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BRIEF DEFINITIVE REPORT

Anti–IFN-γ autoantibodies underlie disseminated *Talaromyces marneffei* infections

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Talaromyces marneffei **causes life-threatening opportunistic infections, mainly in Southeast Asia and South China.** ***T. marneffei*** **mainly infects patients with human immunodeficiency virus (HIV) but also infects individuals without known immunosuppression. Here we investigated the involvement of anti–IFN-γ autoantibodies in severe** ***T. marneffei*** **infections in HIV-negative patients. We enrolled 58 HIV-negative adults with severe** ***T. marneffei*** **infections who were otherwise healthy. We found a high prevalence of neutralizing anti–IFN-γ autoantibodies (94.8%) in this cohort. The presence of anti–IFN-γ autoantibodies was strongly associated with HLA-DRB1*16:02 and -DQB1*05:02 alleles in these patients. We demonstrated that adult-onset acquired immunodeficiency due to autoantibodies against IFN-γ is the major cause of severe** ***T. marneffei*** **infections in HIV-negative patients in regions where this fungus is endemic. The high prevalence of anti–IFN-γ autoantibody–associated HLA class II DRB1*16:02 and DQB1*05:02 alleles may account for severe** ***T. marneffei*** **infections in Southeast Asia. Our findings clarify the pathogenesis of** ***T. marneffei*** **infection and pave the way for developing novel treatments.**

Introduction

Talaromyces (*Penicillium*) *marneffei* is an important intracellular fungal pathogen that can cause severe systemic infection. It is a thermally dimorphic fungus, presenting septate hyphae at 25°C and transforming to the pathogenic yeast morphology at 37°C during infection. It is endemic to Southern China, Taiwan, Thailand, Laos, Vietnam, Northeast India, and Hong Kong and is almost exclusively restricted to Southeast Asia (Vanittanakom et al., 2006). *T. marneffei* infections in humans are supposed to occur through inhalation of *T. marneffei* conidia in the environment. Upon entering the human body, this fungal pathogen can replicate in macrophages in yeast form to cause infection, ranging from local infection in skin and lungs to severe systemic infection (Cao et al., 2019). *T. marneffei* infections usually occur in immunocompromised individuals with impaired cell-mediated immunity, including secondary immunodeficiency due to HIV infection, cancer, and immunosuppressive therapy. However, *T. marneffei* infections can also occur in HIV-negative individuals with no obvious immunosuppression (Kauffman et al., 2014; Ramos-e-Silva et al., 2012). The factors underlying a host susceptibility to this infection are unknown, but a potential immunodeficiency is suspected. For example, children with certain inborn genetic disorders, such as cytochrome B-245 β chain (*CYBB*) and cluster of differentiation 40 ligand (*CD40L*) mutations, or signal transducer and activator of transcription-1 (*STAT1*) gain-of-function mutations, are susceptible to severe *T. marneffei* infection, suggesting T cell–macrophage immunity has a critical role in controlling *T. marneffei* infection (Kamchaisatian et al., 2006; Lee and Lau, 2017; Lee et al., 2014).

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The production of neutralizing anti–IFN-γ autoantibodies is an emerging adult-onset immunodeficiency restricted to specific regions of the globe, including Hong Kong, Thailand, and Taiwan (Browne et al., 2012a; Chi et al., 2013, 2016; Döffinger et al., 2004; Lee et al., 2013; Patel et al., 2005). Sporadic cases have also been reported in Japan, the Philippines, Vietnam, Laos, and other Southeast Asian countries (Chan et al., 2014; Patel et al., 2005; Tanaka et al., 2007). Most patients with neutralizing anti–IFN-γ autoantibodies suffer from disseminated infections with nontuberculous mycobacteria (NTM; Browne et al., 2012a). Other opportunistic infections, such as *T. marneffei* infection, have also been observed in a few NTM infection patients with anti–IFN-γ autoantibodies (Pruetpongpun et al., 2016; Tang et al., 2010).

We showed previously that anti–IFN-γ autoantibodies in adults with disseminated NTM infections are strongly associated with two specific HLA class II alleles: HLA-DRB1*16:02/DQB1*05:02 and HLA-DRB1*15:02/DQB1*05:01 (Chi et al., 2013; Ku et al., 2016). HLA-DRB1*16:02/DQB1*05:02 is a specific haplotype commonly found in populations in South China and Taiwan, whereas -DRB1*15:02/DQB1*05:01 is more common in patients from Southeast Asia (Middleton et al., 2003). The specific distribution of these risk-associated HLA haplotypes accounts for the geographic/ethnic restriction of anti–IFN-γ autoantibody–related diseases. Given the high frequency of the DRB1*16:02/DQB1*05:02 risk haplotype in South China, we hypothesized that this part of the country, particularly Guangxi, would also be a region with high levels of anti–IFN-γ autoantibody production. To test this hypothesis, we analyzed the presence of anti–IFN-γ autoantibodies and HLA class II haplotypes in HIV-negative patients with *T. marneffei* infections from South China.

Results and discussion

Characteristics of patients with *T. marneffei* infections

We enrolled 58 patients suffering from severe *T. marneffei* infections. Their demographic characteristics, proven pathogens, sampling sites (listed in order of occurrence), and treatment outcomes are summarized in Table 1. The patients' mean age was 54.2 yr (range, 22–77), and the study population consisted of 34 men and 24 women. The mean age of the control group was 49.5 yr (range, 23–65), and this control group consisted of 59 men and 48 women. No prior medical condition was reported for 47 of the cases (81.0%). Comorbidities were identified in 11 patients (19.0%), mainly type 2 diabetes mellitus (n = 7), followed by hypertension (n = 3), renal insufficiency (n = 2), thalassemia (n = 1), coronary atherosclerotic heart disease (n = 1), embolism of the pulmonary artery (n = 1), and acute kidney injury (n = 1). None of the patients had a history of cancer, autoimmunity, or any form of immunosuppressive treatment.

Detection of anti–IFN-γ autoantibodies

We evaluated possible adult-onset immunodeficiency due to anti–IFN-γ autoantibodies by performing an indirect ELISA on serially diluted plasma from healthy donors and patients to test for the presence of anti–IFN-γ autoantibodies. Anti–IFN-γ autoantibodies were detected (OD >0.5, in 1:100 dilution) in most patients (55/58 cases; Fig. 1 A). In the inhibition assay, we incubated a fixed concentration (100 pg/ml) of recombinant IFN-γ with serially diluted plasma from the patients. Recombinant human IFN-γ was almost undetectable in the 55 patients defined as anti–IFN-γ autoantibody–positive, even though the plasma was diluted significantly (1:200,000 dilutions for 16 patients; Fig. 1 B). Further analysis showed that most of these autoantibodies were of the IgG1 and IgG4 types (Fig. S1).

We then evaluated these anti–IFN-γ autoantibodies' biological effect by assessing whether they could neutralize the IFN-γ–induced HLA-DR expression and STAT-1 phosphorylation in THP-1 cells (Fig. 1, C–E). Plasma from all anti–IFN-γ autoantibody–positive patients decreased IFN-γ–induced HLA-DR expression and STAT-1 phosphorylation, but no inhibitory effect was observed with plasma from healthy donors or the three antibody-negative patients. IFN-γ had been shown to enhance the clearance of *T. marneffei* in myeloid cells (Sisto et al., 2003). Consistent with this idea, plasma from anti–IFN-γ autoantibody–positive patients could impair the IFN-γ–mediated clearance of *T. marneffei* in THP-1 cells (Fig. S2).

