Aspergillus nidulans Infection in a Patient with Chronic Granulomatous Disease

Aspergillus nidulans is one of the several species of *Aspergillus* with low pathogenicity. The significant infections of *A. nidulans* in human have rarely been reported, almost exclusively in patients with chronic granulomatous disease (CGD). CGD is a primary immunodeficiency disease which results from absence of the NADPH oxidase in the phagocytic cells, leading to recurrent pyogenic infection and granuloma and abscess formation. Here we report a fatal case of *A. nidulans* infection in a six-year-old boy with chronic granulomatous disease. *A. nidulans* was isolated from the culture of a paraspinal abscess and *Aspergillus* was detected in the surgical tissue by *in situ* hybridization. The patient succumbed despite prolonged treatment with high-dose amphotericin B, itraconazole and interferon-α. To our knowledge, this is the first report of *A. nidulans* infection in Korea.

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Key Words : *Aspergillus nidulans, Chronic granulomatous disease, In situ hybridization*

Min Kim, Jong Hee Shin, Soon Pal Suh, Dong Wook Ryang, Chang Soo Park[1], Chan Jong Kim[2], Hoon Kook[2] and Jin Kim[3]

Departments of Clinical Pathology, Pathology[1], and Pediatrics[2], Chonnam University Medical School, Kwangju 501-757, Korea
Department of Parasitology[3], Chonbuk University Medical School, Chonju 561-180, Korea

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Address for correspondence
Jong Hee Shin, M.D., Department of Clinical Pathology, Chonnam University Medical School, Kwangju 501-757, Korea
Tel : (062) 220-5353, Fax : (062) 224-2518

INTRODUCTION

Chronic granulomatous disease (CGD) is characterized by a defect in phagocytic cells that leads to recurrent superficial and deep pyogenic infections. It results from defective production of toxic oxygen metabolites by phagocytic cells and hence ineffective microbial killing (1). While invasive aspergillosis is a common fungal infection in patients with CGD, most cases have been infections with *Aspergillus fumigatus* or *Aspergillus flavus* (2). *Aspergillus nidulans*, a low-grade pathogen, has rarely been implicated in human diseases. Only a few cases have been reported, almost exclusively in patients with CGD (1~3). It was suggested that some factors unique to patients with CGD might favor the growth of *A. nidulans*. Here we add a case of *A. nidulans* infection in a six-year-old boy with CGD. We believe this represents the first case of *A. nidulans* infection in Korea.

CASE REPORT

In September, 1995, a six-year-old boy was admitted to the Chonnam University Hospital because of low grade fever, pneumonia and lump in the right scapular area. He had had multiple episodes of pyogenic infections ranging from skin infection, arthritis, perianal abscess and pneumonia since 5 months of age. He was diagnosed as having CGD based on the nitroblue tetrazolium reduction test, and had been on trimethoprim-sulfamethoxazole prophylaxis. Having a consolidation in right upper lobe, he was treated with vancomycin and aminoglycoside. A CT-guided biopsy of the mass along with repeated blood cultures failed to identify the causative organism. An empiric treatment with amphotericin B at 0.6 mg/kg/day with itraconazole 100 mg/day were initiated when clinical response was judged after 2 weeks of antibiotics. The clinical course was marked by a progression of abscess to the right lung with destruction of thoracic vertebrae (Fig. 1a).

He was transferred to another hospital where he underwent a total laminectomy of T5-T6 with drainage of pus. Although etiologic agent not proven, he was empirically covered with antifungal, antimicrobial and antituberculous medications, all in vain for another 3 months. The repeat chest CT scan showed the enlarged size of the lung abscess in the right lung with newly found abscesses in the left with wide destruction of bone and soft tissue (Fig. 1b).

