

## ORIGINAL ARTICLE

# Adult-Onset Immunodeficiency in Thailand and Taiwan

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## ABSTRACT

**BACKGROUND**

Autoantibodies against interferon- $\gamma$  are associated with severe disseminated opportunistic infection, but their importance and prevalence are unknown.

**METHODS**

We enrolled 203 persons from sites in Thailand and Taiwan in five groups: 52 patients with disseminated, rapidly or slowly growing, nontuberculous mycobacterial infection (group 1); 45 patients with another opportunistic infection, with or without nontuberculous mycobacterial infection (group 2); 9 patients with disseminated tuberculosis (group 3); 49 patients with pulmonary tuberculosis (group 4); and 48 healthy controls (group 5). Clinical histories were recorded, and blood specimens were obtained.

**RESULTS**

Patients in groups 1 and 2 had CD4<sup>+</sup> T-lymphocyte counts that were similar to those in patients in groups 4 and 5, and they were not infected with the human immunodeficiency virus (HIV). Washed cells obtained from patients in groups 1 and 2 had intact cytokine production and a response to cytokine stimulation. In contrast, plasma obtained from these patients inhibited the activity of interferon- $\gamma$  in normal cells. High-titer anti-interferon- $\gamma$  autoantibodies were detected in 81% of patients in group 1, 96% of patients in group 2, 11% of patients in group 3, 2% of patients in group 4, and 2% of controls (group 5). Forty other anticytokine autoantibodies were assayed. One patient with cryptococcal meningitis had autoantibodies only against granulocyte-macrophage colony-stimulating factor. No other anticytokine autoantibodies or genetic defects correlated with infections. There was no familial clustering.

**CONCLUSIONS**

Neutralizing anti-interferon- $\gamma$  autoantibodies were detected in 88% of Asian adults with multiple opportunistic infections and were associated with an adult-onset immunodeficiency akin to that of advanced HIV infection. (Funded by the National Institute of Allergy and Infectious Diseases and the National Institute of Dental and Craniofacial Research; ClinicalTrials.gov number, NCT00814827.)

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THE CONTROL OF INFECTION WITH MYCOBACTERIA, DIMORPHIC MOLDS, AND SALMONELLA depends on the integrity of the interferon- $\gamma$ , interleukin-12, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) pathways, as shown by mendelian defects and iatrogenic inhibition of these pathways.<sup>1,2</sup> Patients with genetic defects tend to present early in life, and the defects often show familial clustering, whereas patients with therapeutic antibody-associated disease have clear risk factors and tend to present in adulthood.

Anticytokine autoantibodies are increasingly recognized as having a role in disease pathogenesis, including susceptibility to infection.<sup>3,4</sup> Since 2004, disseminated nontuberculous mycobacterial and other opportunistic infections involving neutralizing anti-interferon- $\gamma$  autoantibodies have been described in 25 adults without human immunodeficiency virus (HIV) infection, most of whom were from East Asia.<sup>5-13</sup> In large case series from Thailand and Taiwan, descriptions of HIV-uninfected adults with disseminated mycobacterial infection (particularly with rapidly growing mycobacteria), often involving concomitant reactive dermatoses,<sup>14-18</sup> suggested a common syndrome of adult-onset immunodeficiency. To identify a defect that confers a predisposition to infections that are characteristic of advanced HIV infection, we analyzed humoral and cellular function, including assessment for anticytokine autoantibodies, in patients and healthy controls residing in regions where this syndrome appears to have a high prevalence.

## METHODS

### PARTICIPANTS

All patients were seen routinely for their infections at one of four academic centers in Thailand or Taiwan and provided written informed consent according to the protocol, which was approved by the National Institute of Allergy and Infectious Diseases and all local sites. The first author vouches for the completeness and accuracy of the data and for the fidelity of the study to the protocol.

At baseline, complete histories were obtained and physical examinations with routine clinical laboratory tests were performed in all patients. Data were recorded on standardized case-report

forms. Patients had no history of cancer, immunodeficiency, or immune suppression within 4 weeks before enrollment or diagnosis of their infections.

We enrolled the participants in five groups: patients with disseminated, rapidly or slowly growing, nontuberculous mycobacterial infection (group 1); patients with another opportunistic infection (e.g., infection with *Cryptococcus neoformans*, *Histoplasma capsulatum*, or *Penicillium marneffei*; disseminated salmonellosis; or severe varicella-zoster virus infection), with or without nontuberculous mycobacterial infection (group 2); patients with disseminated tuberculosis (group 3); patients with pulmonary tuberculosis (group 4); and healthy controls (group 5).

