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# Pulmonary Fungal Infection in Patients with Healed Tuberculosis or Other Underlying Diseases

**KIM, Sang Jae, Young Pyo HONG and Sung Chin KIM**

(Korean Institute of Tuberculosis, Korean National Tucerculosis Association, Seoul, Korea)

# 肺結核 또는 其他 疾患患者에 있어서의 肺眞菌症에 關한 硏究

金 尙 材·洪 永 杓·金 成 鎭

(大韓結核協會, 結核硏究院)

## ABSTRACT

One hundred and thirteen healed pulmonary tuberculosis patients and 11 patients with other underlying diseases were studied for evidence of pulmonary fungal infection because of persisting hemoptysis or chronic cough. Radiological, mycological and serological investigations revealed that 54 out of 124 patients were evidently infected with one or more species of fungi. *A.fumigatus* was isolated from 4 out of 70 patients whose sera did not react with antigens from this fungus, while it was isolated from 43 out of 47 serological reactors to this fungus. Chest radiography showed a distinct fungus ball in a cyst of one patient and in a preformed cavity in the lung of 17 healed tuberculosis patients and two other patients. The latter two patients were infected with *A. flavus*. Two patients, who were under the long period of immunosuppressive therapy, apparently succumbed to invasive aspergillosis due to *A. fumigatus*. A single or dual infection with *A. flavus*, *A. nidulans*, *A. nidulans var. latus*, *C. albicans*, and *P. boydii* were noticed in some patients without mycetomal shadow on chest radiographs.

Young mycelial extract (ME) of *A. fumigatus* detected antibody in 95.8 percent of the sera from patients infected with this fungus, while the commercial culture filtrate antigen (GL) yielded 78.7 per cent positive result. Culture filtrate antigen,however, was comparable with ME. There was no single antigen with which all the serum specimens reacted. Fractionation of ME resulted in a loss of some activity although it excluded substances that reacted with C-reactive protein. Most reactive and specific precipitinogens distributed in the fraction (FB) which was precipitable at 75 percent saturation with ammonium sulfate and eluted in a second peak in order from gel-filtration and which contained mostly proteinic components. Glycoproteins or polysaccharides rich fractions (FA and ASI) were relatively less effective in detecting antibody.

Demonstration of antibody in the serum from patients using a battery of fungal antigens and of etiologically related fungi from clinical specimens are very useful laboratory procedures for the diagnosis of pulmonary fungal infection which is a common complication of tuberculosis.

## INTRODUCTION

Although the healthy individual is naturally resistant to infection with fungi, which are ubiquitous in nature, a variety of fungal species can procuce pulmonary diseases in the compromised hosts(Kim, 1975; Kim, 1980; Emmons *et al.*, 1977). Atopic individuals are vulnerable to allergic pulmonary mycoses characterized by rhinitis, asthma, and acattered eosinophilic infiltrates without actually invading the tissue(Pepys *et al.*, 1969). Aspergilli and many other fungi produce fungus ball in the cavity or in the ectated bronchus, which have been formed most often by tuberculosis, carcinoma, histoplasmosis, sarcoidosis or bronchiectasis (Kim, 1980; Emmons *et al.*, 1677, BTA, 1970). Invasive form mycoses develop in the immunologically committed individuals and the widely disseminated cases usually result in a fatal outcome (Kim, 1980, Young *et al.*, 1971). Definitive diagnosis can be made by demonstrating the fungus in the affected lung field,even if it is not always possible (Emmons *et al.*, 1977). Isolation of the fungus from sputum specimen is not a sufficient evidence. Demonstration of antibody to etiologically related fungus in serum specimen, therefore, is a very helpful adjunct for the diagnosis of pulmonary mycoses, except for the severely disseminated form of invasive mycoses (Young *et al.*, 1971; Coleman *et al.*, 1972; Kim *et al.*, 1979; Chaparas *et al.*, 1980; Longbottom *et al.*, 1964; Mackenzie *et al.*, 1975). The results of various serodiagnostic tests in patients with various forms of mycoses have been conflicting because of variations in sensitivity,the qualitative nature of test systems, and the wide-spread use of unstandardized antigen preparations (Kim *et al.*, 1978; Kim *et al.*, 1978; Kim *et al.*, 1979; Pepys, 1978). However it has been published elsewhere that mycelial extract (ME) and its fractions of *Aspergillus fumigatus* was effective for the detection of antibody in sera obtained from patients infected with this fungus by a simple immunodiffusion test(Kim *et al.*, 1978; Kim *et al.*, 1978; Kim *et al.*, 1979).