He returned to this hospital, and a repeated culture of subcutaneous abscess revealed a pure growth of *A. nidulans*. A high dose of amphotericin B (1.5 mg/kg/day) mixed in intralipid emulsion, itraconazole (200 mg/day), interferon-α (3 million units three times a week), and granulocyte, macrophage-colony stimulating factor (GM-

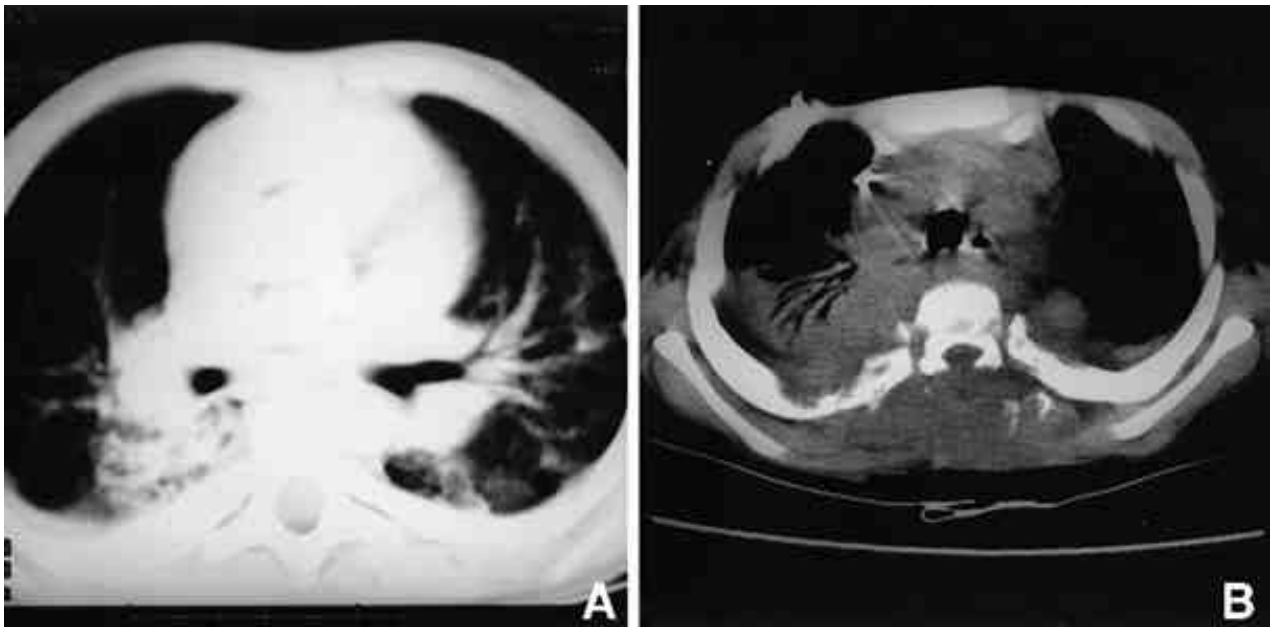

Fig. 1. A : Chest CT scan with lung settings shows patchy and confluent consolidations in both lungs. **B :** Chest CT scan 7 months later shows a mass-like lesion in left and consolidation in right upper lobe with destruction of adjacent ribs. Note laminectomy state of the spine.

CSF, 250 μg) were attempted to maximize antifungal efficacy. Despite aggressive management for about 1 month, he died from clinical deterioration. An autopsy was not granted. A total of 1.8 g of amphotericin B and 15.1 g of itraconazole failed to reverse his clinical course.

Mycologic findings

The fungal isolate obtained from pus aspirated from the paraspinal abscess produced rapidly growing, green-colored downy colonies on SDA agar at 30℃ (Fig. 2). The microscopic characteristics of the isolate by slide cultures were as follows: short, smooth, and brown colored conidiophore, two series (biseriate) of phialide, short columnar headed conidia (Fig. 3), cleistothecia and enmeshed in numerous globose hülle cells (Fig. 4) and lens-shaped ascospores (Fig. 5). On the basis of these features, the isolate was identified as *A. nidulans* (4, 5).

Pathologic findings & *in situ* hybridization

Sections of the mass obtained from the right scapular area showed chronic graulomatous inflammation with numerous multinucleated giant cells on the H-E stain. A few septated branching hyphae elements were detected on the reticulin stain (Fig. 6). *In situ* hybridization (ISH) was performed to detect *Aspergillus* sp. utilizing modified one-hour manual capillary action technology (6) on the Microprobe staining system (Fisher Scientific). Biotin labeled oligonucleotide probe (5'-TCCACACCCTATGG TCGTATGT-3') complementary to *Aspergillus* 5S rRNA was used (7, 8). Positive signals were observed in hyphae as red colour (Fig. 7).

