Review

Histopathology of Deep-Seated Fungal Infections and Detailed Examination of Granulomatous Response Against Cryptococci in Patients with Acquired Immunodeficiency Syndrome

Kazutoshi Shibuya¹, Walter F. Coulson², Shiro Naoe³

¹Department of Pathology, Omori Hospital, Toho University School of Medicine
²Department of Pathology and Laboratory Medicine, UCLA Medical Center
³Department of Pathology, Toho University Ohashi Hospital

Abstract

This study describes general histopathological features of deep-seated mycoses in patients with acquired immunodeficiency syndrome (AIDS) detailed histological examination on cryptococcal lesions with a consideration of morphological modification caused by treatment with highly active antiretroviral therapy (HAART).

In a histopathological review of 164 patients with total human immunodeficiency virus (HIV) infection, the microscopical appearance of esophageal candidiasis which was common in those with single organ involvement revealed necrotic debris containing proliferating hyphae at the site of mucosal erosions without fungal invasion of underlying tissue. The incidence of oral and esophageal candidiasis was followed by that of pulmonary aspergillosis and Candida pneumonia. Nineteen patients including one treated with HAART had generalized cryptococcosis, representing the most common generalized fungal disease. The essential histologic features of the disease were yeast cell proliferation with a histiocytic response, but only minor lymphocytic and neutrophilic components. This was different from those induced by both Candida and Aspergillus infections. Three histologic patterns were recognized in the pulmonary cryptococcal lesions, two of which could be graded with respect to the degree and type of inflammatory reaction. The first was a mild one consisting of small scattered foci of intra-alveolar cryptococcal proliferation with a histiocytic response. The second pattern involved massive cryptococcal infection, which might have been simply more extensive than that in the former. Capillary involvement of alveolar septa was an important common finding in the eighteen patients who had not been treated with HAART. The absence of T cells and decreasing function of antigen-presenting activity in histiocytes were confirmed by immunohistological examination. These findings suggest that the lungs of AIDS patients without HAART offer little resistance to bloodstream dissemination by Cryptococci. The third pattern demonstrated in the patient treated with HAART was characterized by the presence of CD4+ cells, greater response of histiocytes and multinucleated giant cell formation, and lack of massive capillary involvement.

Key words: acquired immunodeficiency syndrome, cryptococcosis, CD4+ cells, highly active antiretroviral therapy, histopathology

Introduction

Deep-seated mycosis occurring in patients with an immunocompromised condition caused by various factors has had a significant impact on contemporary medicine. The worldwide epidemic of acquired immunodeficiency syndrome (AIDS) has become one of the most noteworthy, of which the pathological and clinical features following infection by the human immunodeficiency virus (HIV), and associated with a progressive decrease of cell-mediated immunity due to defective functioning of CD4+ cells¹,². For this reason, certain mycoses have risen dramatically in frequency, particularly systemic cryptococcosis. The incidence of opportunistic fungal infections, including any species of fungus which provokes both localized and generalized disease in AIDS patients, has
been variously reported as 58~81%, of whom 10~20% patients have died as a direct consequence of the fungal infection. It is well recognized that cryptococcal infection in AIDS patients often induces a fatal disease. However, the acceptance of recent antiretroviral therapy among patients has also had a dramatic impact on the epidemiology and clinical characteristics of many opportunistic infections associated with HIV. The present paper describes the histologic features of cryptococcosis and other fungal infections in patients with AIDS, focusing on the differences in inflammatory responses to the various fungal pathogens, and considerable histological modification on cryptococcal lesions noticeably affected by highly active antiretroviral therapy (HAART), as determined at autopsy.

Materials and Methods

The autopsy files of the UCLA Medical Center and the West Los Angeles Veterans Affairs Medical Center were searched for patients with AIDS who had either localized or generalized infection by any species of fungus. The term generalized fungal disease, as used here, is restricted to patients in whom more than two organs were involved by the fungal infection, excluding mucosal lesions.

The histopathological examination to determine organ involvement by fungal pathogen and to know details of tissue responses was carried out on routine hematoxylin and eosin (H & E) preparations, along with selective staining with the periodic acid-Schiff reactions (PAS) and Grocott methods.