In this study,we have investigated 54 cases of pulmonary mycotic infection in patients with healed tuberculosis or other underlying diseases, in regard of special references of laboratory aspects such as isolation of etiologically related fungus from clinical specimens and demonstration of antibody in patient's sera using the commercial and experimental antigen preparations of fungi.

## MATERIALS AND METHODS

Antigens: *A.fumigatus* extract was prepared, as described previously(Kim *et al.*, 1978; Kim *et al.*, 1978; Kim *et al.*, 1979), by mechanical disruption, and fractionated by ammonium sulfate precipitation, gel-filtration, and passage through an anion exchange column. Fractions FA and FB were precipitable at 75 percent saturation with ammonium sulfate, and eluted in order from a Sephadex G-75 column. Fraction ASI was soluble at 75 percent saturation with ammonium sulfate, and bound firmly to a DE-52 column and eluted with 2M sodium chloride. Antigens of other species were prepared from filtrates of 5 weeks stationary culture in Sabouraud's broth medium at 28C. Culture fitrate was separted from mycelia and concentrated by vacuum dialysis using Visking cellulose membrane (1-cm diameter). The concentrated antigens were stored at -20C in 50 percent glycerine. Protein and carbohydrate contents of culture filtrate antigens were analysed by the method of Lowry and associates and by a resorcinol method as described elsewhere (Kim *et al.*, 1978; Kim *et al.*, 1978). The antigen concentrations used for immund-

iffusion tests were 2, 3, 1, 1, 0.5, and 12 mgs per ml of 9 month-old culture filtrate (CF), mycelial extract (ME), FA, FB, FC, ASI, and Greer Laboratory antigen (GL) respectively.

Patients: One hundred and thirteen tuberculosis patients whose main complaints were chronic cough or continued hemoptysis although their sputum specimens were devoid of tubercle bacilli as a result of successful anti-tuberculous chemotherapy, were employed in this study. Seventeen of them had a distinct fungus ball shadow in a cavitary lesion on their chest radiographs and fungal masses were demonstrated in resected specimens from two patients. A distinct mycetomal shadow was seen in chest radiographs of two patients without a noticiable current or past underlying disease. One patient had a fungus ball in a large cyst. Two patients were under the long period of immunosuppressive therapy in order to control a rejection response against transplanted kidney and hepatoma respectively. Additional six patients with nontuberculous underlying diseases or unknown were also included in this study.

Blood was drew from all of those patients (124) and serum was saparated and merthiolated within few hours. Sera were stored at 1 to 4C until used. Fresh spot sputum was collected from patients after rinsing mouth and cultured to isolate the fungi implicated within six hours after collection. In most cases, several serum and sputum specimens were collected from a patient. Open lung biopsy specimen or bronchial washings were obtained from some patients.

Isolation of the fungus: Sputum or other clinical specimen was inoculated on Sabouraud's and blood agar media containing 50 mcg per ml of chloramphenicol and incubated at 28C and 37C for up to 4 weeks. Generic or species identification of the isolated fungi was based on their colonial and microscopic morphology (Ainsworth *et al.*, 1973; Raper *et al.*, 1965).

Immunodiffusion tests: Double immunodiffusion tests were performed basically according to the methods described by Crowle (5) in 1 percent Noble agar containing 1 percent sodium azide on 2.5×7.5cm glass slides. Serum well (3mm diameter) and antigen well (2mm diameter) were punched out of the agar a 3mm apart. After incubation for 72 hours at room temperature the slides were observed before and after washing with 5 percent sodium citrate to dissociate precipitate produced with C-reactive protein.

## RESULTS

Of the 113 patients with healed tuberculosis, sera from 49 patients produced positive precipitin reactions with homemade or commercial fungal antigens and the etioloically related fungi were isolated from sputum or other clinical specimens in 44 patients. Isolation of etiologically related fungus has been failed in 5 patients even if their serial serum specimens continually produced a strong precipitin reaction with fungal antigens tested, Five out of 11 patients with nontuberculous underlying diseases were found to have been infected with fungus as seen in table 1.

**Table 1.** Over-all findings of investigations of 124 patients.

| Underlying diseases | Number of cases investigated | Numbe of cases apparently infected with fungus |
|---|---|---|
| Tuberculosis | 113 | 49 |
| Notuberculous diseases | 7 | 3 |
| Unknown | 4 | 2 |
| Total | 124 | 54 |

Etiologically related or unrelated fungi isolated from sputum or other clinical specimens are listed in table 2.

