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Clinical Significance of Interferon-γ Neutralizing Autoantibodies Against Disseminated Nontuberculous Mycobacterial Disease

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Background. Interferon-γ neutralizing autoantibodies (nIFNγ-autoAbs) are reported in patients with disseminated nontuberculous mycobacteria (NTM) infection and may function by increasing the infection risk. Notwithstanding, the prevalence of nIFNγ-autoAbs as well as the clinical presentation, diagnosis, and natural history of disseminated NTM infection in these patients is poorly understood.

Methods. In this retrospective observational study, data and sera for 331 Japanese subjects with mycobacterial infection were collected and analyzed. IFNγ-autoAb titers in sera were quantified using an enzyme-linked immunosorbent assay; neutralizing capacity was evaluated via flow cytometry.

Results. Disseminated NTM was identified in 50 human immunodeficiency virus–uninfected patients. Of these, 30 of 37 (81%) immunocompetent patients had an increased nIFNγ-autoAb titer whereas only 1 of 13 (7.7%) immunodeficient patients had an increased nIFNγ-autoAb titer ($P < .0001$, χ^2 test). Presenting symptoms were nonspecific and NTM infection was not included in the differential diagnosis in most cases. All patients with disseminated NTM and an increased serum nIFNγ-autoAb level received prolonged antimicrobial therapy. In 6 cases when antibiotic treatment was discontinued, NTM infection recurred and required resumption of antibiotic therapy for infection control. The mortality rate was 3.2% in disseminated NTM patients with nIFNγ-autoAbs and 21% in those without.

Conclusions. nIFNγ-autoAbs were present in most patients with disseminated NTM infection without a diagnosis of clinical immunodeficiency. Diagnosis of disseminated NTM requires a high degree of suspicion and can be improved by measuring serum nIFNγ-autoAb titer. Long-term antibiotic therapy helps prevent recrudescent NTM infection.

Keywords. disseminated NTM; interferon-γ neutralizing autoantibodies; ELISA; flow cytometry; prophylaxis.

Nontuberculous mycobacteria (NTM) cause a broad spectrum of infections in humans, including pulmonary, osteoarticular, and skin infection, as well as disseminated infections. The nature and extent of infection is thought to depend mainly on the immunological status of the host. Increased susceptibility to severe infection with mycobacteria is often caused by genetic defects that impair interferon-gamma (IFN-γ)– and interleukin 12 (IL-12)–mediated immunity [1]; this is termed Mendelian susceptibility to mycobacterial disease [2]. According to the clinical manifestations of Mendelian susceptibility to mycobacterial disease, it is clear that the IFN-γ/IL-12 axis plays a critical role in the biological defense against NTM infection. Over the past decade, studies have reported that impaired IFN-γ signaling due to IFN-γ neutralizing autoantibodies (nIFNγ-autoAbs) is associated with severe disseminated infections with intracellular pathogens, especially NTM [3–8]. However, whether nIFNγ-autoAbs increase the risk of or cause disseminated NTM infection is not known. Notwithstanding, nIFNγ-autoAbs are an acquired autoimmune factor that are considered to regulate disease susceptibility [9]. A few phenotypes associated with nIFNγ-autoAbs have been investigated in Taiwan [10]; however, a comprehensive investigation of its clinical implications has not been attempted thus far. Therefore, we conducted an observational retrospective study to clarify the clinical significance of nIFNγ-autoAbs in disseminated NTM disease.

MATERIALS AND METHODS

Subjects

From May 2012 to October 2016, we screened for the presence of nIFNγ-autoAbs in the sera of 331 adult Japanese subjects with mycobacterial infection recruited from various regions in Japan. In each experiment, sera from healthy volunteers without any relevant disease served as controls. Pulmonary NTM

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infection was diagnosed on the basis of the diagnostic guidelines of the Japanese Respiratory Society and the Japanese Society for Tuberculosis [11]. A diagnosis of pulmonary NTM was confirmed when NTM species were detected in sputum cultures more than twice or when NTM was cultured from specimens collected via bronchoscopy. Disseminated NTM disease was diagnosed when samples from >1 lesion or when clinically sterile samples such as blood and bone marrow yielded positive cultures for NTM. Pulmonary tuberculosis was defined as a positive culture for *Mycobacterium tuberculosis* from sputum. Site of involvement was defined as the location of the lesion where the NTM species were actually detected pathologically or via culture, or where suspected via computed tomography, magnetic resonance imaging, or fludeoxyglucose positron emission tomography. The medical records of subjects with disseminated NTM were reviewed and extracted into a standardized format by their attending physicians. Each patient's prognosis was recorded from diagnosis until the end of 2016. This study was performed with the approval of the Ethics Committee at the School of Medicine, Niigata University (approval number 1413), and complied with the Declaration of Helsinki. All subjects provided written informed consent.