To compare the histology of pulmonary cryptococcal infections, sections of pulmonary lesions obtained from a group of non-AIDS patients were employed; the group comprised seven patients without any immunosuppressed condition, ranging in age from 21 to 73 years (mean, 43.7) who had a wedge biopsy or lobectomy for their incidental abnormal shadows on chest roentgenograms. In addition, a standard peroxidase/antiperoxidase technique was used for the antibodies to CD45RO, L26, HLA-DR and IL-1 beta, and the antigen-antibody complexes were visualized with diaminobenzidine. Sections of pulmonary cryptococcal lesions from both immunocompetent and AIDS patients were employed. The former, showing the typical features of peripheral pulmonary granulomas, was chosen from the group of non-immunosuppressed patients as control histology. Further examination was performed: a confocal laser scanning microscope equipped with argon was applied for three-dimensional observation and selective H & E stained sections were examined by concise morphometric analysis.

Results

Autopsies were performed on 164 AIDS patients who had died during the period from 1983 to 1998 in the UCLA Medical Center and from 1982 to 1991 in the West Los Angeles VA Medical Center.

1. Localized disease

The prevalence of localized infection associated with oral candidiasis, esophageal candidiasis, pulmonary aspergillosis, candidial pneumonia, cryptococcal meningoencephalitis, and renal candidiasis (pyelonephritis) was 7.3, 6.1, 4.9, 3.0, 2.4, and 0.6%, respectively, among this group. Lesions of esophageal candidiasis were characterized by necrotic debris at the site of erosions with underlying infiltrates of chronic and a few acute inflammatory cells. Hyphae proliferated in the necrotic debris, but did not invade tissue in any of these individuals.

As mentioned, though most central nervous system cryptococcal infections were found as a part of a generalized disease among our cases, in four the infection was localized in the central nervous system at the time of autopsy. One of them had a history of anti-fungal chemotherapy for generalized disease, but the remaining three had not been treated for any fungal infections. Lesions consisted of multiple small cysts containing many encapsulated yeasts, in cortical and subcortical areas, which were also present in the adjacent subarachnoidal space. There was a histiocytic response which varied from case to case, but necrosis and neutrophilic infiltrates were not encountered.

Localized lung disease was associated with two fungal species, Aspergillus and Candida. The essential feature of the pulmonary lesions with both fungi was purulent bronchopneumonia, in which bronchioles and alveoli were filled with necrotic debris, neutrophils, and proliferating fungi. Alveolar septa often showed coagulative necrosis and disruption, but lymphocytes and fibrosis were not present. Bacteria were occasionally observed within the necrotic debris. With Aspergillus infection, the mucosa was focally necrotic and eroded with readily visible hyphae.

2. Generalized disease

Nineteen (11.6%) cases of generalized cryptococcosis were found to cause the most common
generalized fungal infection among the 164 autopsies. Much less commonly encountered were generalized histoplasmosis, coccidioidomycosis and candidiasis. Those prevalence was 1.9, 1.2, and 1.2%, respectively. Granulomatous lesions were demonstrated in the lungs of individuals with generalized histoplasmosis and coccidioidomycosis, but were poorly formed in coccidioidomycosis (Fig. 1). Besides, most of those affected with generalized cryptococcosis had wide-spread lesions. The lung being the organ most commonly involved (94.7%). Lymph nodes, spleen, and the central nervous system were also frequently involved. Yeast cell proliferation, a histiocyte response, and minor lymphocytic infiltration, but

---

**Fig. 1.** Pulmonary lesion of generalized coccidioidomycosis, showing spherules and loosely aggregated macrophages (PAS reaction, X400)

**A** Images obtained by a process of confocal laser scanning microscope reveal a focus of histiocytic aggregation in a patient with AIDS without treatment of HAART. Whereas histiocytes phagocytizing many yeast cells are aggregated in alveoli, no evidence of cytoplasmic fusion is found with an indication of the border of each cytoplasm clearly represented. At left is a computer-added image generated from fluorescence by argon laser, and at right is a monochromic image transferred from the right.

**B** Three-dimensional representation of the intensity of the image of figure 1-A. Border of cytoplasm is demonstrated as a continuous peak, Z-axis, height of the image indicates strength of fluorescence in each point. At left is a computer-added image generated from fluorescence by argon laser, and at right is a reversed monochromic image transferred from the left.
an absence of neutrophils and eosinophils were common findings. However, a minor neutrophilic infiltrate was infrequently observed in lung, liver, and kidney. The classical granulomas, usually present in primary cryptococcosis in immunocompetent patients, were not observed in our cases.

