SEROLOGICAL STUDIES ON ASPERGILLUS FUMIGATUS

by

MASAO FUKUI & JUNICHI YASUDA

Department of Bacteriology, Research Institute for Microbial Diseases
Osaka University, Osaka, Japan

(25.VII.1960)

INTRODUCTION

At present, serological studies on pathogenic fungi appear to be at a turning point toward a promising future. Species-specific antigen-antibody reactions are now introduced into the taxonomy of fungi, which hitherto had been based only on morphological findings. Also, the role of serological reactions in the diagnosis of mycoses has come to be reevaluated.

Until recently, no one attempted to study the serology of fungi either for taxonomic identification or for practical diagnosis, because of the great difficulty in obtaining a high titer immune serum and of too poor specificity. Although considerable improvements have been achieved in the serology of the species Candida chiefly by Japanese mycologists, in the case of Asp. fumigatus the difficulties have not yet been overcome. With sera of aspergillosis patients, NICAUD (1929) failed to demonstrate agglutination of the conidia. Using rabbit immune sera, MATSUMOTO (1929) obtained positive precipitation with culture filtrate antigen, but negative data with extracts of fungous cells. Using sera of rabbits immunized with cell sap of Asp. fumigatus and of Cephalosporium acremonium, HENRICI failed to show precipitation of homologous cell saps. However, he obtained positive results by precipitin tests with Asp. fumigatus cell sap and the sera of the rabbits infected with its conidia. MATSUMOTO also observed positive complement fixation with culture filtrate antigen and the rabbit immune sera, though he could not clearly demonstrate its correlation to the taxonomy based on morphology. Aspergillin, devised by the National Institutes of Health (U.S.A.) for skin test antigen, has not yet been widely accepted because of its irritative effect and its poor specificity.

Since one of the present authors (M.F.) isolated a strain of Asp. fumigatus from a lung cavity of a penguin in 1953, the biological
properties of the isolated mold from the standpoint of medical microbiology have been studied. It should be mentioned that by disintegrating cell walls through repeated cryolysis, the authors were able to obtain from the *Asp. fumigatus* pellicle a substance which was highly toxic to mice. Applying the same procedure to the formalin-killed mycelia of *Asp. fumigatus* and using the extract so obtained as an immunizing antigen the authors were able to obtain high-titer and highly specific rabbit immune sera. With these immune sera the specificity of various antigens prepared from the mold have been studied, the details of which are given in the following chapters.

I. PREPARATION OF IMMUNE SERUM

In our experiments *Asp. fumigatus* HANSHIN strain (isolated by one of the authors), No. 4040 strain\(^1\) and No. 4140 strain\(^1\) were used.

Three loopfuls of the conidia were inoculated into 130 ml of Czapek's fluid medium and cultivated at 37° C for 3 days, shaking the culture continuously. By this method, the mold does not develop conidia and grows to form a mass of mycelia. Formalin was added to 3 % and the culture was left at room temperature for another 3 days. After confirming the death of the mold, the mycelia were collected and ground with a mortar and pestle until pasty in appearance. The paste was then dried in chilled acetone, 0.5 mg of the acetone dried powder suspended in 5 ml of physiological saline frozen by immersing the tube into chilled acetone and then thawed in a water bath at 37° C. After this procedure was repeated 15 times, the supernate was separated by centrifugation. This extract will hereafter be called ME (an abbreviation for mycelial extract). 0.5 ml of ME (started from 50 mg of dried mycelia) could be injected into mice without giving any toxic effect.

Two rabbits were intravenously immunized with ME and its residue to a concentration of 0.5 mg per ml. The doses given were 0.5 ml for the first injection and 1 ml for the second to the tenth injection. Allowing 10 days to elapse after the final injection, the animal was bled and the serum separated. After inactivation at 56° C for 30 minutes, the sera were stored at −60° C. No animal died during the immunization.

a) Precipitation

The immune serum was tested by the precipitin test (ring-method) against the ME and the conidial extract (CE). Conidial extract was prepared as follows: 20 loopfuls of conidia were suspended in 1 ml of saline and repeated cryolysis was performed as in the case of the suspension of dried mycelia. The suspension was then filtrated to eliminate the remaining conidia and the debris.

\(^1\) delivered from the Institute for Fermentation (Osaka).