We used a bead-based multiplex system to screen for the presence of autoantibodies on five other cytokines (GM-CSF, IL-6, IL-17A, IL-12, and IL-23), which have been linked to immune dysregulation and infection (Ku et al., 2020). Anti–GM-CSF autoantibodies were found in one patient (case 28), but these autoantibodies to GM-CSF were nonneutralizing, and this patient has no anti–GM-CSF autoantibody–related disease (Table 1 and Fig. S3). No other anti-cytokine autoantibodies were observed in the remaining *T. marneffei*–infected patients. 39 HIV patients (19 of whom had *T. marneffei* infections) were screened for anti–IFN-γ autoantibodies, and all were negative. In addition, we performed whole-exome sequencing with the DNA from the 50 anti–IFN-γ autoantibody–positive and 3 anti–IFN-γ autoantibody–negative cases. Neither reported pathogenic nor homozygous/compound heterozygous-predicted deleterious single-nucleotide polymorphisms (SNPs) were identified in Mendelian susceptibility to mycobacterial disease (MSMD)–associated genes (*IL12RB1*, *IL12B*, *ISG15*, *SPPL2A*, *IRF8*, *TYK2*, *IFNGR1*, *IFNGR2*, *STAT1*, *NEMO*, *CYBB*, *IL23R*, *IFNG*, or *IL12RB2*) and chronic mucocutaneous candidiasis-associated genes (*IL17F*, *IL17RC*, *IL17RA*, *ACT1*, *CARD9*, *STAT3*, *RORC*, and *MAPK8*; Bustamante, 2020; Kerner et al., 2020; Li et al., 2017, 2019). The three heterozygous SNPs (*IL12RB2* c.G1003T, p.D335Y; *SPPL2A* c.T995C, p.L332P; and *IFNGR2* c.C115T, p.R39C) identified in the three patients (cases 1, 11, and 33, respectively) were predicted as probably damaging. Nevertheless, the immunodeficiency by autosomal dominant inheritance of *IL12RB2* or *SPPL2A* has never been reported, and the frequency of *IFNGR2* c.C115T is relatively common (∼0.07%) in East Asians. Thus, these SNPs are unlikely to cause the immunological defect observed, and no unifying genetic theory could be found for this patient cohort. Together, we demonstrated that neutralizing anti–IFN-γ autoantibodies were present in the plasma of 55/58 (94.8%) patients with severe *T. marneffei* infections.

Table 1. **Characteristics of the 58 patients with *T. marneffei* infection**

ID	Age	Sex	Medical history	Comorbid conditions	Co-infection	Culture specimen yielding *T. marneffei*	Days required for diagnosis	Number of recurrences	Follow-up (mo)	Antibiotics used	Outcome[a]
1	57	F	Anti-TB treatment; cough and expectoration; left hand herpes; lymph node (neck, jaw) enlargement for 1 yr; recurrent fever		CMV, NTM, HBV, *C. albicans*	Bone marrow	218	2	35	CLA, EMB, ITC, MXF	Cured
2	59	F	Anti-TB treatment; anterior chest erythema, nodules, and swelling for 4 mo; cervical lymph node enlargement for 4 mo; recurrent fever; weight loss		MAC	Skin lesion	145	1	37	ITC, RMP	Cured
3	47	F	Chest pain for 9 mo; cough and expectoration; recurrent fever; skin abscess; weight loss		*B. cepacia*, NTM, VZV	Pus (skin)	205	4	29	EMB, INH, LVX, PZA	Death
4	54	M	Hepatomegaly; multiple lymph nodes (neck, mediastinum, retroperitoneum); recurrent fever for 1 mo; weight loss		HBV	Skin lesion	59	1	38	ITC	Death
5	59	F	Erythema, papules and pustules (Sweet's syndrome); lymph node enlargement for 8 mo; weight loss		NTM	Skin lesion	108	1	62	CEF, ITC	Death
6	49	M	Anti-TB treatment; fever, cough and expectoration for 2 yr; lymph node enlargement; weight loss	DM, H/T		Skin lesion, pleural tissue	923	Unknown	28	EMB, INH, RMP, VRC	Unknown
7	57	M	Cough and expectoration for 1 mo; fever; weight loss	RI		Alveolar lavage fluid	42	Unknown	31	CEF, FLC, ITC, LVX, MXF, VRC	Unknown
8	31	F	Anti-TB treatment; chest pain; cough and expectoration for 5 mo; lymph node enlargement (neck); weight loss			Alveolar lavage fluid, sputum, liver tissue	40	Unknown	29	CEF, CLA, EMB, FLC, INH, MXF, PZA, RMP	Unknown
9	62	F	Anti-TB treatment; lumbosacral pain for 2 mo; recurrent fever; skin abscess; weight loss			Pus (bone)	488	1	39	AMB	Cured
10	40	F	Anti-TB treatment; fever with lymph node enlargement (neck, groin) for 4 mo; joint pain; weight loss			Bone marrow	375	5	72	AMB, FOX, ITC, LVX	Persistent infection

Table 1. **Characteristics of the 58 patients with *T. marneffei* infection (*Continued*)**

ID	Age	Sex	Medical history	Comorbid conditions	Co-infection	Culture specimen yielding *T. marneffei*	Days required for diagnosis	Number of recurrences	Follow-up (mo)	Antibiotics used	Outcome[a]
11	42	F	Anti-TB treatment; erythema, papules, and pustules over entire body (Sweet's syndrome); lymph node enlargement for >6 mo; recurrent fever; weight loss			Pus (clavicle)	319	4	26	AMB, ITC	Cured
12	58	F	Anti-TB treatment; cough, expectoration, and fever for 6 mo; multiple masses; weight loss			Pus (skin)	611	1	41	AMB, ITC, LVX, VRC	Cured
13	49	F	Anti-TB treatment; cough, expectoration, and fever for 8 mo; lymph node enlargement (supraclavicular, mediastinum, pelvic cavity, groin); weight loss		*K. pneumoniae*, *A. veronii*	Pus (joint)	119	0	25	VRC	Death
14	40	M	Acute generalized pustular disease; cough, expectoration and fever for 3 mo			Alveolar lavage fluid	30	2	13	CLA, VRC	Persistent infection
15	51	F	Anti-TB treatment; chest pain; cough, expectoration, and fever for 7 d; lymph node enlargement (mediastinum); skin mass; weight loss			Alveolar lavage fluid, lung tissue	273	5	13	AMB, CEF, FOX, INH, ITC, LVX, PZA, RMP	Persistent infection
16	51	M	Cough, expectoration for 6 mo; recurrent fever for 3 mo; skin abscess; weight loss			Pus (bone)	208	1	10	VRC	Persistent infection
17	50	M	Fever; cough and expectoration for 5 mo; swelling of the right neck lymph node for 3 mo (neck and mediastinum)			Blood	119	2	11	ITC	Persistent infection
18	51	M	Fever and cough for 4 mo; lympho-adenopathy for 3 yr (mediastinum, supraclavicular)		HBV	Lung tissue	261	0	10	CEF, ITC	Persistent infection
19	57	M	Anti-TB treatment; cough, expectoration, and fever for 1 mo; weight loss		NTM	Blood, sputum (lung)	34	3	37	EMB, FLC, FOX, INH, LVX, MXF, PZA, RMP, SXT, VRC	Persistent infection

