J Infect Chemother (2004) 10:138–145
DOI 10.1007/s10156-004-0315-5

REVIEW ARTICLE

Kazutoshi Shibuya · Tsunehiro Ando · Chikako Hasegawa
Megumi Wakayama · Shigeharu Hamatani
Tsutomu Hatori · Tadashi Nagayama · Hiroko Nonaka

Pathophysiology of pulmonary aspergillosis

Received: April 6, 2004

Abstract A description of the pathophysiology of aspergillosis is followed by a review of investigational considerations of animal models. Because a large body of invasive *Aspergillus* infection occurs as opportunistic infection, there is a large spectrum of the histopathological feature of lesions demonstrated at the site of infection. Histopathology of the lesions can be understood as a phenotypical representation of interaction between lowered defense mechanisms in the host and the virulence of invading fungi. Detailed observations with a consideration of previous pathological knowledge of infection and inflammation provide much important information useful in predicting the pathophysiology of the patient. Moreover, experimental studies can also provide much insight to elucidate pathogenesis of the infection that emerges from the clinical and pathological investigations. The importance of pathophysiology should be emphasized to understand the implications of radiographic images, clinical symptoms, and laboratory dates. By reviewing these, especially computed tomography (CT) images, we can see that they accurately mirror the histological features of the lesion that can be recognized as a phenotypical representation of pathophysiology of *Aspergillus* infection. This is also confirmed by the reports emphasizing the importance of CT scans to identify hallmark clinical signs and symptoms of the disease.

K. Shibuya (✉) · C. Hasegawa · S. Hamatani · T. Hatori · T. Nagayama · H. Nonaka
Department of Pathology, Omori Hospital, Toho University School of Medicine 6-11-1 Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan
Tel. +81-3-3762-4151; Fax +81-3-3767-1567
e-mail: kaz@med.toho-u.ac.jp

T. Ando
Department of Internal Medicine and Infectious Control, Japanese Red Cross Medical Center, Tokyo, Japan

M. Wakayama
Department of Pathology, Toho University Ohashi Hospital, Tokyo, Japan

Key words Pulmonary aspergillosis · Histopathology · Neutropenia · HIV · Neutrophile · Leukemia

Inflammation and repair

Injury, inflammation, and repair have been the hallmarks of pathology. Knowledge of the basic phenomena, as well as the consequences, complications, and nuances of these processes, constitutes the basis for understanding many diseases. Inflammation is the host cell and tissue reaction to any injury. An injury agent or a damaged cell and normal inflammatory, homeostatic, and immune responses are the essential ingredients needed for inflammation to occur. Inflammatory response may be provoked by any of the agents previously discussed. Individual response to injury may vary widely for any given injurious agent, however, owing to the unique set of genetic, nutritional, physical, infectious, chemical, hormonal, and immune factors that make up that individual's internal and external environment. Inflammatory and reparative processes are generally simultaneous, but one phase usually dominates when tissue is examined microscopically at any given time after injury. The inflammatory response varies depending upon the particular agent, the tissue, and the individual host characteristics. Accordingly, the infectious disease can be recognized as an inflammatory response caused by an microorganism as an injury agent. Histopathological changes demonstrated at the site of infection and altered structures are generally epitomized by extensively complicated interaction between causative microbes and tissue response. In cases of opportunistic infection, especially in those with invasive fungal infections, tissue response against some pathogenic fungi must be lowered, but both cause and degree of decreasing functioning of defense mechanisms vary from cases to case. Given these facts, lesions produced by an invasion of pathogenic fungi can be understood as a phenotypical expression emerging from interactions between the invasiveness of causative fungi and the lowered defense mechanisms of the host. In this article, histopathological features are examined

in several important forms of invasive *Aspergillus* infection to provide reference to specific mechanisms of lowered functioning of immunity or defense mechanisms in hosts, and so that pathogenesis and pathophysiology may both be conducted.

Aspergillosis

Immunocompromised hosts have continued to increase in number in recent years due to an increasing in number of patients with chemotherapy, HIV infection, organ transplantation, and long-term administration of immunosuppressants for example. Under these circumstances, invasive fungal infections have been attracting public attention as opportunistic infections in the immunocompromised host for many years.[1,2] The overall incidence of invasive fungal infections, especially *Candida albicans* infections, found at autopsies is tending to decrease, largely owing to the introduction of new triazole antifungal agents. On the other hand, the proportion of aspergillosis to invasive fungal infections continues to increase. In particular, invasive pulmonary aspergillosis is often diagnosed only at autopsy because of the difficulty in diagnosing its rapid progression, and the restricted options of useful antifungals.[3] Furthermore, there are background factors related to the difficulty in identifying this disease, such as the lack of clinical symptoms and chest X-ray findings, and the low rate of isolation of fungi from sputum and other specimens obtained from the respiratory tract.[4] In addition, the general health of the patients is often so poor that invasive laboratory tests are restricted, usually making it more difficult to establish histopathological and cytological diagnoses. Under such circumstances, there is an urgent need to elucidate pathological mechanisms underlying pulmonary aspergillosis to establish diagnostic methods and develop safer and more definitive therapies.

