Recurrent of Disseminated Mycobacterium avium Complex Disease in a Patient with Anti-Gamma Interferon Autoantibodies by Reinfection

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We report a case of recurrent disseminated Mycobacterium avium complex (DMAC) disease with anti-gamma interferon autoantibodies. To our knowledge, this is the first reported case caused by reinfection with a separate isolate of M. avium. DMAC disease activity was monitored using serum IgG antibody titers against lipid antigens extracted from a MAC strain.

CASE REPORT

A 65-year-old woman was admitted to an outside hospital with subcutaneous panniculitis. Chest radiography revealed infiltration of the right upper lobe, and Mycobacterium avium was cultured from respiratory secretions. The patient was transferred to Keio University Hospital for definitive diagnosis and treatment. On admission, the patient’s chief complaint was severe cough. She had a temperature of 38.3°C and marked hepatosplenomegaly on examination. The white blood cell (WBC) count and alkaline phosphatase (ALP) concentration were 36,100/μl and 1,771 IU/liter, respectively. Human immunodeficiency virus (HIV) antibody testing with an enzyme immunoassay was negative. Chest computed tomography (CT) showed an infiltrate in the right upper and middle lobes of the lung with angiogram sign and bilateral pleural effusions. M. avium was isolated from multiple samples, including sputum, pleural effusion, bronchoalveolar lavage fluid, bone marrow, and liver biopsy tissues. A bone marrow biopsy showed inflammation with granulomata. This case satisfied the diagnostic criteria for nontuberculous mycobacterial disease established by the American Thoracic Society (ATS) and the Infectious Disease Society of America (IDSA) (1), and we diagnosed this patient with disseminated Mycobacterium avium complex (DMAC) disease. Antimycobacterial therapy was initiated with four antibiotics: rifampin (RFP) at 450 mg/day, ethambutol (EB) at 750 mg/day, clarithromycin (CAM) at 800 mg/day, and kanamycin (KM) at 750 mg three times per week. After the initiation of antimycobacterial therapy, the WBC and ALP concentration were decreased compared with the values on admission. Pleural effusions decreased, and the pulmonary infiltrates improved. The patient was discharged on day 84 after admission. Two months after discharge, the WBC and ALP concentration had decreased to 6,800/μl and 474 IU/liter. KM was switched to levofloxacin (LVX) at 400 mg/day due to concern about the cumulative dose approaching 40 g. M. avium was not isolated from any of the sputum cultures after discharge, and antimycobacterial therapy (EB, 750 mg/day; CAM, 800 mg/day; RFP 450 mg/day; and LVX, 400 mg/day) was continued for approximately 2 years. Six months after the cessation of therapy, the patient again felt fatigued and feverish. The WBC and ALP concentration were again elevated, and she was readmitted to Keio University Hospital 9 months after the cessation of therapy (30 months after her first discharge). On readmission, the WBC and ALP concentration were elevated to 13,900/μl and 497 IU/liter, respectively. A bone marrow biopsy was performed, and M. avium was again isolated from her bone marrow. Abdominal magnetic resonance imaging (MRI) was performed and revealed nonspecific inflammation of the cervix. We suspected cervicitis caused by M. avium and obtained a biopsy sample from the cervix. Based on the culture and the pathological findings, we diagnosed recurrent DMAC disease. Antimycobacterial therapy with EB at 750 mg/day, CAM at 800 mg/day, RFP at 450 mg/day, and gatifloxacin (GAT) at 400 mg/day was started. The WBC and ALP concentration decreased gradually, and the cervicitis improved after the initiation of antimycobacterial therapy. She was again discharged on four antibiotics for DMAC disease. The antimycobacterial therapy has been continued for more than 5 years, and she has been free of recurrence.

In order to determine whether DMAC disease developed due to relapse or reinfection after the cessation of therapy, we compared the strains of M. avium isolated on the first admission with those obtained on the second admission. The M. avium strains we isolated were analyzed by a variable-number tandem repeat typing.
method using the \textit{M. avium} tandem repeat loci (MATR-VNTR). MATR-VNTR was performed as described previously (2). Briefly, the clinical isolates of \textit{M. avium} were cultured at 37°C for 3 weeks in Middlebrook 7H9 liquid medium supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment. PCR amplification was performed by using DNA extracted from the clinical isolates and the specific primers for MATR loci. Gel electrophoresis was performed on the PCR products, and the numbers of repetitions of various MATR loci of each strain were determined, compared with those of \textit{M. avium} ATCC 19698. \textit{M. avium} isolated on the first and second hospital admissions had different numbers of repetitions on 4 MATR loci, indicating that the two specimens were different \textit{M. avium} strains (Table 1). To confirm the two isolates were separate strains, the \textit{M. avium} isolates from each hospital admission were analyzed using restriction fragment length polymorphism-based pulsed-field gel electrophoresis (RFLP-PFGE) of their genomic DNA. RFLP-PFGE was performed as described previously (3). Genomic DNA was digested using the restriction enzyme XbaI or AseI. The patterns of restriction fragments were analyzed by PFGE and were identified as different strains as each showed different RFLP patterns (Fig. 1). In addition, there were also distinctly different drug susceptibility test results between the strain isolated on the first admission and that on the second admission (data not shown). These results indicate the patient developed a second episode of DMAC disease by infection with another strain of MAC.

