INTERNAL MEDICINE

□ CASE REPORT □

Mycobacterium shigaense Causes Lymph Node and Cutaneous Lesions as Immune Reconstitution Syndrome in an AIDS Patient: The Third Case Report of a Novel Strain Non-tuberculous Mycobacterium

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Abstract

A 40-year-old man complaining of progressive body weight loss was diagnosed to have acquired immunodeficiency syndrome. Within 2 weeks after the initiation of combination antiretroviral therapy, he developed fever, massive cervical lymphadenopathy and a protruding subcutaneous abscess. A lymph node biopsy and abscess drainage revealed non-caseous granuloma and mycobacterium. The mycobacterium belonged to Runyon II group, but it showed no matches to any previously reported species. According to sequence analyses, the strain was identified as *Mycobacterium shigaense*. After six months of antimycobacterial treatment, the lesions were all successfully cured. This is the third case report of the novel mycobacterium, *M. shigaense*, presenting in associatioin with immune reconstitution syndrome.

Key words: acquired immunodeficiency syndrome (AIDS), non-tuberculous mycobacteria (NTM), immune reconstitution syndrome (IRS), lymphadenitis, *Mycobacterium shigaense*

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Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous bacteria found in the soil, water, plant or animals. While person-to-person transmission is rare, they sometimes cause life threatening disease in compromised hosts (1). Especially in acquired immunodeficiency syndrome (AIDS) patients, *Mycobacterium avium complex* (MAC) often causes disseminated infection (2). The advent of combination antiretroviral therapy (cART) has greatly improved the prognosis of AIDS patients, but at the same time, unfavorable excessive inflammation, *i.e.*, immune reconstitution syndrome (IRS) has emerged as a result of strong and rapid immune restoration (3).

MAC is one of the most common etiologic pathogen for IRS, but it is also well known that NTM other than MAC, for example, *M. genavense, M. fortuitum, M. xenopi* or *M. kansasii* are often seen in AIDS patients (1, 4). Besides, in addition to conventional culture methods, recent progress in molecular biology has offered new insight into many other species of NTM (5-8). In AIDS patients, too, cases with newly identified mycobacteria such as *M. triplex* (9), *M. lentiflavum* (10), or *M. parascrofulatum* (11) have been reported.

Mycobacterium shigaense is a novel NTM strain (12). There have only been two case reports so far, and little is known about this organism. We herein report the third

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Table 1. Laboratory Findings at First Visit.

WBC	2,500 /μL	TP	8.3 g/dL	Fe	77 mg/dL
Neut	65 %	ALB	4.5 g/dL	UIBC	180 mg/dL
Eosin	9.6 %	AST	27 U/L	IgG	1,761 mg/dL
Baso	0 %	ALT	23 U/L	IgM	76 mg/dL
Lymph	20.6 %	LDH	304 U/L	IgA	385 mg/dL
Mono	4.8 %	ALP	476 U/L	CRP	0.32 mg/dL
RBC	448×10^6 /μL	γ-GTP	35 U/L	Ferritin	531.4 ng/mL
Hb	13.3 g/dL	CHE	405 U/L	sIL2-Rec	600 U/mL
Ht	38.6 %	T-BIL	0.71 mg/dL		
PLT	12.8×10^4 /μL	D-BIL	0.16 mg/dL	HIV-1RNA	1,400,000 copies/mL
		NA	142 mEq/L		
CD3	54 %	CL	107 mEq/L	β-D-glucan	<5.0 pg/mL
CD19	24 %	K	4.2 mEq/L		
CD4	2 %	UN	10.1 mg/dL	CMV antigenemia	(-)
CD8	44 %	CRE	0.7 mg/dL	HBs antigen	(-)
CD4 count	10 /μL	UA	5.7 mg/dL	HCV antibody	(-)
		T-CHO	198 mg/dL	TPLA	(-)
		TG	191 mg/dL	RPR	(-)
		AMY	92 U/L		
		CPK	45 mg/dL		

CMV: cytomegalovirus, RPR: rapid plasma reagin test, sIL2-Rec: soluble interleukin-2 receptor, TPLA: Treponema pallidum latex agglutination test

known case of *M. shigaense*, causing IRS soon after the initiation of cART.