Histopathology of pulmonary aspergillosis

At present, it is generally accepted that pulmonary aspergillosis is classified into three types: allergic bronchopulmonary aspergillosis (ABPA), fungus ball type pulmonary aspergillosis, and invasive pulmonary aspergillosis (IPA).[5] Other clinical entities have been proposed, for example, chronic necrotizing pulmonary aspergillosis and a semi-invasive form of aspergillosis proposed as a transitional form between the latter two types.[6,7] However, the pathophysiological independence of their entities has not been confirmed histopathologically. A case initially diagnosed as pulmonary aspergillosis of fungus ball type characterized by noninvasive proliferation of hyphae in a preexisting cavity in the lung may transform into the invasive pulmonary disease when the defense mechanisms of the host are lowered by the innate course of the underlying disease or by induced immunosuppression. However, a few histopathological studies have been reported conducting these varieties of clinical and radiological features. In this article, we describe the histopathological characteristics of pulmonary aspergillosis, with an emphasis on the invasive form of the disease, through detailed studies of autopsies. We also discuss the progression process of the pulmonary lesions.

Allergic bronchopulmonary aspergillosis (ABPA)

Colonization of *Aspergilli* can cause an allergic response in particular individuals who have clinical symptoms of chronic obstructive pulmonary disease.[5] Cytological examination of bronchopulmonary lavage can be recommended as one of the most useful procedures for diagnosis of the disease, as it demonstrates clusters of hyphae with dichotonous bifurcation and numerous eosinophils in mucin. Mucosa obtained by forceps biopsy histologically shows alterations corresponding to allergic bronchitis, but invasion by fungal elements is not necessarily found (Fig. 1).

Noninvasive form and other related diseases

The noninvasive form of pulmonary aspergillosis has been named aspergilloma and is pathologically defined as the development of a fungal ball in a preexisting cavity usually caused by old tuberculosis or cystic diseases of the lung. Hyphae tightly align themselves in a radial pattern inside the ball, which develops in a cavity whose wall is usually either eroded or covered with metaplastic epithelium from the respiratory tract. The inflammatory infiltrate is essentially consistent with the wall observations, and no hyphal invasion occurs when the patient is immunocompetent (Fig. 2). From this definition, the entity may be somewhat controversial because the histopathological definition of the disease requires the exclusion of invasive fungal proliferation into the lung tissue as an essential feature of the infectious disease.

The semi-invasive form of aspergillosis[6] is still incompletely understood. However, as previously mentioned, this form may be largely accepted as the state transformed from the noninvasive form into the invasive pulmonary disease when the defense mechanisms of the host are lowered by the innate course of the underlying disease or by induced immunosuppression. The cavity wall is usually eroded and invaded by the elongated hyphae. Both acute and chronic inflammatory infiltrates are seen with an association of fibrosis and necrosis in various degrees. Blood vessels are usually involved and occluded by an invasion that may cause hemoptysis (Fig. 3).

Invasive pulmonary aspergillosis (IPA)

We have been conducting histopathology and pathophysiology of invasive pulmonary aspergillosis through autopsies

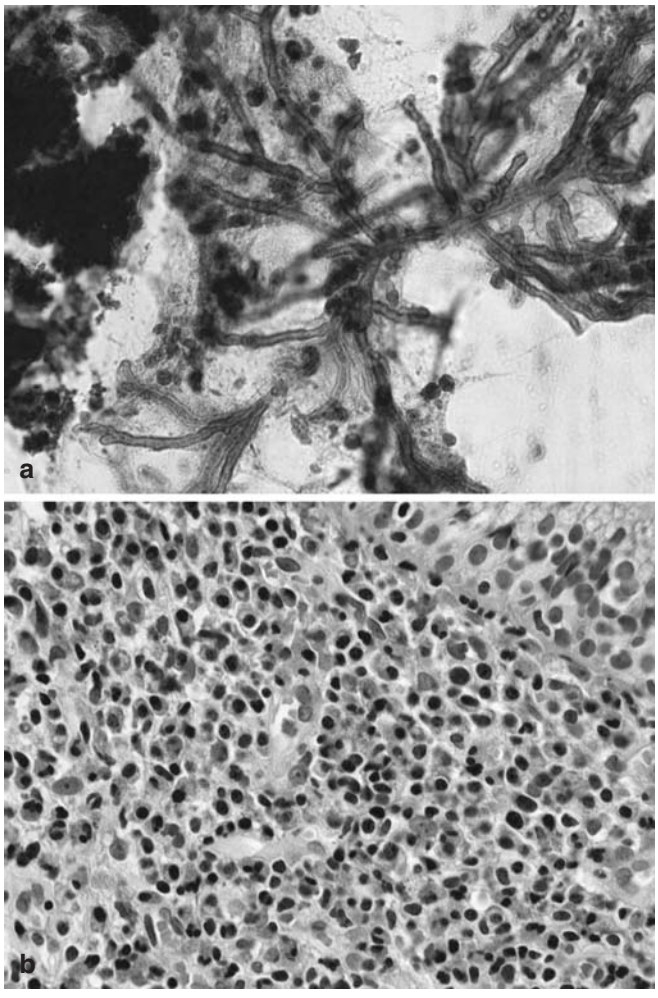

Fig. 1. Allergic bronchopulmonary aspergillosis. **a** Cytological specimen of broncho-alveolar lavage shows a cluster of hyphae indicating dochotonomous bifurcation (Papanicolaou, ×400). **b** Histological specimen of the transbronchial biopsy demonstrates dense inflammatory infiltrate comprising lymphocytes and eosinophils. The basement membrane is mildly thickened and covered with stratified columnar epithelium (Hematoxylin-Eosin, ×400)

