Retraction:

Pleural Cryptococcosis with Idiopathic CD4 Positive T-lymphocytopenia

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Pleural Cryptococcosis with Idiopathic CD4 Positive T-lymphocytopenia

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Abstract

A 19-year-old man was admitted to our hospital because of chest pain. He was diagnosed as having pleural cryptococcosis by pleural biopsy. His CD4 positive T-lymphocyte count was low (<300 µl) and there was no evidence of human immunodeficiency virus infection. He was successfully treated with fluconazole. However, his CD4 positive T-lymphocyte counts remained low after the recovery and he was diagnosed as idiopathic CD4 positive T-lymphocytopenia. Pleural cryptococcosis is rare and its predisposing condition is still controversial. To our knowledge, this is the first case of pleural cryptococcosis associated with idiopathic CD4 positive T-lymphocytopenia.

Key words: CD4 lymphocytes, Cryptococcus neoformans, CD95 (Fas/Apo-1)

Introduction

The most common manifestation of Cryptococcus neoformans infection is meningitis followed by pulmonary involvement (1). Among pulmonary infections, pleural cryptococcosis is extremely rare, and, for the early diagnosis, it is important to pay attention to immuno-deficientive status such as acquired immuno-deficiency syndrome (AIDS) (2).

On the other hand, idiopathic CD4 positive T-lymphocytopenia (ICL) is a recently described syndrome characterized by a significant depression in the number of circulating CD4 positive T-lymphocytes in the absence of human immuno-deficiency virus (HIV) infection (3). ICL causes some opportunistic infections including Cryptococcus neoformans (4). However, there has been no report of pleural cryptococcosis associated with ICL. Here, we report the first case of pleural cryptococcosis associated with ICL. We propose that ICL can become one of the predisposing conditions of pleural cryptococcosis.

Case Report

A 19-year-old man was admitted to our hospital because of chest pain. One month before admission, he developed left sided chest pain. Two weeks before admission, he went to another hospital. A chest X-ray film and chest computed tomography (CT) showed thickness of left pleura. He was treated with some antibiotics (piperacillin, minocycline and imipenem) with unsuccessful results. His chest pain persisted and he was admitted to our hospital. He had been in good health except for appendicitis 3 years before admission, and had never traveled abroad nor abused drugs. He had smoked a pack of cigarettes per day for 2 years.

Laboratory findings on admission showed leukocytopenia (white blood cell counts was 2,980/µl and CD4+ lymphocyte counts was 292/µl). The amount of pleural effusion was too small to aspirate for laboratory tests. Natural killer cell activity was normal and serum immunoglobulin levels were in normal range. Lymphocyte blastogenesis against PHA was normal range. Purified derivative tuberculin skin test was negative. The antibodies for HIV and human T-lymphotrophic virus type-I (HTLV-I) were negative in serum. Cryptococcus antigen was negative in blood and in cerebrospinal fluid. Cultures of his sputum, blood, urine and cerebrospinal fluid were all negative for fungi. Chest CT still showed left pleural thickness (Fig. 1). Thoracoscopic pleural biopsy was performed for a definitive diagnosis. The biopsy specimen revealed pleural thickness with numerous infiltrates that was identified as budding encapsulated yeast forms, compatible with cryptococcus, and some inflammatory cells (Fig. 2). The diagnosis of pleural cryptococcosis was made and anti-fungal therapy was begun (fluconazole).
Three weeks after the start of therapy, his chest pain disappeared. He was treated with fluconazole (400 mg/day) for three months. After the treatment, the abnormal shadow on his chest X-ray film almost disappeared (Fig. 3). However, the lymphocyte counts were still low and CD4 positive T lymphocyte counts were under 300/cu mm on more than one separate examination (Fig. 4). He is in good health 3 years after admission. His CD4 positive T lymphocyte counts still remain low and the antibody for HIV is still negative. During the entire clinical course, he never received immunosuppressive drugs and he showed no evidence of malignant tumor.

**Flow cytometry analysis**

The expression of cell surface CD95 (Fas/APO-1) antigens was evaluated by flow-cytometry analysis. One hundred thousand cells were suspended in 50 μl of cold PBS containing 0.1% sodium azide, 10 ng/ml BSA and 20 μg/ml of human IgG, incubated for 10 minutes on ice, and with mouse monoclonal anti-CD95 antibody and FITC-conjugated CD4 antibody for an additional 15 minutes on ice. Cells were washed with PBS, and incubated with PE-conjugated goat anti-mouse IgG for 15 minutes on ice. The cells were washed with PBS, and subsequently analyzed by flow cytometry using a FACScan (Becton Dickinson, San Jose CA, U.S.A.). Results were processed using the CellQuest software (Becton Dickson). In our patient, 80.5% CD4 cells were positive for CD95 while 6.2±10.2% CD4 cells were positive for CD95 (Fig. 5).

**Measurement of soluble Fas and Fas ligand in serum**

We measured serum soluble Fas and Fas ligand concentrations in serum using an ELISA kit purchased from R&D Systems, Minneapolis, MN.

