A Case of Penicillium marneffei Infection in an AIDS Patient
—The First Case in Japan—

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Abstract

A 38-year-old Japanese AIDS patient developed papular lesions which rapidly increased in number, eroded and crusted, and spread over not only skin but also the mucosal surface. High fever, sore throat, malaise and hepatosplenomegaly were also noted, and he died despite 2 months of intensive treatment. An autopsy revealed numerous histiocytes infected with Penicillium marneffei in the lymph nodes, liver, spleen, bone marrow, skin, and mucosal surface of the oral cavity to the pharynx. This case is thought to be the first Japanese case of penicilliosis marneffei.

Key words: penicilliosis marneffei, Japanese case, AIDS, opportunistic infection

Penicillium marneffei is a dimorphic fungus which is endemic in southeast Asia, and the first human case was described in 1973¹. Instances of patients infected with P. marneffei have been reported in both healthy and immunocompromised hosts². The number of cases is rapidly increasing, especially among residents of southeast Asia, as the number of the patients infected with human immuno-deficiency virus (HIV) increases, but in Japan there has been no case reported to date. We have experienced an acquired immunodeficiency syndrome (AIDS) patient with high fever and papulovesicular rash, who later was discovered as having had P. marneffei infection. We believe this was the first case in Japan.

Report of the case

A 38-year-old man had been abroad many times on business to Africa, Mexico and Pakistan, each for several months. He had malaria 3 times when in Africa, but was otherwise healthy and had no history of blood transfusion. He had never married, but was heterosexual, and in 1994 had a vacation in Thailand. He had an episode of fever and lymphadenopathy in 1986 but the tests for HIV antigens were negative at that time.

He was diagnosed with AIDS in July, 1994, based on long-standing fever, malaise and leucopenia. The laboratory data (Table 1) showed low WBC count (2300/mm³), low percentage of lymphocyte population (7%, the absolute number 161/mm³), and that the number of CD4 positive lymphocytes was 7/mm³. Tests for HIV antibodies are shown in Table 2. The antibodies for Pneumocystis carinii and cytomegalovirus were negative. Treatment with azidothymidine (AZT) and didanosine (ddI) was begun. Clarithromycin, fluconazole and pentamidine isetionate were added for possible opportunistic infections.

He consulted the Clinic of Dermatology on July 7th. At that time he had seborrhoic dermatitis on his scalp, perleche and thrush on his oral mucosal surface, and scaly erythema on his inner thighs and scrotum (spores and hyphae of Candida positive on KOH preparation), and scales on both soles (hyphae of Trichophyton positive on KOH preparation). Treatment with antifungal cream and lotion cleared these lesions in about a week, but small vesicles and erosions with a red flare, appeared on his lips and body, and Tzanck smear test revealed viral multinucleated giant cells. These erosions disappeared after intravenous aciclovir treatment.

In August his general condition became better and he was discharged on August 29th. His condition remained good for a while but a few months
after the discharge anorexia and weight loss appeared which led to the discontinuance of the anti-HIV drugs. In May, 1995, he was readmitted to the hospital because of bloody stool and fever. PCR of the biopsy specimen from the ileocecal area showed Mycobacterium tuberculosis but not Mycobacterium avium complex, cytomegalovirus, nor herpes simplex virus. Also detected were a tubercle in the right lung field on a plain X-ray film, and a cold abscess in the pelvic cavity on CT scan. In June, high fever together with right axillar lymph node swelling, appeared and he was referred to the dermatology clinic.

His right axillar lymph nodes were swollen to thumb head size, elastic soft, and no tenderness was noted. Pink papules appeared to be grouping on his right chest wall to the right side, and larger papules, with infiltration and crusts on top, were seen on the right side of the neck and abdominal wall (Fig. 1). He also suffered from sore throat, and physical examination revealed small ulcers on both palatine tonsils and swollen erythematous epiglottis.

At the end of June, the small pink papules rapidly began to grow in size and number, to form infiltrated papules with crust formation, and the ulcers with red flare on his nasopharyngeal region became numerous. His cervical, axillar, and inguinal lymph nodes were swollen but not tender. At that time we were unable to make a diagnosis.

Bloody stool and diarrhea stopped after antituberculous drugs were given but his high fever and sore throat remained unchanged. General condition of the patient became worse despite the treatments and therapies, and the number of his CD4 positive lymphocytes was 1/mm$^3$. A left retinal hemorrhage occurred in August, and Foscarnet was given for the possible cytomegalovirus infection. Pancytopenia, renal failure and respiratory failure appeared, chest X-p showed diffuse cloudy shadows, and moist rale was heard in both lung fields. Skin eruptions became numerous, body temperature rose above 40°C, watery diarrhea appeared frequently and Methycillin-resistant Staphylococcus aureus (MRSA) was detected from the patient’s blood culture. Additional Vancomycin could no longer ease the discomfort of patient and he died on August 25th.

**Autopsy results**

The liver swelled to a weight of 3400 g, the spleen to 1240 g, and lymph nodes showed systemic enlargement. These organs were diffusely and massively infiltrated with Penicillium-infected histiocytes which destroyed the normal architecture. The adrenal glands, kidneys, bone marrow, lungs and stomach were also infiltrated with these Penicillium-laden histiocytes, and the skin eruptions and mucosal lesions were due to the Penicillium infection. There were numerous MRSA-infected purulent lesions in the kidneys which by themselves could prove fatal. The lesions of tuberculosis in the lungs and intestine were almost cured. There was a residual lesion of Mycobacterium infection in the peritoneum.