3. Histopathology of pulmonary cryptococcosis

To elucidate the histopathological characteristics of cryptococcal lesions in patients with AIDS, particularly the relationship in histological features and reactivation of CD4+ cells induced by HAART, a more detailed histopathological examination of pulmonary lesions was performed.

3-1) Pulmonary lesions in AIDS patients

Three histologic patterns emerged from the study. In fifteen individuals, lesions were small, consisting of intra-alveolar proliferations of *Cryptococcus* with a histiocytic response. The architecture was unaltered, but involved alveoli were mildly expanded by both proliferating *Cryptococcus* and reacting histiocytes. *Cryptococcus* were present in capillaries in all fifteen cases. However, the number of intra-alveolar lesions varied from case to case. In five, lesions were scarce, while in ten cases, there was focal proliferation of *Cryptococcus* with a major histiocytic response. *Cryptococcus* were widely distributed in the lung, involving many alveoli, and were accompanied by histiocytes and multinucleated giant cells which were loosely aggregated (Fig. 2). Most of the giant cells were of foreign body type with less than ten nuclei per cell. Typical Langhans giant cells were not represented. *Cryptococcus* were seen as extra- and intracellular yeast cells with budding forms in both positions (Fig. 3).

In three of the remaining four cases, there was a massive proliferation of *Cryptococcus* in both expanded alveoli and the capillary/interstitium,
and septa were destroyed. A histiocytic and giant cell response was present, as well as focal hemorrhage (Fig. 4). On the assumption that these two patterns represented progressive severity, they were correlated with the number of organs involved in each individual, but no particular association was found.

In the remaining one case, which had been treated with HAART consisting of zidovudine, lamivudin, and indinavir, however, a significant difference in histopathology was recognized. The lesion, which kept to the third pattern in our group, demonstrated features characterized by a presence of lymphocytic infiltrate, much greater response of histiocytes and multinucleated giant cell formation, and lack of massive capillary involvement. Whereas foci of dense cryptococcal proliferation were distributed throughout the lung, they were encompassed with fibroblasts and reacting histiocytes with most considerable multinuclear giant cell formation, but no giant cells of typical Langhans type were found (Fig. 5).

3-2) **Histology of pulmonary cryptococcosis in non-AIDS patients without immunological dysfunction**

Circumscribed granulomas were seen in all seven cases without any history of immunological dysfunction. The lesion was composed of compactly aggregated histiocytes and multinucleated giant cells, including both Langhans and foreign body type, with numerous intracytoplasmic organisms (Fig. 6).

3-3) **Comparative immunohistological study on the lesions developed in those with AIDS treated with and without HAART and those without apparent immunological dysfunction**

Lung sections from a lesion in an AIDS case without HAART showing cryptococcal proliferation with loosely aggregated reactive histiocytes and multinucleated giant cells, a lesion demonstrated in a case with HAART, and the typical granulomas developed in a 33 year old immunocompetent male were used for this study. CD45RO positive small round cells were visible in the typical granuloma developed in the immunocompetent individual. Histiocytes and foreign body giant cells with numerous intracytoplasmic organisms were strongly positive for both HLA-DR and IL-1 beta. There were a few small aggregates of L26 positive cells in the lesion. In the AIDS case with HAART, CD45RO...
Discussion

Before the era of HAART, fatal opportunistic infections such as cryptococcosis, mycobacterial infection and Pneumocystis pneumonia, usually developed in patients with AIDS. Among them, oral and esophageal candidiasis and generalized cryptococcosis are regarded as particularly important complications of the disease. The first issue which should be stated here is that the present autopsy study confirms this.

In AIDS-related esophageal candidiasis, one important point provided by this study is that while necrotic debris was present at the site of mucosal erosions, the underlying tissue was not invaded by proliferating hyphae. There were also very few cases with generalized candidiasis, and their pulmonary lesions showed prominent necrosis and neutrophilic infiltration. These facts suggest that neutrophils may play an important role restricting candidal infection, in individuals with terminal HIV infection. Localized pulmonary aspergillosis also occurred in those with single organ involvement, and also featured purulent bronchopneumonia with necrosis. Although it was reported recently that production of IL-4 by CD4+ cells may be one major factor discriminating susceptibility and resistance to experimental Aspergillus infection, no cases we observed had generalized aspergillosis. Patients with AIDS appear to prevent bloodstream dissemination of inhaled conidia of Aspergillus sp. by inducing a non-specific purulent inflammation in the lung, the primary site of infection. This notion is supported by a previous report emphasizing that the defense mechanism against Aspergillus is mainly dependent on the function of neutrophils and macrophages.

