Serological studies of a case of fatal craniofacial mucormycosis

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Abstract

Counter-immunoelectrophoresis and indirect immunofluorescence tests were used in a fatal case of craniofacial mucormycosis.

The indirect immunofluorescent test was rapid and more sensitive than counter-immunoelectrophoresis and provided estimation of serum antifungal antibodies (IgA, IgG and IgM).

The high serum IgA titer (comparable to IgM) found in this case of acute infection suggests a role of IgA antibodies which needs further investigation.

Introduction

The use of an indirect immunofluorescent (IF) test and counter-immunoelectrophoresis (CIE) was investigated in a case of craniofacial mucormycosis. The indirect immunofluorescent test was more sensitive than counter-immunoelectrophoresis, and it also permitted the determination of different serum immunoglobulins directed against fungal antigens. High titer of serum IgA and IgM and a low titer of serum IgG were observed. The high titer of serum IgA antibody suggests the possible importance of this antibody against the fungus in human infection. The use of sections prepared from paraffin-embedded fungal cultures offers a rapid and sensitive test for serologic diagnosis of infection.

Mucormycosis is an acute, rapidly progressing, opportunistic fungal infection caused by members of the Mucoraceae family (1). Among the most common organisms causing human infection are those which belong to the genera Rhizopus, Mucor and Absidia. The rapid rate of growth of the fungus and its extensive involvement of the vital organs have made early detection of the infection of prime importance, as the patient may be treated and possibly cured if the infection with the fungus is detected early (1, 2, 8). Current diagnostic methods include tissue biopsy and culture of organism, which both require time and may yield false negative results. Jones & Kaufman using immunodiffusion (5), and Smith (9) using counter-immunoelectrophoresis have claimed a high degree of specificity for their serological tests. We employed counter-immunoelectrophoresis and immunofluorescent tests to detect antibodies in the serum of a patient with craniofacial mucormycosis. The indirect immunofluorescent test was more sensitive and specific than counter-immunoelectrophoresis, and showed a high titer of serum IgA antibodies against the fungus.

Materials and methods

Antigen preparation

Cultures of Rhizopus species, Mucor pusillus, Absidia corymbifera and Syncephalastrum species were maintained on Sabouraud’s dextrose agar slant...
at 30 °C temperature. Exoantigens were prepared by adding 5 ml of 1:5 000 dilution of merthiolate in saline solution directly to the culture and incubating for 24 hours at 30 °C (3). The solution was then decanted and centrifuged at 10 000 g for 10 minutes to remove particulate matter. The supernatant was used as the exoantigen preparation. Total antigens of each were prepared by homogenizing the mycelium of the fungi with a Dounce homogenizer. The mixture was centrifuged at 10 000 g for 10 minutes and the supernatant was collected and used as total antigen. Lowry's method of protein determination was used (7). Protein concentration in the different antigen preparations for counter-immunoelectrophoresis was adjusted to the same level by phosphate buffered saline.

**Counter-immunoelectrophoresis**

The Hyland kit for CIE (085-015) was used in all studies. Sera to be examined were placed in separate wells. The antigen preparations were then placed in parallel rows next to the sera on the cathode side. Electrophoresis was carried out in a constant current of 23 mA for 1 h in 0.075 M barbital buffer, pH 8.3. The plate was then soaked for 1 h in 0.1 M acetate buffer, pH 4.3, to reduce the nonspecific reaction of the C-reactive protein with the polysaccharides in the antigen preparation. The plate was drained and examined visually, photographed by indirect background illumination.

**Immunofluorescence studies**

Fluorescein isothiocyanate (FITC) conjugates of rabbit anti-human IgA, IgD, IgE, IgG and IgM immunoglobulins, prepared against the heavy chains of immunoglobulins from Atlantic Antibodies (Westbrook, Maine), were used in all tests. Optimal dilutions of labelled antisera for detection of serum antibodies with mucormycosis were found to be 1:80 for IgG, 1:20 for IgA and 1:10 for IgM. Anti-IgD and anti-IgE sera were used without dilution.

The indirect immunofluorescent technique (6) was used to detect the presence of antibodies against different fungal antigens. The fungi were grown on Sabouraud's dextrose agar plates for 24–36 h at 30 °C. The agar with the aerial hyphae was cut into 0.5 cm cubes. Some of the blocks were quick-frozen in Cryoquick (polyethylene glycol) and sectioned in the cryostat. The sections were placed on microscopic slides and used without fixation. The other blocks were fixed in chloroform, embedded in paraffin, and sectioned. The sections were then deparaffinized in xylene, and rehydrated through graded dilutions of ethyl alcohol in phosphate buffered saline at pH 7.4. The sections were incubated with various dilutions of sera at room temperature for 30 minutes in a moist chamber. They were then washed three times in 0.01 M phosphate-buffered saline, pH 7.4, and incubated in a humidified chamber with FITC conjugates of rabbit sera at room temperature for 30 minutes. They were washed three times in phosphate-buffered saline, pH 7.4, and mounted in a 1:1 mixture of glycerol and phosphate-buffered saline.

A direct immunofluorescence technique was used to examine the formalin-fixed, paraffin-embedded surgical specimens of infected tissues of the patient to demonstrate the in vivo binding of various antibodies to the invading fungus. FITC conjugates of rabbit antisera of human immunoglobulins (IgA, IgD, IgE, IgG, and IgM) were applied directly to deparaffinized sections containing invading fungus and then incubated in a moist chamber at room temperature for 30 minutes. The sections were then washed three times with phosphate-buffered saline, pH 7.4, and mounted in glycerol phosphate-buffered saline (1:1) solution.

All sections were examined with a Zeiss RA-38 fluorescent microscope using epi-illumination and oil immersion dark-field illumination. A 100 Watt halogen lamp was used for illumination in combination with the FITC interference filter and 500 nm barrier filter.

**Enzymic treatments of exoantigens preparations**

Seven different enzymes were used to treat the fungal exoantigen preparations before counter-immunoelectrophoresis, to determine the nature of the *Rhizopus* exoantigen responsible for the precipitant bands (Table 1).

The exoantigen preparations contained 550 mg protein/ml.

**Source of sera**

A 50-year old diabetic with a kidney transplant was found to have a mucormycotic ulcer on the hard palate. The infection rapidly progressed with