Measurement of Interferon-γ (IFN-γ) Neutralizing Autoantibodies Concentration and Neutralizing Capacity Against IFN-γ

Serum IFNγ-autoAb concentration (including both neutralizing and nonneutralizing antibody species) was quantified by using an enzyme-linked immunosorbent assay as reported previously [12] in both patients and healthy controls. The relative titer of IFNγ-autoAbs for subjects was described as a ratio to the optical density compared to that of a healthy control. A ratio of >2-fold was considered increased (positive).

The neutralizing capacity for IFN-γ was evaluated using a newly developed method. One million Jurkat cells were inoculated with 90 μL of sera that was diluted 100-fold with phosphate-buffered saline. Then, 10 μL of 100 ng/mL recombinant human IFN-γ (Wako Co Ltd, Gunma, Japan) was added and subsequently incubated at 37°C for 15 minutes. Cells were fixed with 4% paraformaldehyde. After washing, the cells were permeabilized with 95% methanol on ice for an hour. The permeabilized cells were incubated with 20 μL of antimouse phospho-signal transduction and activator of transcription 1 (STAT1) antibody (Alexa Fluor 647 Mouse Anti-STAT1 (pY701), BD Biosciences). Positive cells with phosphorylated STAT1 were identified using FACSCalibur (BD Bioscience) and analyzed using CellQuest Pro software (BD Bioscience). Serum from a healthy control was analyzed concurrently to attain a normal value. The STAT1-phosphorylation index was calculated as the mean fluorescence intensity of cells primed with 10 ng/mL IFN-γ minus that of unprimed cells divided by that of unprimed cells, and then multiplied by 100. An index <0.3-fold that of the control indicated the presence of neutralizing capacity against IFN-γ.

Statistical Analysis

Data were analyzed using SigmaPlot software version 12.3 (Systat Software, San Jose, California) software. The data for the 2 groups were compared using the *t* test or Mann-Whitney *U* test for continuous variables and χ^2 test or Fisher exact test for categorical data. A *P* value <.05 was considered to indicate statistical significance.

RESULTS

Screening of Interferon-γ Neutralizing Autoantibodies Titers in Clinical Specimens

We recruited 331 Japanese subjects with mycobacterial disease including 189 with pulmonary tuberculosis, 91 with pulmonary NTM, and 51 with disseminated NTM. Thirty-eight subjects (11.5%) had an increased serum IFNγ-autoAb level (Figure 1A). We also evaluated the serum IFN-γ neutralizing capacity in 138 subjects including all subjects with increased serum IFNγ-autoAbs. Thirty one of 138 subjects had increased serum IFNγ-autoAbs and a confirmatory increase in serum IFN-γ neutralizing capacity (Figure 1B), supporting the conclusion that they had increased functional, neutralizing IFNγ-autoAbs. The prevalence of nIFNγ-autoAbs in patients with mycobacterial disease was 9.4% (31/331). All 31 subjects with nIFNγ-autoAbs had disseminated NTM. On the other hand, 7 subjects with nonneutralizing IFNγ-autoAbs had localized mycobacterial infection. A detailed classification of the participants is shown in Supplementary Figure 1.

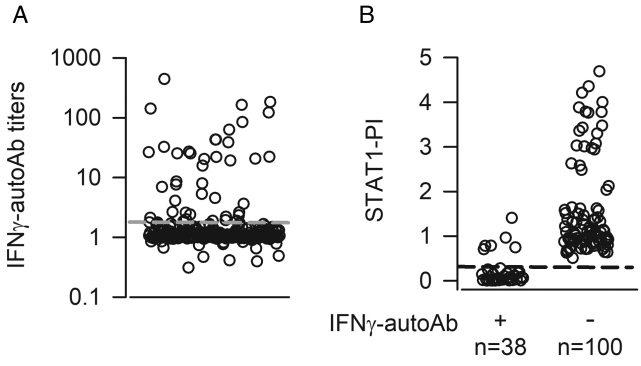


Figure 1. Evaluation of interferon-γ autoantibody (IFNγ-autoAb) titers and neutralizing capacities in the sera of patients with mycobacterial disease. *A*, IFNγ-autoAb titers in the sera of 331 patients with mycobacterial disease were measured. A titer >2-fold that of the control was defined as a positive result (dashed line indicates the threshold). Thirty-eight patients had a positive IFNγ-autoAb titer value. *B*, Neutralizing capacity to exogenous IFN-γ was evaluated among 138 subjects, including all IFNγ-autoAb–positive subjects. Data for antibody positivity and negativity are described separately. An index <0.3-fold that of the control indicated the presence of neutralizing capacity against IFN-γ. Thirty-one subjects with IFNγ-autoAbs harbored a neutralizing capacity against IFN-γ. The y-axis in the figures shows the fold increase in titers compared to that of the control. Abbreviations: IFNγ-autoAb, interferon-γ autoantibody; STAT1-PI, STAT1-phosphorylation index.