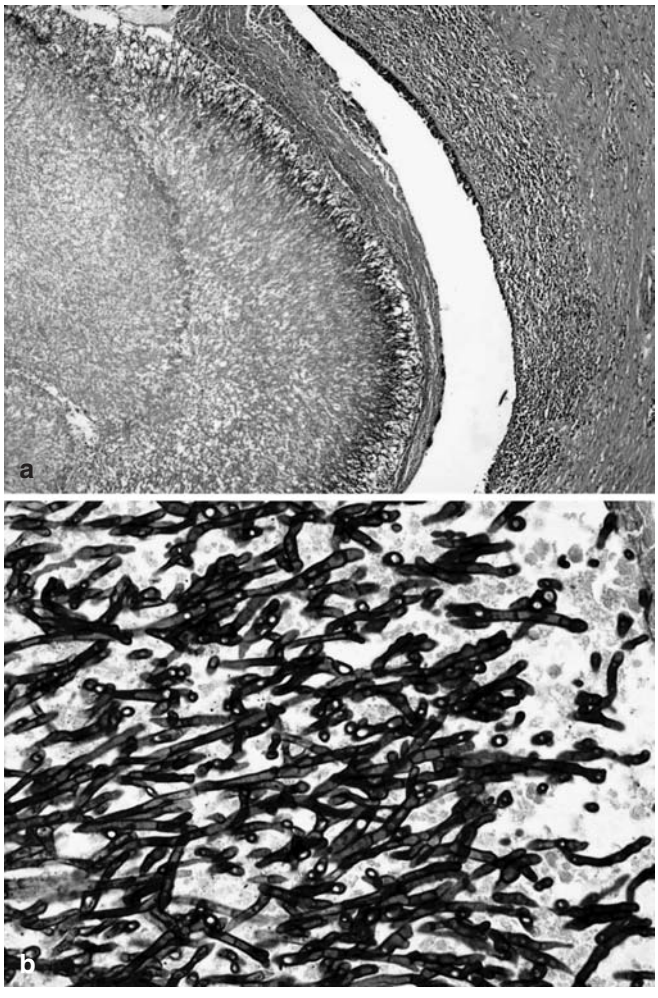

Fig. 2. Noninvasive pulmonary aspergillosis, aspergilloma. **a** Cavity is filled by a fungus ball in which hyphae align themselves in a radial pattern. Cavity wall is entirely covered with metaplastic epithelium and infiltrated with chronic inflammatory cells (Hematoxylin-Eosin, ×20). **b** There are densely intertwined septated hyphae at the periphery of the ball (GMS, ×400)

in a 64-patient group that can be classified according to two patterns that emerged during the study. One group was discrete nodule (DN), consisting of well-demarcated and round-shaped coagulation necrosis in which numerous hyphae aligned in a radial pattern. A circumferential band of hemorrhage surrounds the area of coagulation necrosis. Less apparent is any type of inflammatory infiltrate in this pattern that usually occurs in a patient with severe bone marrow suppression or agranulocytosis[4,8] (Fig. 4). The second pattern was fused lobular consolidation (FLC), which corresponds to usual bronchopneumonia histologically characterized by the filling of acute inflammatory exudates with a fungal proliferation in alveoli. The gross feature of this pattern is a fusion of lobular consolidation. Necrosis present in FLC is usually colliquative and may be induced by neutrophilic infiltration. This can produce a cavity at the center of a region when the bronchi involved in the necrosis play a role in drainage. Patients indicating FLC showed a considerable response of neutrophils recognized as a first defense line against *Aspergillus* infection[8] (Fig. 5).

Using two radiographic images for each case – initial alterations and the pattern just before the patient's death – correlations between histopathological features and the radiographic patterns were studied. Shadows documented as patchy/nodular or irregular infiltrate were found as the most common initial radiographic alterations in our patient group.[9] Each abnormal shadow enlarged gradually or rapidly, and most of the patchy/nodular shadows transformed into irregular infiltrates. However, some of the closest parallel findings between initial radiographs and histopathological findings at autopsy were that DN was discovered in patients who initially showed patchy/nodular shadows, and FLC was found in patients who had irregular infiltrates, as defined by interpretation of radiographic images.[9,10] In addition, based on the study, the halo sign recognized as one of the important hallmarks of IPA may mirror a band of

