CASE REPORT

DISSEMINATED *MYCOBACTERIUM AVIUM* AND RECURRENT *SALMONELLA* GROUP D INFECTION IN A PATIENT WITH AUTOANTIBODIES TO INTERFERON-GAMMA

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Abstract. We reported a case of a 40-year-old woman who presented with prolonged fever for 1 month, left sternoclavicular arthritis, anemia, multiple cervical lymphadenopathy and hepatosplenomegaly. She had a previous history of recurrent *Salmonella* group D septicemia. Computed tomography of her chest and abdomen revealed left sternoclavicular (SC) arthritis, left subscapular collections, hepatosplenomegaly, and multiple hypodensed lesions in the spleen. Blood, synovial fluid and bone marrow for mycobacterial cultures identified *Mycobacterium avium* by real-time PCR and reverse hybridization. Cell mediated immunodeficiency investigations were strongly positive for autoantibodies to interferon-gamma (IFN-γ) by ELISA technique. During the third week of antimycobacterial therapy, she developed an acute generalized pustular eruption. Skin biopsy showed leukocytoclastic vasculitis; drug allergy was suspected. The pustular eruption resolved with steroid treatment and discontinuation of levofloxacin and clarithromycin. She was discharged home after 8 weeks of hospitalization with azithromycin, rifampicin and ethambutol.

Keywords: *Mycobacterium avium, Salmonella* group D, septicemia, autoantibodies, IFN-γ

INTRODUCTION

Disseminated *Mycobacterium avium* complex (MAC) infection is commonly considered an opportunistic infection occurring in HIV infected patients with advanced disease or in other less frequently seen conditions, such as during immunosuppressive therapy or hereditary or acquired T-cell deficiency (Rossi *et al*, 2001; Doffinger *et al*, 2005). Immunity against nontuberculous mycobacterial (NTM) infection, including MAC, is provided by T-helper 1 lymphocytes, cytokines, such as interferon(IFN)-γ and interleukin (IL)-12, and with macrophages (Ottenhoff *et al*, 2002; Doffinger *et al*, 2005). Several pa-

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tients with unexplained nontuberculous mycobacterial infections have been found to have autoantibodies to IFN-γ, making them susceptible to intracellular pathogen infections (Kampmann *et al*, 2005; Patel *et al*, 2005).

In a case series published in 2011 (Kampitak *et al*, 2011), of 16 patients with autoantibodies to IFN-γ, disseminated NTM infection or MAC co-infection were seen in 9 of 16 patients. We report a case of recurrent *Salmonella* septicemia and disseminated *M. avium* infection in a patient with autoantibodies to IFN-γ.

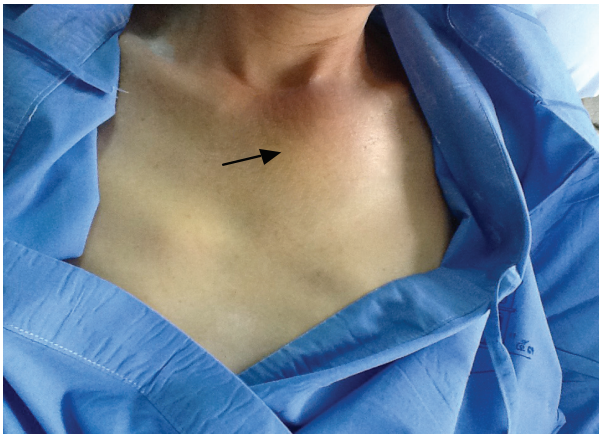

Fig 1–Swelling, tenderness and redness around left sternoclavicular joint (arrow).

CASE REPORT

A 40-year-old Thai woman was referred from Nan Provincial Hospital to Siriraj Hospital, Bangkok, Thailand on March 20, 2012 with a history of prolonged fever and left sternoclavicular (SC) arthritis for 1 month. She was no previous history of underlying disease. Six months prior to this admission, she was hospitalized three times at the provincial hospital for recurrent *Salmonella* group D septicemia and steroid responsive immune hemolytic anemia. All 3 episodes of salmonellosis were resolved by treatment with ceftriaxone and Co-trimoxazole. She was readmitted to Nan Provencial Hospital for one month with recurrent fever and coexisting left sternoclavicular pain. Recurrent salmonellosis was initially suspected. However, all aerobic blood cultures were negative. Her symptoms did not respond to ceftriaxone treatment. Abdominal computed tomography showed hepatosplenomegaly, a few small enhancing nodules 0.5 cm in size in the spleen, and a small pancreatic node. After 3 weeks of antimicrobial treatment, she was referred to Siriraj Hospital for further management.