Distribution of Disseminated Nontuberculous Mycobacteria Subjects With Interferon-γ Neutralizing Autoantibodies

After excluding 1 subject from further analysis because of human immunodeficiency virus (HIV) infection, we identified 50 subjects with disseminated NTM. The rate of nIFNγ-autoAb positivity in these 50 subjects with disseminated NTM was 62%. Thirty-seven subjects were clinically immunocompetent, defined as formerly without obvious immunodeficiency and a history of receiving immunosuppressive agents, and the rest of the 13 subjects had obvious immunodeficiency. Among patients with disseminated NTM, 30 of 37 immunocompetent subjects (81.1%) and 1 of 13 immunodeficient subjects (7.8%) had nIFNγ-autoAbs. The distribution of subjects with nIFNγ-autoAbs was significantly different between these groups ($P < .001$). Thus, nIFNγ-autoAbs are strongly associated with disseminated NTM infection among previously immunocompetent patients.

Comparison of Clinical Features Between Disseminated Nontuberculous Mycobacteria Patients With and Without Interferon-γ Neutralizing Autoantibodies

Clinical Characteristics of the Patients and Mycobacterial Species Present

We compared disseminated NTM manifestations between subjects with (n = 31) and without (n = 19) nIFNγ-autoAbs (Table 1). Age at disseminated NTM infection onset was higher in subjects with nIFNγ-autoAbs than in those without nIFNγ-autoAbs. No differences in terms of sex and body mass index were observed. The concentrations of C-reactive protein, as well as the white blood cell count, were higher in subjects with nIFNγ-autoAbs. Although antinuclear autoantibodies that indicate autoimmune disease were detected in 2 patients with disseminated NTM with nIFNγ-autoAbs, no case satisfied the criteria of collagen vascular disease. The site of disease involvement varied; however, the lungs were the most common lesion site. Past history of concomitant infection in subjects with nIFNγ-autoAbs included 1 case of nontyphoidal *Salmonella* infection, 1 case of cytomegalovirus infection, and 2 cases of varicella zoster infection.

Various mycobacterial species were detected. There was no significant difference in bacterial species distribution between the 2 groups (Table 2). The most frequently isolated slow-growing mycobacteria were *Mycobacterium avium* complex, detected in 26 (70.3%) and 13 (68.4%) subjects with and without nIFNγ-autoAbs, respectively. *Mycobacterium abscessus* complex was the most frequent rapid-growing mycobacteria.

Host Immunological Status

Among the 30 individuals with nIFNγ-autoAbs (96.8%) and 7 individuals without nIFNγ-autoAbs (36.8%), we did not

Table 1. Clinical Characteristics of Subjects With Disseminated Nontuberculous Mycobacterial Infection With or Without Interferon-γ Neutralizing Autoantibodies

	With nIFNγ-AutoAbs (n = 31)		Without nIFNγ-AutoAbs (n = 19)		
Characteristics	No.	Value	No.	Value	*P* Value
Demographics					
Age, y, median (IQR)	31	66 (63–69)	19	51 (39–61)	<.01
Sex, female/male, No.		16/15		7/12	NS
BMI, kg/m^2, median (IQR)	30	19.4 (17.5–21.7)	17	19.8 (16.6–20.4)	NS
Laboratory data, median (IQR)					
Total protein, g/dL	30	7.4 (6.8–7.9)	18	6.8 (6.0–7.5)	NS
Albumin, g/dL	30	2.8 (2.0–3.0)	15	3.0 (2.5–3.5)	NS
Hemoglobin A1c, %	26	5.8 (5.4–6.2)	13	5.3 (5.1–5.9)	NS
CRP, mg/dL	30	6.6 (4.8–12.7)	18	4.6 (1.0–8.1)	<.05
WBC count, cells/μL	30	12 010 (9450–16 525)	18	6700 (4100–8050)	<.01
Neutrophils, %	29	80 (68.8–84.8)	18	78.5 (67.7–83.9)	NS
Lymphocytes, %	29	11.3 (8.8–22.1)	18	12 (5.8–23.0)	NS
Site of involvement, No. (%)					
Lung	31	19 (61.3)	19	11 (57.9)	NS
Bone/joints	31	18 (58.1)	19	7 (36.8)	NS
Lymph nodes	31	15 (48.4)	19	6 (31.6)	NS
Bone marrow/blood	31	15 (48.4)	19	5 (26.3)	NS
Skin	31	8 (25.8)	19	4 (21.1)	NS
Spleen/liver	31	6 (12.9)	19	2 (5.3)	NS
Muscle	31	4 (12.9)	19	3 (15.8)	NS
Pericardium	31	0	19	3 (15.8)	<.05
Urinary organ	31	2 (6.5)	19	0	NS
Genitals	31	1 (3.2)	19	0	NS
Central nervous system	31	1 (3.2)	19	0	NS
Eye	31	1 (3.2)	19	0	NS