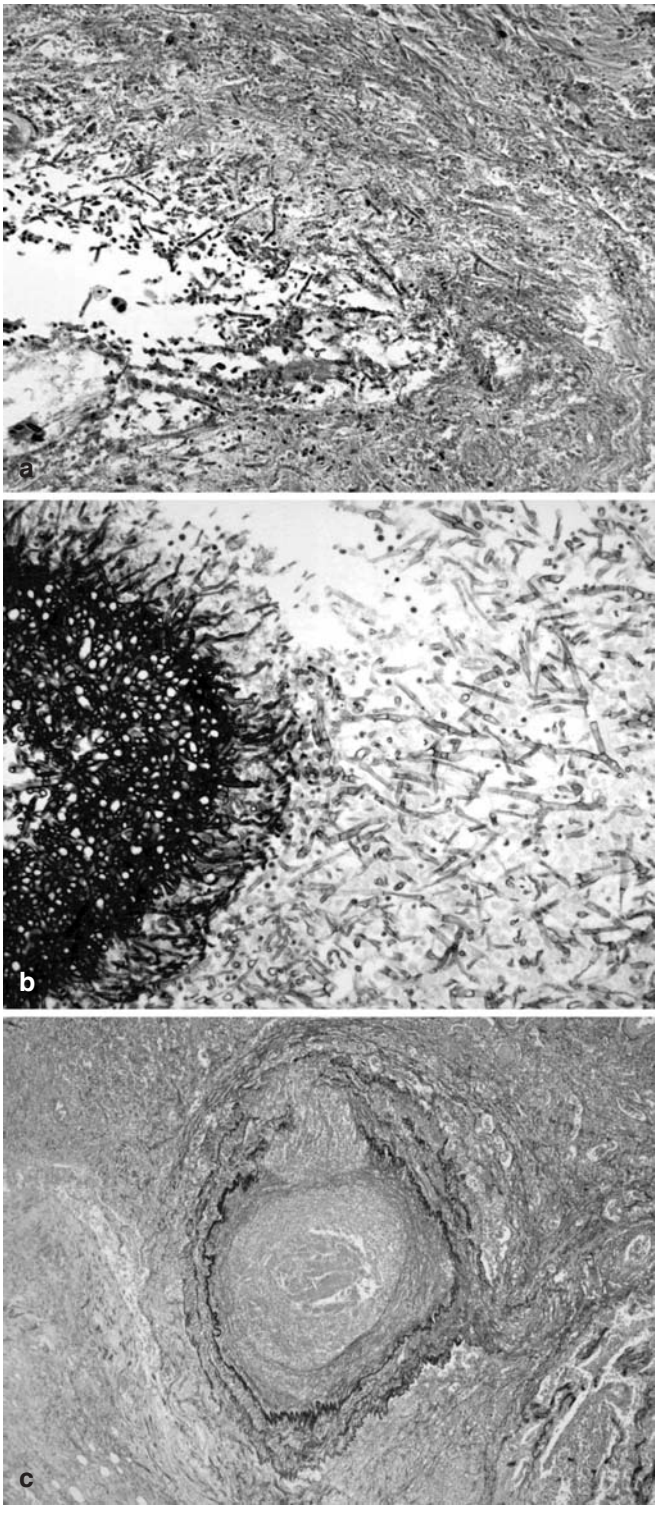

Fig. 3. Semi-invasive pulmonary aspergillosis. **a** Cavity wall is eroded with a penetration of elongated hyphae (Hematoxylin-Eosin, ×100). **b** Hyphae invade into the cavity wall (GMS, ×200). **c** A vein involved by the invasion is occluded by concentric fibrous intimal thickening (Elastica, ×200)

hemorrhage surrounding DN developed in a patient with agranulocytosis. On the other hand, cavities and peripheral air crescents may be caused by the exclusion of colliquative necrosis produced by neutrophilic infiltrate against the invading fungi.

Aspergillus and animal models

As mentioned above, aspergillosis can be defined as damage to the tissue of humans or animals caused by several species of *Aspergillus*. Damage may be caused by invasion of tissues, colonization of cavities, or allergic mechanisms, resulting in the diseases varying in severity and clinical course, which may also depend upon the organs affected and the host. *Aspergilli* are abundant in the environment.

The genus *Aspergillus* now includes more than 200 recognized species, all of which are worldwide in distribution. Their conidia are produced in great abundance and are readily disseminated by the wind. Although the conidia are frequently inhaled by humans, most human aspergillosis are thought to be caused by the *A. fumigatus* group.[11,12] Although an individual with apparently normal defense mechanisms may rarely be infected by *A. fumigatus*, systemic aspergillosis followed by a pulmonary infection occurs in individuals whose resistance is decreased by various conditions.[6,12,13] From clinical observations it has been concluded that neutropenia and high doses of corticosteroids are the two major risk factors for invasive aspergillosis[13,14] and that the combination may act synergistically to break down natural resistance.[13] IPA is mainly implicated by *A. fumigatus*, with *A. flavus* the next most important pathogen.[12]

A major aim of experimental study with animal models of aspergillosis is to gain insight into the pathogenesis of human aspergillosis. However, an experimental model has also been used for evaluation of drug efficacy, elucidation of virulent factors of *Aspergilli*, assessment of new diagnostic procedures involving serological, molecular biological, and histopathological examination, and immunological analysis of the infection.

A large number of studies have been conducted, using a variety of experimental models of the disease. Fatal experimental infections leading to multiple organ involvement have been most frequently produced in mice with an intravenous injection of conidia suspension. This type of murine model has been demonstrated to be effective in evaluating antifungal agents, although rabbits, rats, monkeys, sheep, ducks, and chickens have also been employed as models for this type of experimental aspergillosis.[15] Since mice, rabbits, and other laboratory animals normally show high resistance to *Aspergillus* infection, their host defense needs to be lowered by the administration of immunosuppressants or by using some other way to expose the subjects to *Aspergillus* inoculums to secure the establishment of persistent and invasive infections in these animals. Animals with predisposition to experimental *Aspergillus* infection and which are often used to produce animal models of systemic aspergillo-