Case Report

A 40-year-old man who had been treated for recurrent oral candidiasis came to our hospital because of general malaise and progressive body weight loss, losing 10 kg in a half year. He was bisexual, having no habit of intravenous drug use. While he had no history of any sexually transmitted diseases, he had experienced herpes zoster 5 years before, but he had never taken any human immunodeficiency virus (HIV) antibody tests.

At presentation, he had low grade fever, gum bleeding and tinglingness in the posterior cervical region. He complained of no particular respiratory and gastrointestinal symptoms. Physical examination revealed oral candidiasis, gingivitis and slight lymphadenopathy involving the cervical, submandibular, left postauricular and inguinal nodes (measuring 4-5 mm in diameter, without tenderness). The liver and spleen were not palpable. Folliculitis was seen on the trunk and extremities. An HIV screening test was positive and a Western blot test was done, and it proved to be positive. Blood examination results were as follows (Table 1); HIV-RNA 1,400,000 copies/mL, CD4 count 10/μL, slight pancytopenia, and hypergloburinemia were seen. The serum alkaline phosphatase (ALP) level and lactate dehydrogenase (LDH) level were slightly elevated. With an extremely low CD4 count and wasting syndrome, he was diagnosed as having AIDS. Therefore, a thorough examination regarding opportunistic infections and sexually transmitted diseases was done. Plasma (1->3) β-D-glucan, cytomegalovirus antigenemia, HBs-antigen, hepatitis C virus (HCV) antibody, and Treponema pallidum latex agglutination test, and rapid plasma reagin test were negative. Blood, fecal, sputum, and urine cultures were done, but no particular microbes including bacteria, mycobacteria, fungi, and protozoa were detected. A computed tomography (CT) scan showed slight splenomegaly and lymph node swelling in the neck areas. Lymphadenopathy was not detected in the thorax or abdomen. No lung lesions were found in a CT scan. Brain MRI, esophagogastroduodenoscopy and total colonoscopy showed no abnormalities. Pneumocystis pneumonia (PCP) was not suspected, and sulfamethoxazole 800 mg/trimethoprim 160 mg was administered as PCP prophylaxis. Oral azithromycin 1,200 mg weekly was also started as prophylaxis for the MAC infection. At that time, no respiratory symptoms were observed, and mycobacterium was not detected in a smear. However, after four weeks of incubation mycobacterium was detected in a sputum culture, but polymerase chain reaction (PCR) tests for *M. tuberculosis* and MAC were negative.

After the completion of fluconazole therapy for oral candidiasis, cART was started with abacavir, lamivudine and darunavir/ritonavir. On day 11 (of cART), fever, tender submental lymph node swelling and trismus developed. On day 18, his temperature rose to 40°C and cervical lymph node (LN) swelling became more conspicuous with severe tenderness (Fig. 1). The serum ALP level was 618 U/L and MAC-IRS was suspected. On day 28, we performed a biopsy of the submental lymph node. At this time, the CD4 count was 94/μL and the serum HIV-RNA level had declined to 1,700 copies/mL.

The sampled lymph node had swollen to 2 cm in diameter and the surface of section was gray-white with focal yellowish area. Histologically, non-caseous granuloma was seen (Fig. 2a), and relatively thin bacilli were detected in Ziehl-Neelsen staining (Fig. 2b). Mycobacterium was isolated after 8.8 days in supplemented Middlebrook 7H9 broth liquid culture (BacT/ALERT® MP for mycobacterium, Biomerieux, USA) and after 11 days in 7H11 agar culture. In 2% Ogawa egg slant medium (Kyokuto Pharmaceutical Industrial, Tokyo, Japan), it was scotochromogenic with an intense yellow