Each quartile value was compared by using *t* test or Mann-Whitney *U* test as appropriate. Each distribution was compared by using Fisher exact test.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; nIFNγ-autoAbs, interferon-γ neutralizing autoantibodies; NS, not significant; WBC, white blood cell.

Table 2. Distribution of Nontuberculous Mycobacterial Species in Subjects With or Without Interferon-γ Neutralizing Autoantibodies

Species	With nIFNγ-AutoAbs	Without nIFNγ-AutoAbs	*P* Value
Slow-growing mycobacteria			
M. avium complex	26 (70.3)	13 (65.0)	
M. gordonae	2 (5.4)	0 (0)	
M. kansasii	1 (2.7)	3 (15.0)	
M. mantenii	1 (2.7)	0 (0)	
M. colombiense	1 (2.7)	0 (0)	
M. genavense	1 (2.7)	0 (0)	
M. marinum	0 (0)	1 (5.0)	
Rapid-growing mycobacteria			
M. abscessus complex	3 (8.1)	1 (5.0)	
M. fortuitum	2 (5.4)	1 (5.0)	
M. chelonae	0 (0)	1 (5.0)	NS
Slow-growing/rapid-growing mycobacteria	32/5	17/3	NS
Superinfection	6 (16.2)	1 (5.0)	NS

Data are presented as No. (%). Superinfection was defined as >1 isolate detected in the same subject. Each distribution was compared by using χ^2 test or Fisher exact test as appropriate. *P* values <.05 were defined as significant.

Abbreviations: nIFNγ-autoAbs, interferon-γ neutralizing autoantibodies; NS, not significant.

identify any predisposing factors for immunodeficiency, and no drugs that would cause immunosuppression were administered (Supplementary Figure 2*A*). Only 1 subject with nIFNγ-autoAbs was considered to have acquired immunodeficiency because he had been in complete remission after receiving chemotherapy for malignant lymphoma (Supplementary Figure 2*B*). On the other hand, 5 subjects and 1 subject without nIFNγ-autoAbs were diagnosed with acquired and congenital immunodeficiency, respectively, according to their medical history. Overall, no subjects with nIFNγ-autoAbs were administered immunosuppressive drugs before disease onset (Supplementary Figure 2*C*). In contrast, 9 subjects with nIFNγ-autoAbs received immunosuppressants after disease onset (Supplementary Figure 2*D*), because the initial diagnosis included an inflammatory disease or malignant lymphoma.

Initial Manifestations and Diagnosis

The primary manifestations of patients with disseminated NTM with nIFNγ-autoAbs were nonspecific, which made the diagnosis difficult (Supplementary Figure 3*A*). In most cases, the attending physicians did not include NTM infection in the differential diagnosis (Supplementary Figure 3*B*). Only 3 cases were suspected to be mycobacteriosis (NTM disease or tuberculosis). The mean period from disease onset to diagnosis in both groups were similar: 4 months (interquartile range [IQR], 2–8 months) in subjects with nIFNγ-autoAbs and 3 months (IQR, 1–4 months) in subjects without IFNγ-autoAbs. In 5 subjects with nIFNγ-autoAbs, the period from disease onset to diagnosis was more than a year; however, all subjects without nIFNγ-autoAbs were diagnosed with disseminated NTM infection within a year (Supplementary Figure 3*C*).