In groups 1, 2, and 3, disseminated disease was defined as infection in two noncontiguous, sterile sites, at least one of which was extrapulmonary. Patients in groups 1 and 2 were eligible for enrollment if they had an active disseminated opportunistic infection or a history of culture-proven disseminated opportunistic infection. In groups 1 and 2, HIV testing was performed with the use of up to three different rapid enzyme immunoassays, as specified by World Health Organization guidelines.<sup>19</sup> Group 3, which was composed of patients with disseminated tuberculosis, was an exploratory group that was not included in the predefined statistical analysis. HIV testing was performed in group 3, and all patients were HIV-negative.

Patients with pulmonary tuberculosis, who were recruited as controls with mycobacterial disease (group 4), had culture-proven tuberculosis or smear-positive results for acid-fast bacilli and an appropriate response to directed antituberculous therapy. They were not routinely screened for HIV in the absence of an overt clinical suspicion of HIV infection, since Thailand and Taiwan are regions with a high burden of tuberculosis.<sup>20</sup> Infections were categorized as active or inactive at enrollment on the basis of clinical evidence, including the ongoing need for antimicrobial agents. Healthy controls (group 5) were anonymized blood donors who were enrolled from one site each in Thailand and Taiwan. They provided written informed consent separately and were compensated for their participation. Only age, sex, and race or ethnic group were recorded for participants in group 5; HIV testing was not performed.

# CLINICAL LABORATORY TESTS AND IMMUNOPHENOTYPING

Blood specimens were separated at each local site into plasma and peripheral-blood mononuclear cells (PBMCs) by means of density-gradient centrifugation. PBMCs were stimulated as described previously for assessment of cell-intrinsic interferon- $\gamma$  synthesis and response.<sup>10</sup> Immunophenotyping by means of flow cytometry was performed at the local site, but raw data were analyzed centrally with the use of FSC Express, clinical version 3 (De Novo Software), and FlowJo, version 9.1 (Treestar) (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org). Clinical laboratory tests included a complete blood count with a differential count, assessment of liver and kidney function, antinuclear antibody testing, and quantitative measurement of serum immunoglobulin levels.

# ANTICYTOKINE AUTOANTIBODIES

The detection of autoantibodies against cytokines with the use of Luciferase Immunoprecipitation Systems has been reported previously.<sup>4</sup> Autoantibodies were evaluated against 41 targets: interferons  $\gamma$ ,  $\alpha 1$ ,  $\beta 1$ ,  $\epsilon$ ,  $\lambda 1$ ,  $\lambda 3$ , and  $\omega$ ; interleukins  $1\alpha$  and  $1\beta$ ; the interleukin-1 receptor antagonist; interleukins 2, 3, 4, 6, 7, 8, 10, 12p35, 12p40, 15, 17A, 17F, 18, 21, 22, 23p19, 27p28, 32, and 33; Epstein-Barr virus-induced gene 3 protein (interleukin-27b); granulocyte colony-stimulating factor (G-CSF); granulocyte-macrophage colony-stimulating factor (GM-CSF); TNF- $\alpha$ ; tumor necrosis factor  $\beta$ ; B-cell-activating factor; a proliferation-inducing ligand; the Fas ligand (FasL); the CD40 ligand; erythropoietin; transforming growth factor  $\beta$ ; and the extracellular domain of the CD4 receptor. Additional methodologic details are described in the Supplementary Appendix; a detailed protocol and video describing the Luciferase Immunoprecipitation Systems technique are also included in an article by Burbelo et al.<sup>21</sup>

Anti-interferon- $\gamma$ -specific autoantibody isotype and IgG subclasses were determined with the use of a particle-based assay, as described previously<sup>22</sup>; total IgG subclasses were determined with the use of the Bio-Plex isotype kit (Bio-Rad Laboratories) according to the manufacturer's instructions. Interferon- $\gamma$ -specific IgG was purified by fractionating total IgG on protein G columns (Ab

SpinTrap, GE Healthcare) and applying the total IgG fraction to an interferon- $\gamma$  column.