On admission, the patient appeared chronically ill, had marked pallor, a body temperature of 38.3ºC, a heart rate of 102/min, a respiratory rate of 16/min and a blood pressure of 92/54 mmHg. Multiple cervical lymphadenopathy and hepatosplenomegaly were detected. A grade 4 systolic ejection murmur was heard at the left upper parasternal border, compatible with isolated pulmonary artery dilatation seen on transthoracic echocardiography. Left sternoclavicular arthritis was still identified (Fig 1). Her other findings were unremarkable. A complete blood count revealed severe anemia with a hemoglobin and hematocrit of 4.1 g/dl and 13%, respectively, a white blood cell count of 30,450/mm^3 (neutrophils 70%, lymphocytes 15%) and a platelet count of 80,000/mm^3. A direct Coombs' test was negative. She had an elevated alkaline phosphatase level (608 U/l), hyperbilirubinemia (total bilirubin 1.8 mg/dl, direct bilirubin 1.6 mg/dl) and azotemia (BUN 63.4 mg/dl, creatinine 4.0 mg/dl). Chest radiography showed bilateral interstitial infiltrations. Her HIV antibody test was negative.

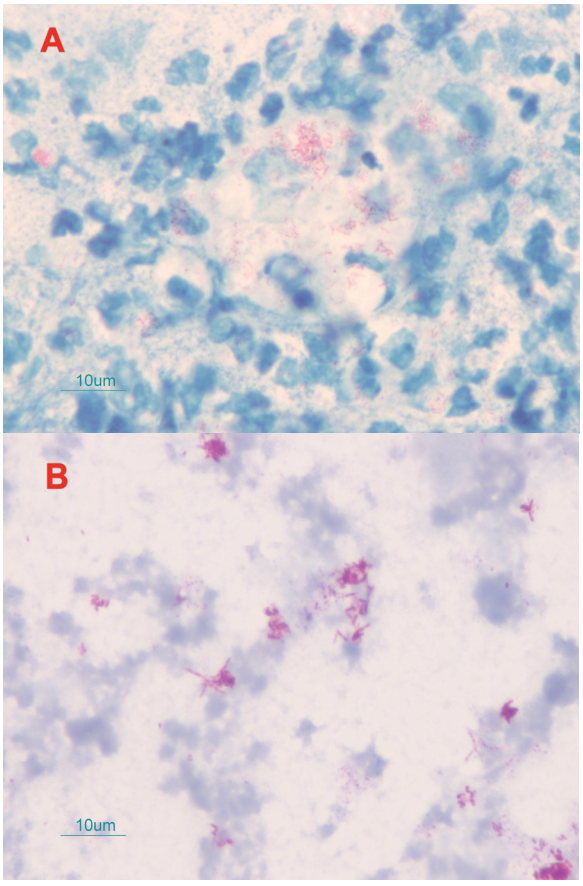

Fig 2–Left sternoclavicular joint aspiration (2A) and bone marrow aspiration (2B) with numerous acid-fast bacilli.

