaginalis* was even isolated when both direct examination and specific cultures were negative. Nevertheless, as has been observed by Gentry et al. (3), *Trichomonas vaginalis* never grows in cell culture if specimens are frozen prior to inoculation. Initially, *Trichomonas vaginalis*-induced cell damage was interpreted as HSV-CPE with rounding and clumping of cells. After prolonged incubation complete cell-destruction was observed, mimicking a toxic effect. Careful examination with a microscope of the unfixed and unstained cells of the duplicate culture revealed numerous "motile cells". Examination of a drop of the culture suspension under greater magnification (× 400) with a conventional microscope showed *Trichomonas vaginalis* with its typical morphology and motility. We therefore suggest that "toxic" cultures on McCoy or other cell monolayers used for isolation of *Chlamydia trachomatis* be examined for the presence of *Trichomonas vaginalis*, especially if unfrozen specimens were inoculated.

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Meningitis due to *Cryptococcus neoformans* biovar *gattii* in a Zairean AIDS Patient

Sir,

Cryptococcosis is one of the most frequently diagnosed opportunistic infections in African AIDS patients. The steep rise in its incidence has greatly contributed to recognition of the AIDS epidemic in Central Africa (1, 2). *Cryptococcus neoformans* is a heterogeneous species which comprises two biovars: the classical biovar *neoformans* and the biovar *gattii*. The latter variety was first described in Zaire in 1970 (3), The two varieties can be differentiated on the basis of serotype (4, 5), physiology (6, 7, 8), morphology (3) and epidemiology (9).

In a recent communication (10) the biovars of 47 clinical isolates of *Cryptococcus neoformans* received from Zaire between 1951 and 1985 were reviewed. While the majority (6/7) of isolates received before 1969 belonged to the biovar *gattii*, all 40 isolates received after 1969 belonged to the biovar *neoformans*. Of the latter, 35 were from AIDS cases. Apart from that all of 30 strains isolated from Rwandese patients with proven AIDS and typed in our laboratory belonged to the biovar *neoformans*. The apparent abscence of *Cryptococcus neoformans* biovar *gattii* in patients with AIDS has also been observed in the USA (11, 12). That this might not be an absolute rule, is demonstrated by the following observation.

A 28 year old heterosexual Zairean man, worker in the copper mine of Musoshi at the Zairean-Zambian border, was admitted to the Sendwe General Hospital in Lubumbashi, Zaire, on 4 April 1986. He had a history of intermittent paroxysms of severe headache with sudden onset two days earlier. Physical examination revealed no abnormality, except for mental confusion accompanying the attacks of headache. He appeared to be in good physical condition: he was 174 cm tall with a body weight of 82 kg. Blood pressure and pulse rate were normal. He was febrile and remained so during his entire hospital stay in which periods of perfect lucidity alternated with episodes of drowsiness and mental confusion. Ophthalmoscopy revealed a bilateral fixed mydriasis and a normal fundus. Haemoglobin, total and differential leucocyte counts were within normal limits. A thick blood film was negative for malaria and a stool smear was negative for parasites. Urine sediment revealed numerous red and white blood cells. There was slight albuminuria and a urine culture was negative for bacteria. Spinal tap yielded a clear cerebrospinal fluid with 5/mm^3 lymphocytes, 55 mg/dl protein and 67 mg/dl glucose. An India ink preparation was positive for heavily encapsulated yeasts, which comprised both normal spherical and elongated rod-shaped cells. Culture on Sabouraud Dextrose agar at 37 °C yielded rapid growth of mucoid colonies showing the same microscopic features in the primary culture. As soon as cryptococcosis was diagnosed, therapy was begun with itraconazole, a triazole drug under clinical investigation (Janssen Pharmaceutica, Belgium), at a daily oral dose of 200 mg. The patient showed rapid improvement but after 12 days of treatment he developed intracranial hypertension. Because of progressive clinical deterioration, the patient was taken home by his family. He expired on 26 April less than one month after his first complaints.

Serum from the patient was sent to the Institute of Tropical Medicine in West Berlin for HIV antibody testing and was found both by ELISA and western blot analysis to be positive, confirming that he had suffered from AIDS. The *Cryptococcus* culture was sent to Belgium where it was identified as biovar *gattii* on the basis of the typical yeast morphology and the distinctive growth on selective canavanine-glycine-bromothymol blue agar (8). This was further confirmed by serotyping. The strain belonged to serovar B, the predominant serovar of the biovar *gattii* in Central Africa (9).

To our knowledge this is the first report on isolation of *Cryptococcus neoformans* biovar *gattii* from a patient with AIDS and also the first documented isolation of this variety in Central Africa in 17 years. This observation forces us to reconsider the hypothesis that only the classical biovar *neoformans* of the fungus is involved in patients with AIDS. Meanwhile, the temporary disappearance of the biovar *gattii* from Zaire since 1969 remains as mysterious as before.

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