Serological diagnosis of pulmonary aspergillosis*

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Abstract

Methods for the preparation of antigens from clinically isolated cultures of Aspergillus were standardized. Sera from 25 suspected cases of pulmonary aspergillosis were tested against antigens prepared by us, from 4 strains of A. fumigatus and one strain of A. flavus, using the Ouchterlony double diffusion and immunoelectrophoretic techniques.

Of the 25 sera tested, 18 reacted positively with antigens of A. fumigatus, one with A. flavus and 2 with both these species. Antigens of two non-pathogenic Aspergilli included in the study failed to react with any of the sera. Our antigen preparations gave more numerous as well as sharper precipitin lines than the commercial Bencard antigens which were used for comparison. Moreover, mycelial antigens from 48 to 96 h old cultures revealed precipitin lines comparable to that of the routine, 4 week old culture filtrate antigens, thus suggesting that the incubation period for obtaining antigens could be cut down considerably.

Introduction

Aspergillus organisms have been associated with human disease as tissue invaders, cavity colonizers and antigenic stimulators of the immune system leading to hypersensitivity syndrome. Of the 150-odd species, relatively few have been ascribed to disease-producing organisms. A. Fumigatus Fresenius is the species usually associated with disease, though other Aspergillus species, such as A. flavus Link and the niger group ('A. niger') have been described as possible causal organisms (3, 5, 13). Several cases of pulmonary aspergillosis reports from India show that Aspergillus fumigatus, A. flavus and A. niger are the dominant pathogenic species and among these A. fumigatus is considered to be the chief causal agent of this disease (3, 11, 12, 13).

In the diagnosis of aspergillosis, culture of the sputum may not always be reliable since there is often no communication between the aspergilloma and the bronchi. The presence of precipitating antibodies against extracts of organisms of the genus Aspergillus, and the species A. fumigatus in particular, in the sera of patients suffering from pulmonary aspergillosis was reported by Pepys et al. (9). With the advancement in serological methods, Ouchterlony's double diffusion test has been increasingly used in the diagnosis of pulmonary aspergillosis (1, 3, 6).

Even though pulmonary aspergillosis is apparently common in India, facilities for sero diagnosis are generally lacking. The required antigens have to be imported. The present study was therefore undertaken with a view to prepare standard antigens to test the sera of suspected cases of pulmonary aspergillosis. Strains of the species A. fumigatus and A. flavus, being known to be pathogenic, were mainly used. The antigens were compared with commercial Bencard antigens (Bencard, Brentford,
Table 1. Source of fungi and types of antigens used.

<table>
<thead>
<tr>
<th>No.</th>
<th>Fungi</th>
<th>Accession No.</th>
<th>Obtained from</th>
<th>Source Isolated from</th>
<th>Type of antigen (CFT) 4 wk</th>
<th>% of protein CFT</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aspergillus fumigatus</em></td>
<td>FA</td>
<td>Sputum</td>
<td>Tuberculosis Sanatorium, Tambaram, Madras</td>
<td>CFT</td>
<td>0.07</td>
<td>0.53</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus fumigatus</em></td>
<td>FJ</td>
<td>Sputum</td>
<td>Tuberculosis Sanatorium, Tambaram, Madras</td>
<td>CFT</td>
<td>0.07</td>
<td>0.58</td>
</tr>
<tr>
<td>3</td>
<td><em>Aspergillus fumigatus</em></td>
<td>FR</td>
<td>Sputum</td>
<td>Tuberculosis Sanatorium, Tambaram, Madras</td>
<td>CFT</td>
<td>0.05</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td><em>Aspergillus fumigatus</em></td>
<td>SP-285</td>
<td>Sputum</td>
<td>Vallabhbhai Patel Chest Institute, University of Delhi</td>
<td>CFT</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td><em>Aspergillus flavus</em></td>
<td>A. fl</td>
<td>Sputum</td>
<td>Personal collection of Dr. Indira Kalyanasundaram</td>
<td>CFT</td>
<td>0.05</td>
<td>0.35</td>
</tr>
<tr>
<td>6</td>
<td><em>A. candidus</em></td>
<td>MUBL 186</td>
<td>Wheat</td>
<td>Culture Collection of Madras University Botany Laboratory, Madras-5</td>
<td>CFT</td>
<td>0.07</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><em>A. chevalieri</em></td>
<td>MUBL 372</td>
<td>Rice</td>
<td>Culture Collection of Madras University Botany Laboratory, Madras-5</td>
<td>CFT</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Bencard (B)</td>
<td>1</td>
<td>Commercially obtained from Bencard, Brentford, England</td>
<td>-</td>
<td>CFT</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>A. fumigatus</em></td>
<td>2</td>
<td>Commercially obtained from Bencard, Brentford, England</td>
<td>-</td>
<td>CFT</td>
<td>0.30</td>
<td>-</td>
</tr>
</tbody>
</table>

England) by double diffusion and immuno-electrophoresis. In order to exclude errors owing to nonspecific reactions, two non-pathogenic species of *Aspergillus* namely, *A. candidus* Link and *A. chevalieri* (Mangin) Thom and Church, isolated by our colleagues from stored grain, were also included in the study. Antigens were prepared by the standard method involving culture filtrates; since this proved time-consuming owing to the prolonged incubation required, an attempt was made with mycelial antigens, with a view to cut down the incubation time (4).

Materials and methods

Sources of antigens and sera

The fungi and their sources are presented in Table 1. Sera from suspected cases of pulmonary aspergillosis were obtained from Government Tuberculosis Sanatorium, Tambaram, Madras, and they are referred to in the text by arabic numerals, as serial numbers from 1 to 25.

Antigens from culture filtrate (CFT)

Glucose peptone broth (Glucose-20.0 g; Peptone-10.0 g and water – 1000 ml) was used for growing the fungi. Conidial suspension prepared from 3 day old cultures on glucose peptone agar slants was used for inoculation and the cultures were grown at 37 °C for 4 weeks in static condition (5). The culture fluid was freed from spores and mycelium by filtration through four layers of cheese cloth followed by centrifugation at 12 000 g for 30 minutes. The culture filtrate was then dialysed