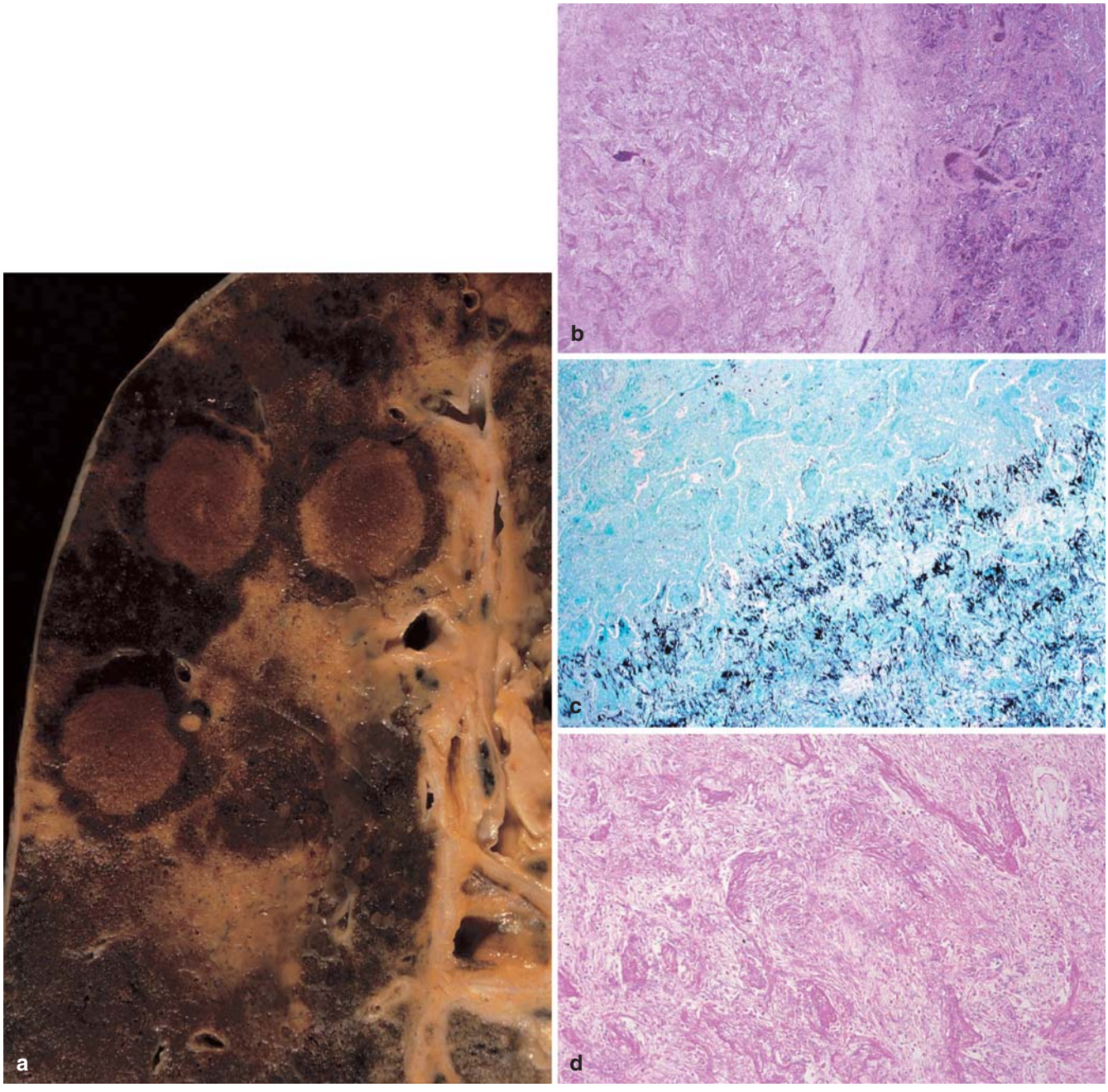

Fig. 4. Invasive pulmonary aspergillosis, discrete nodule. **a** There are sharply demarcated nodules surrounded by hemorrhage on a section of lung. **b** Nodule comprises coagulation necrosis of the lung tissue (*left*) and acute hemorrhage usually accompanies the necrosis and may mimic a halo sign on CT (Hematoxylin-Eosin, ×100). **c** Zone formation (mimicking annual ring) of hyphae aligned in a radial pattern at the periphery of the nodule (GMS, ×40). **d** No inflammatory infiltrates are accompanied in the lesion (Hematoxylin-Eosin, ×200)

sis are: those with alloxin-induced diabetes,[16] those fed deficient diets,[17] and those treated with immunosuppressive drugs such as corticosteroids, cyclosporine A, and cyclophosphamide.[18] Species of animal, routes of infection, and immunosuppressive agents must be chosen carefully. Models of *Aspergillus* infection have been mainly produced in mice, rabbits, and rats.

Mouse models. The most frequently used animal model of aspergillosis is that produced in the mouse, as it has various merits for experimental studies. In addition to the convenience of handling in laboratories, various inbred strains, in which immunological and genetic defects are clearly known, are easily obtainable. Inbred mice can be the most suitable animals for analyzing defense mechanisms and cytokine cascades of the host against *Aspergillus* sp. In the past, mice lethally infected by intravenous inoculation of a conidial suspension were often used to evaluate the efficacy of novel drugs on the basis of mortality and organ cultures, as well as to test the virulence of different strains of *Aspergilli*.[15] More