Table 1. **Characteristics of the 58 patients with *T. marneffei* infection (*Continued*)**

ID	Age	Sex	Medical history	Comorbid conditions	Co-infection	Culture specimen yielding *T. marneffei*	Days required for diagnosis	Number of recurrences	Follow-up (mo)	Antibiotics used	Outcome[a]
20	60	F	Anti-TB treatment; recurrent fever; repeated enlargement of the right axillary lymph nodes for >20 yr; subcutaneous mass; weight loss			Skin lesion, pus	360	1	27	CLA, EMB, INH, ITC, MXF, RMP, VRC	Persistent infection
21	52	F	Anti-TB treatment; recurrent fever, cough, and expectoration for 8 mo; subcutaneous mass; weight loss		CMV	Alveolar lavage fluid, skin lesion	268	2	37	AZ, FLC, MXF, VRC	Unknown
22	41	M	Anti-TB treatment; hepatosplenomegaly; lymph node enlargement (ear, neck, and submaxillary); recurrent fever and cough for 4 mo; skin ulcers; weight loss		VZV	Blood	207	1	42	CEF, EMB, FLC, ITC, LVX, PZA, RMP, VRC	Unknown
23	29	M	Anti-TB treatment; fever, cough, and expectoration for 1 mo; hepatosplenomegaly; prolonged fever for 5 mo; weight loss		TB	Pus (skin)	73	2	38	EMB, FLC, ITC, LVX, PZA, RMP	Unknown
24	46	M	Fever and lymph node enlargement for 3 mo; hepatosplenomegaly; weight loss		*Clonorchis sinensis*, VZV	Pus (skin)	124	1	37	CEF, FLC, ITC, LVX, MXF	Unknown
25	57	M	Chest pain; cough and expectoration for 2 mo; lymph node enlargement (supraclavicular, neck) for 6 mo; recurrent fever; weight loss			Pleural tissue	85	Unknown	35	AMB, CEF, LVX, MXF, VRC	Unknown
26	64	F	Anti-TB treatment; cough and cervical lymph node enlargement for 10 mo; weight loss			Lymph node	283	1	44	AMB	Cured
27	50	F	Anti-TB treatment; cough and expectoration for >1 yr; recurrent fever for 2 mo; weight loss	DM, H/T		Blood	55	2	41	CEF, FLC, ITC, VRC	Death
28	68	M	Anti-TB treatment; cough, expectoration, and fever for 1 mo; lymph node enlargement (armpit, mediastinum, groin); recurrent fever; weight loss	DM	*E. cloacae*	Lung tissue	116	1	7	AMB, CEF, IPM, LVX	Death

Table 1. **Characteristics of the 58 patients with *T. marneffei* infection (*Continued*)**

ID	Age	Sex	Medical history	Comorbid conditions	Co-infection	Culture specimen yielding *T. marneffei*	Days required for diagnosis	Number of recurrences	Follow-up (mo)	Antibiotics used	Outcome[a]
29	67	M	Erythema, papules, and pustules over entire body (Sweet's syndrome); lymph node enlargement for 2 yr; recurrent fever for 2 yr		*Salmonella* spp.	Bone marrow, blood	515	Unknown	22	CEF, ITC, LVX, VRC	Unknown
30	65	M	Cough and expectoration for 5 mo; fever for 2 mo; hepatomegaly; lymph node enlargement (armpit, groin); skin lesions			Bone marrow	91	1	33	CEF, MXF, VRC	Cured
31	44	M	Anti-TB treatment; chest pain; cough and expectoration; lymph node enlargement (mediastinum); recurrent fever for 9 mo; weight loss		*Mycobacterium abscessus*	Sputum (lung)	312	1	27	IPM, LZD, MXF, VRC	Cured
32	22	M	Anti-TB treatment; hip pain for 8 mo; weight loss	Pyogenic osteomyelitis, thalassemia		Blood	166	8	45	VRC	Death
33	77	M	Anti-TB treatment; neck mass for 10 mo; lymph node enlargement (cervical and pulmonary portal) for 10 mo; weight loss			Pus, skin lesion	417	1	13	LVX, INH, ITC, VRC	Persistent infection
34	70	F	Anti-TB treatment; fever; cough and expectoration; cervical lymph node enlargement for 1 mo; weight loss		VZV	Lymph node	91	4	29	LVX, FOX, VRC, ITC, RMP, EMB, INH, CAS, MXF, VA	Death
35	64	M	Anti-TB treatment; cough and expectoration for 3 mo; recurrent fever for 1 mo; weight loss			Alveolar lavage fluid	91	5	18	AMB, ITC, TG, CDZ, ETM, VA, FLC, IPM	Persistent infection
36	65	M	Anti-TB treatment; recurrent fever and cough for 1 yr; Sweet's syndrome; weight loss	DM, coronary atherosclerotic heart disease	*Salmonella* spp., TB	Pus (joint)	305	5	21	INH, RMP, EMB, MXF, AMB	Death
37	53	M	Anti-TB treatment; recurrent fever and abdominal pain; weight loss			Blood	365	0	24	CLI, EMB, MEM, TEC, CAS, VRC	Death
38	61	M	Recurrent fever, cough, and abdominal pain; weight loss		*Salmonella* spp.	Lung tissue	210	1	16	LVX, DOX, CHL	Death
39	56	M	Recurrent fever and cough; lymph node enlargement; Sweet's syndrome; weight loss			Lymph node	30	2	19	LVX, FLC, VRC, AMB	Cured

Table 1. **Characteristics of the 58 patients with *T. marneffei* infection (*Continued*)**