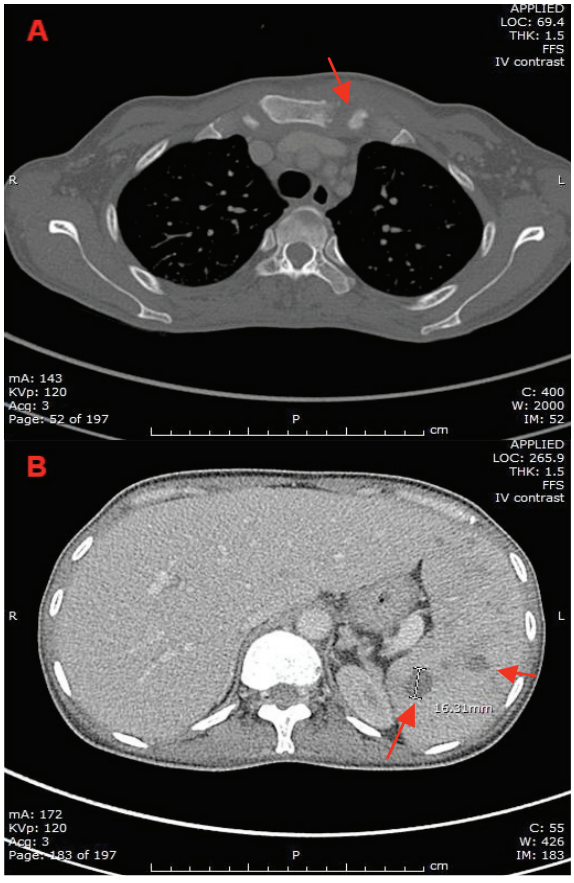

Fig 3–Computed tomography of chest (3A) and abdomen (3B) revealing a periarticular osteolytic lesion of the left SC joint (arrow), hepatosplenomegaly, and multiple, varying in size, hypodensed splenic lesions (two arrows).

Intravenous imipenem/cilastatin was started empirically to treat presumed severe sepsis. However, numerous acid-fast bacilli were detected from the left sternoclavicular joint and on bone marrow aspiration (Fig 2). Therefore, rifampicin (450 mg/day), levofloxacin (750 mg/day), clarithromycin (1,000 mg/day), and ethambutol (1,000 mg/day) were started to cover both disseminated *M. tuberculosis* and NTM infection. Imipenem/cilastatin was discontinued after 10 days of treatment. All mycobacterial cultures from the left SC joint, bone marrow, and blood yielded *Mycobacterium avium* by real-time PCR and reverse hybridization methods with Innogenetics/Auto-LiPA 48 (Innogenetics NV, Ghent, Belgium). Chest and abdominal computed tomography also revealed periarticular osteolytic lesion of left SC joint, a left subscapular collection, hepatosplenomegaly, and multiple, varying in size, hypodense splenic lesions (Fig 3). Investigations were conducted evaluating causes of T-lymphocyte immune dysfunction. The CD4 T-lymphocyte

count was 1,247 cell/μl. Intradermal tests for tuberculin and tetanus toxoid were non-reactive. Phytohemagglutinin stimulated peripheral blood mononuclear cell (PBMC) response, IFN-gamma receptor and interleukin-12 receptor tests were all within normal limits. Autoantibodies to interferon-gamma were strongly positive by enzyme-linked immunosorbent assay (ELISA).

Azotemia and severe anemia were corrected by intravenous fluid replacement therapy and blood transfusion, respectively. The creatinine level gradually decreased from 4.0 to 1.1 mg/dl in 3 days. Intravenous amikacin 700 mg/day was substituted for rifampicin owing to progressive hyperbilirubinemia in the first week of treatment, whereas levofloxacin, clarithromycin and ethambutol were continued. The fever gradually subsided within 2 weeks. However, intermittent high grade fever reappeared, and an acute generalized pustular eruption of the patient's skin developed during the third week of antimycobacterial therapy. Skin biopsy showed leukocytoclastic vasculitis (LCV) and was negative for mycobacteria on tissue culture. A consultant dermatologist postulated either the clarithromycin or levofloxacin were possible causes of the pustular eruption. The fever and skin lesions resolved with discontinuation of clarithromycin and levofloxacin, along with a 3-week course of intravenous dexamethasone a 15 mg/day. Azithromycin and rifampicin were sequentially reintroduced after remission of the skin lesions. Eventually, azithromycin (500 mg/day), rifampicin (300 mg/day), and ethambutol 1,000 mg/day were used to treat the infection along with oral prednisolone at 50 mg/day to control the cutaneous pustulosis. Patient was discharged home from the hospital after 8 weeks of hospitalization with no fever, no relapse of skin lesions, and improving hepatosplenomegaly; she was seen 1 month later at follow-up. Prednisolone was slowly tapered to a dose of 5 mg/day on 23 July, 2012.

DISCUSSION

Mycobacterium avium complex (MAC), a slow growing mycobacteria, is common in the environment, including in water and soil, and around animals (Horsburgh, 1996). MAC is comprised of 2 taxa: *M. avium* and *M. intracellulare*. However, new species of *M avium* have been proposed: *M. avium*, *M. silvaticum*, and *M. paratuberculosis* (Shin *et al*, 2010). MAC colonies have a heterogeneous morphology and slow growth. Molecular methods can be used to identify MAC. Several novel molecular assays, such as real time-PCR and reverse hybridization, have proved useful to reduce delay in diagnosis.