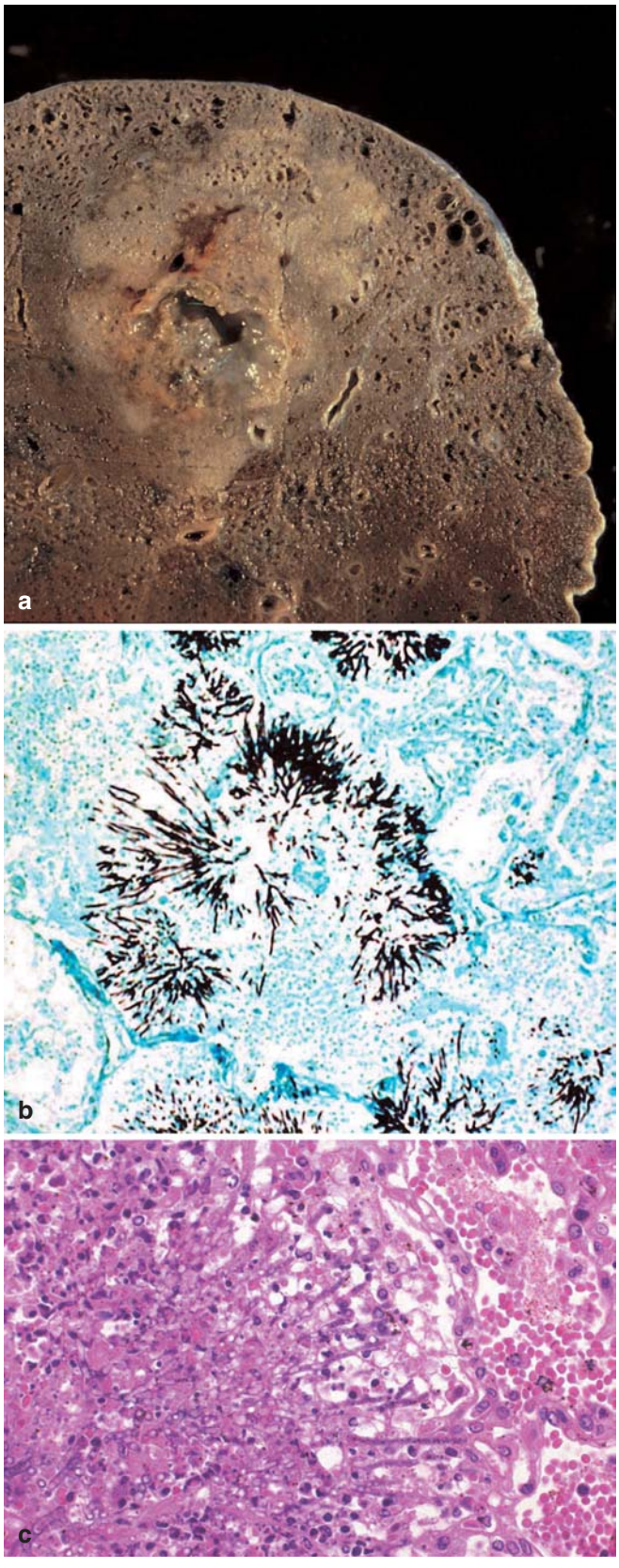

Fig. 5. Invasive pulmonary aspergillosis, solid nodular consolidation. **a** The lesion composed of a fusion of solidified lobules is seen on a section of lung. Necrotic cavity is usually present at the center. **b** An eccentric growth of fungus is present in each alveolus (GMS, ×200). **c** Alveoli involved by the invasion of hyphae are filled with neutrophils and fibrin (Hematoxylin-Eosin, ×400)

recently, however, such intravenous models have been less frequently used.[19] Currently, the route of infection chosen most frequently is the respiratory tract because it reflects the natural route of *Aspergillus* infection in humans.[14,20] Most of these murine models, usually reported as IPA models, are developed in animals pretreated with some immunosuppressive or myelotoxic agents to induce granulocytopenia. Mostly, mice are intranasally inoculated with a conidial suspension, but airborne models in which mice are infected by aerosol aspiration of *Aspergillus* conidia have also been reported.[20] If the usage of experimental infection were limited to an induction of primary lesions in lungs, these intranasal models might be satisfactory. Recently, several *A. fumigatus* strains genetically different from their parental wild-type strains in term of genes encoding putative virulence factors have been developed and produced by gene-disruption techniques and applied to produce experimental murine models of pulmonary infection in which a conidial suspension is inoculated by the intranasal route.[14,20–23] However, regardless of such applications of molecular biological approach, it has not been made clear

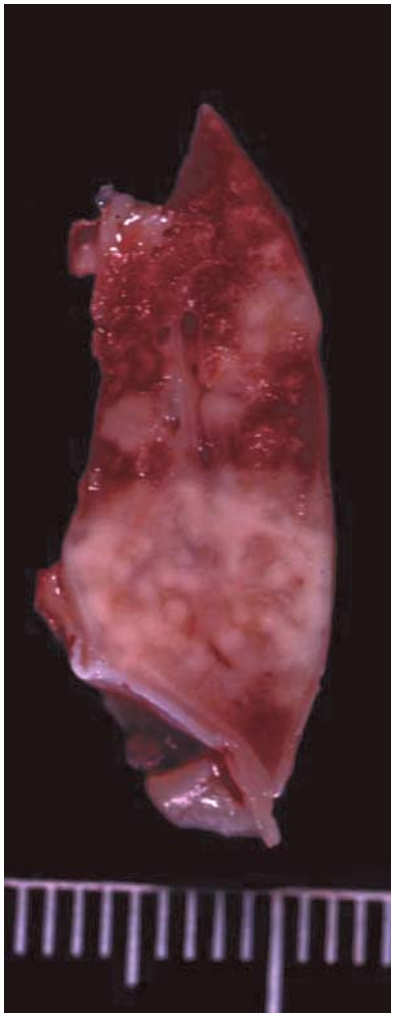

Fig. 6. Experimental pulmonary aspergillosis in rat. There is a fused lobular consolidation in part of the lower lobe, mimicking that of human disease