ID	Age	Sex	Medical history	Comorbid conditions	Co-infection	Culture specimen yielding *T. marneffei*	Days required for diagnosis	Number of recurrences	Follow-up (mo)	Antibiotics used	Outcome[a]
40	33	F	Anti-TB treatment; fever; chest pain, cough, and expectoration for 4 mo; Sweet's syndrome; weight loss			Alveolar lavage fluid	515	1	13	PTZ, CLI, RMP, INH, EMB, AMB, VRC	Unknown
41	59	M	Fever and cough for 20 d; weight loss	RI	VZV	Sputum, alveolar lavage fluid	75	0	17	VRC, MEM, LZD, TEC, CDZ, AMB, MXF, DOX, AN, ACV	Unknown
42	63	M	Neck mass for 1 mo; fever for 19 d; weight loss		EBV	Blood	60	1	14	LVX, CDZ, MEM, VA, VRC, AMB, PTZ, LZD	Persistent infection
43	50	F	Anti-TB treatment; recurrent cough for 7 mo; recurrent fever for 5 mo; weight loss		VZV	Blood, alveolar lavage fluid	240	0	15	PSL, LVX, TCF, MXF, INH, RMP, EMB, PZA, AMB, ACV	Persistent infection
44	63	M	Anti-TB treatment; cough and expectoration for 8 mo	DM		Alveolar lavage fluid	210	0	8	CDZ, LVX, SCF, AMB	Persistent infection
45	64	M	Cough and expectoration for 4 mo; fever for 7 d; weight loss	Embolism of pulmonary artery, acute kidney injury	VZV, *A. baumannii*, *K. pneumoniae*	Lung tissue	150	0	12	PTZ, MXF, AMX, LVX, CDZ, SCF, CLI, FLC, IPM, VRC, LZD, SXT, MEM, VA, CAS, AMB, ACV, TG	Persistent infection
46	50	F	Anti-TB treatment; fever; right supraclavicular mass for 3 mo		*Aspergillus*	Blood, lymph node	151	0	9	INH, RPT, EMB, PZA, FOX, VRC	Persistent infection
47	68	M	Recurrent fever and cough for 8 mo	DM, H/T		Blood	240	0	12	CDZ, CLI, SCF, MXF, VA, VRC	Death
48	72	F	Recurrent fever for 6 mo		EBV, CMV	Lymph node	181	1	11	TG, AN, SCF, GCV, LVX, VRC	Death
49	49	F	Anti-TB treatment; neck mass for 3 mo; fever for 15 d; weight loss		TB	Alveolar lavage fluid	89	2	15	INH, RMP, EMB, PZA, PTZ, LVX, AMB	Cured
50	45	M	Anti-TB treatment; recurrent fever, cough, and expectoration for 3 mo; respiratory distress and asthma for 2 mo; weight loss			Alveolar lavage fluid	90	0	15	CDZ, MXF, MEM, VRC	Cured
51	57	M	Recurrent fever and cough for 1 mo		*K. pneumoniae*	Lung tissue	29	1	20	CLI, TCF, VA, VRC, AMB	Persistent infection

Table 1. **Characteristics of the 58 patients with *T. marneffei* infection (*Continued*)**

ID	Age	Sex	Medical history	Comorbid conditions	Co-infection	Culture specimen yielding *T. marneffei*	Days required for diagnosis	Number of recurrences	Follow-up (mo)	Antibiotics used	Outcome[a]
52	52	F	Anti-TB treatment; repeated enlargement of cervical lymph node; recurrent cough and expectoration		*M. abscessus*	Lymph node	62	1	23	ITC, CXM, RMP, EMB, INH	Persistent infection
53	45	M	Anti-TB treatment; recurrent cough and expectoration; weight loss		*C. albicans*		120	1	14	PSL, MXF, PTZ, LZD, INH, FLC, RMP, EMB	Persistent infection
54	51	M	Cough, expectoration, and fever for 1 mo	DM	VZV, *Salmonella* spp.	Blood	28	1	18	AMB, VRC, ITC	Persistent infection
55	68	M	Recurrent fever, cough, and expectoration for 1 mo; weight loss		*C. albicans*	Alveolar lavage fluid	29	1	14	CDZ, MXF, PTZ, ETM, FLC	Unknown
A	69	M	Cough and expectoration for 5 d; hepatosplenomegaly; recurrent fever for 1 mo; weight loss		HBV, *C. albicans*	Blood	30	Unknown	33	CEF, VRC	Unknown
B	62	F	Chest pain for 1 yr; recurrent fever for 3 mo; skin ulcers			Pus (skin)	83	Unknown	19	AMB, VRC	Unknown
C	47	F	Anti-TB treatment; cough and expectoration; hepatomegaly; recurrent fever; swollen lymph nodes for 3 mo			Lung tissue	1,213	1	27	LVX, MXF, VRC	Cured

ACV, acyclovir; AMB, amphotericin B; AMX, amoxicillin; AN, amikacin; AZ, azithromycin; CAS, caspofungin; CDZ, cefodizime; CEF, ceftibuten; CHL, chloramphenicol; CLA, clarithromycin; CLI, clindamycin; CXM, cefuroxime; DM, diabetes mellitus; DOX, doxycycline; EMB, ethambutol; ETM, etimicin; F, female; FLC, fluconazole; FOX, cefoxitin; GCV, ganciclovir; H/T, hypertension; HBV, hepatitis B virus; INH, isoniazid; IPM, imipenem; ITC, itraconazole; LVX, levofloxacin; LZD, linezolid; M, male; MAC, *Mycobacterium avium* complex; MEM, meropenem; MXF, moxifloxacin; PSL, piperacillin/sulbactam; PTZ, piperacillin/tazobactam; PZA, pyrazinamide; RI, renal insufficiency; RMP, rifampin; RPT, rifapentine; SCF, sulbactam/cefoperazone; SXT, trimethoprim-sulfamethoxazole; TCF, tazobactam/cefoperazone; TEC, teicoplanin; TG, tigecycline; VA, vancomycin; VRC, voriconazole.
[a]The definition of cure is "the patient has no sign or no recurrence of *T. marneffei* infection during the follow-up period."

Clinical manifestations in the 55 patients with anti–IFN-γ autoantibodies

In the 55 patients with anti–IFN-γ autoantibodies (cases 1–55; Table 1), common clinical features of *T. marneffei* infections included fever (85.5%), cough (80.0%), weight loss (78.2%), and lymphadenopathy (76.4%; Table 2). Multiple organ involvement was observed in these patients. The lungs were affected most frequently (100%), followed by the lymph nodes, skin, bones/joints, liver, and spleen (78.2%, 47.3%, 23.6%, 14.5%, and 9.1%, respectively; Table 2). Seven patients presented reactive skin lesions, five patients had Sweet's syndrome, and two had generalized pustulosis. Laboratory investigations revealed leukocytosis, thrombocytosis, anemia, and high $CD4^+$ and $CD8^+$ counts (Table 3). Before and after *T. marneffei* infections, 30 patients (54.5%) were also infected with other opportunistic pathogens, including NTM (n = 7; 12.7%), varicella zoster virus (VZV; n = 8; 14.5%), CMV (n = 3; 5.5%), *Candida albicans* (n = 3; 5.5%), *Mycobacterium tuberculosis* (tuberculosis [TB]; n = 3; 5.5%), *Salmonella* spp. (n = 4; 7.3%), *Klebsiella pneumoniae* (n = 3; 5.5%), EBV (n = 2; 3.6%), *Burkholderia cepacia* (n = 1), *Aeromonas veronii* (n = 1), *Acinetobacter baumannii* (n = 1), *Aspergillus* (n = 1), and *Enterobacter cloacae* (n = 1; Table 1). Based on computed tomography and/or biopsy findings similar to those for TB, anti-TB antibiotics were given to some patients before *T. marneffei* infections were confirmed. Except for 10 cases of confirmed mycobacterial infections, TB infections were excluded by additional computed tomography scans, staining for acid-fast bacilli, and/or nonresponse to anti-TB treatment.

