
THE CYTOTOXIC ACTIVITY IN THE CULTURE FILTRATE OF *ASPERGILLUS FUMIGATUS* AND ITS ACTIVE COMPONENTS

Kamei K, Watanabe A, Sekine T, Hashimoto Y, Ochiai E, Higurashi H
Res Center for Pathogenic Fungi & Microbial Toxicoses, Chiba University, Chiba, Japan

Aspergillosis is the leading cause of death in hematological malignant diseases. The causative agent, *Aspergillus fumigatus*, is also known to cause serious infections in apparently immunocompetent individuals. The virulence factor(s) of this fungus, however, is yet to be known. To help determine the virulence factor and the mechanism of the infection, the biological activities of culture filtrate of *A. fumigatus* were examined.

Conidia of *A. fumigatus* were cultured at 37°C in RPMI 1640 in 24-well microplates. The culture filtrate was collected, and stored at -80°C. Murine peritoneal cells were collected from the peritoneum of BALB/c mice, and human peripheral polymorphonuclear neutrophils were separated from the venous blood of healthy volunteers. To determine the cytotoxic activity of the culture filtrates, murine peritoneal macrophages or human peripheral polymorphonuclear neutrophils were cultured with or without the filtrate, and the viability of the neutrophils was determined using TetraColor One colorimetric system. Morphological changes were also examined under microscopy. To know the biological activity of the filtrate in vivo, the filtrate was injected intraperitoneally with conidia of *A. fumigatus* into 6 week-old male ddY mice. Control mice received either the fungus or the filtrate alone. When the survival rate of these mice was compared, the survival of the mice injected with both the fungus and the filtrate was significantly lower. Pathological examination of the mice confirmed the massive growth of *A. fumigatus*. These findings suggest that the activity of the filtrate might contribute to the development of the infection by causing a breach of the defense system.

To learn of the active substance(s) in the culture filtrate, the filtrate was analyzed using gas-chromatography mass-spectrometry. Analysis of the filtrate using gas-mass spectrometry disclosed gliotoxin and some other unknown components. The content of gliotoxin in the filtrate, however, was too low to exhibit the potent cytotoxic activity shown in our study. On the other hand, one fraction of the filtrate that contained no gliotoxin showed strong cytotoxic activity, which indicates that a component(s) other than gliotoxin plays a major role in the activity of the filtrate.

The mechanism of infection by *A. fumigatus* has been a big mystery for decades. The fungus is capable of causing chronic infections in apparently healthy patients, and aggressive, fatal diseases in immunocompromised patients. The treatment methods we are able to use today are of limited efficacy, and a new therapeutic strategy has been long awaited.

Although a number of reports have been made on the activity of *A. fumigatus* culture filtrate, little has been known about its cytotoxic activity against neutrophils. In a previous study we found that highly aerated conditions contributed to the development of strong cytotoxicity. In the well ventilated environment of the lung, the fungi are also exposed to a higher content of oxygen and this fact may help to exert strong toxicity from the fungi.

Whether the fungus is able to produce the active substance(s) at the site of infection is not yet known, and the precise significance of the substance(s) in the development of the disease is still at large. Our study, however, suggests that the activity of the filtrate may play an important role in vivo.