Review

Pathological Spectrum of Invasive Pulmonary Aspergillosis
—Study of Pulmonary Lesions of 54 Autopsies and the Relationship Between Neutrophilic Response and Histologic Features of Lesions in Experimental Aspergillosis—

Kazutoshi Shibuya¹, Tsunehiro Ando¹, Megumi Wakayama¹, Masayoshi Takaoka², Katsuhiro Uchida³, Shiro Naoe¹

¹Department of Pathology, Toho University Ohashi Hospital, 2-17-6 Ohashi Meguro-ku, Tokyo 153
²ICAM Co. Ltd., 3-28-14 Tokiwadai Itabashi-ku, Tokyo 174
³Research Center for Medical Mycology, Teikyo University, 339 Otsuka, Hachioji, Tokyo 192-02

Abstract

In addition to a histological study on experimental pulmonary aspergillosis in rats, pulmonary lesions from 54 autopsies of invasive pulmonary aspergillosis were examined. Three distinct patterns were seen in the lesions of autopsied lungs. The pathological characteristics of each pattern were affected by three important factors: the width and type of necrosis, the distribution of fungi and the degree of the neutrophilic response. The neutrophilic response might play an important role in creating a cavity in the center of the lesion as well as transforming from coagulation necrosis to colliquative necrosis. Furthermore, cytotoxic agents released by the aspergilli and local ischemia might be important factors which modify the features of lesions.

Key words: Invasive pulmonary aspergillosis, pathology, autopsy, animal model, Aspergillus fumigatus.

Introduction

Various factors have resulted in an increase in the number of immunocompromised patients. For this reason, certain mycoses have risen dramatically in frequency. Thus, invasive pulmonary aspergillosis (IPA) has come an important opportunistic fungal infection, and this disease is a serious and prevalent problem in contemporary medicine. The variety of clinical and radiological manifestations of this disease may depend on the immunological capacity of the host¹. Namely, a fulminant form of invasive pulmonary aspergillosis has been seen in patients with prolonged agranulocytosis². On the other hand, chronic necrotizing pulmonary aspergillosis³ and some other clinical forms of pulmonary aspergillosis¹ have been reported which may occur in mildly immunocompromised patients, such as patients with diabetes mellitus, those on low-dose steroid therapy and so on.

To examine the pathologic features of IPA, we reviewed the pulmonary lesions of 54 autopsies who had IPA. In addition, a histopathological study on experimental pulmonary aspergillosis in rats was carried out to elucidate the development of these lesions.

Materials and Methods

1. Pathological study of autopsies

Formalin-fixed, paraffin-embedded sections of lungs from 54 autopsied patients who had IPA were stained with hematoxylin and eosin (H&E), periodic acid-Schiff reaction (PAS) and for elastin. In addition, sections were also stained with Grocott methenamine silver. Light microscopic examination was performed on these sections.

2. Experimental study

Animals: Sprague-Dawley rats (SD-I), 220 to 250 g in weight, female, were obtained from Charles River Japan Inc., Kanagawa, Japan. The rats were housed one per cage in hardwood sawdust bedding and given free access to tap water and
chow (Oriental Yeast Inc., Tokyo, Japan). These animals were subcutaneously injected with ampicillin (Takeda Pharmaceutical Co., Ltd.; Osaka, Japan) at a dose of 20 mg/kg of body weight on days -7, -5, -3, -1, 3, 5 and 10.

Immunosuppression: Rats were immunosuppressed with cyclophosphamide (Shionogi Pharmaceutical Co., Ltd.; Osaka, Japan) at a dose of 25 mg/kg of body weight and prednisolone (Mitaka Pharmaceuticals Co., Ltd.; Tokyo, Japan) at a dose of 25 mg/kg of body weight injected subcutaneously on days -7, -5, -3, -1, 3, 5 and 10.

Organism: A. fumigatus TIMM1776, isolated from the autopsied lungs of patients with generalised aspergillosis, were employed in this study. Cultures of A. fumigatus TIMM1776 were maintained on Sabouraud glucose agar slants. Before preparation of inocula for intratracheal injection into rats, the fungi were transferred on Sabouraud agar slants, incubated at 27°C for 48 hrs.