We followed disease progression in 43 patients with anti–IFN-γ autoantibodies. 13 died despite antimicrobial treatment (Table 1), and 19 suffered recurrent *T. marneffei* infections or subsequently developed other opportunistic infections, both

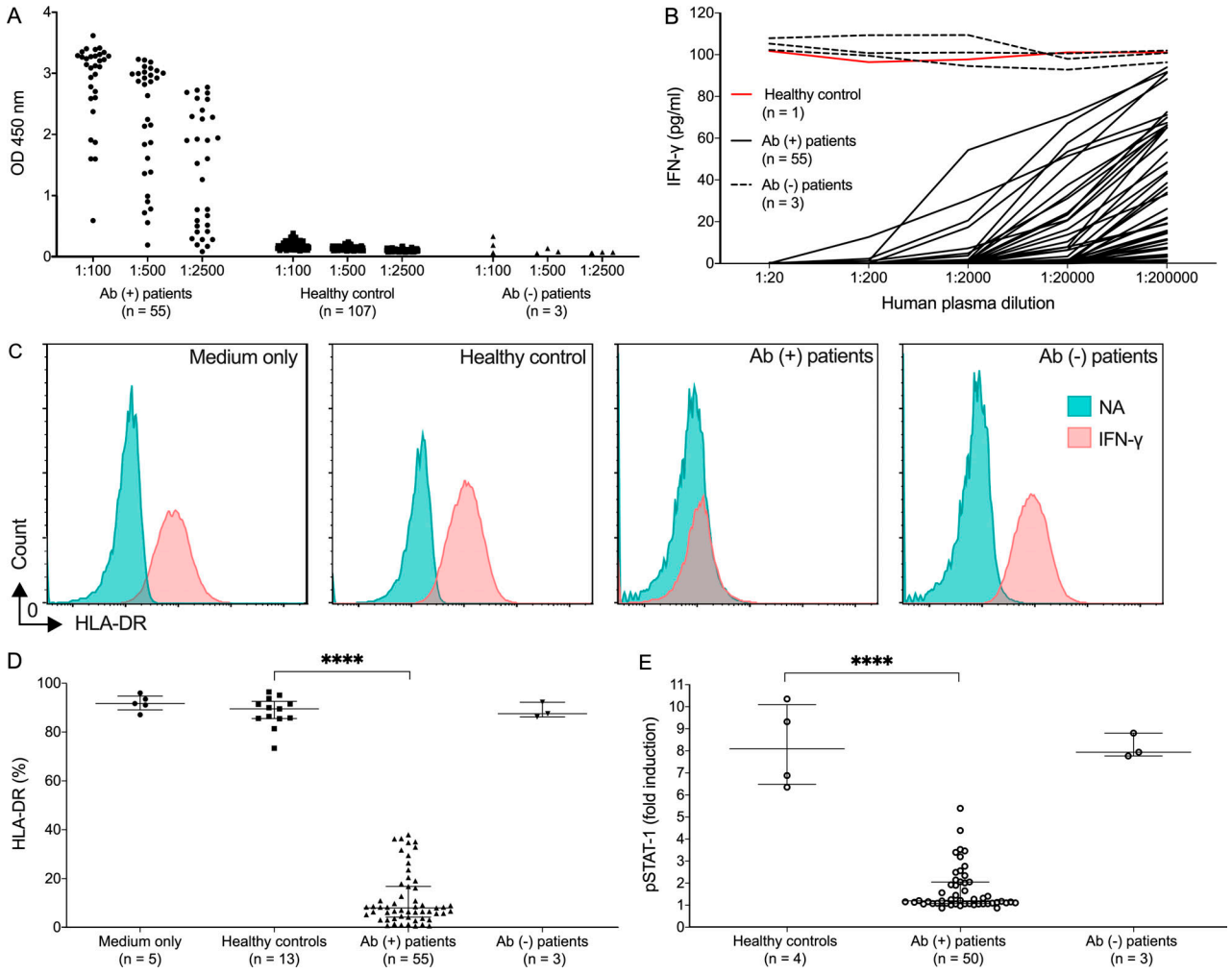

Figure 1. **Anti–IFN-γ autoantibodies in plasma of patients with severe *T. marneffei* infection. (A)** An IFN-γ–immobilized plate was used to detect antibodies against IFN-γ in plasma. After applying serially diluted patient plasma, peroxidase-conjugated anti-human IgG was added to detect and quantify the presence of human autoantibodies against IFN-γ. Low OD values were obtained with plasma from healthy donors (107 cases, filled square) and three antibody-negative patients (Ab [−] patients, filled triangle), while high values (OD > 0.5 in 1:100 dilution) were detected in 55 antibody-positive patients (Ab [+] patients, filled circle). **(B)** Plasma from anti–IFN-γ autoantibody–positive patients interfered with the detection of human IFN-γ. Serially diluted patient plasma samples were respectively incubated with a fixed concentration (100 pg/ml) of IFN-γ. The amount of remaining unbound IFN-γ was detected by peroxidase-conjugated anti–IFN-γ antibodies. **(C–E)** Anti–IFN-γ autoantibodies from *T. marneffei*–infected patients showed neutralizing activity in vitro. IFN-γ–neutralizing activity was addressed by detecting the impact of patient plasma on IFN-γ–induced HLA-DR expression of THP-1 cells (C and D) and STAT-1 phosphorylation (pSTAT-1) in THP-1 cells (E). **(C)** THP-1 cells without IFN-γ were used as a negative control (blue peaks) as compared with those with IFN-γ induction (red peaks). **(D)** A scatter plot summarizing the result of HLA-DR expression with all the patient plasma samples in this cohort is shown. **(E)** STAT-1 phosphorylation in THP-1 cells was detected by flow cytometry. The result was shown as fold induction measured by the ratio of mean fluorescence intensity relative to those of cells without IFN-γ activation. All results are representative of at least two independent experiments. **(D and E)** Values represent median with interquartile range. The statistical analysis was performed by Mann–Whitney test. ****, P < 0.0001. NA, not activated.

requiring long-term antimicrobial treatment and severely affecting the patients' quality of life. Only eight patients were cured after antimicrobial treatment (cure was defined as sustained resolution 6 mo after the end of treatment; Ouyang et al., 2017), and three patients sustained resolution for >3 mo after the end of treatment. Mortality was higher in these patients than it was in the HIV-positive patients investigated in a previous study (Kawila et al., 2013).

HLA alleles associated with anti–IFN-γ autoantibodies

We showed previously that the HLA-DRB1*16:02/DQB1*05:02 and DRB1*15:02/DQB1*05:01 haplotypes are strongly associated with the presence of anti–IFN-γ autoantibodies (Ku et al., 2016). Therefore, we analyzed the HLA–class II molecules DRB1 and DQB1 in patients with *T. marneffei* infections. In total, 16 DQB1 alleles and 25 DRB1 alleles were detected in patients and healthy controls (Table 4 and data not shown). We showed that DRB1*16:02 (n = 47; 85.4%) and DQB1*05:02 (n = 48; 87.3%) were more frequent in patients with anti–IFN-γ autoantibodies than in the control population (22.4% in controls for DRB1*16:02, and 43.9% for DQB1*05:02). Additionally, we found that 98.2% of the anti–IFN-γ autoantibody–positive patients carried the DRB1*15:02 or DRB1*16:02 alleles. The presence of the DRB1*16:02 or DQB1*05:02 allele was strongly associated with severe *T. marneffei* infections, and also associated with the

Table 2. **Clinical features of *T. marneffei* infections in patients with anti–IFN-γ autoantibodies**