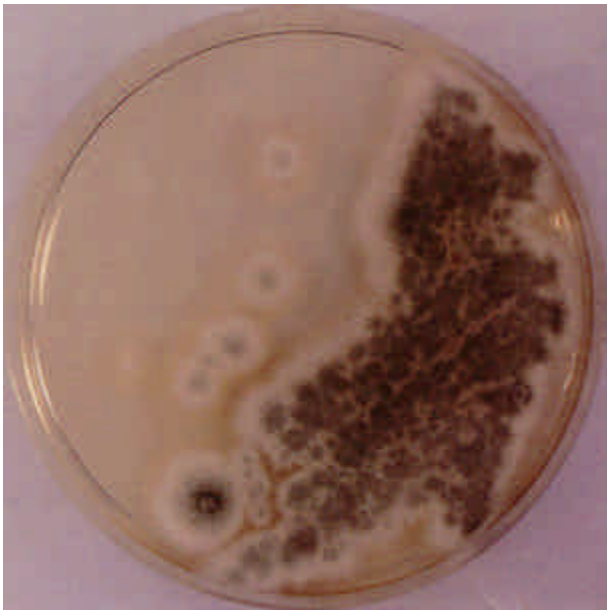

Fig. 2. Green colonies of *A. nidulans* on SDA after 7 days' culture.

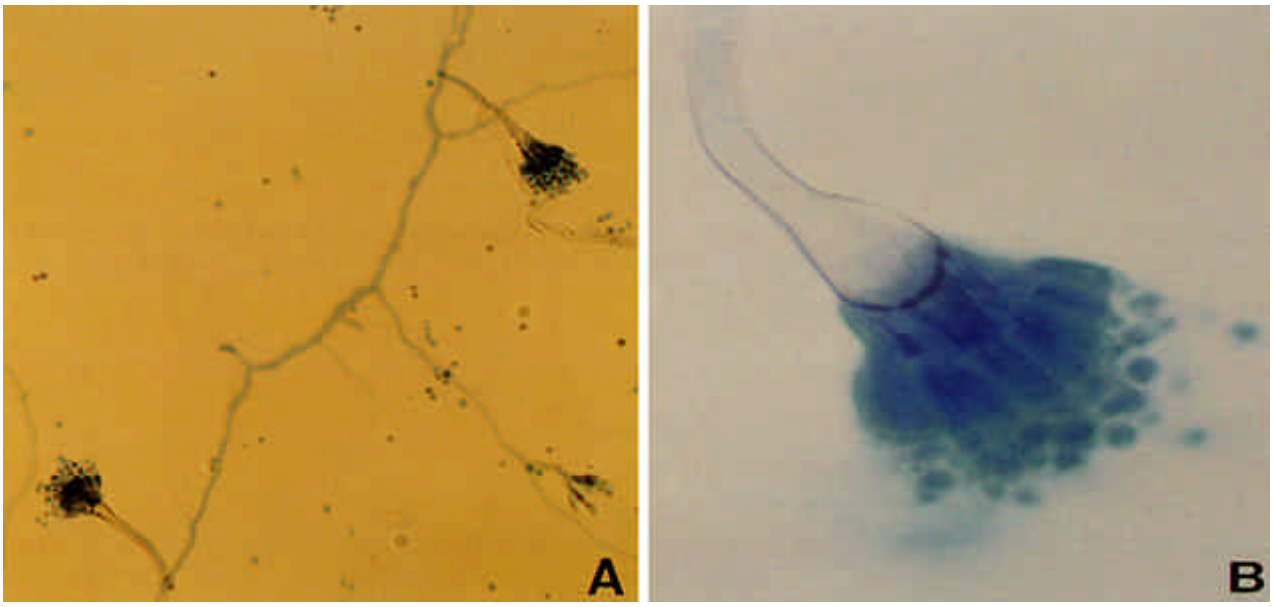

Fig. 3. Several short, brown colored conidiophore on hypae, columnar head conidia (A, ×100) and biseriate phialides (B, ×1,000) in slide culture (lactophenol cotton blue stain, SDA).