Preparation of inocula: Agarose beads were prepared by a modification of the method of Cash and co-workers. Conidia were harvested as a suspension in phosphate-buffered saline (PBS), pH 7.0, and this suspension was diluted with an appropriate volume of the same PBS to give a final concentration of 10^7 spores/ml, after determination of the concentration of spores with a hemocytometer. Next 2.5 ml of 2% (weight vol. -1) agarose (Type I, Lew EEO SIGMA) in PBS was melted at 48°C, and 0.5 ml of conidial suspension was added. 15 ml of heavy mineral oil (Heavy white oil, SIGMA), warmed to 48°C, was vigorously stirred with a magnetic spin bar, and 3 ml of melted agarose, with and without conidia, was added. The oil-agarose mixture was cooled rapidly by placing crushed ice around the vessel, while stirring was continued for approximately 5 min. During this time, agarose droplets solidified into beads. These were washed twice with 0.25% Tween 80 in PBS to facilitate removal of the mineral oil. Beads with over 100 μm and under 50 μm in diameter were filtered off by nylon mesh #150 and #300, respectively. This was followed by 4 washes in PBS alone. Beads with a diameter of 50 μm to 100 μm were suspended in PBS to give a final concentration of 4 × 10^4 beads/ml.

Infection model: On day 0, rats were anesthetized by intra-abdominal injection of 70 mg/kg of pentobarbital sodium (Abbot Laboratories Ltd.; Chicago, USA) and a small cervical incision was made to expose the trachea. A 22-gauge catheter (Terumo Inc., Tokyo, Japan) with a caving on the top and the tubing trimmed to approximately 4 cm was introduced through the incision and advanced to the left main bronchus. 0.1 ml of the inoculum was gently injected. The catheter was withdrawn, and the skin incision was closed with sutures.

Histopathological study: Infected animals were killed on the first, third, fifth, 10th and 13th days after inoculation. The lungs were fixed with formalin and paraffin embedded sections were stained with H&E, PAS-elastica and Grocott methenamine silver. In addition, approximately 300 μm-thick sections of formalin-fixed lungs from the mice killed on the 13th day after inoculation were cleared with xylene and stained with Victoria blue after dehydration.

Results

Study of autopsies

Most of the lungs examined had wide-spread aspergillous lesions, and the features of vascular involvement by hyphal growth of fungi were commonly seen (Fig. 1). In addition, the bronchial mucosa was occasionally invaded by fungi. In these lesions, a uniform band of coagulation necrosis of the parenchyma, without invasion of hyphae, was demonstrated between the involved erosive mucosa and viable lung tissue, which usually shows a packed infiltrate of neutrophils (Fig. 2).

Three morphologic patterns, however, emerged from the present study based on the particular relationship between the type of necrosis and the features of fungal growth in the lesions. The first pattern, pattern I, was characterized by scattered discrete and round nodules, encompassed by hemorrhage, which often measured more than 3 cm in diameter. These nodules consisted of both many proliferating hyphae and coagulation necrosis of the lung, involving septa, bronchioli and blood vessels. No inflammatory cell infiltration was seen in such lesions (Figs. 3, 4). In addition, none of the lesions classified as pattern I had a cavity. The autopsies indicated pattern I involved various kinds of malignant hematopoietic disorders. In pattern II, the lesion consisted of scattered intra-alveolar proliferation of aspergilli with a neutrophilic response. Hyphae were aligned in a radial pattern in each alveoli, and the alveoli around the fungi were filled with necrotic tissue containing neutrophils (Fig. 5). Septa were occasionally invaded by the hyphae. By the naked eye examination, the lesions of pattern II show ill-defined and fused nodules on the cut surface of the lung with and without hemorrhage (Fig. 6). Ill-defined cavities were very rarely seen in the center of these lesions. In pattern III, the lesion was characterized by a cavity developed in wide-spread coagulation necrosis, occasionally involving...
one segment or more. A woven band consisted of a palisading of hyphae was seen on the cavity wall, but most of the necrotic area was not invaded by the aspergilli (Figs. 7, 8).