# INTERFERON- $\gamma$ -INDUCED SIGNALING AND CYTOKINE PRODUCTION

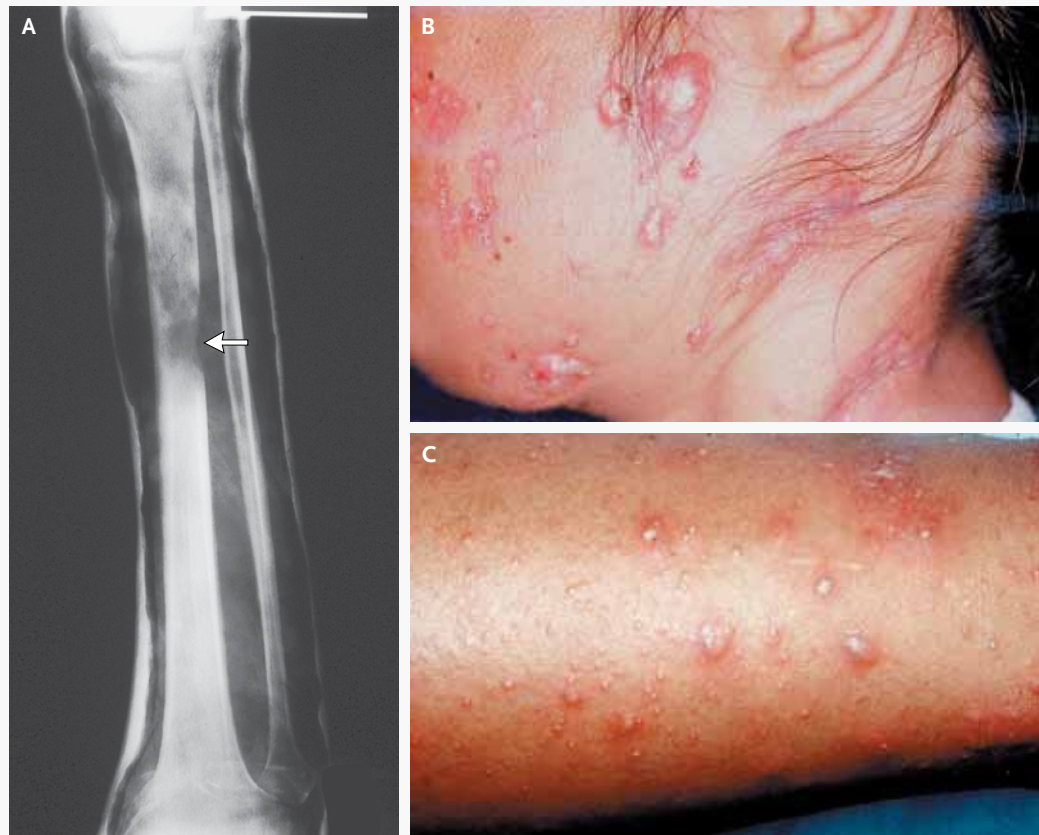
PBMCs (at a concentration of  $1 \times 10^6$  cells per milliliter) were cultured in complete RPMI 1640 medium consisting of 2 mM glutamine, 20 mM HEPES buffer, 100 U of penicillin per milliliter, 100  $\mu$ g of streptomycin per milliliter, and 10% patient or control plasma. Cultures were left unstimulated or were stimulated with interferon- $\gamma$  (1000 U per milliliter, InterMune) or interferon- $\alpha 2b$  (1000 U per milliliter, Schering) for 15 minutes at 37°C. Monocytes were identified with CD14+ surface staining. Intranuclear staining was performed as described previously<sup>11</sup> with the use of anti-phospho-signal transducer and activator of transcription 1 (STAT1) (tyrosine 701) antibody (BD Pharmingen).

Data were collected with the use of FACSCanto flow cytometry (BD Biosciences) and analyzed with the use of FlowJo (Treestar). The methods for the detection of interferon- $\gamma$ -induced TNF- $\alpha$  are described in the Supplementary Appendix.

# STATISTICAL ANALYSIS

Group differences were examined with the use of Fisher's exact test for categorical variables and with the use of analysis of variance for continuous variables. Mean differences between each group of patients and the group of healthy controls were examined by means of Wald tests, with Holm's procedure used to correct for multiple comparisons. Tests for between-group differences in the 41 anticytokine autoantibodies used a Bonferroni-adjusted P value ( $P=0.0012$ ). Skewed laboratory data were log-transformed, and counts were offset by one half to avoid logarithms of zero.