Treatment and Prognosis

The median duration of observation was 36 months (IQR, 9–54 months) and 12 months (IQR, 6–33 months) among disseminated NTM subjects with and without nIFNγ-autoAbs, respectively. Various antibiotic regimens were administered to suppress disease activity in both groups (Table 3 and Supplementary Table 1). Median treatment duration for subjects with nIFNγ-autoAbs was 21 months (IQR, 6.25–39 months), and for subjects without nIFNγ-autoAbs was 11 months (IQR, 2.5–18 months). Antibiotic treatment was terminated in 6 subjects (once in 5 cases and twice in 1 case) with nIFNγ-autoAbs owing to disease remission. One case (case 10) without applicable precise data was excluded from further analysis. The median treatment period was 24 months (IQR, 14.5–49 months) in these 5 cases. NTM disease recurrence was reported in all cases of treatment cessation, and the median period until recurrence was 10.5 months (IQR, 4–18.5 months). Among subjects with nIFNγ-autoAbs, surgical drainage was performed in 10 subjects and recombinant IFN-γ was administered in 2 subjects. Two patients were administered rituximab (anti-CD20 antibodies) to reduce nIFNγ-autoAb production via B lymphocyte depletion.

One subject with nIFNγ-autoAbs died due to disseminated NTM within 36 months, which was the median observation period for the nIFNγ-autoAb–positive group. Moreover, during this period, 3 nIFNγ-autoAb–negative subjects (15.7%) died. Two deaths were caused by NTM infection and another due to aspiration pneumonia. Kaplan-Meier survival analysis showed survival rates to be significantly higher for subjects with nIFNγ-autoAbs than those without nIFNγ-autoAbs (Figure 2).

DISCUSSION

Herein, we reported the clinical significance of nIFNγ-autoAbs against disseminated NTM and differences between subjects with and those without nIFNγ-autoAbs. The most significant clinical finding from our study was that 62% of subjects with disseminated NTM and without HIV infection and 81.1% of subjects with disseminated NTM and without obvious immunodeficiency retained nIFNγ-autoAb positivity. Moreover, nIFNγ-autoAbs were not detected in any subject with pulmonary NTM and pulmonary tuberculosis but only those with disseminated NTM. Therefore, disseminated NTM was the most significant phenotype associated with nIFNγ-autoAbs, in accordance with previous reports [6, 10, 13]. Our data are consistent with those of Browne et al, who reported that 81% of disseminated NTM subjects with normal CD4 lymphocyte counts retained nIFNγ-autoAb positivity [6]. These results suggest that nIFNγ-autoAbs commonly exist in disseminated NTM patients without obvious immunodeficiency. Therefore, nIFNγ-autoAbs should be qualified in such patients to elucidate the pathophysiology.

Table 3. Antibiotic Regimen and Clinical Course in Subjects With Disseminated Nontuberculous Mycobacteria With Interferon-γ Neutralizing Autoantibodies

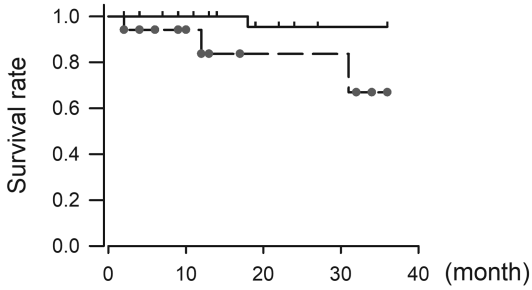
Patient	NTM Species	Used Antibiotics	Duration of Observation From Onset, mo	Duration of Antibiotic Treatment[a], mo	Antibiotic Cessation	Outcome
1	*M. avium*	CAM, RFP, EB, SM, MFLX	22	18	–	Alive
2	MAC, *M. gordonae*	CAM, RFP, SM, LVFX	175	12	+	Alive
3	*M. avium*	CAM, RFP, EB	44	40	–	Alive
4	*M. avium*	CAM, RFP, EB	57	56	–	Alive
5	*M. abscessus* complex	CAM, IPM, AMK, MFLX, LVFX, LZD	42	39	–	Dead
6	*M. intracellulare*	CAM, RFP, SM, MFLX	42	41	–	Dead
7	*M. avium, M. intracellulare*	CAM, RFP, EB	54	46	–	Alive
8	*M. gordonae, M. mantenii*	CAM, RFP, EB	41	37	–	Alive
9	*M. abscessus* complex, MAC	CAM, RFP, EB, AZM, MEPM, CPFX, AMK	18	1	–	Dead
10	*M. fortuitum*	CAM, LVFX	69	NA	+	Alive
11	*M. avium*	CAM, KM, EB, RFP, LVFX	176	24	+	Alive
12	*M. avium*	AZM, RFP, EB, SM, CPFX, RBT, STFX, MFLX	36	32	–	Alive
13	*M. abscessus* complex	CAM, IPM, AMK, MINO	19	11	–	Alive
14	*M. avium*	CAM, RFP, EB, KM	172	74	+	Alive
15	*M. avium*	CAM, RBT, STFX, SM	40	39	–	Dead
16	*M. avium*	CAM, RFP, EB	9	3	–	Alive
17	*M. fortuitum, M. avium*	CAM, EB, STFX	4	4	–	Alive
18	*M. avium*	CAM, RFP, EB	48	24	+	Alive
19	*M. kansasii*	CAM, RFP, INH	72	24	–	Alive
20	*M. avium*	CAM, RFP, EB, SM, LVFX	54	32	–	Alive
21	*M. avium*	CAM, RFP, EB	27	12	–	Alive
22	MAC	CAM, RFP, EB, AMK, STFX	14	12	–	Alive
23	MAC	CAM, RFP, EB, SM	11	7	–	Alive
24	*M. avium*	CAM, RFP, EB	36	10	–	Alive
25	*M. avium*	CAM, RFP, EB, LZD	2	17	+	Alive
26	*M. genavense*	NA	2	NA	NA	Alive
27	*M. avium*	CAM, RFP, EB, SM	7	0.1	–	Alive
28	*M. colombiense*	CAM, RFP, EB, CPFX	7	4	–	Alive
29	*M. intracellulare*	CAM, RFP, EB	8	3	–	Alive
30	*M. intracellulare*	CAM, RFP, EB	8	1	–	Alive
31	*M. avium, M. intracellulare*	CAM, RFP, EB, RBT	36	36	–	Alive