Clinical features	Number (n = 55)	Percentage (%)
Symptoms/signs		
Fever	47	85.5
Leukocytosis	46	83.6
Cough	44	80.0
Weight loss	43	78.2
Lymphadenopathy	42	76.4
Anemia	34	61.8
Thrombocytosis	30	54.5
Cutaneous or subcutaneous lesion	28	50.9
Misdiagnosed as TB	28	50.9
Malaise	25	45.5
Arthritis or arthralgia	19	34.5
Lymphopenia	13	23.6
Abdominal pain or diarrhea	11	20.0
Chest pain	11	20.0
Dyspnea	11	20.0
Hepatomegaly	9	16.4
Thrombocytopenia	7	12.7
Splenomegaly	6	10.9
Hemoptysis	5	9.1
Osteomyelitis	2	3.6
Organ involvement		
Lung/pleura	55	100.0
Lymph node	43	78.2
Skin	26	47.3
Bone/joints	13	23.6
Liver	8	14.5
Spleen	5	9.1

Table 3. **Laboratory findings for the 55 patients with anti–IFN-γ autoantibodies**

Laboratory examination	Median (range)
Hemoglobin (g/liter)	91.7 (15.6–119.9)
White blood cell count (×10^3/μl)	16.11 (4.67–37.67)
Absolute neutrophil count (×10^3/μl)	11.99 (2.71–30.63)
Absolute lymphocyte count (×10^3/μl)	2.16 (0.36–7.95)
Platelet count (×10^3/μl)	359.6 (21.3–869.6)
Aspartate aminotransferase (units/liter)	20.5 (4–88)
Alanine aminotransferase (units/liter)	20.5 (3–150)
$CD4^+$ cell count (cells/mm^3)	757 (32–2314)
$CD8^+$ cell count (cells/mm^3)	565 (61–2124)
IgG (g/liter)	24.34 (8.27–54.68)
IgA (g/liter)	2.93 (1.013–7.307)
IgM (g/liter)	0.9 (0.179–2.595)
Natural killer cell (%)	17.55 (0.078–42.9)

production of anti–IFN-γ antibodies with odds ratios (ORs) of 20.32 (95% CI, 8.46–48.81; P = 1.92 × 10^{-14}; corrected *P* value [*Pc*] = 4.80 × 10^{-13}) and 8.75 (95% CI, 3.63–21.11; P = 1.13 × 10^{-7}; *Pc* = 1.8 × 10^{-6}), respectively (Table 4).

T. marneffei is considered an opportunistic fungal pathogen specifically found in South China and Southeast Asia (Vanittanakom et al., 2006). Here, we showed that, in addition to the previously reported NTM infections, anti–IFN-γ autoantibodies were highly prevalent in the patients with severe *T. marneffei* infections from the Guangxi region. We further demonstrated that these patients carried the HLA-DRB1*16:02/DQB1*05:02 haplotype, which was associated strongly with anti–IFN-γ autoantibodies, as we showed previously (Chi et al., 2013). Overall, our results suggest that autoantibodies against IFN-γ are the main etiology of *T. marneffei* infections in HIV-negative individuals, in which the risk HLA–class II alleles HLA-DRB1*16:02 and -DQB1*05:02 are highly prevalent.

The strong association between anti–IFN-γ autoantibodies and HLA-DRB1*16:02/DQB1*05:02 observed in this study and our previous work (Chi et al., 2013) suggests a pathogenic role of this HLA haplotype in anti–IFN-γ autoantibody generation. The geographic distribution of HLA-DRB1*16:02/DQB1*05:02 and *T. marneffei* largely overlaps in Guangxi, Guangdong, and Yunnan (provinces in South China; Ku et al., 2016). The high epidemicity of *T. marneffei* infections in these regions, in which bamboo rats serve as a reservoir of this species (Cao et al., 2011), is likely increased markedly by the high prevalence of anti–IFN-γ autoantibodies and the underlying HLA haplotype (Deng et al., 1988; Jiang et al., 2019; Lee and Lau, 2017). Indeed, severe *T. marneffei* infections are generally considered opportunistic infections found in a few patients with anti–IFN-γ autoantibodies (Chan et al., 2013; Tang et al., 2010). We further provided a large cohort of patients with *T. marneffei* infections, demonstrating that anti–IFN-γ autoantibody production is the major etiology of *T. marneffei* infections in previously healthy adults.

The discovery of this acquired IFN-γ deficiency in patients with *T. marneffei* infection suggests IFN-γ has a role in combating this fungal infection in humans. The IFN-γ's biological role in humans has mostly been elucidated by studies of children with MSMDs (Bustamante et al., 2014; Casanova and Abel, 2002). MSMD patients with defects in the IFN-γ circuit mainly present with severe to lethal infections, and saprophytic nontuberculosis mycobacteria is one of those opportunistic pathogens. Similar to mycobacteria, *T. marneffei* is capable of replicating in human macrophages (Roilides et al., 2003). IFN-γ plays a key role in activating phagocytes to clear engulfed pathogens. Impairment of IFN-γ function may disturb the clearance of intracellular pathogens from various phyla, not only intracellular bacteria. Consistent with this view, a severe *T. marneffei* infection was recently reported in a Thai child with IFNGR1 deficiency (Lee and Lau, 2017). Therefore, IFN-γ-mediated macrophage activation and microbicidal activity are critical to control *T. marneffei* infections. Nevertheless, the detailed

Table 4. **Comparison of the HLA alleles carried by anti–IFN-γ autoantibody–positive patients and healthy controls**

HLA alleles	Number carrying the allele		OR	95% CI	P value	*Pc*
	Patients (*n* = 55)	Controls (*n* = 107)				
DRB1*16:02	47	24	20.318	8.457–48.813	1.921×10^{-14}	4.803×10^{-13}
DRB1*15:02	10	10	2.156	0.838–5.547	0.105	2.625
DRB1*15:01	9	31	0.480	0.210–1.097	0.078	1.950
DRB1*12:02	2	20	0.164	0.037–0.731	0.008	0.200
DQB1*05:02	48	47	8.754	3.631–21.107	1.126×10^{-7}	1.802×10^{-6}
DQB1*05:01	6	9	1.333	0.449–3.959	0.603	9.648
DQB1*02:01	2	22	0.146	0.033–0.645	0.004	0.064
DQB1*03:01	5	23	0.365	0.131–1.021	0.048	0.768
DRB1*16:02 or *15:02	54	34	115.941	15.389–873.527	9.364×10^{-16}	1.498×10^{-14}

CI, confidence interval.

mechanism by which IFN-γ contributes to the control of *T. marneffei* in vivo remains to be determined. Further studies in humans and animal models are warranted to address this question.

In our cohort, only 12.7% of patients (7/55) with anti–IFN-γ autoantibodies experienced NTM infections (Table 1). NTM infections are the most common clinical manifestations of anti–IFN-γ autoantibody disease; however, the low NTM infection rate in our cohort might be important for future consideration. NTM are common saprophytes that are found in diverse natural ecosystems. Therefore, the rate of microbial exposure is unlikely to account for the lower prevalence of NTM infections compared with *T. marneffei* infections in patients with anti–IFN-γ autoantibodies in Guangxi (Claeys and Robinson, 2018). We speculate that *T. marneffei* is more pathogenic than NTM, and anti–IFN-γ autoantibodies' neutralizing effects might be less potent in patients with *T. marneffei* infections. Whereas, residual IFN-γ activity may be sufficient to control the dissemination of NTM infections in these patients. Further studies are needed to test this hypothesis.