**Table 2.** Fungi isolated from sputum or other clinical specimens of 123 patients

| Fungi isolated | *53 cases apparently infected with fungus | 70 cases probably not infected with fungus |
|---|---|---|
| *A. fumigatus* | 43(81.1) | 4 ( 5.7) |
| *A. flavus* | 2( 3.8) | 3 ( 4.3) |
| *A. nidulans* group | 1( 1.9) | 2 ( 2.7) |
| *A. ochraceus* group | 0 | 3 ( 4.3) |
| *A. versicolor* group | 0 | 3 ( 4.3) |
| *Penicilium* spp. | 0 | 9 (12.9) |
| *Petriellidium boydii* | 1( 1.9) | 0 |
| *Candida albicans* | 5( 9.4) | 10(14.3) |
| Unidentified yeast like fungi | 0 | 10(14.3) |
| Unidentified filamentous fungi | 0 | 8(11.4) |
| No growth | 5( 9.4) | 38(54.3) |

Some patients contained more than one species of fungi in their sputum, therefore total of listed cases does not consist with total number of cases studied.

Numbers in parentheses represent percent.

*In this column we did not include the patients from whom etiologically unrelated fungi were isolated.

A variety of fungi were encountered in the sputum specimens from the patients without pulmonary fungal infection. *Candida albicans* and unidentified yeast-like fungi were most commonly isolated from the sputum specimens of those patients. *C. albicans*, however, caused

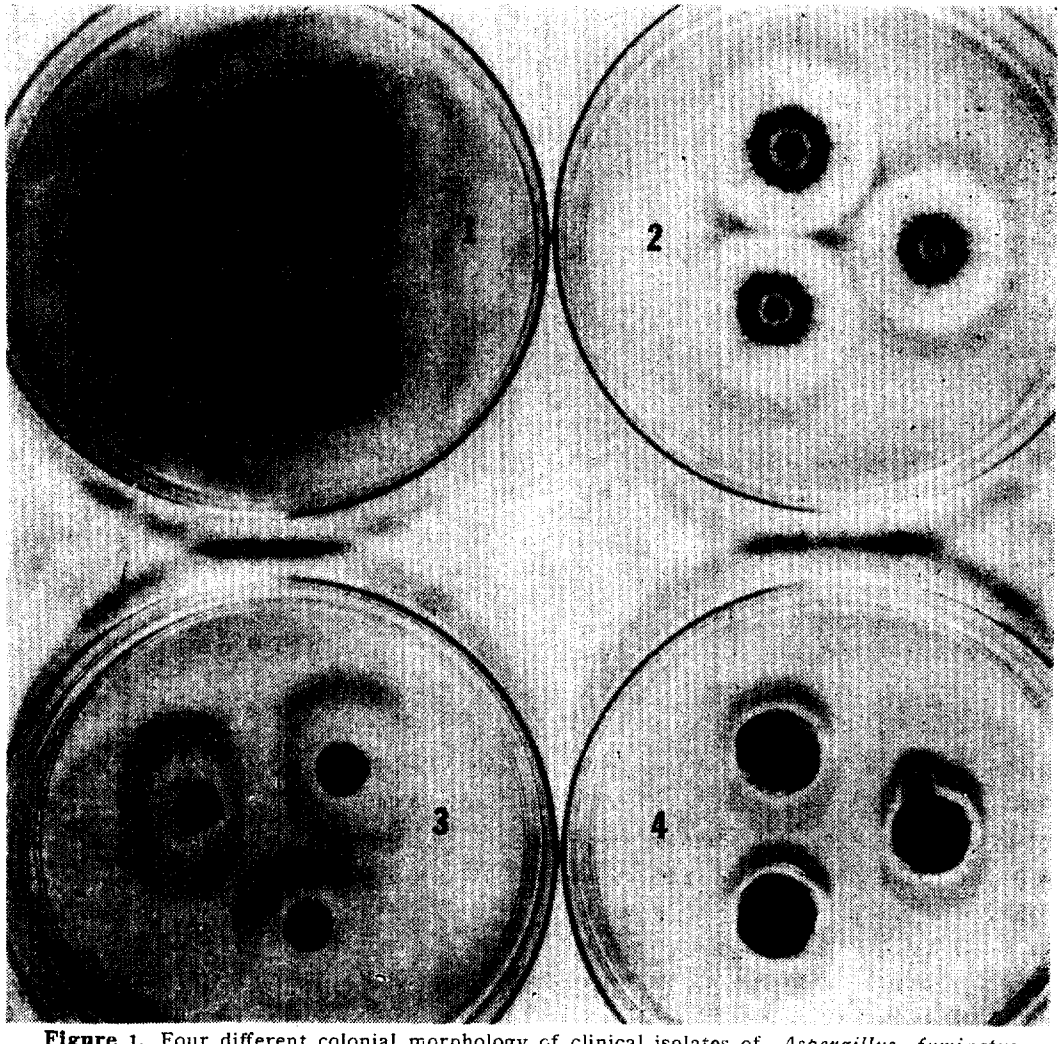


**Figure 1.** Four different colonial morphology of clinical isolates of *Aspergillus fumigatus*, grown on Czapek's solution agar for 5 days at 30C