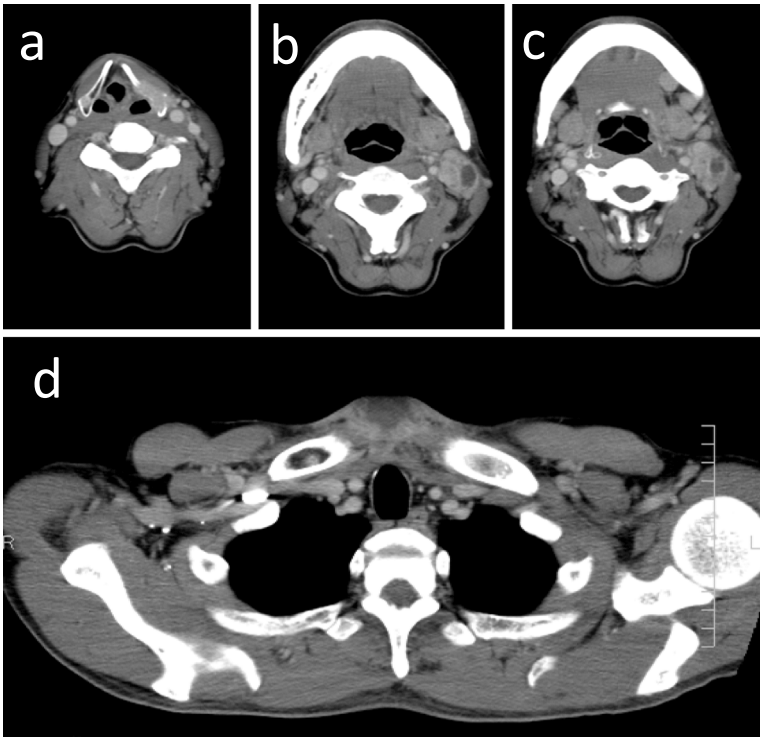

Figure 1. **Neck CT findings at day 28 of cART. a: Osteolytic invasion into the thyroid cartilage can be seen. b, c: Multiple cervical lymphadenopathy contains abscess lesions. d: Subcutaneous abscess can be seen in front of the manubrium sterni.**

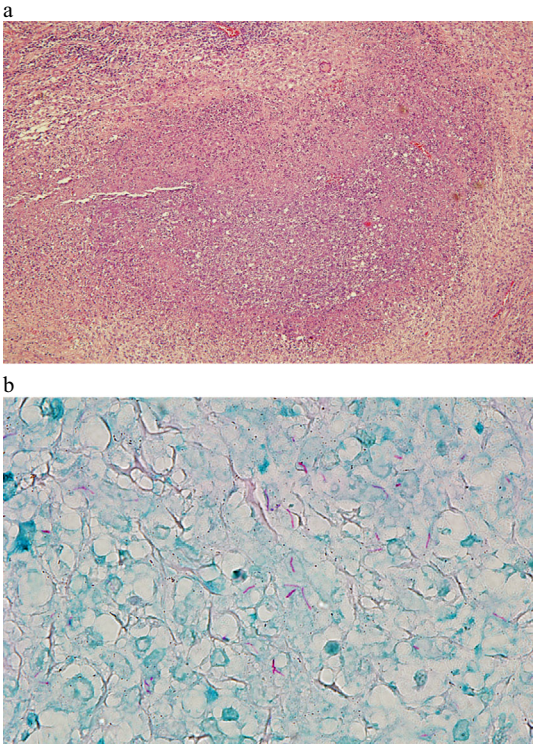

Figure 2. **Histological findings after a lymph node biopsy. a: Granuloma is seen (Hematoxylin and Eosin staining, 40×). b: Thin acid fast bacilli are detected (Ziehl-Neelsen stain, 1,000×).**

color in both light and dark conditions (Fig. 3). Therefore, the strain was thought to belong to the Runyon II group.

Figure 3. **Scotochromogenic colonies of the *Mycobacterium shigaense* sub-cultured on 2% Ogawa egg slant medium.**

DNA-DNA hybridization (DDH Mycobacteria® Kyokuto Pharmaceutical Industrial) was done, but there were no matches with any of the 18 mycobacterial species.