Abbreviations: AMK, amikacin; AZM, azithromycin; CAM, clarithromycin; CPFX, ciprofloxacin; EB, ethambutol; INH, isoniazid; IPM, imipenem; KM, kanamycin; LVFX, levofloxacin; LZD, linezolid; MAC, *Mycobacterium avium* complex; MEPM, meropenem; MFLX, moxifloxacin; MINO, minomycin; NA, not applicable; NTM, nontuberculous mycobacteria; RFP, rifampicin; RBT, rifabutin; SM, streptomycin; STFX, sitafloxacin.

[a]Duration of antibiotics treatment was described as the period before antibiotics cessation among the cases with the episode.

Another clinically significant finding from our study was that establishing a precise diagnosis was very difficult. We explored 3 possibilities. First, individuals with nIFNγ-autoAbs were considered to be formerly immunocompetent because they did not have predisposing factors for immunodeficiency. Disseminated NTM disease was the first severe infectious disease for almost all subjects with nIFNγ-autoAbs. Second, nonspecific initial manifestations confused the attending physicians; some subjects were tentatively diagnosed with inflammatory diseases due to unknown causes by their attending physicians. As a result, 9 subjects with nIFNγ-autoAbs received immunosuppressants after disease onset. On the other hand, no subject without nIFNγ-autoAbs received immunosuppressants after disease onset. Some previous studies reported multiple NTM-induced bone lesions in immunocompetent subjects [14, 15], which were clinically similar to our cases. There is a possibility that several subjects with nIFNγ-autoAbs may have been included in these reports but were simply overlooked. Third, the method used to evaluate nIFNγ-autoAbs varies widely. According to our data, measurement of neutralizing capacities was useful for diagnosis. All of the 7 subjects with IFNγ-autoAbs without neutralizing capacity did not have a disseminated NTM infection. This result supports the findings of several previous reports demonstrating that anticytokine autoantibodies exist in healthy subjects [16, 17]. Therefore, neutralizing capacity was more important than the antibody concentration itself to determine predisposition to a disease.

Most subjects with nIFNγ-autoAbs presented with nonspecific initial symptoms, similar to HIV-infected patients [18, 19]. Gastrointestinal symptoms, such as diarrhea, are common in HIV-infected patients with disseminated NTM because the digestive tract is a major entry point for NTM species. Our



Number at risk					
nIFNγ–autoAb (+)	31	26	20	17	17
nIFNγ–auto Ab (-)	17	9	5	5	2

Figure 2. Kaplan-Meier estimation of survival for patients with disseminated nontuberculous mycobacteria (NTM) with and those without interferon-γ neutralizing autoantibodies (nIFNγ-autoAbs). Survival rate after disseminated NTM onset among subjects with (solid line) and those without (dashed line) nIFNγ-autoAbs ($P < .05$). Abbreviations: nIFNγ-autoAb, interferon-γ neutralizing autoantibody.

results implied that the airway might be the most likely entry point owing to the predominance of lung lesions and missing gastrointestinal lesions. The lesion site has generally shown to vary [10, 20, 21] in disseminated NTM subjects with and those without nIFNγ-autoAbs. However, the distribution of lesion site did not differ between subjects with and those without nIFNγ-autoAbs, indicating that nIFNγ-autoAbs did not contribute to organ-specific susceptibility to NTM infection.