**Table 3.** Double immunodiffusion analysis of serum specimens from patients with pulmonary mycoses and cultural findings of sputum or other specimens

| Patients | Antigens *Aspergillus fumigatus* | | | | | | | | | | | Cultural findings | Specific clinical diagnosis and complications |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GL | CF | ME | FA | FB | FC | ASI | AV | AN | CA | PB | | |
| 77-1729 | 1 | 4 | 2 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | AF & CA | M+TB |
| 78-1663 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| 77-118 | 0 | 4 | 1 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | AF | M+TB |
| 77-1216 | 2 | 4 | 4 | 2 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | AF | M+TB |
| 78-1718 | 3 | 5 | 4 | 3 | 4 | 1 | 2 | 1 | 0 | 1 | 0 | AF | M+TB |
| F-11 | 1 | 2 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | AF | M+TB |
| F-15 | 3 | 7 | 4 | 4 | 4 | 1 | 5 | 0 | 0 | 0 | 0 | AF | M+TB |
| 79-1884 | 1 | 3 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| 80-1480 | 5 | 4 | 2 | 2 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| F-18 | 0 | 2 | 3 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| 79-2288 | 3 | 5 | 3 | 2 | 3 | 2 | 3 | 0 | 0 | 1 | 0 | AF | M+TB |
| 78-1706 | 1 | 2 | 2 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | AF & Y | M+TB |
| 80-2088 | 1 | 4 | 2 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| 77-1170 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | AF | M+TB |
| 81-274 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | AF | M+TB |
| 80-1833 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| 80-398 | 2 | 2 | 2 | 2 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | AF | M+TB |
| 78-1534 | 3 | 7 | 3 | 2 | 6 | 2 | 2 | 0 | 0 | 0 | 0 | AF | TB |
| 79-1898 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF | TB |
| 77-13 | 3 | 3 | 1 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | AF | TB |
| 79-1978 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF & CA | TB |
| 77-1332 | 1 | 2 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| 80-171 | 3 | 5 | 3 | 2 | 4 | 1 | 2 | 0 | 0 | 0 | 0 | AF | TB |
| 78-679 | 3 | 2 | 2 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | AF | TB |
| 80-354 | 3 | 2 | 1 | 1 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| 79-1031 | 1 | 2 | 1 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| 80-995 | 1 | 2 | 1 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| F-17 | 3 | 7 | 4 | 4 | 2 | 2 | 2 | 1 | 0 | 1 | 0 | AF | TB |
| 80-2077 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF | TB |
| 78-1797 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 0 | 1 | 0 | 0 | AF | TB |
| F-22 | 2 | 3 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| F-23 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF | TB |
| 80-1920 | 1 | 2 | 2 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | AF & CA | TB |
| F-31 | 0 | 2 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| 79-2076 | 1 | 4 | 2 | 3 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| 81-386 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | AF | TB |
| 80-788 | 1 | 5 | 2 | 3 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | AF | TB |
| 79-1355 | 1 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | AF | TB |
| 80-1423 | 2 | 2 | 1 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | AF | TB |
| 77-553 | 2 | 5 | 3 | 4 | 3 | 2 | 3 | 0 | 0 | 0 | 0 | AF | TB |
| F-20 | 2 | 2 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | CA | TB |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 79-1680 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | TB |
| 80-1815 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | | TB |
| F-89 | 1 | 5 | 3 | 2 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | | TB |
| HY-261816 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF | M+C |
| LKH | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | AF | IA |
| NOJ | 3 | 3 | 2 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | AF | IA |
| 77-1174 | 0 | 1 | 0 | | | | | 4 | 0 | 5 | 0 | AV & CA | TB |
| F-9 | 0 | 0 | 0 | | | | | 0 | 0 | 0 | 0 | AV | M |
| PMH | 0 | 0 | 0 | | | | | 3 | 0 | 1 | 0 | not done | M |
| 79-985 | 0 | 0 | 0 | | | | | 0 | 1* | 0 | 0 | ANL | TB |
| F-15 | 0 | 0 | 0 | | | | | 1 | 2 | 1 | 0 | | TB |
| 77-1425 | 0 | 0 | 0 | | | | | 3 | 0 | 2 | 4 | PB & CA | TB |
| 81-48 | 0 | 0 | 0 | | | | | 2 | 0 | 3 | 0 | CA | TB |

Numbers in table represent number of precipitin bands. For explanation of antigen terms, see *Materials and methods*. Abbreviations: AF=*A. fumigatus*, ANL=*A. nidulans* var. *latus*, AV=*A. flavus*, C=cyst, CA=*C. albicans*, IA=invasive aspergillosis, M=fungus ball, PB=*Petriellidium boydii*, TB =tuberculosis, Y=unidentified yeasts.