Thus, there was a striking histological difference between Aspergillus or candidal and cryptococcal infection in lungs of our cases with AIDS. No purulent inflammatory responses were observed in the cryptococcal lesions examined in this study. This result may be supported by the previous investigation which concluded that cryptococcal polysaccharides, especially glucuronoxylomannan, can cause shedding of L-selectin from the surface of neutrophils, and this may prevent neutrophils from attaching to the endothelial cell surface. On the other hand, it has been reported recently that eosinophils may be one of the effector cells against Cryptococcus neoformans, and tissue eosinophilia was experimentally induced in lungs of infected mice. In such a case, depletion of CD4+ cells ablates IL-5 production by lung leukocytes in vitro and eosinophil recruitment in vivo. The present study revealed that none of the cryptococcal lesions was associated with eosinophilic infiltration, and this is consistent with depletion of CD4+ cells in HIV infection.

Cryptococcal infection has been recognized as a primary deep-seated fungal infection in immunocompetent hosts. The disease is usually asymptomatic, and although typical granulomas develop in the lung, this type of infection is thought to be self-limiting and benign. However, the incidence of opportunistic cryptococcal infection has been rising in recent years, due in large part to increasing numbers of immunocompromised patients. Infection with Cryptococcus neoformans is still a life-threatening disease often occurring in patients with AIDS. In the past, many studies on cryptococcal infection in patients with AIDS have been reported, most of them concerned with clinical, microbiological, and immunological aspects, but few with the histology of human disease. In this report, histologic features are emphasized, focusing on the pulmonary lesions found in 18 patients who died of AIDS. Four distinct histologic types of pulmonary cryptococcosis were classified by McDonnell and Hatchins as peripheral pulmonary granuloma, granulomatous pneumonia, intracapillary/interstitial involvement, and massive pulmonary involvement, respectively, without special reference to a specific underlying disease. Cryptococcal infection of the lungs in patients with AIDS took the form of intracapillary/interstitial or massive pulmonary involvement. Peripheral granulomas and granulomatous pneumonia were not encountered in patients with AIDS.

The majority of our cases had lung lesions characterized by alveoli containing proliferating Cryptococci, reactive histiocites, and multinucleated giant cells, and organisms were not seen proliferating within the bronchial mucosa. On the other hand, capillary involvement was ubiquitously demonstrated in the lesions, whereas organisms were limited to relatively few alveoli. The lung is commonly considered as the portal of infection, and might be expected to reflect this by manifesting an intra-alveolar proliferation of...
inhaled yeasts without capillary involvements. However, we found five cases with generalized disease of whom the histologic features were characterized by a few lesions of intra-alveolar proliferation of Cryptococcus and widespread intracapillary involvements without fibrous thickening of involved septa. In such a case, the intracapillary form may represent hematogenous dissemination of inhaled yeasts to which an extremely weak inflammatory response might be induced in alveoli in patients with terminal HIV infection. It has been reported that acute-phase mortality from cryptococcosis among AIDS patients with pneumonia was 42%\(^6\). Thus, the evidence of such a pattern is most likely explained by the rapidity of onset of vascular involvement, also leading to generalized disease. In addition, the histological alteration of such a pattern might be expected to manifest a normal chest roentgenogram which had been reported as a common roentgenographic finding of pulmonary and/or generalized cryptococcosis in patients with AIDS\(^{23}\). However, there is still the possibility that the form reflects hematogenous dissemination from another organ in which primary infection was induced. This may explain the evidence of patients with central nervous system cryptococcal infection without pulmonary lesions at the time of autopsy.