To identify this isolate, sequence analyses were performed, targeting fragments of the 16S ribosomal RNA (16S rRNA, 269 bp), RNA polymerase B (*rpoB*, 315 bp), and heat shock protein 65 (*hsp65*, 401 bp) genes. In a 16S rRNA sequence, although this isolate best matched with *M. simiae* with 99.7% homology, it also matched several reference strains with more than 98.5% homology (*M. lentiflavum, M. triplex, M. genavense, M. intermedium, M. scrofulaceum, M. kansasii* and *M. gastri*). The sequence analysis showed a high homology rate with Mycobacterium sp. UN-

Table 2. Antibiotic Susceptibility Tests.

	Minimal Inhibitory Concentration (μg/mL)
clarithromycin (CAM)	16
rifampicin (RFP)	2
rifabutin (RBT)	0.25
ethanbutol (EB)	4
isoniazid (INH)	16
kanamycin (KM)	64
amikacin (AMK)	>16
streptomycin (SM)	>128
ciprofloxacin (CPFX)	>32
levofloxacin (LVFX)	>32

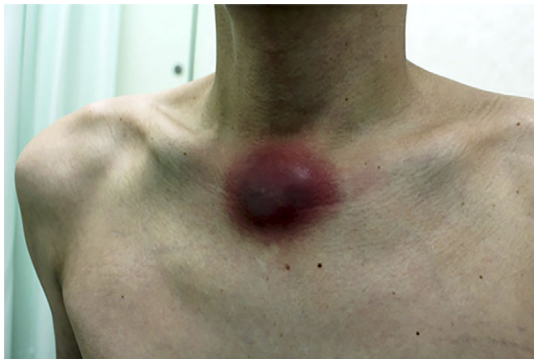

Figure 4. Cutaneous lesions at day 31 of cART.

152, (GeneBank accession no. AB547401) reported as *M. shigaense*; 100% in 16S rRNA, 100% in *rpoB*, and 94% in *hsp65*. This strain was thus identified to be *M. shigaense*. Later the strain was proven to grow at 25°C, 30°C and 37°C, but not at 42°C. A niacin test was negative. Table 2 shows the results of antimicrobial susceptibility tests (Broth-MIC NTM®; Kyokuto Pharmaceutical Industrial). Clarithromycin, ethanbutol and rifabutin seemed to be moderately susceptible, while the others were not susceptible.

An antimycobacterial regimen was started on day 25 of cART. The regimen contained rifabutin (RBT) 150 mg, ethanbutol (EB) 750 mg, and clarithromycin (CAM) 800 mg. Isoniazid (INH) 300 mg was also added until the subspecies was identified.

Soon after initiation of the regimens, the fever subsided, but lymph node enlargement continued. A subcutaneous abscess with a protruding erythematous lesion appeared on the sternum and grew to 5 cm in diameter within a week (Fig. 4). Drainage was performed, and again, mycobacterium was found in Ziehl-Neelsen stain, and it was later identified to be *M. shigaense*. Thus, the final diagnosis was immune reconstitution syndrome caused by the novel mycobacterium, *M. shigaense*. Though we could find mycobacterium in the biopsy or drainage specimens, it took over 4 months to correctly identify this strain. Meanwhile, INH was continued.

The serum ALP level normalized at 12 weeks, HIV-RNA turned to negative at 16 weeks, and the CD4 count recovered to over 100/μL at 27 weeks after the initiation of cART (Fig. 5). Blood, urine and stool cultures for Mycobacterial were repeatedly done, but those were all negative. The skin lesions and lymphadenopathy all gradually improved, and the antimycobacterial regimen was stopped after 6 months. Since then, the patient has been followed up without any recurrence of NTM disease for more than a year.