The presented case was diagnosed as having disseminated *M. avium* infection with the Innogenetics/Auto-LiPA 48, which can identify several species of mycobacteria including tuberculosis from colonies grown on liquid or solid media (Tortoli *et al*, 2003). Before the advent of AIDS, the most common presentation of MAC infection was pulmonary disease, including upper lobe fibrocavitary or tuberculosis-like infection, nodular bronchiectasis or a solitary nodule; which are commonly found in non-immunocompromised persons (Field *et al*, 2004). It is difficult to differentiate NTM lung disease from pulmonary tuberculosis by clinical and radiological findings only. Age greater than 40 years, non-smoking status, previous TB treatment, absence of a pleural effusion or the presence of middle or lower lobe involvement suggest NTM lung infection. The isolation and identification

of the causative organism is mandatory for a correct diagnosis (Koh *et al*, 2006). Other mycobacterial syndromes include cervical lymphadenitis and disseminated disease which usually occur in patients with advanced HIV infection (Gordon and Horsburgh, 2010). MAC is the most common NTM infection involving HIV infected patients whose CD4 count has usually below 100 cell/mm^3 (Jordan, 1991; Nightingale *et al*, 1992). The organism is an intracellular organism that preferably infects circulating monocytes (Doffinger *et al*, 2004); however, disseminated MAC infection is uncommon in non-HIV patients. The presented case was previously healthy and had a negative serological test for HIV infection. She had recurrent *Salmonella* group D infection, then developed disseminated *M. avium* infection. With the recurrent multiple disseminated intracellular pathogen infections, the patient was suspected of having dysfunction of the intracellular pathogen clearance pathways. Interleukin 12 and interferon gamma (IL-12-IFN-γ) play a major role in combating these infections (Doffinger *et al*, 2005). IFN-γ, secreted by activated Th1 cells gives a positive feedback signal to both macrophages and CD4+ T-cells to eradicate intracellular microorganisms (Qu *et al*, 2011). Several studies demonstrated autoantibodies to IFN-γ are associated with disseminated NTM infection, especially in Asian populations (Doffinger *et al*, 2004; Hoflich *et al*, 2004; Kampmann *et al*, 2005; Patel *et al*, 2005; Tanaka *et al*, 2007; Baerlecken *et al*, 2009; Koya *et al*, 2009; Kampitak *et al*, 2011).

Of 129 non-HIV infected Thai patients diagnosed with disseminated NTM infection, only 12% had underlying diseases, such as diabetes mellitus, malignancy and thalassemia (Chetchotisakd *et al*, 2007); the most commonly affected organs were lymph nodes (89%), skin and soft tissue (26%), lungs (19%), bones and joints (16%), blood (15%), and the spleen (9%). The reported case presented with sternoclavicular infection arthritis and subscapular infection. The study by Chetchotisakd *et al* (2007) found non-typhi *Salmonella* was the most common co-infection with NTM (39.5%). Preexisting reactive skin diseases also associated with disseminated NTM infections include Sweet syndrome (70%), pustular psoriasis (7%), acute generalized exanthematous pustulosis (AGEP) (6%), and erythema nodosum (5%); rapidly growing mycobacteria (75%) were the predominant NTM organism in the study (Chetchotisakd *et al*, 2007). None of the cases in the above study had conditions associated with acquired immunodeficiency, such as receipt of steroids or immunosuppressive agents, advanced cancer, malnutrition, radiotherapy, or chronic renal disease. In the presented case, a pustular eruption occurred after 21 days of antimycobacterial treatment. Even though the primary cutaneous presentaion of the presented patient was similar to AGEP, the pathological findings were compatible with LCV. A histopathological study of AGEP in 102 patients, found most had sub-or intra-corneal pustules (41%), intraepidermal pustules (20%), or combined pustules (38%) and vasculitis was generally absent (Halevy *et al*, 2010). LCV is related to circulating immune complexes with many causes, such as medication, collagen vascular diseases, paraproteinemia and, rarely, neoplasias (Lowry *et al*, 1994). In the presented case, drug hypersensitivity due to levofloxacin and/or clarithromycin were considered more likely than reactive skin disease. However, both conditions are treated with steroid therapy. The patient's skin lesions responded well to high

dose IV dexamethasone and no relapses occurred after dose de-escalation. Additionally, the disseminated disease was controllable with a successful rechallenge with azithromycin combined with ethambutol and rifampicin without a relapse of pustular lesions.