Data regarding the mortality rate due to disseminated NTM infection are scarce. Some studies have reported these deaths to be associated with HIV infection [19, 22]. Kobayashi et al reported that during 2000–2013, 7 of 24 patients (29%) from a single hospital in Japan with HIV infection and disseminated NTM died [19]. Data from Oregon in the United States showed that 19 of 37 (51.4%) subjects with disseminated NTM and HIV infection died between 2007 and 2012; the median survival period was 0.3 months after being diagnosed with disseminated NTM [22]. Prognosis was generally very poor. In our study, 3 subjects without nIFNγ-autoAbs died (mortality rate, 15.7%) and 1 subject with nIFNγ-autoAbs died (mortality rate, 3.2%) during the observation period. Nonetheless, the survival rates for subjects with and those without nIFNγ-autoAbs was significantly higher than that for subjects with HIV infection (Figure 2). These data suggest that disseminated NTM with nIFNγ-autoAbs shows a relatively favorable prognosis under appropriate antimicrobial therapy.

Long-term maintenance antibiotic therapy for mycobacterial infection is necessary and mandatory in HIV-infected patients [23]; however, the usefulness of such therapy for patients with nIFNγ-autoAbs has remained unclear thus far. In 6 cases wherein the antibiotic treatment was discontinued, NTM recurrence was observed, which required resumption of antibiotics. In Taiwan, Chi et al reported that 9 of 45 patients with disseminated NTM and nIFNγ-autoAbs achieved remission after >500 days of antimicrobial therapy; 4 of these 9 cases involved recurrent NTM infection [10]. Although the period from termination of antimicrobial therapy to recurrence of NTM infection was not mentioned, a high recurrence rate was indicated. In a few of our cases for which long-term observation was conducted, remission was maintained for ≥5 years with continuous antimicrobial therapy while maintaining nIFNγ-autoAb positivity (data not shown). In such cases, it might be difficult to terminate antimicrobial therapy completely; moreover, prophylaxis, as in HIV-infected individuals, is important.

We acknowledge some limitations to our study. First, the number of subjects was small. An epidemiological study of autoimmune pulmonary alveolar proteinosis induced by anti–granulocyte macrophage colony-stimulating factor autoantibodies reported that the prevalence of the disease was quite rare (6.2 per million) [24]. Therefore, disseminated NTM with nIFNγ-autoAbs could also be assumed to be rare. Second, our study design was retrospective in nature. Longitudinal prospective cohort studies should be conducted to clarify the disease course in clinical settings. Third, selection bias was existent because this study was retrospective in nature and involved convenience samples. We did not conduct a nationwide investigation. Also, our study included cases wherein the attending physician knew that the pathological condition was induced by nIFNγ-autoAbs. The possibility of overlooking numerous cases was also taken into consideration.