MAC has trehalose monomycolate (TMM-M) and apolar-glycopeptidolipid (GPL), which are the major cell surface antigens and are specific for \textit{M. avium}. Previously we reported that the serodiagnosis by antibodies against these antigens was useful (4). We have examined the levels of anti-TMM-M antibodies and anti-GPL antibodies sequentially after the patient’s first hospital discharge (Fig. 2). After the cessation of antimycobacterial therapy, the levels of these antibodies increased gradually as the WBC and ALP concentration concurrently became elevated. The levels of anti-TMM-M antibodies and anti-GPL antibodies increased to optical densities (ODs) of 2.817 and 2.895 on readmission and decreased gradually after the initiation of antimycobacterial therapy. These results indicated that the levels of anti-TMM-M antibodies and anti-GPL antibodies reflected the disease activity in this patient.

DMAC disease mostly occurs in immunocompromised hosts, such as patients with acquired immune deficiency syndrome (AIDS) and patients with underlying malignancy or inherited or therapeutic immunodeficiency. Although this patient had never received immunosuppressive therapy, nor was she ever diagnosed with HIV or malignancy, we strongly speculated that a defective function in gamma interferon (IFN-\(\gamma\)) signaling caused her DMAC disease after a subsequent whole-blood IFN-\(\gamma\) release assay (QuantiFERON TB 2G assay) detected very low levels of IFN-\(\gamma\), even with mitogen (phytohemagglutinin) stimulation. A high titer of autoantibodies to IFN-\(\gamma\) was identified in her serum,

### TABLE 1 Comparison of MATR-VNTR results from the \textit{M. avium} strains isolated on the first admission and second admissions

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<tr>
<th>Isolate</th>
<th>No. of repetitions of MATR locus</th>
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<tr>
<td></td>
<td>1</td>
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<td>1st admission</td>
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<td>2nd admission</td>
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*The number of repetitions of each MATR locus is indicated, compared with that in \textit{M. avium} ATCC 19698.*
and this case was diagnosed as DMAC disease due to autoantibody-related defective function of IFN-γ.

Recently, several case reports have described an adult-onset immunodeficiency due to the production of IFN-γ autoantibodies and DMAC disease (5, 6, 7). However, we have found no reports to date that discuss recurrent DMAC disease with IFN-γ autoantibodies, and it remains unclear whether recurrent DMAC diseases in patients with IFN-γ autoantibodies are more commonly due to reinfection or relapse. Although pulmonary and disseminated MAC diseases might have different pathogeneses, reinfection with a different MAC isolate (73%) was more common than true relapse from the same MAC isolate (27%) among 25 microbiologic recurrences in patients with MAC pulmonary disease on antibiotic therapy (8). Reinfection with a different MAC isolate (75%) was also more common than true relapse (25%) among 74 microbiologic recurrences of the patients with MAC pulmonary disease after completion of therapy. VNTR typing of Mycobacterium bovis BCG showed that of the 12 VNTR loci, only 1 locus was polymorphic, with variable numbers of perfectly identical repeats among the three M. bovis BCG strains (M. bovis BCG Japan, M. bovis BCG Glaxo, and M. bovis BCG Pasteur) (9). This suggests that VNTR loci of mycobacteria are quite stable for a few decades. Based on these reports as well as our microbiological evaluation of clinical isolates, this case was considered to be attributable to recurrent DMAC disease by reinfection of distinct M. avium strains.

A recent report described the recurrence of pulmonary MAC infection due to reactivation of disease 3 months after the end of chemotherapy (10), and another described how 27 of 120 patients (22.5%) with MAC pulmonary disease had a polyclonal infection (11). Although it is difficult to completely rule out the possibility of persistent polyclonal MAC infection or major strain change during the clinical course, the fact that cultures yielded only a single isolate at each admission that varied significantly in their antibiotic susceptibilities, VNTRs, and RLFP patterns makes this unlikely in this patient. Whole-genome sequencing of bacteria has progressed greatly in recent years and would be useful to confirm the differentiation of the two bacterial isolates.

The clinical presentation of DMAC disease is hindered by nonspecific systemic symptoms and bacterial growth in deep tissues, complicating diagnostic sample collection and making it difficult to evaluate disease activity and to predict recurrence. Figure 2 showed that the levels of anti-TMM-M antibodies and anti-GPL antibodies were elevated with the recurrence and coincided with changes of inflammatory severity of inflammation speculated by the WBC and ALP concentration, indicating that measurement of the levels of anti-TMM-M antibodies and anti-GPL antibodies may be useful for the evaluation of disease activity of DMAC disease.

In this case, recurrence developed only 6 to 9 months after the completion of 2 years of antitubercular therapy. IFN-γ autoantibodies likely suppressed anti-IFN-γ-related immunity, conferring a high risk of DMAC recurrence in this patient. Our retrospective evaluation of the patient’s clinical course after discovering circulating anti-IFN-γ autoantibodies highlights the importance of early screening for a deficiency in TH1-mediated immunity in otherwise immunocompetent individuals diagnosed with DMAC disease. This patient was likely reexposed to MAC and reinfected after clearance of the initial infection; therefore, our data suggest that patients with anti-IFN-γ autoantibodies will likely need an antibiotic prophylaxis regimen after completing their antibiotic course for DMAC disease.

ACKNOWLEDGMENTS

T.N. received Japan Society of the Promotion of Science KAKENHI grant no. 26860772 and a grant from the Ohyama Health Foundation. The authors have no conflicts of interest.

REFERENCES