**Discussion**

*Cryptococcus neoformans* infection rarely occurs in healthy individuals. Although if it does, pulmonary involvement commonly manifests with pulmonary nodules (5). Conversely, diffuse interstitial infiltrates, alveolar consolidation, ground glass shadow and mediastinal or hilar lymphadenopathy are most commonly seen in cases of immunocompromised hosts with a disease such as AIDS (1). Specifically, pleural abnormality is reported to be the least common pulmonary manifestation of *Cryptococcus neoformans* infection and its frequency was reported to be 0/10 (6), 1/12 (7) and 3/14 (8). Some predisposing conditions of pleural cryptococcosis were previously reported such as malignant lymphoma, diabetes mellitus, renal failure, treatment with immunosuppressive drugs (9) and infection with HTLV-I (10), however, to the best of our knowledge, ICL has not been reported as a predisposing condition.

In 1992, the diagnostic criteria for ICL were defined by the Centers for Disease Control and Prevention (CDC) as follows (11): 1) CD4 positive T-lymphocyte depletion (absolute CD4 positive T lymphocyte level <300/cu mm or <20% of total lymphocytes on more than one trial), 2) no serological evidence of HIV infection, and 3) no defined immunodeficientive status or therapy associated with T-cell depletion. We believe that this case fulfilled the criteria for ICL for the following reasons: 1) The patient showed no evidence of HIV infection and had never received immunosuppressive drugs; 2) he suffered from an opportunistic infection (*Cryptococcus neoformans*); 3) the CD4 positive T lymphocyte count was below 300/cu mm on more than 2 occasions and was still below 300/cu mm 3 years after admission.

ICL is a rare disease with an incidence of 0.25% among blood donors (12) and 1.5% in HIV-seronegative homo-
Pleural Cryptococciosis with ICL

Figure 2. Hematoxylin-eosin staining of the biopsied specimen of pleura showing granuloma formation with infiltration of inflammatory cells (A: original manifestation ×150, B: original manifestation ×400). And Grocott staining of the biopsied specimen showing well-encapsulated yeast forms characteristic of Cryptococcus neoformans (C: original manifestation ×150, D: original manifestation ×400). The arrow indicates yeast forms.

Figure 3. Chest X-ray film 3 months after the treatment with fluconazole.

Figure 4. Clinical course of the present case.
sexual men (13). In Japan, only 19 cases have been reported and *Mycobacterium tuberculosis* is the most frequent pathogen followed by atypical mycobacterium (14–17) while *Pneumocystis carinii* or *Cryptococcus neoformans* are the common pathogens of ICL in other countries (3, 18). There are some reports of ICL cases with cryptococciosis infection in the central nervous system and pulmonary parenchyma (19), however there are no reports of ICL cases with pleural cryptococciosis infection as seen in the present case.

Granulomatous inflammation evoked by cryptococcus is uncommon and is not observed in immunosuppressed individuals (20). Granuloma formation indicates an intact cell-mediated immunity resulting in localization of the disease (20, 21). In the present case, natural killer cell activity and lymphocyte blastogenesis against PHA were normal. These results indicate that our case had an intact cell-mediated immunity despite the low number of CD4 positive lymphocytes. This is an interesting point and we think this might explain why ICL cases do not have disseminated cryptococciosis infection. Further accumulation of cases addressing this point is necessary to clarify the detailed immune mechanism in ICL.

Laurence et al reported a possible association of CD4 positive lymphocyte apoptosis with ICL (22) and Roger et al reported an ICL case with high CD95 (Fas/APO-1) (23). CD95 (Fas/APO-1) is a member of the tumor necrosis factor (TNF)/nerve growth factor superfamily that can directly transduce an apoptotic death signal on trimerization with Fas ligand (CD95L) or Fas-specific Abs (25, 26). Fas is expressed on the surface of various cell types, including T cells, B cells, and macrophages, as well as cells of the liver, spleen, lung, testis, heart, brain, and intestine (27, 28). Apoptosis provides one mechanism for the regulation of peripheral CD4 positive T-cell homeostasis. Recent studies suggested that T-cell apoptosis is associated with T-cell activation by either CD3 positive cross-linking, phorbol myristate acetate, or phytohemagglutinin (25, 26, 29–32). This occurs through a MHC nonrestricted monocyte-dependent mechanism (33). The requirement for T-cell activation seems to be a common feature of monocyte-dependent apoptosis mediated by Fas-FasL interaction (30, 34, 35). Laurence et al suggested that patients with ICL linked to clinical immune suppression have evidence for accelerated T cell apoptosis *in vitro* (22) and Roger et al reported an ICL case in which CD4 positive T lymphopenia was correlated with an overexpression of CD95 (Fas receptor) together with spontaneous and Fas-induced apoptosis (24). The result of the present patient was compatible with previous reports. Therefore, we propose the possibility that “primed” Fas positive CD4 positive lymphocytes of ICL may interact with activated monocytes that express FasL, resulting in apoptosis that leads to preferential deletion of CD4 positive T cells and immune dysfunction in ICL.

In summary, we report the first case of pleural cryptococciosis in association with ICL. ICL should be considered as one of the predisposing conditions of pleural cryptococciosis. CD95 (Fas/APO-1) may be associated with the CD4 positive T lymphocyte depletion in ICL; in this regard,
further studies addressing this point may be necessary to clarify the pathogenesis of ICL.

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