**Experimental study**

On the first day after inoculation, the lungs of infected rats showed settling of the agar beads in the alveoli. Conidia and early elongation of hyphae was seen in these beads, which are accompanied by a minor response of polymorphonuclear leukocytes as well as histiocytes (Fig. 9). On the third day after inoculation, elongated hyphae penetrated through the septa from the beads in the alveoli, and polymorphonuclear leukocytic infiltration around the beads became prominent. However, fusion of each minute nodular lesion around the beads and necrosis were not very apparent. On the fifth day, the hyphae aligned themselves in a radial pattern and were accompanied by a histiocytic response and polymorphonuclear leukocytic infiltration. Nodular lesions were demonstrated in the periphery of the infected lobe, and they consisted of fused, small infected foci (Fig. 10). The inoculated beads were clearly observed in each alveoli. The involved alveoli and bronchiolar spaces were filled with infiltrated polymorphonuclear leukocytes and a small amount of necrotic debris, including nuclear dusts. A small area of coagulation necrosis was seen in the center of the lesion. Larger lesions, involving one or more segmental areas of the lung, had developed in the rats on both the 10th and 13th days after inoculation. These lesions consisted of widely spread necrosis.
coagulation necrosis, and radial or palisading arrangements of elongating hyphae appeared in periphery of the lesion. The larger pulmonary architecture, containing bronchial or bronchiolar walls and arteries, was occasionally penetrated by elongated hyphae. Bronchial mucosal lesions were rarely seen in these rats. Coagulation necrosis was seen in the center of the lesions, which are infiltrated with a small number of inflammatory cells. Pleurae in contact with the lesions were also involved and penetrated (Fig. 11). Thick sections of the lung cleared with xylene indicated continuous development of coral-like emboli in both arteries and veins (Fig. 12).

**Discussion**

In a previous study, we reported the histopathological features of IPA which had developed in patients with and without malignant hematologic disorders. A large round nodule of numerous hyphae without an inflammatory cell response and frequent fungal emboli were the characteristic feature of IPA in patients with neutropenia. On the contrary, patients showing a neutrophilic response manifested a long-standing aspergillus infection in lungs, and the histologic features of those lesions were central liquefaction necrosis, proliferating hyphae aligned in a radial pattern around the lesion and prominent neutrophilic infiltration. The primary focus of IPA has been recognized as the alveoli, and this fact was also confirmed by the present studies, which included both examinations of autopsied lungs and on animal model. In addition, three morphologic patterns...
were seen in the aspergillus lesions developed in human lungs (Fig. 13). Pattern I might be a result of hematogenous dissemination of fungi followed by primary infection in the lungs, because the lesions had an well defined round shape and their distribution was random. Furthermore, this fact may support the hypothesis that most of the patients showing this pattern had a malignant hematopoietic disorder as an underlying disease. On the contrary, both patterns II and III showed inflammatory cell responses, necrosis and prominent fungal proliferation, but the essential difference was that a bronchopneumonic distribution of the lesion
was seen only in pattern II (Fig. 14). An important characteristic of pattern III was wide-spread coagulation necrosis, which included randomly-scattered aggregates of hyphae (Fig. 15). This necrosis seen in pattern III might be a result of ischemia as a sequel to fungal emboli, and the necrotic parenchyma was consequently invaded by fungi. Based on the results of the present experimental study, the extension of the aspergillus lesions in the lungs might have two different ways. One is the direct invasion from the primary focus usually seen in alveoli, and the other is the wide-spread coagulation necrosis caused by the continuous growth of coral-loke fungal emboli. On the other hand, band-like coagulation necrosis was occasionally seen between the mucosa involved by fungi and the viable parenchyma. Because such necrosis had an orderly thickness, this feature might be a result of cytotoxic effect of the fungi. In addition, if the host retains the capacity to mount a neutrophilic response, the central necrosis might transform from coagulation necrosis to colliquative necrosis.
the inoculum. The common procedures for immuno-
suppression were treatment with steroids\(^7\)\(^-\)\(^8\),
cyclophosphamide\(^9\) or gamma irradiation\(^9\), and
animals were infected by intravenous\(^6\)\(^,\)\(^10\) or
intratracheal\(^12\)\(^,\)\(^13\) inoculation and by inhalation of
dry spores\(^16\) or spore suspensions\(^15\). The longest
duration of continuous experimental infection of
the lungs in the models developed to date has been
15 days\(^16\), but the duration in most models is
less than 10 days\(^12\)\(^,\)\(^13\). In addition, few studies
have described in detail the pathology of the develop-
ment of lesions in experimental IPA. Foci of aspergillus infection in mice and rats, especially,
developed in the bronchial or bronchiolar mucosa
in the existing models. In the present study, agarose
beads containing conidiae of Aspergillus fumigatus
were used to accurately deliver to the alveoli. Then
time-course histological examination was performed
on the lungs of infected rats treated with low-dose
immunosuppressive agents to evaluate the exten-
sion of IPA. As a result, colliquative necrosis was
observed in the lesions which developed in rats more
than 10 days after infection. This finding suggests
that a neutrophilic response plays an important role
in inducing colliguation of the involved tissue.

