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 HLA-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 homozgyous/compound heterozygous-predicted deleterious single-nucleotide polymorphisms (SNPs) were identified in Mendelian susceptibility to mycobacterial disease (MSMD)–associated genes (*IL12RB1, IL12B, ISG5, SPPL2A, IRF8, TYK2, IFNGRI, IFNGR2, STAT1, NEMO, CYBB, IL23R, IFNG, or IL12RB2*) and chronic mucocutaneous candidiasis-associated genes (*IL17F, IL17RC, IL1VRA, 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.T995C, p.L332P) 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.
| 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* |
|----|-----|-----|-----------------|---------------------|--------------|----------------------------------------|-----------------------------|------------------------|----------------|----------------|----------|
| 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 |
| 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 |
|----|-----|-----|-----------------|--------------------|--------------|---------------------------------|-----------------------------|----------------------|--------------|----------------|---------|
| 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 | 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 |
| 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*              |
|----|-----|-----|---------------------------------------------------------------------------------|--------------------|--------------|----------------------------------------|-----------------------------|------------------------|----------------|---------------------------------------|-----------------------|
| 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, FL, 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, FL, 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, FL, 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, FL, 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, FL, 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            | AMB, CEF, IPM, LVX                    | Death                |
| 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* |
|----|-----|-----|-----------------|--------------------|--------------|----------------------------------|---------------------------|-----------------------|----------------|----------------|----------|
| 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 | | 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 |
| 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* |
|----|-----|-----|-----------------|--------------------|--------------|--------------------------------------|----------------------------|-------------------------|----------------|----------------|----------|
| 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 |  |  | 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                        | LVX, CDZ, 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 |

*Outcome can be Cured, Persistent infection, or Unknown.
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 syndrome, and two had genital ulcers. 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 mycobacterial infections or 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

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 |
|----|-----|-----|-----------------|--------------------|--------------|---------------------------------------|-----------------------------|------------------------|----------------|----------------|---------|
| 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 | Blood | 120 | 1 | 14 | PSL, MXF, PTZ, LZD, INH, FLC, RMP, EMB | Persistent infection |
| 54 | 51 M | Cough, expectoration, and fever for 1 mo | DM | 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, EMT, 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) | Unknown | 83 | | 19 | AMB, VRC | Unknown |
| C | 47 F | Anti-TB treatment; cough and expectoration; hepatosplenomegaly; recurrent fever; swollen lymph nodes for 3 mo | Lung tissue | Lungs | 1,213 | 1 | 27 | LVX, MXF, VRC | Cured |

ACV, acyclovir; AMB, amphotericin B; AMX, amoxicillin; AN, amikacin; AZ, azithromycin; CAS, caspofungin; CDZ, cefodizime; CEF, cefixime; CHL, chloramphenicol; CLA, clarithromycin; CLI, clindamycin; CXM, cefuroxime; 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.

*The definition of cure is “the patient has no sign or no recurrence of T. marneffei infection during the follow-up period.”
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 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).

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.

HLA alleles associated with anti–IFN-γ autoantibodies
We showed previously that the HLA-DRBI*16:02/DRB1*05:02 and DRB1*15:02/DRBI*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 DRBI and DQBI in patients with T. marneffei infections. In total, 16 DQBI alleles and 25 DRBI alleles were detected in patients and healthy controls (Table 4 and data not shown). We showed that DRBI*16:02 (n = 47; 85.4%) and DQBI*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 DRBI*16:02, and 43.9% for DQBI*05:02). Additionally, we found that 98.2% of the anti–IFN-γ autoantibody–positive patients carried the DRBI*15:02 or DRBI*16:02 alleles. The presence of the DRBI*16:02 or DQBI*05:02 allele was strongly associated with severe T. marneffei infections, and also associated with the
production of anti–IFN-γ antibodies with odds ratios (ORs) of 20.32 (95% CI, 8.46–48.81; \( P = 1.92 \times 10^{-14} \); corrected \( P \) value \( [P_c] = 4.80 \times 10^{-13} \)) and 8.75 (95% CI, 3.63–21.11; \( P = 1.13 \times 10^{-7} \); \( P_c = 1.8 \times 10^{-6} \)), 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 (Rolildes 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 IFNGRI 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 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 (×10^12/µl) | 16.11 (6.7–37.67)    |
| Absolute neutrophil count (×10^9/µl) | 11.99 (2.71–30.63)  |
| Absolute lymphocyte count (×10^12/µl) | 2.16 (0.36–7.95)    |
| Platelet count (×10^12/µl)   | 359.6 (213.8–869.6)   |
| Aspartate aminotransferase (units/liter) | 20.5 (4–88)      |
| Alanine aminotransferase (units/liter) | 20.5 (3–150)     |
| CD4+ cell count (cells/mm³)  | 757 (32–2314)         |
| CD8+ cell count (cells/mm³)  | 565 (61–2124)         |
| IgG (g/liter)                | 24.34 (8.27–54.68)    |
| IgA (g/liter)                | 2.93 (1.03–7.307)     |
| IgM (g/liter)                | 0.9 (0.179–2.595)     |
| Natural killer cell (%)      | 17.55 (0.078–42.9)    |
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 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.

### 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 (95% CI) |
|-------------|---------------------------|-------------|---------|------------|
| **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.
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 × 10⁶ 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 × 10⁵ 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 P< 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 infection https://doi.org/10.1084/jem.20190502 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 used to amplify HLA-DQ81 and -DRB1 exon 2.

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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.
Table S1 is provided online and shows the primer set used to amplify HLA-DQβ1 and -DRB1 exon 2.

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.