Discussion

Mycobacterium shigaense is a novel NTM strain firstly reported by Nakanaga et.al in 2011. Its nucleotide sequence resembles that of *M. simiae*, but it can be distinguished by the temperature it grows (namely, not at 42°C, while *M. simiae* does grow at 42°C), the presence of colony pigmentation in the dark (while *M. simiae* is not pigmented in the dark) and negativity for the niacin test (12). There has been two case reports of *M. shigaense* so far (Table 3) (12, 13). The first case was a severely immunocompromized male with Hodgkin lymphoma under chemotherapy. Cutaneous nodules on the back were the main lesions, and CAM and INH were administered for 12 months. The skin lesions often became exacerbated and he finally died due to other opportunistic infections (12). The second case was an immunocompetent female who had papules, nodules and purulent lesions in the face. They were simple chronic skin lesions and were successfully treated with 6 months' medication with CAM and moxifloxacin (13). Both of the cases showed solely cutaneous lesions, but in our case, lymphadenopathy was prominent. Probably the underlying immunodeficiency due to AIDS might explain the reason for the differences in location and the clinical course.

The clinical picture of this case resembles that of disseminated MAC infection in AIDS patients (14, 15). Nowadays, it is well known that non-tuberculous mycobacteria other than MAC cause dissemination and IRS (11, 16, 17). The major risk factors of NTM-IRS development are a low CD4 count or high plasma HIV-RNA at cART initiation (18) and rapid decline in HIV-RNA after cART (19). Our case exactly matches both of these clinical pictures and risk factors of NTM-IRS. First, the main lesions were in the lymph nodes. Second, all the blood culture samples repeatedly taken before and after cART administration were negative for mycobacteria. And third, the onset of IRS occurred at around 2 weeks after cART initiation, with a much higher CD4 count and a dramatically decreased plasma HIV-RNA level in comparison to the values at initial presentation.

It is interesting to note, however, that unlike MAC cases, the three *M. shigaense* cases including ours demonstrated no lung lesions throughout the whole clinical course. Besides, the mycobacterium was not the cause of death in the first case and the latter two cases were cured with treatment. This is somewhat different from other NTM. Unlike disseminated MAC infection which often requires a longer duration of antimycobacterial treatment, our case did not experience either any recurrence or a refractory course even after the cessation of antimycobacterial regimens. This might be attributed to either the nature of this organism, the early ad-

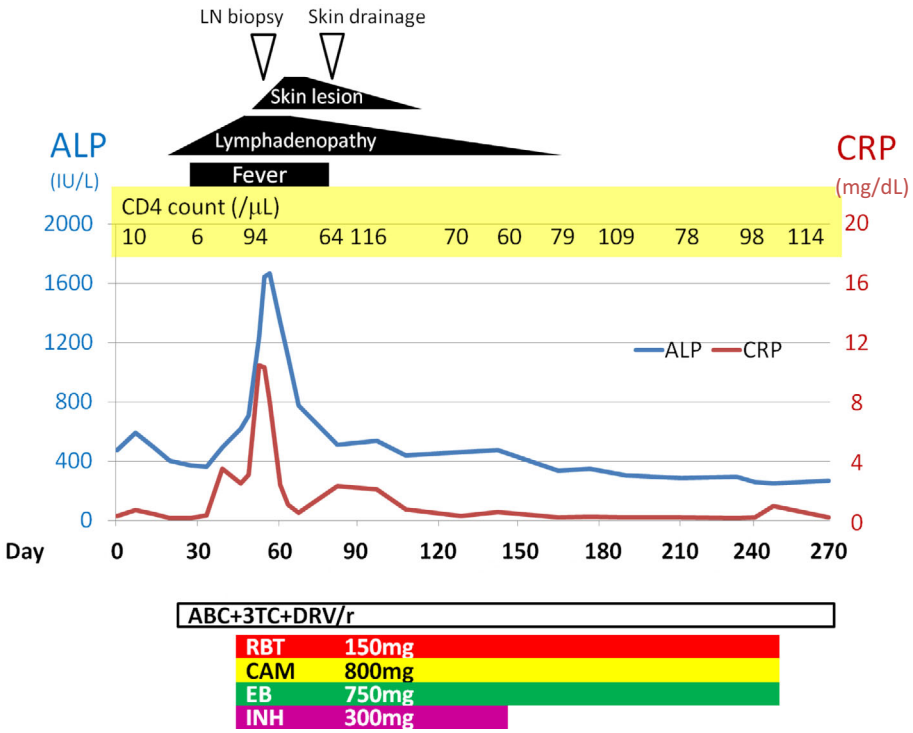

Figure 5. **Clinical course. ABC: abacavir, ALP: alkaline phosphatase, CAM: clarithromycin, CRP: C-reactive protein, DRV/r: darunavir/ritonavir, EB: ethanbutol, INH: isoniazid, 3TC: lamivudine, RBT: rifabutin**