Persistent, life-threatening infections have been observed in a significant portion of our patients, even in those undergoing anti-fungal antibiotic treatment. B cell–depletion treatments, such as anti-CD20 monoclonal antibody therapy, were applied in anti–IFN-γ autoantibody patients who failed to response to antibiotics (Browne et al., 2012b). By eliminating the antibody-producing B cells, the titer of autoantibody decreased, and the endogenous IFN-γ–mediated immunity against pathogens was restored. This indicates that administering anti-CD20 antibody therapy may be useful for treating anti–IFN-γ–positive patients who suffer from recurrent *T. marneffei* infections.

In conclusion, we show that the adult-onset acquired immunodeficiency, mediated by anti–IFN-γ autoantibodies, is the most important factor underlying severe *T. marneffei* infections in HIV-negative patients. Based on our findings, we strongly recommend screening for anti–IFN-γ autoantibodies in HIV-negative patients suffering from severe *T. marneffei* infections, even if they do not have NTM infections. These findings not only improve our understanding of the pathogenesis of this disease but may also point out the anti–IFN-γ autoantibody–producing B cells as novel therapeutic targets for these patients in the near future.

Materials and methods

Study population

We conducted a 5-yr (from January 2013 to June 2018) prospective, cross-sectional, case-control study at the First Affiliated Hospital of Guangxi Medical University. The inclusion criteria for cases were (1) over the age of 18 yr; (2) negative for anti-HIV antibodies and no obvious immunosuppressed condition, such as malignant tumors and organ transplantation; (3) presented with histologically and culture-proven *T. marneffei* infections; and (4) provided informed consent for participation. Among the 174 patients with *T. marneffei* infection identified during this period, 108 cases were excluded due to HIV infection (101 cases) or other immunosuppressed conditions (7 cases). Informed consent was obtained from 58 of the 66 remaining cases. We followed these patients after first diagnosis and/or during the active stage of *T. marneffei* infection. Complete histories were obtained, and physical examinations, including routine clinical laboratory tests, were performed on all patients. The healthy control group was recruited from the physical examination center from the same hospital. The Institutional Ethics Review Board of the First Affiliated Hospital of Guangxi Medical University approved the study, which was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

Determination of autoantibodies against IFN-γ

Anti–IFN-γ autoantibodies in plasma were measured by indirect ELISA according to a modified version of a previously described procedure (Chi et al., 2013). Briefly, 100 µl of 2 µg/ml recombinant human IFN-γ (R&D Systems), in carbonate buffer (pH 9.5), were added to each well in a 96-well flat-bottomed MaxiSorp plate (Nunc), and the plate was incubated overnight at 4°C.

The next day, the wells were washed three times with wash buffer (0.05% Tween-20/PBS); 100 µl of blocking buffer (5% human albumin in PBS) were then added to each well, and the plate was incubated for 2 h at room temperature. Plasma samples were diluted serially with blocking buffer. Diluted plasma (100 µl) was added to each well, and the plate was incubated for 2 h at room temperature. The plate was washed four times with wash buffer, and 100 µl of horseradish peroxidase–conjugated goat anti-human IgG (1:5,000 diluted; Invitrogen) were added to each well. The plate was incubated for 1 h at room temperature and then washed four times with wash buffer. Next, 100 µl of tetramethylbenzidine (Sigma-Aldrich) were added to each well and incubated for 15 min at room temperature. Tetramethylbenzidine stop solution (100 µl; Southern Biotech) was added to each well to stop the reaction, and OD was determined at 450 nm. Samples with OD values >0.5 were considered positive for antibodies against IFN-γ, and this result was confirmed by inhibition assays and functional tests. ELISA was performed in duplicate for all samples.

IFN-γ inhibition assay

Plasma samples were serially diluted (1:20; 1:200; 1:2,000; 1:20,000, and 1:200,000) and incubated with recombinant human IFN-γ at a final concentration of 100 pg/ml for 3 h at room temperature. IFN-γ levels were determined with a human IFN-γ ELISA kit (BD Biosciences) according to the manufacturer's instructions. Inhibition assays were performed in duplicate for all samples.

Functional test for autoantibodies against IFN-γ

The autoantibodies' neutralizing activity against IFN-γ was assessed by evaluating their ability to reduce the IFN-γ–induced HLA-DR expression on THP-1 cells. THP-1 cells were cultured in complete RPMI-1640 medium containing 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco). THP-1 cells (2×10^5 cells/ml) were incubated with human recombinant IFN-γ (10 ng/ml, R&D Systems), in the presence or absence of plasma (10 µl), from anti–IFN-γ autoantibody–positive patients or healthy donors for 24 h at 37°C. HLA-DR expression was measured with a PE–anti–HLA-DR antibody (555812; BD Biosciences) by flow cytometry (FACS Canto II; BD), and the results were analyzed with FlowJo VX software. To measure STAT-1 phosphorylation, 2×10^5 peripheral blood mononuclear cells were placed in 200 µl of RPMI-1640 with 10% FBS (Geneteks) and 1% penicillin/streptomycin. Cells were then stimulated with 20 IU or 200 IU IFN-γ in RPMI-1640, which were preincubated with 20% normal plasma or patient plasma for 20 min at room temperature. After 30 min of stimulation in a 37°C incubator, cells were fixed and permeabilized using lysing solution (BD). The PE-phospho-STAT1 (pY701) antibody (BD PharMingen) was applied, and data were collected and analyzed with a FACSVerse flow cytometer and FACSuite software (BD Biosciences).

HLA typing

Blood from all participants was collected into EDTA for DNA extraction with the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. The HLA-DQB1 and HLA-DRB1 polymorphisms in exon 2 of the target genes were investigated using a sequence-based typing method (Al-Hussein et al., 2002; Perz et al., 2007). All primers were synthesized by Shanghai Health (Table S1). The sequencing results were compared with the latest known allele sequences from an authoritative database (the human major histocompatibility complex section of the international immunogenetics database, ImMunoGeneTics/HLA) to identify the allele that was present in each subject (Robinson et al., 2001).

Statistical analysis

The proportions of HLA allele carriage were determined by direct counting. The allelic OR, 95% confidence interval, and P values for two-tailed tests were obtained with SPSS (version 22.0, IBM). We corrected for multiple testing by calculating *Pc* using the Bonferroni method. A value of $Pc < 0.05$ was considered significant.

Online supplemental material

Fig. S1 shows IgG subtypes of anti–IFN-γ autoantibody in our cohort. Fig. S2 provides evidence through *T. marneffei* killing assay that patients' plasma with anti–IFN-γ autoantibody obviously blocked the IFN-γ clearance of *T. marneffei*. Fig. S3 shows the autoantibodies against GM-CSF found in case 28 plasma. Table S1 provides the primer set we used to amplify HLA-DQB1 and -DRB1 exon 2.