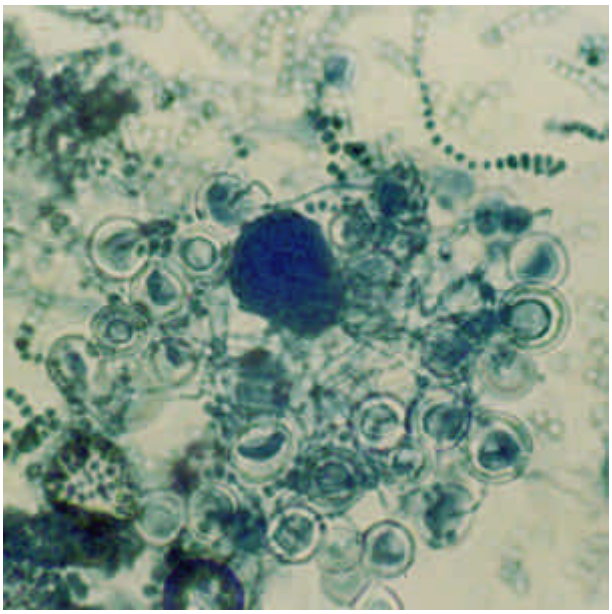

Fig. 4. A cleistothecia enmeshed in numerous globose hülle cells in slide culture (lactophenol cotton blue stain, ×400).

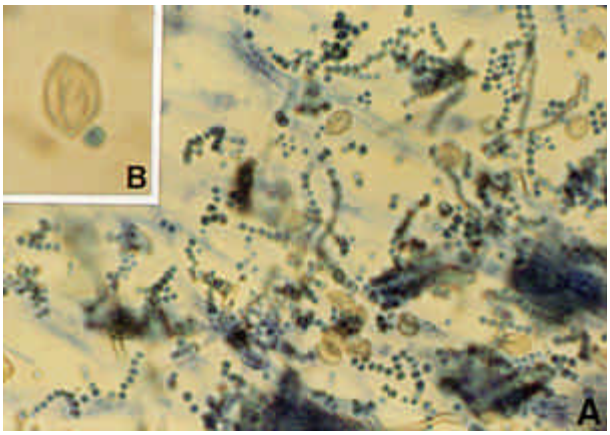

Fig. 5. Scattered lense shaped ascospores (A, ×100) and an ascospore with equatorial crests (B, ×1,000) in slide culture (lactophenol cotton blue stain).

DISCUSSION

Of the hundreds of recognized species of *Aspergilli*, only about 20 have been verified as causing infections in human and animals. The most important fungus is *A. fumigatus*, which accounts for more than 90% of human infections (9). The other fungi are *A. flavus*, *A. niger*, and *A. terreus*. Although *A. nidulans* is one of the commonly encountered *Aspergillus* species in soil, there have been only a few reported cases of human infections due to *A. nidulans* (10, 11).

Risk factors for invasive aspergillosis include granulocytopenia in leukemic patients, high-dose corticosteroid treatment and immunocompromized state following marrow transplantation (5). Significant human infection with *A. nidulans* were mostly found in patients with CGD (1, 3, 12), except for a case of chronic necrotizing pulmonary infection in a 55-year-old man without evidence of immunodeficiency (10). As *A. nidulans* has not been reported in patients with risk factors other than

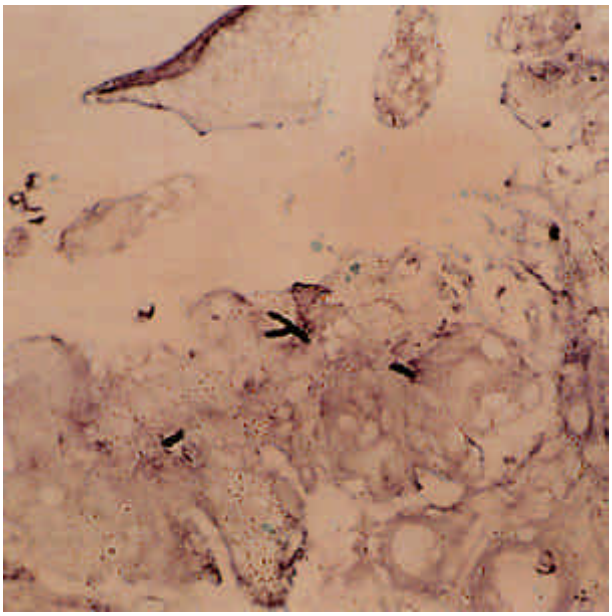

Fig. 6. Tissue section of a lesion showing only a few branching hyphae (reticulin stain, ×100).