Table 3. **Three Cases of *M. shigaense* Infection Reported So Far.**

	case 1	case 2	case 3 (present case)
Location	Shiga, Japan	Jiangsu, China	Shiga, Japan
Age/Sex	55/M	56/F	40/M
Comorbidities	Hodgkin lymphoma (chemotherapy) erythroderma (prednisone)	none	AIDS
Lesions	skin (back)	skin (cheek)	cervical lymph nodes skin (breast)
Susceptible antibiotics	RFP, RBT, CAM, EB, quinolones, aminoglycosides	RFP, RBT, CAM, EB, quinolones, aminoglycosides	RFP, RBT, CAM, EB (intermediate)
Treatment	CAM+INH 12 months	CAM+MFLX 6 months	CAM+EB+RBT+INH 4 months →CAM+EB+RBT 2 months
Outcome	died of other disease (CMV infection, bacteremia)	complete remission	complete remission

CAM: clarithromycin, CMV: cytomegalovirus, EB: ethanbutol, INH: isoniazid, MFLX: moxifloxacin, RBT: rifabutin, RFP: rifampicin

ministration of antimycobacterial agents, or the rapid immune restoration by cART.

We administered RBT+CAM+EB, the standard regimen for disseminated MAC infection which can also be applied to many other NTM species (20). The regimen was intended to cover major Runyon II mycobacteria, *i.e. M. xenopi*, *M. ulcerans*, *M. szulgai*, and *M. gordonae*. In addition, because *M. kansasii* and other species requiring INH treatment were considered in the differential diagnosis, we added INH until those species could be ruled out. After 6 months of treatment, the infection had almost completely improved, though this isolate had a low susceptibility to the antimycobacterial agents. In comparison to the previous two cases, this strain showed lower minimal inhibitory concentration (MIC) to aminoglycosides and quinolones. Rifamycins, CAM and EB were relatively susceptible, though the MIC was higher than

in the first case. Even so, the chemotherapy might have been effective, because usually no correlation has been shown between the susceptibility results and the clinical outcome for antimycobacterial agents (20). It is also possible to speculate that rapid immune restoration by cART may have led to the elimination of NTM.

The detection of mycobacterium in the sputum and abnormal elevation of serum ALP level (which is a common finding in cases with disseminated MAC infection of AIDS patients) were the only findings suggestive of NTM infection before the onset of IRS. While the other liver enzymes were close to normal levels, the ALP level peaked to 1,664 U/L on day 30, and then declined after treatment. As is often the case with AIDS patients, the patient was already on cART when mycobacterium was detected in the sputum after four weeks of culture. Although azythromycin was prescribed for prophylaxis, we could not prevent IRS caused by this NTM. To better predict the onset of a disseminated infection or IRS of NTM, an ALP elevation might be considered as an early marker and this had also been reported in the pertinent literature (21).

In conclusion, we experienced the case of an AIDS patient who developed *M. shigaense* lymphadenitis and skin lesion as IRS within 2 weeks after initiation of cART. He was treated with drainage and 6 months of antimycobacterial therapy. Owing to the rapid immune restoration by cART, the disease has been cured without any complications. Ours is the third case report of this novel mycobacterium, showing that this organism causes IRS in AIDS patients, just like other NTM does.

With the development of immunosuppressants and massive chemotherapies, the problems of opportunistic NTM infections are no longer limited to AIDS patients. We should keep in mind that a prompt diagnosis is needed for NTM infections in those compromised hosts. Further case reports of such rare species are needed to establish appropriate management strategies to treat such patients.

Author's disclosure of potential Conflicts of Interest (COI).
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