Acknowledgements

Authors would like to thank Dr Hideyo
Yamaguchi (Professor in chief, Department of
Microbiology, Teikyo University, School of
Medicine), Dr Takehisa Nagayama (Chief of the
Department of Pathology, Koritsu Showa
Hospital), Dr Tamiko Takemura (Chief of the
Department of Pathology, Japan Red Cross
Hospital), Dr Takehisa Nagayama (Chief of the
Department of Pathology, Koritsu Showa
Medical Center) and the research staffs of both the
department of pathology, Toho University Ohashi
Hospital and the research center for medical
mycology, Teikyo University.

Reference

1) Gefter WB: The spectrum of pulmonary
2) Guiot HFL, Fibbe WE, van’t Wout JW: Risk
factors for fungal infection in patients with ma-
lignant hematologic disorders: Implications for
empirical therapy and prophylaxis. Clin Infect
3) Binder RE, Faling LJ, Pugatch RD, Mahasaen
C, Snider GL: Chronic necrotizing pulmonary
aspergillosis: A discrete clinical entity. Medicine
4) Cash HA, Woods DE, Mc Cullough B, Johanson
WG, Bax JA: A rat model of chronic respiratory
infection with Pseudomonas aeruginosa. Am Rev
5) Ando T, Wakayama M, Takahashi K, Shibuya
K, Naoe S: Histopathological study on pulmonary
aspergillosis-focusing on the comparison between
patients with and without malignant hematopoietic
disorders-. Proceedings of the third China-Japan
International Congress of Mycology, Hangzhou,
China, 9/21-23, 1995, International Academic
Publishers.
6) Pitt JI: The current role of Aspergillus and
penicillium in human and animal health. J Med
7) Dixon DM, Polak A, Walsh TJ: Fungus dose-
dependent primary pulmonary aspergillosis in
8) Eisen DJ, Biddinger PW, Rhodes JC: Experimen-
9) Kolattukudy PE, Lee JD, Rogers LM, Zimmerman
P, Ceselski S, Fox B, Stein B, Copelan EA: Evidence for possible involvement
of elastolytic serine protease in aspergillosis. Infect
10) Sabetta JR, Miniter P, Andriele VT: The diag-
nosis of invasive aspergillosis by an enzymelinked
immunosorbent assay for circulating antigen. J
11) Scheffer A, Douglas H, Braude A: Selective pro-
tection against conidia by mononuclear and against
mycelia by polymorphonuclear phagocytes in
resistance to Aspergillus: observations of these two
lines of defense in vivo and in vitro with human and
1982.
12) Spreadbury CL, Krausz T, Pervez S, Cohen J:
Invasive aspergillosis: Clinical and pathological
features of a new model. J Med Vet Mycol 27:
13) Williams DM, Weiner MH, Drultz DJ:
Immunologic studies of disseminated infection
with Aspergillus fumigatus in the nude mouse. J
Infect Dis 143: 726–733, 1981.
14) Kohary MH, Chase T Jr, Macmillan JD:
Correlation of elastase production by some strains
of Aspergillus fumigatus with ability to cause
pulmonary invasive aspergillosis in mice. Infect
bronchoalveolar macrophage defense against
Rhizopus oryzae and Aspergillus fumigatus. J Infect
16) Smith JM, Tang CM, Noorden S, Holden DW:
Virulence of Aspergillus fumigatus double mutants
lacking restrictocin and alkaline protease in a low-
dose model of invasive pulmonary aspergillosis.