**Histological examination**

Histological examination of the skin lesions showed numerous histiocytic infiltrations from...
papillary dermis to the subcutis. These histiocytes contained round to oval spores 2-3 μm in diameter. These spores were clearly demonstrated with PAS or Grocott stain, but as they were so numerous, they could be seen even in HE stained sections. HE stained the cytoplasm of the spores, whereas PAS or Grocott stained their cell wall, therefore in HE stained sections the spores seemed somewhat smaller with a blurred border.

Some spores lay free in the tissue. These had the same diameter, but some of them became elongated to form short tubes like "sausages" with central septa (binary fission) (Fig. 2), which is a characteristic feature that differentiates this organism from *Histoplasma capsulatum*. The antibody against *P. marneffei* showed cross reaction between *H. capsulatum*, so the morphological differentiation is of value.

Figure 3 showed the spores stained with antibody against *P. marneffei* (courtesy of Dr. Hotchi). The antibody was made by the procedure described elsewhere and precipitin titer was ×16. Staining titer for the indirect immunoperoxidase method for *P. marneffei* was ×2000, and for *H. capsulatum* ×500. The antibody was then absorbed with the spores of *H. capsulatum* to exclude the cross reaction as described, and the staining titer of the antibody after absorption for *P. marneffei* was ×500 (appropriate staining titer ×100), and for *H. capsulatum* ×0.

**Discussion**

Penicilliosis marneffei is thought to be an indicator disease for AIDS in Thailand, and the disease is expected to spread to Japan as number of AIDS patients is rapidly increasing in this country. They are thought to have been infected by HIV about 10 years earlier, when oversea tours were very popular. The progress in transportation made a formerly endemic infectious agent pandemic. The patient described here was thought to have been infected by HIV by 1986, and by *P. marneffei* in about 1994, which remained asymptomatic until the number of his CD4 positive lymphocytes became below 20/μm³. He had had many opportunistic infections, and had recovered from many of them but finally MRSA and *P. marneffei* overtook him.

He did not develop Kaposi's sarcoma. The now common sarcoma was formerly found sporadically in elderly males of eastern Europe, but now the disease is firmly associated with the later stages of HIV infection, and is much commoner in homosexuals than others at risk. Endemic cases have found in Africa. The disease is also seen among patients with non-HIV induced immunosuppression, such as transplant patients. Considering that the present case had been to Africa and Asia, the reason he did not develop Kaposi's sarcoma is probably because he was heterosexual.

Apart from the patients, *P. marneffei* was isolated from the viscera of bamboo rats, and soil from the bamboo rat burrow. It is still a question whether the bamboo rats are natural reservoirs for transmission, or are coincidental hosts. However, it is thought that the conidia of *P. marneffei* in the environment are taken in through inhalation, and the initial infection takes place in the lung, since it is statistically proved that exposure to soil is a more risky factor than exposure to or consumption of bamboo rats, and a Chinese woman developed *P. marneffei* osteomyelitis after working with the Vietnamese workers. The infected remains asymptomatic until he is immunosuppressive, but the disseminated infection was reported among immunologically normal persons, and the incubation period has not yet been determined.

In immunocompromised hosts *P. marneffei* infec-
tion is fatal. Histopathological reactions following this infection are classified into 3 types: granulomatous, suppurative, and anergic. The anergic reaction is seen in compromised hosts, with histiocytes packed with living organisms, and many scattered extracellular yeast-like cells and 'sausage' forms proliferating in binary fission, but no other inflammatory cells such as PMNs or lymphocytes are seen around them. P. marneffei is one of the facultative intracellular parasites, and the reaction indicates a progressive and disseminated infection. So, early and accurate diagnosis is necessary for the successful prevention and treatment of penicilliosis marneffei. Clinically, the patients showed no specific symptoms—fever, malaise, weight loss, anemia, lymphadenopathy and hepatosplenomegaly. A more specific sign was the skin eruptions—papules with central necrosis. The culture from the clinical specimens such as pus, skin scrapings, sputum, biopsy specimen (bone marrow aspiration, skin biopsy) and blood readily gives growth in a mycelial phase on Sabouraud dextrose agar. The direct skin touch smear or the pus in Wright stain demonstrates oval or elliptical fungal elements.

Since histoplasmosis and penicilliosis showed similar clinical and histological manifestations, a way of differentiation between them other than morphology has been sought. A PCR method to detect P. marneffei was developed and we have tried this, though without results to date. The enzyme antibody method can demonstrate the pathogen but care should be taken with the cross reaction.

As for treatment, Vanittanakom & Sirisanthana recommended amphotericin B 0.6 mg/kg/day for 2 weeks, followed by 400 mg of itraconazole per day orally for the next 10 weeks, and for secondary preventive treatment itraconazole 200 mg/day for the rest of their lives. The fungus is also susceptible to miconazole and ketoconazole, but fluconazole given to our patient seemingly had no effect on Penicillium. The high mortality rate is due to the failure of making a timely diagnosis, the earlier the treatment begins the better is the response to therapy.

Since many diseases show strange features in immunocompromised patients, and these patients are also often infected by numerous uncommon opportunistic pathogens, clinically atypical or peculiar eruptions should be cultured or biopsied in the early stage.

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References