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Author contributions: J. Guo, X.-Q. Ning, J.-Y. Ding, H.-P. Shih, C.-L. Ku, and C.-W. Cao designed the experiments. J. Guo, X.-Q. Ning, and J.-Y. Ding conducted the experiments. J. Guo, X.-Q. Ning, Y.-Q. Zheng, J.-Y. Ding, H.-P. Shih, H.-T. Ting, Y.-F. Lo, I.Y.-F. Chang, C.-F. Yeh, K.-H. Tu, Y.-H. Tsai, C.-L. Ku, and C.-W. Cao analyzed and/or interpreted the data. N.-N. Shi, F.-Y. Wu, Y.-K. Lin, G. Liang, X.-C. Lu, J.-L. Kong, K. Wang, Y.-B. Lu, Y.-J. Fu, R. Hu, T.-M. Li, K.-S. Pan, X.-Y. Li, C.-Y. Huang, and C.-W. Cao provided samples and performed clinical diagnoses and patient follow-ups. C.-L. Ku and C.-W. Cao supervised the study. X.-Q. Ning, J. Guo, J.-Y. Ding, Y.-H. Tsai, C.-L. Ku, and C.-W. Cao wrote the manuscript and designed the figures, to which all other authors contributed. All of the authors revised the manuscript.

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Supplemental material

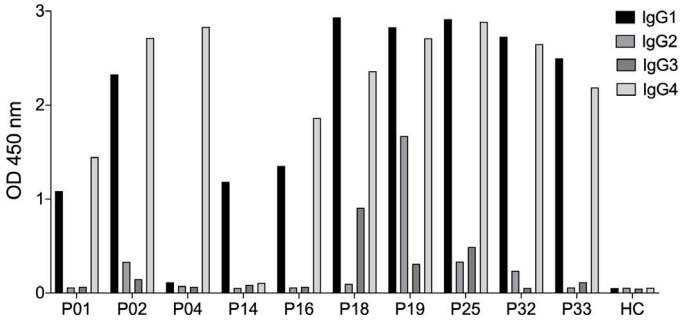

Figure S1. **Determination of anti–IFN-γ autoantibody IgG subtypes.** Representative bar graph shows the IgG subclass of IFN-γ–reactive antibodies from selected patient plasma (1:1,000 diluted) as determined by indirect ELISA. The assays were performed in duplicate independently. HC, healthy control.

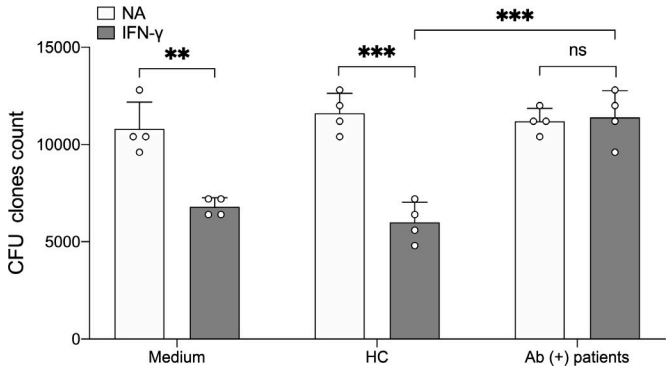

Figure S2. **Plasma from patients with anti–IFN-γ autoantibodies inhibited the IFN-γ–mediated clearance of *T. marneffei* in THP-1 cells.** THP-1 cells were stimulated with 20 ng/ml PMA for 48 h followed by resting for 12 h in refreshed medium. The differentiated cells were incubated with plasma in the absence or presence of IFN-γ (50 ng/ml) for 24 h. The cells were reseeded and co-cultured with *T. marneffei* yeasts (multiplicity of infection, 0.05) for 2 h. The cells were washed twice, then further cultured in RPMI-1640 medium containing 10% FBS, 1% penicillin/streptomycin, and 0.03 µg/ml amphotericin B for 48 h, followed by cell lysis and fungal CFUs counting. The results were shown as mean with SD obtained from two independent experiments. Statistical analysis was performed with the Student *t* test. **, P < 0.01; ***, P < 0.001. HC, healthy control; NA, not activated; ns, not significant.

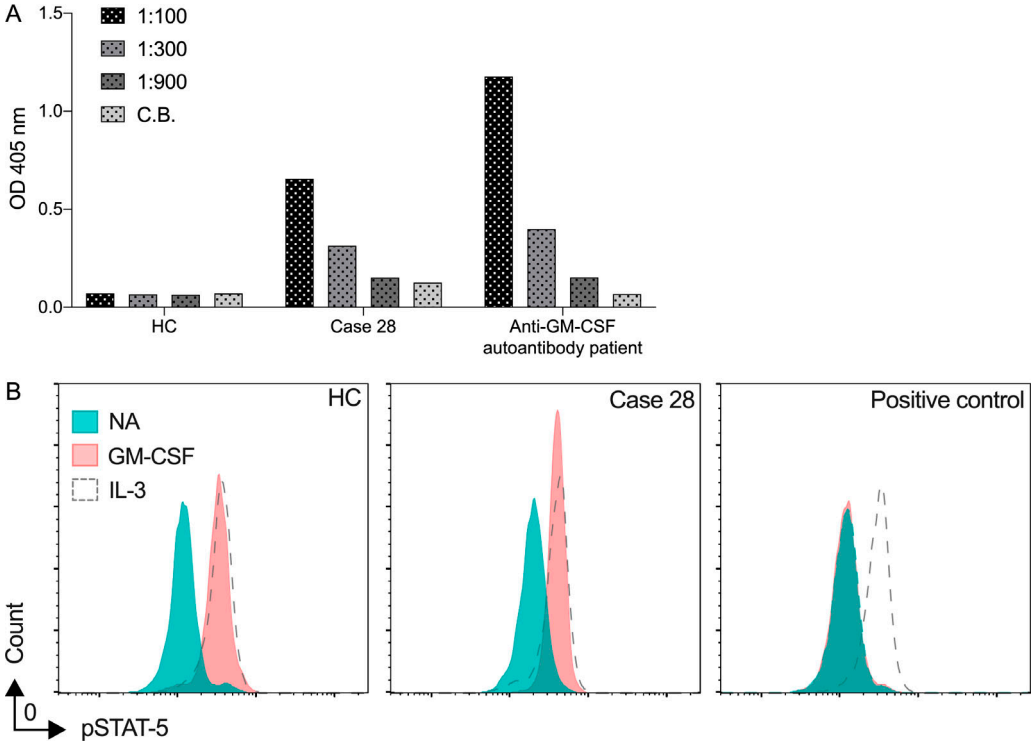

Figure S3. **The plasma of case 28 contains nonneutralizing autoantibodies against GM-CSF. (A)** The autoantibodies against GM-CSF were detected by indirect ELISA with serially diluted plasma. C.B., coating buffer only; HC, healthy control. **(B)** GM-CSF neutralizing activity of the plasma was performed by STAT-5 phosphorylation (pSTAT-5) assay with peripheral blood mononuclear cells from healthy volunteers. IL-3 treatment was used as a positive control for pSTAT-5. Plasma from one anti–GM-CSF autoantibody patient served as positive control (Kuo et al., 2017). The assays were performed in duplicate independently. NA, not activated.

Table S1 is provided online and shows the primer set used to amplify HLA-DQB1 and -DRB1 exon 2.