*Sera from this patient reacted only with antigon prepared with *A. nidulans* var *latus* isolated from the patient own sputum specimens.

pulmonary infection in 9.4 percent of the patients apparently infected with fungi. *A. fumigatus* was isolated from 5.7 percent of the patients not infected with fungi, while this fungus was demonstrated in the specimens of 81.1 per cent of the patients with pulmonary fungal infection. Variation in colonial and/or microscopic morphology of clinical isolates of *A. fumigatus* has been observed in many cases as seen in figure 1. Penicilli were also commonly encountered in the sputum specimens but did not cause pulmonary infection in this study.

Precipitin reactions of the serum specimens from the patients, who have been apparently infected with fungi, with the commercial and experimental preparations of *Aspergills* and other fungal antigens are shown in table 3, along with cultural findings of their clinical specimens and with their probable clinical diagnosis and underlying diseases. Sera from 47 patients produced positive precipitin reaction with *A. fumigatus* antigens and this fungus was isolated from the specimens of 43 cases. Repeated tests of serially collected serum and sputum specimens produced positive result in most cases. *A. fumigatus* was not isolated from 4 patients although their sera showed a strong reaction with antigens from this fungus. A distinct fungus ball was seen in chest radiographs of 18 patients whose sera produced positive precipitin reaction with *A. fumigatus* antigens and whose sputum specimens yielded positive culture of *A. fumigatus*. Seventeen of them had a fungus ball in a preformed cavity due to tuberculosis and one patient in a large cyst. Sera from two patients, who were under the long period of immunosuppressive therapy, showed a positive reaction with *A. fumiatugs* antigens and this fungus was demonstrated in an open lung biopsy specimen of patient LKH and in sputum specimens of patient NOJ, suggesting that they were suffered from invasive aspergillosis. Sera from patient 77-1174 and PMH produced a strong precipitin reaction with *A. flavus* antigens. The former patient's serum also reacted with *C. albicans* antigen and both fungi *A. flavus* and *C.albicans* were isolated repeatedly from his sputum specimens. The latter patient had a fungus ball in his chest