what the most important virulent determinant of *Aspergilli* in pulmonary lesions is. The histopathological findings of pulmonary lesions in mice have been infrequently reported but appear to be more or less different from that seen in the corresponding human disease, namely IPA. As previously mentioned, the essential histopathological features of IPA in humans can be classified according to patterns which are composed by both a proliferation of *Aspergilli* and by various degrees of exudative response in alveoli. However, in our experience, the development of most experimental lesions in the lungs of infected mice initiates from conidia settling on the bronchial mucosa. This histological difference between mice and humans may be due to the anatomical simplicity of the bronchial tree in mice and the hydrophobicity of *Aspergillus* conidia. Accordingly, when animal models are needed with histopathological features as well as pathophysiological states more similar to those of human IPA, larger animals with more complex bronchial trees should be chosen. Besides, the intraperitoneal route of inoculation has been used to induce fatal infection in mice, particularly those treated with immunosuppressive agents. However, this route of infection has recently been used as a primer to be followed by respiratory tract exposure to conidia intended to elucidate the immunological response[24] or eosinophilopoiesis in bone marrow,[25] as these are closely related to the pathogenesis of ABPA.

Rabbit models. Next to the mouse, the rabbit has been most frequently used to generate a model of *Aspergillus* infection. Recently, rabbits are commonly used to produce a primary pulmonary infection to elucidate the pathogenesis of IPA,[19] to evaluate the clinical usefulness of newly developed antifungal agents,[26,27] and to establish and test new serodiagnostic procedures.[28] The rabbit may be large enough to accommodate a laryngoscope to inoculate a conidial suspension by insertion of catheter.[18] This may be an important merit because a short cervical incision, which is usually performed to expose the trachea of the animals treated with immunosuppressive drugs, increases the risk of bacterial infection. Furthermore, rabbit models are advantageous over mouse or rat models in that a rabbit is much larger, making it possible to perform serial sampling of serum from an infected individual, detailed histopathological observation of pulmonary lesions,[18] and cytological examination of bronchoalveolar lavage specimens.[29] In this sense, rabbits infected by respiratory tract injection of conidial suspension are considered suitable IPA models. Besides, rabbits infected by intravenous injection of a conidial suspension also provide experimental models for some specific forms of systemic aspergillosis such as endophthalmitis[30] and endocarditis.[15]

Rat models. The rat is another species of laboratory animal that has often been used for experimental investigations of aspergillosis because it is of a suitable size for producing a primary pulmonary infection by *Aspergilli*.[17,31,32] We recently reported a new rat IPA model which was produced by an intratracheal injection of agarose beads containing *A. fumigatus* conidia, accurately delivered to the alveoli[4] (Fig. 6). The histopathological findings of the pulmonary lesions obtained in this rat model are closely similar to those for IPA in humans. In addition, the development of fungal emboli, which is also known to be characteristic of the pathology of IPA, is clearly demonstrated.

Conclusion

The present article described pathophysiology of aspergillosis followed by a review of investigational considerations of animal models. Since a large body of invasive aspergillus infection occurs as opportunistic infection, there is a large spectrum of the histopathological feature of lesions demonstrated at the site of infection. Histopathology of the lesions can be understood as a phenotypical representation of interaction between variously lowered defense mechanism of the host and virulence of invading fungi. Details observation with a consideration of previous pathological knowledge regarding infection and inflammation provides a lot of important information to predict pathophysiology of the patient. Moreover, experimental studies can also provide a lot of insights to elucidate pathogenesis of the infection that largely emerged from the clinical and pathological investigations. An importance of pathophysiology should be emphasized to know the implications of radiographic images, clinical symptoms, and laboratory dates. By reviewing of studies reported, the first, especially CT images accurately mirror the histological features of the lesion that can be recognized as a phenotypical representation of pathophysiology of aspergillus infection. This is also confirmed by the reports emphasizing the importance of CT scans to identify hallmark of clinical signs and symptoms of the disease.

References

1. Shibuya K, Coulson WF, Naoe S. Histopathology of deep-seated fungal infection and detailed examination of granulomatous response against cryptococci in patients with acquired immunodeficiency syndrome. Jpn J Med Mycol 2002;43:143–51.
2. Wakayama M, Shibuya K, Ando T, Oharaseki T, Takahashi K, Naoe S, et al. Deep-seated mycosis as a complication in bone marrow transplantation patients. Mycoses 2002;45:146–51.
3. McNeil MM, Nash SL, Hajjeh RA, Phelan MA, Conn LA, Plikaytis BD, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. Clin Infect Dis 2001;33: 641–7.
4. Shibuya K, Ando T, Wakayama M, Takaoka M, Uchida K, Naoe S. 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. Jpn J Med Mycol 1997;38:175–81.
5. Shibuya K, Yamaguchi Y, Naoe S. Animal models of *A. fumigatus* infections. In: Brakhage AA, Jahn B, Schmidt A, editors. *Aspergillus fumigatus*, contribution to microbiology, vol 2. Basel: Karger; 1999. p. 130–8.
6. Gefter WB. The spectrum of pulmonary aspergillosis, J Thorac Imaging 1992;7:56–74.
7. Binder RE, Faling LJ, Pugatch RD, Mahasaen C, Snider GL. Chronic necrotizing pulmonary aspergillosis: A discrete clinical entity, Medicine 1982;61:109–24.