On the other hand, there is a striking difference in the histological features of cryptococcal lesions in AIDS patients with and without HAART. This can be summarized by the presence of lymphocytic infiltrate, much greater response of histiocytes and multinucleated giant cell formation, and lack of massive capillary involvement. This form might be transformed from massive capillary involvement that might have been previously produced in the patient by primary cryptococcal infection, as a sequel to the administration of HAART. In fact, CD4+ cells were visible at the periphery of each nodule consisting of dense cryptococcal proliferation. Accordingly, recovering and reactivation of CD4+ cells induced by HAART can activate histiocytic response against Cryptococcus while prominent multinucleated giant cell formation. A hallmark of infection with Cryptococcus neoformans is depression of the immune system characterized by poor inflammatory responses and loss of delayed-type hypersensitivity and antibody responses\(^{26}\). We found discrete granuloma consisting of compactly aggregated giant cells and histiocyte, being strongly positive for HLA-DR as well as IL-1 beta, in all seven immunocompetent individuals, most likely as a sequel to normal functioning of all considerable defense mechanisms against Cryptococcus. Development of a T cell-mediated pulmonary inflammatory response is critical for clearance of Cryptococcus\(^{29}\), and this has been demonstrated in murine cryptococcosis and supported by data from several human studies\(^{2,26,30}\). Although it has been discussed that humoral immunity was elicited by cryptococcal capsular polysaccharide\(^{31,32}\), none of the histopathological hallmarks of activated humoral immunity, e.g. reactive lymphadenitis, lymphoid follicular hyperplasia of bronchial mucosa, and so on, were represented in the cases we studies.

The present study showed an absence of CD4+ cells in pulmonary lesions using immunohistochemistry, as well as a recovery of them at the focus of the individual treated with HAART. Furthermore, the expression of IL-1 beta and HLA-DR was weak in histiocytes and multinucleated giant cells, when compared with granulomatous lesions in immunocompetent individuals. Alveolar macrophages are recognized as a first line of defense against cryptococcal infection, and it has been reported that human alveolar macrophages from normal subjects play a significant role in antigen-presentation to T-cells, while their effector function seems to be less relevant, at least in the afferent arm of the immune response to this yeast\(^{33}\). The study is consistent with decreased antigen-presenting activity of histiocytes in pulmonary cryptococcal infection\(^{34}\), in turn reducing the number of T-cells in the lesion, and consequently inducing. The important event, however, from findings of this study, is that the phagocytic activity of histiocytes reacting towards Cryptococcus was unaffected in AIDS patients, and phagocytosis was commonly present. This histological characteristic may be supported by a previous report indicating that bronchoalveolar lavage cells from early HIV-infected individuals did not have an intrinsic defect in fungistasis of Cryptococcus\(^{35}\). In addition to the lack of typical Langhans giant cells, the reactive histiocytes and multinucleated giant cells reveal that while there is mostly normal phagocytic function, there is a decrease in the ability to kill Cryptococcus. The essential feature of the pulmonary lesions in AIDS patients is the proliferation of Cryptococcus with reactive histiocytosis and a much lesser lymphocytic infiltration, very possibly the morphological response to cryptococcal infection in patients with manifest T-cell dysfunction. We wish to emphasize the absence of typical granuloma formation, the extended capillary involvement and the minimal lymphocytic response in cryptococcal disease in AIDS patients.
Further, reactivation of CD4+ cells induced by HAART can transfer the histological feature of the cryptococcal lesions from predominant massive capillary involvement to granuloma-like formation in the presence of CD4+ cells.

Acknowledgments

We thank Dr. Harrison Latta, Dr. Klaus Lewin, Dr. Scott Nelson, Dr. Joan Mao (Department of Pathology, UCLA CHS), Dr. Jerome S. Wollman MD (Department of Pathology, VA Wadsworth Medical Center), Dr. Kazuyoshi Kawakami (First Department of Medicine, University of Ryukyus), Dr. Stuart Levitz (Medicine and Microbiology, Boston University School of Medicine), Dr. Megumi Wakayama (Department of Pathology, Toho University Ohashi Hospital), and Dr. Yoshiro Sawae (Department of Medicine, Kyushu University) for their valuable advice.

References

14) Huffnagle GB, Boyd MB, Street NE, Lipscomb MF: IL-5 is required for eosinophil recruitment, crystal deposition, and mononuclear cell recruitment during a pulmonary Cryptococcus neoformans infection in genetically susceptible mice (C57BL/6). J Immunol 160: 2393-2400, 1998.
28) Blackstock R, Casadevall A: Presentation of cryptococcal capsular polysaccharide (GXM) on activated antigen-presenting cells inhibits the T-suppressor response and enhances delayed-type hypersensitivity and survival. Immunology 92:

この論文は、第45回日本医真菌学会総会の“シンポジウムⅡ：難治性深在性真菌症－新しい観点から－”において発表されたものです。