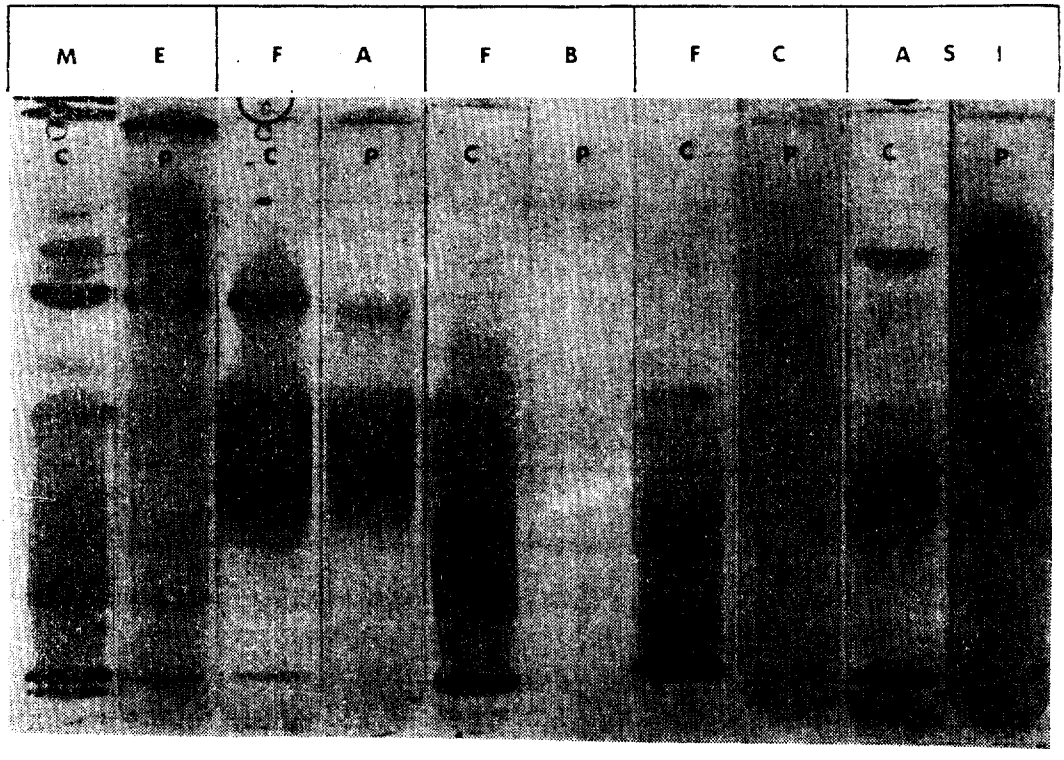


**Figure 2.** Two-dimensional immunoelectrophoresis of young mycelial extract (ME) of *Aspergillus fumigatus*. Eight mg of ME were first separated electrophoretically in a direction to the left of the origin. Electrophoresis was then conducted so that the saparated antigens migrated into agar containing rabbit anti-*A. fumigatus* hyperimmune serum (from bottom to top).

radiograph.F-9 patient was also infected with *A. flavus* because this fungus was repeatedly isolated from her sputum specimens collected at different times for a period of over one and half years although her sera continuously showed a negative precipitin reaction prepared even with antigen with clinical isolate from her sputum specimen. A distinct fungus ball was seen in her chest radiograph. but she had no history of tuberculosis *A. nidulans* var. *latus* was continuously isolated from sputum specimens of patient 79-385 and his sera weakly reacted only with antigen prepared with this fungus. Serum specimen of F-13 patient showed a strong reaction with *A. nidulans* antigen but the fungus was not isolated from sputum specimens. Sera from patient 77-1425 showed a strong precipitin reaction with *P. boydii*, *A. flavus*, and *C. albicans* antigens and *P. boydii* and *C. albicans* were continuously isolated from his sputum specimens. Patient 81-43 produced three precipitin bands with *C. albicans* antigen and two weak bands with *A. flavus* antigen and his sputum cultures yielded *C.albicans* continuously. The majority of cases of aspergillosis are due to *A. fumigatus*. Immunodiffusion reactions of serum specimens from patients with aspergillosis caused by *A. fumigatus*, with commercial and experimental antigen preparations of *A. fumigatus* are summarized in table 4. There is no antigen with which all test sera produced positive precipitin reaction. ME detected antibody in the serum of largest number of patients (95.8 percent) and comparable results were obtained with CF (9 month-old culture filtrate antigen) by det-

**Table 4.** Immunodiffusion reaction of serum specimens from patients with aspergillosis caused by *A.fumigatus* with commercial and experimental antigen preparations of *A. fumigatus*

| Antigens | Number of cases shown positive reaction | Per cent(%) |
|---|---|---|
| GL | 37 | 78.7 |
| CF | 43 | 91.5 |
| ME | 45 | 95.8 |
| FA | 35 | 74.5 |
| FB | 41 | 87.2 |
| FC | 16 | 34.0 |
| ASI | 33 | 70.2 |
| Total number of cases tested | 47 | 100.0 |

For explanation of antigen terms, see *Materials and Methods*.

ecting precipitins in 91.5 percent of the cases. At least 52 precipitinogens have been demonstrated in ME by 2-dimensional immunoelectrophoresis using rabbit anti-*A. fumigatus* hyperimmune serum as shown in figure 2. The commercial antigen GL detected antibody in 78.7 percent of sera tested. Fractionation of ME might resulted in a loss of some activity because at least three cases (F-23, 80-2077, HY261816) failed to react with and fractions derived from ME. Lower concentration (1 mg or less than 1 mg per ml) of the fractions used for immunodiffusion tests might diluted out some reactive components present in relatively higher concentration (3 mg per ml) of ME. On the contrary, patient 77-1170 reacted with FB but not with ME, suggesting that some active components could be concentrated in FC by fractionation. FB fraction detected antibody in 41 out of 47 serum samples, while FA,FC, and ASI detected antibody in 35, 16, and 33 serum specimens respectively. As seen in figure 3, FB is a protein rich fraction and contains more reactive components than the other fractions. FC fraction comprising smaller molecules was inferior to