8. Shibuya K, Takaoka M, Uchida K, et al. Histopathology of experimental invasive pulmonary aspergillosis in rats: Pathological comparison of pulmonary lesions induced by specific virulent factor deficient mutants. Microbiol Pathog 1999;27:123–31.
9. Shibuya K. Chest radiographs closely mirror pulmonary Aspergillosis, recognizing image patterns can improve estimate of prognosis. Mycology observer 2001;10:6–7.
10. Caillot D, Casanovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. J Clin Oncol 1997;15:139–47.
11. Raper KB, Fennel DI. The genus *Aspergillus*, Williams & Wilkins, Baltimore, 1965.
12. Kwon-Chung KJ, Bennett JE. Medical Mycology, Lea & Febiger, Philadelphia, 1992.
13. Guiot HFL, Fibbe WE, van't Wout JW. Risk factors for fungal infection in patients with malignant hematologic disorders: Implications for empirical therapy and prophylaxis. Clin Infect Dis 1994; 18:525–32.
14. Smith JM, Tang CM, Van Noorden S, Holden DW. Virulence of *Aspergillus fumigatus* double mutants lacking restriction and an alkaline protease in a low-dose model of invasive pulmonary aspergillosis. Infect Immun 1994;62:5247–54.
15. Miyaji M, Yamaguchi H. Animal models in medical mycology, CRC, Boca Raton, Florida, 1987.
16. Ford S, Friedman L. Experimental study of the pathogenecity of *Aspergilli* for mice. J Bacteriol 1967;94:928–33.
17. Niki Y, Bernard EM, Edwards FF, Schmitt HJ, Yu B, Armstrong D. Model of recurrent pulmonary aspergillosis in rats. J Clin Microbiol 1991;29:1317–22.
18. Berenguer J, Allende MC, Lee W, Garrett K, Lyman C, Ali NM. Pathogenesis of pulmonary aspergillosis: granulocytopenia versus cyclosporin and methylpredonisoloe-induced immunosuppression. Am J Respir Crit Care Med 1995;152:1079–86.
19. Jensen HE, Aalbaek B, Hau J, Latge JP. Detection of galactomannan and complement activation in the pregnant mouse during experimental systemic aspergillosis. APMIS 1996;104:926–32.
20. Tang CM, Cohen J, Krausz T, Van Noorden S, Holden DW. The alkaline protease of *Aspergillus fumigatus* is not a virulence determinant in two murine models of invasive pulmonary aspergillosis. Infect Immun 1993;61:1650–6.
21. Mellado E, Specht CA, Robbins PW, Holden DW. Cloning and characterization of chsD, a chitin synthase-like gene of *Aspergillus fumigatus*. FEMS Microbiol Lett 1996;143:69–76.
22. Mondon P, De Champs C, Donadille A, Ambroise-Thomas P, Grillot R. Variation in virulence of *Aspergillus fumigatus* strains in a murine model of invasive pulmonary aspergillosis. J Med Microbiol 1996;45:186–91.
23. Frosco MB, Chase T Jr, Macmillan JD. The effect of elastase-specific monoclonal and polyclonal antibodies on the virulence of *Aspergillus fumigatus* in immunocompromised mice. Mycopathologia 1994;125:65–76.
24. Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. J Exp Med 1997;185:1089–99.
25. Murali PS, Kurup VP, Guo J, Fink JN. Development of bone marrow eosinophilia in mice induced by *Aspergillus fumigatus* antigens. Clin Immunol Immunopathol 1997;84:216–20.
26. George D, Miniter P, Andriole VT. Efficacy of UK-109496, a new azole antifungal agent, in an experimental model of invasive aspergillosis. Antimicrob Agents Chemother 1996;40:86–91.
27. Berenguer J, Ali NM, Allende MC, Lee J, Garrett K, Battaglia S, et al. Itraconazole for experimental pulmonary aspergillosis: comparison with amphotericin B, interaction with cyclosporin A, and correlation between therapeutic response and itraconazole concentrations in plasma. Antimicrob Agents Chemother 1994;38: 1303–8.
28. de Repentigny L, Kilanowski E, Pedneault L, Boushira M. Immunoblot analyses of the serologic response to *Aspergillus fumigatus* antigens in experimental invasive aspergillosis. J Infect Dis 1991;163:1305–11.
29. Chilvers ER, Spreadbury CL, Cohen J. Bronchoalveolar lavage in an immunosuppressed rabbit model of invasive pulmonary aspergillosis. Mycopathologia 1989;108:163–71.
30. McGuire TW, Bullock JD, Bullock JD Jr, Elder BL, Funkhouser JW. Fungal endophthalmitis. An experimental study with a review of 17 human ocular cases. Arch Ophthalmol 1991;109:1289–96.
31. Cicogna CE, White MH, Bernard EM, Ishimura T, Sun M, Tong WP, et al. Efficacy of prophylactic aerosol amphotericin B lipid complex in a rat model of pulmonary aspergillosis. Antimicrob Agents Chemother 1997;41:259–61.
32. Kurtz MB, Bernard EM, Edwards FF, Marrinan JA, Dropinski J, Douglas CM, et al. Aerosol and parenteral pneumocandins are effective in a rat model of pulmonary aspergillosis. Antimicrob Agents Chemother 1995;39:1784–9.