In summary, we reported the practical clinical manifestations of disseminated NTM patients with nIFNγ-autoAbs in Japan. Disseminated NTM infection harboring nIFNγ-autoAbs could account for the majority of subjects with disseminated NTM without obvious underlying immunosuppression. Diagnosis of disseminated NTM itself is difficult because the initial symptoms and physical findings are nonspecific; moreover, the concept of an autoimmune disease induced by a severe infection is not well recognized. For the diagnosis of disseminated NTM, not only measurement of antibody titers but also serum neutralizing capacity against exogenous IFN-γ is important. Although long-term antimicrobial therapy is required for treatment, the prognosis is more favorable than that in disseminated NTM patients with an HIV infection or without nIFNγ-autoAbs. However, continuation of antibacterial therapy may be necessary for prophylaxis. Although disseminated NTM infection accompanied with nIFNγ-autoAbs is very rare in clinical settings, it involves the possibility of misdiagnosis as an unknown inflammatory disease or malignant disease.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. van de Vosse E, van Dissel JT, Ottenhoff TH. Genetic deficiencies of innate immune signaling in human infectious disease. Lancet Infect Dis **2009**; 9:688–98.
2. Filipe-Santos O, Bustamante J, Chapgier A, et al. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. Semin Immunol **2006**; 18:347–61.
3. Kampmann B, Hemingway C, Stephens A, et al. Acquired predisposition to mycobacterial disease due to autoantibodies to IFN-gamma. J Clin Invest **2005**; 115:2480–8.
4. Patel SY, Ding L, Brown MR, et al. Anti-IFN-gamma autoantibodies in disseminated nontuberculous mycobacterial infections. J Immunol **2005**; 175:4769–76.
5. Koya T, Tsubata C, Kagamu H, et al. Anti-interferon-gamma autoantibody in a patient with disseminated *Mycobacterium avium* complex. J Infect Chemother **2009**; 15:118–22.
6. Browne SK, Burbelo PD, Chetchotisakd P, et al. Adult-onset immunodeficiency in Thailand and Taiwan. N Engl J Med **2012**; 367:725–34.
7. Hase I, Morimoto K, Sakagami T, Kazumi Y, Ishii Y, van Ingen J. Disseminated *Mycobacterium gordonae* and *Mycobacterium mantenii* infection with elevated anti-IFN-γ neutralizing autoantibodies. J Infect Chemother **2015**; 21:468–72.
8. Nishimura T, Fujita-Suzuki Y, Yonemaru M, et al. Recurrence of disseminated *Mycobacterium avium* complex disease in a patient with anti-gamma interferon autoantibodies by reinfection. J Clin Microbiol **2015**; 53:1436–8.
9. Lin CH, Chi CY, Shih HP, et al. Identification of a major epitope by anti-interferon-γ autoantibodies in patients with mycobacterial disease. Nat Med **2016**; 22:994–1001.
10. Chi CY, Lin CH, Ho MW, et al. Clinical manifestations, course, and outcome of patients with neutralizing anti-interferon-γ autoantibodies and disseminated nontuberculous mycobacterial infections. Medicine **2016**; 95:e3927.
11. Committee on Management of Nontuberculous Acid-Fast Bacterial Infection of the Japanese Society for Tuberculosis and the Section on Tuberculosis and Infection, the Japanese Respiratory Society. Guideline for diagnosis of non-tuberculous acid-fast bacterial infection of the lung—2008 [in Japanese]. Kekkaku **2008**; 83:525–6.
12. Shima K, Sakagami T, Tanabe Y, et al. Novel assay to detect increased level of neutralizing anti-interferon gamma autoantibodies in non-tuberculous mycobacterial patients. J Infect Chemother **2014**; 20:52–6.
13. Valour F, Perpoint T, Sénéchal A, et al; Lyon TB Study Group. Interferon-γ autoantibodies as predisposing factor for nontuberculous mycobacterial infection. Emerg Infect Dis **2016**; 22:1124–6.
14. Shimizu H, Mizuno Y, Nakamura I, Fukushima S, Endo K, Matsumoto T. Vertebral osteomyelitis caused by non-tuberculous mycobacteria: case reports and review. J Infect Chemother **2013**; 19:972–7.
15. Suzuki T, Murai H, Miyakoshi N, Hongo M, Itoi E, Shimada Y. Osteomyelitis of the spine caused by *Mycobacterium avium* complex in an immunocompetent patient. J Orthop Sci **2014**; 18:490–5.
16. Watanabe M, Uchida K, Nakagaki K, et al. Anti-cytokine autoantibodies are ubiquitous in healthy individuals. FEBS Lett **2007**; 581:2017–21.
17. Uchida K, Nakata K, Suzuki T, et al. Granulocyte/macrophage-colony-stimulating factor autoantibodies and myeloid cell immune functions in healthy subjects. Blood **2009**; 113:2547–56.
18. Grinsztejn B, Fandinho FC, Veloso VG, et al. Mycobacteremia in patients with the acquired immunodeficiency syndrome. Arch Intern Med **1997**; 157:2359–63.
19. Kobayashi T, Nishijima T, Teruya K, et al. High mortality of disseminated non-tuberculous mycobacterial infection in HIV-infected patients in the antiretroviral therapy era. PLoS One **2016**; 11:e0151682.
20. Kampitak T, Suwanpimolkul G, Browne S, Suankratay C. Anti-interferon-γ autoantibody and opportunistic infections: case series and review of the literature. Infection **2011**; 39:65–71.
21. Wongkulab P, Wipasa J, Chaiwarith R, Supparatpinyo K. Autoantibody to interferon-gamma associated with adult-onset immunodeficiency in non-HIV individuals in northern Thailand. PLoS One **2013**; 8:e76371.
22. Varley CD, Ku JH, Henkle E, Schafer SD, Winthrop KL. Disseminated nontuberculous mycobacteria in HIV-infected patients, Oregon, USA, 2007–2012. Emerg Infect Dis **2017**; 23:533–5.
23. Pierce M, Crampton S, Henry D, et al. A randomized trial of clarithromycin as prophylaxis against disseminated *Mycobacterium avium* complex infection in patients with advanced acquired immunodeficiency syndrome. N Engl J Med **1996**; 335:384–91.
24. Inoue Y, Trapnell BC, Tazawa R, et al; Japanese Center of the Rare Lung Diseases Consortium. Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. Am J Respir Crit Care Med **2008**; 177:752–62.