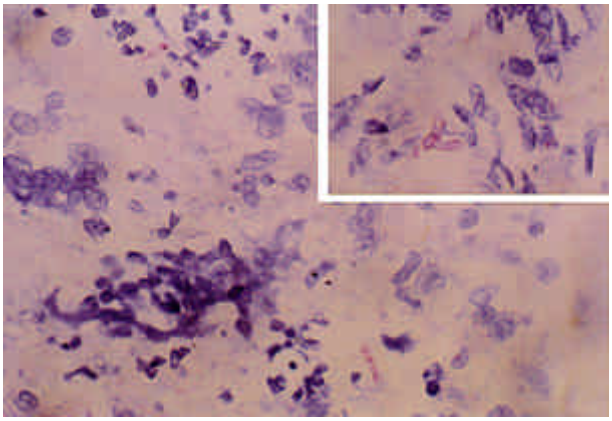

Fig. 7. ISH using *Aspergillus* specific oligonucleotide probe: Positive reactions are seen in hyphae element as red color (hematoxylin counterstain, ×100 and ×400).

those listed above, White et al. (1) suggested some unique factor, still uncertain, in patients with CGD might favor the growth of *A. nidulans*.

As the primary defect of CGD is the NADPH oxidase deficiency in the phagocytic cells, leading to recurrent pyogenic infections and with granuloma and abscess formation, conventional strategies consisting of prophylactic trimethoprim-sulfamethoxazole and aggressive infection control measures have not always been successful (13). Recently, an *in vitro* and *in vivo* study demonstrating increased respiratory burst activity of neutrophils and macrophage from patients with CGD by interferon-γ (14, 15) prompted randomized studies evaluating the safety and effectiveness of long-term interferon-γ therapy in patients with CGD (16,17). In this case, interferon-α was used instead because of ready availability.

High-dose amphotericin B has still been the gold standard treatment for invasive aspergillosis, while recent evidence showed that itraconazole may have significant activity against aspergillosis. In addition, GM-CSF was proven to augment antifungal activity *in vitro* and *in vivo*. Despite these endeavors, he was unable to overcome his illness. The best regimen for invasive *A. nidulans* infection, however, has yet to be determined.

When hyphae elements that branch dichotomously at acute (45°) angles are present in tissue sections, a presumptive diagnosis for aspergillosis can be made, but it should always be confirmed by culture or other techniques. Hyphae of other opportunistic hyaline molds such as *Fusarium* species and *Pseudallescheria boydii* may resemble those of aspergilli (5). An ISH technique using *aspergillus* specific nucleic acid sequence was used for detecting *aspergilli* in tissues in this study. We used a twenty-two base oligonucleotide probe complementary to nucleic acid 1 to 22 of *Aspergillus* 5S ribosomal RNA 3' terminally labeled with biotin. Positive signals were detected with a linear or aggregated pattern in the tissue section. The isolate in this case was identified easily as *A. nidulans* in the respect of morphologic characteristics such as 1) blue, green or yellow-green colored colonies, 2) columnar shaped conidial heads, 3) short, brown, smooth walled conidiophores, 4) biseriate phialides on upper surface, and 5) commonly presented cleistothecia which is enmeshed in globose hülle cell (4, 5). Even though both *A. versicolor* and *A. ustus* often have hülle cells, that in *A. versicolor* has radiating heads and colorless conidiophores, while that in *A. ustus* is elongated shape rather than lens shape in *A. nidulans*.

In summary, we report *A. nidulans* infection in a six-year-old boy with CGD. *A. nidulans* infection was diagnosed by characteristic morphologic features of the isolate as well as ISH using *Aspergillus* specific probe in a tissue section.

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