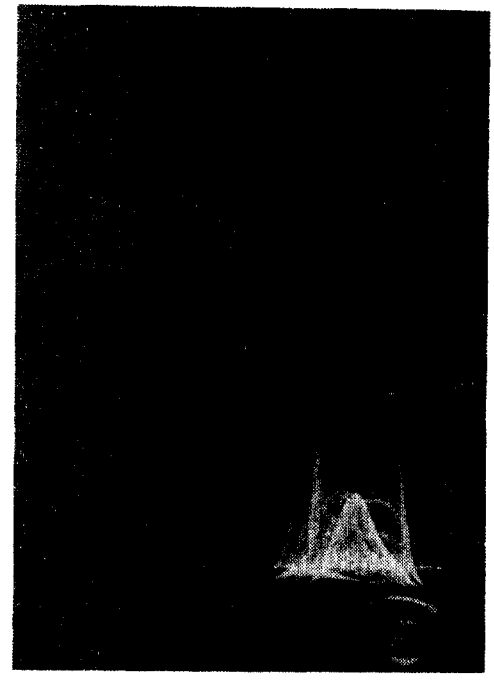

**Figure 3.** Analytic disc gel electrophoresis of *Aspergillus fumigatus* fractions. Bottom of tubes were connected to the anode. C=stained for proteins with Coomassie brilliant blue. P=stained for polysaccharides and glycoproteins by the periodic acid-Schiff (PAS) reaction.

the other fractions for detecting antibody in serum from patients. Fraction FA produced a precipitin band that did form a line of identity with ASI fraction in many cases. Both fractions are rich of glycoproteins and/or polysaccharides as seen in figure 3. Patient 79-1898 did not react with ME and it's frations but reacted with culture filtrate antigens CF and GL. On the contrary, CF and GL missed two cases whose sera reacted with ME or it's fractions.

## DISCUSSION

A variety of fungi frequently colonize in a preformed cavity in the lung due to tuberculosis or other lung diseases (Kim, 1980; Emmons *et al.*, 1977; BTA,1970). Most common species involved in this type of fungal infection

was *A. fumigatus* (Kim, 1980; BTA, 1970). and, in this study, 47 out of 54 patients with pulmonary mycoses were found out to have been infected with this fungus. Although a definitive diagnosis can be made by demonstrating the fungus from the resected fungus-balls, the radiological, mycological, and serological studies can establish a satisfactory diagnosis before surgery. Nearly 100 percent of aspergilloma cases produce easily detectable precipitating antibody to *Aspergillus* antigens and etiologic fungus could be demonstrated in the sputum specimen of more than 80 percent of them (Kim, 1980). Yeast-like fungi and penicilli were frequently isolated from the sputum specimens but they were rarely involved in the pulmonary infection except for *C. albicans*. In this study, *C. albicans* infection has been noticed in at least 5 patients of whom 4 patients seemed to have a mixed infection with other fungi. *A. flavus* is a second common species involved in the pulmonary infections in this study. Patient F-9 is an unusual case because she had a distinct fungus ball in her chest radiograph and excreted *A. flavus* in her sputum specimens continuously over one and half years but her sera repeatedly failed to react with *A. flavus* antigens prepared with both type strain and clinical isolate from her sputum specimens. Contamination of sputum with this fungus from her environment can be ruled out because the same fungus was not isolated from air and house dusts of her home, collected from time to time for over one year. What factors impaired her antibody response to this fungus was not determined.

Demonstration of precipitating antibody in the serum specimens has been found being a useful adjunct in establishing diagnosis of pulmonary fungal infection, in conjunction with cultural examination of clinical specimens. However, a substantial number of the cases might be missed if reliance of serologic tests is based solely upon antigens derived from only one species. There is also great heterogeneity in antibody response to fungal antigens in infected humans, thus it is necessary to use not only a battery of fungal antigens but sometimes antigen prepared with clinical isolate from patient own specimens. Sera from patient 79-385 reacted weakly with *A. nidulans* var. *latus* antigen prepared with clinical isolate from his sputum specimens but not *A. nidulans* antigen. Serum specimens of patient HY261816 did not react with antigen derived from clinical isolate from his sputum but with CF and ME. Although no single antigen of *A. fumigatus* detected antibody in all test sera from patients with aspergillosis caused by this fungus, young mycelial extract (ME) was most reactive preparation of the reagents tested. Fractionation of ME resulted in a loss of some activity even if this procedure excluded substances that reacted with C-reactive protein. CRP reaction was frequently observed with CF, ME and GL. Most of reactive components distributed in protein-rich FB fraction.

We must not override cultivation of the fungi from clinical specimens because it not only substantiates serological diagnosis of fungal infection but also permits to screen the cases infected with organisms whose antigen was not included in serological tests or serological non-reactors such as patient F-9.