Kampitak *et al* (2011) reviewed 16 non-HIV infected patients who suffered from opportunistic infections associated with interferon-γ autoantibodies. Disseminated MAC infection was identified in 9 patients with a similar history to the reported case who had strongly positive autoantibodies to IFN-γ. However, the condition may be underestimated because of incomplete immunological investigation of disseminated NTM infection in a non-HIV infected patient. Some patients were co-infected with both MAC and other mycobacteria, such as *M. abscessus* or *M. chelonae*. Most patients were adult Asians without a previous history of an immunodeficiency disorder and had multiple sites of infection such as soft tissue, bone, joints, lymph nodes, and lungs. None of patients developed reactive skin disease during the course of the MAC infection. Although the presented patient had taken a low dose of prednisolone for immune hemolytic anemia, the strongly positivity antibodies against IFN- γ were probably an important contributing factor in the disseminated MAC infection. The presented case had a normal CD4+ count, normal phytohemagglutinin stimulated response and normal IFN-γ and IL-12 receptors; therefore, IFN-γ autoantibodies were deemed to be the problem. Detection of IFN-γ autoantibodies was performed by human IFN-γ antigen coated enzyme-linked immunosorbent assay (ELISA). The IFN-γ autoantibodies in this patient's plasma can inhibit IFN-γ production by PBMC. This patient's PBMC produced undetectable levels of IFN-γ. However, the patient's PBMC had a normal IFN-γ response when stimulated with both PHA and normal plasma. The results suggest the patient's PBMC had the ability to produce a normal amount of IFN-γ while the activity of IFN-γ was inhibited by the patient's autoantibodies. Many healthy persons may have low levels of IFN-γ autoantibodies which are not active against cytokine production (Patel *et al*, 2005).

Both specific treatment of opportunistic infections and immunological therapy for the autoantibodies were mandatory in this presented patient. The preferred antimycobacterial regimen for disseminated MAC, includes clarithromycin, rifabutin and ethambutol; whereas the alternative agents are aminoglycosides (amikacin or streptomycin) and fluoroquinolones (Gordon and Horsburgh, 2010). The presented case received intravenous amikacin, oral levofloxacin, oral clarithromycin and oral ethambutol as an intensive regimen for mycobacteremia and disseminated infection and then azithromycin, ethambutol, and rifampicin were used for maintenance therapy. Because rifabutin is unavailable in Thailand, rifampicin was alternatively used. Rifampicin has evidence for use with standard combination antibiotics to treat MAC lung disease and gives a favorable microbiologic response (Sim *et al*, 2010). Azithromycin is an alternative to clarithomycin in cases of disseminated MAC infection in AIDS patients (Koletar *et al*, 1999). The treatment of IFN-γ autoantibodies is not well established. Recently, anti-CD-20 or rituximab has been used as adjunctive treatment for relapsed or poorly responsive disseminated NTM infection associated with IFN-γ autoantibodies (Browne *et al*, 2012). Browne *et al* (2012) reported 4 patients who had received rituximab in 8-12 doses during

the first year as adjunctive therapy for refractory disease (Browne *et al*, 2012). After rituximab therapy, outcomes improved by clearance of infection and reduction in both inflammatory markers and IFN-γ autoantibody levels. However, recurrence of disease was commonly found; subsequent, additional doses were required. Intravenous immunoglobulin (IVIG) (Koya *et al*, 2009), plasmapheresis with pulse cyclophosphamide (Baerlecken *et al*, 2009) and IFN-γ (Kampmann *et al*, 2005; Browne *et al*, 2012) have also been reported as additional treatment, but the treatment outcomes varied among the cases. In the presented case, a combination of antimycobacterial drugs and a tapering dose of steroids were continued without recurrence of symptoms. To our knowledge, there is no curative treatment for pathogenic IFN-γ autoantibodies. The disease gives unfavorable outcomes. Most patients have persistent or relapsing infection (Patel *et al*, 2005; Koya *et al*, 2009; Kampitak *et al*, 2011). Further pathophysiologic studies are needed to clarify optimal treatment to improve clinical outcomes.

In conclusion, autoantibodies to IFN-γ should be considered in an HIV-negative adult who presents with a severe *M. avium* infection. The clinical features usually manifests as disseminated infection and chronic recurrent infection. A long-term combination of antimycobacterial agents is the mainstay of treatment. Immunosuppressive and/or immunomodulatory agents as adjunctive therapy to prevent relapse of disease need to be further studied to optimize outcomes.

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