## 적 요

發病力이 비교적 큰 病原性眞菌은 免疫機轉에 현저한 缺陷이 없는 사람에게도 致命的 全身性眞菌症을 일으키지만, 여러가지 理由로 인해 抵抗力이 弱化된 個體에 있어서는 發病力이 약한 眞菌類에 의해서도 局所 또는 全身性眞菌症이 發病한다. 結核이나 其他疾患으로 損傷된 肺臟의 空洞 또는 擴張된 氣管枝內에 各種 眞菌類의 胞

子가 吸入되어 眞菌腫을 형성하는 症例가 우리나라에서도 희귀하게나마 보고된 바 있다. 그러나 手術前 診斷을 가능케하는 各種 檢査方法들이 未洽하고 病原性眞菌類의 同定 및 分類가 가능한 檢査室이 거의 없어 그러한 肺眞菌症의 診斷이 매우 부진한 상태에 있다.

著者 등은 1980年 1月부터 1981年 3月까지 結核研究院 附屬醫院 및 서울市內 數個 綜合病院에서 登錄治療中에 있는 환자 가운데서 結核은 현저하게 治癒되었으나 계속해서 間歇的 喀血 또는 血痰을 呼訴하면서 胸部 X-線寫眞上에 fungus ball 陰影이 분명하게 보이는 患者 등 113명과 結核以外의 疾患을 앓거나 기타 이유로 그와 비슷한 症狀을 가지 患者 11명을 대상으로 肺眞菌感染與否를 觀察하였다. 胸部X-線寫眞, 喀痰培養 및 血淸學的檢査 등을 통하여 124명 중 54명이 各種眞菌類에 感染되었음을 알 수 있었다. 그 가운데서 47명의 患者가 *Aspergillus fumigatus*에 의해 感染되었다. *A. fumigatus*의 菌體 또는 培養濾液抗原에 대해 沈降抗體를 가지고 있는 患者의 喀痰으로부터 *A. fumigatus*가 繼續해서 分離培養되는데 비해서 이 菌에 感染되지 않아 血淸에서 沈降抗體가 證明되지 않는 患者 70명 중에서는 4명의 喀痰에서 소수의 菌이 일시적으로 分離培養되었다. 따라서 이 菌에 의한 感染與否가 菌分離培養 및 double immunodiffusion에 의한 沈降抗體 檢出結果와 잘 일치하고 있다. 胸部X-線寫眞上에 분명한 fungus ball이 보이는 患者가 20명이었는데 그중에서 結核으로 인해 생긴 空洞內에 *A. fumigatus* 의해 形成된 경우가 17例였다. 1例는 *A. fumigatus*가 囊腫性病變內에 fungus ball을 形成하였고 다른 2例는 밝혀지지 않은 원인으로 생긴 空洞性病變內에 *A.flavus*가 fungus ball을 형성하고 있었다. *A. fumigatns*에 의해 감염된 患者 중 1例는 移植한 腎臟에 대한 拒否反應을 抑制하기 위해 그리고 다른 1例는 肝癌때문에 오래동안 免疫抑制劑를 사용해온 患者들로서 그들의 血淸으로부터 이 菌에 대한 特異抗體가 檢出되고 그리고 肺生檢組織과 喀痰으로부터 菌이 分離培養되므로서 이들이 invasive aspergillosis를 앓고 있음을 알 수 있었다. 그밖에 *A. nidulans* 및 *A.nidulans* var. latus에 依한 感染이 各各 1例, *A. flavus*와 *Candida albicans*에 의해 二重感染된 例가 1例, *C. albicans*에 의한 感染이 1例, 그리고 *Petriellidium boydii*와 *C. albicans*에 의해 二重感染된 例가 1例였다.

血淸檢査結果는 使用된 抗原의 性狀에 크게 영향을 받는다. *A. fumigatus*에 感染된 患者의 血淸으로 이 菌의 各種抗原製品을 比較한 결과를 보면 대체로 가장 反應力이 큰 抗原成分들은 活發하게 增殖하고 있는 어린 菌絲를 磨碎하여 抽出한 抗原製品(ME)에 가장 많이 分布되어 있었다. Greer Laboratories로부터 購入한 培養濾液抗原(GL)은 劣等한 製品이었지만 9個月間 培養한 培養濾液抗原(CF)은 우수하였다. 가장 反應力이 크고, 他菌群이나 菌種과 交叉反應이 적고, C-reactive protein과 反應하는 多糖類가 없는 抗原成分들은 75% ammonium sulfate飽和溶液에서 沈澱되고 Sephadex G-75 columne에서 두번째로 流出되는 蛋白成分(FB)들이었다. Glycoproteins나 多糖類가 많은 分屑(FA 및 ASI)은 비교적 反應力이 낮았다.

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