The normal range for the anti-interferon- $\gamma$ -autoantibody concentration was defined by the 99th percentile for the patients with pulmonary tuberculosis (group 4) and the healthy controls (group 5) combined and was estimated with the use of the log-normal distribution. Outlying concentrations were classified as positive for anti-interferon- $\gamma$  autoantibodies. Differences in biologic function of antibodies and in interferon- $\gamma$ -induced phospho-STAT1 production according to interferon- $\gamma$ -autoantibody status were examined with the use of the Wilcoxon rank-sum test.



**Figure 1. Common Disease Manifestations in Patients with Anti-Interferon- $\gamma$  Autoantibodies.**

Panel A shows osteomyelitis (arrow) due to infection with *Cryptococcus neoformans*. Panel B shows a disseminated *Mycobacterium abscessus* infection. Panel C shows neutrophilic dermatosis.

Statistical tests were two-sided and, unless otherwise noted, performed at the 0.05 level. Statistical analysis was performed with the use of R software (www.r-project.org).

## RESULTS

### PARTICIPANTS

We enrolled 204 persons over a period of 6 months: 52 patients with disseminated nontuberculous mycobacterial infection (group 1); 45 patients who had other opportunistic infections, with or without nontuberculous mycobacterial infection (group 2) (Fig. 1); 9 patients with disseminated tuberculosis (group 3); 49 patients with pulmonary tuberculosis (group 4); and 48 healthy controls (group 5); 1 patient was withdrawn because of ineligibility. The majority of patients in groups 1 through 4

had active infection at the time of enrollment (Fig. S1 in the Supplementary Appendix).

Sex distribution did not differ significantly between groups (Table 1). The median age of patients with any opportunistic infection was 50 years (range, 18 to 78), whereas the median age of patients with pulmonary tuberculosis and healthy controls was 43 and 38 years, respectively ( $P < 0.001$ ). No familial clustering was noted in any group. Coexisting conditions that were restricted to groups 1 and 2 included reactive dermatoses and lymphatic obstruction ( $P < 0.001$  and  $P = 0.002$ , respectively, for the comparisons with groups 3 and 4). There were modest between-group differences in hemoglobin levels, total white-cell counts, absolute neutrophil counts, and percentage of monocytes (Table S1 in the Supplementary Appendix).

**Table 1. Clinical Characteristics of the 203 Participants.\***

| Characteristic  | Group 1<br>(N=52) | Group 2<br>(N=45) | Group 3<br>(N=9) | Group 4<br>(N=49) | Group 5<br>(N=48) | P Value† |
|---|-------------------|-------------------|------------------|-------------------|-------------------|----------|
| Age — yr  |                   |                   |                  |                   |                   | <0.001   |
| Median  | 50                | 49                | 38               | 43                | 38                |          |
| Range   | 18–78             | 22–69             | 21–74            | 18–77             | 21–62             |          |
| Male sex — no. (%)  | 21 (40)           | 17 (38)           | 3 (33)           | 28 (57)           | 22 (46)           | 0.32     |
| Anti-interferon- $\gamma$ autoantibody–positive — no. (%) | 42 (81)           | 43 (96)           | 1 (11)           | 1 (2)             | 1 (2)             | <0.001   |
| Associated conditions — no.                               |                   |                   |                  |                   |                   |          |
| Lymphatic obstruction                                     | 3                 | 9                 | 0                | 0                 | —                 | 0.002    |
| Pain or neuropathy  | 4                 | 3                 | 0                | 2                 | —                 | 0.86     |
| Hypercalcemia   | 4                 | 3                 | 1                | 1                 | —                 | 0.37     |
| Erythema nodosum  | 3                 | 3                 | 0                | 1                 | —                 | 0.76     |
| Exanthematous pustulosis                                  | 5                 | 1                 | 0                | 0                 | —                 | 0.09     |
| Pustular psoriasis  | 0                 | 2                 | 0                | 0                 | —                 | 0.20     |
| Neutrophilic dermatosis                                   | 21                | 19                | 0                | 0                 | —                 | <0.001   |

\* Patients in group 1 had disseminated, rapidly growing or slowly growing, nontuberculous mycobacterial infection. Patients in group 2 had other opportunistic infections (e.g., *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Penicillium marneffei*, disseminated salmonellosis, or severe varicella–zoster virus infection) with or without nontuberculous mycobacterial infection. Patients in group 3 had disseminated tuberculosis. Patients in group 4 had pulmonary tuberculosis. Group 5 was composed of healthy controls.

† P values were determined with the use of Fisher's exact test for categorical variables and analysis of variance (F-test) for continuous variables.

Rapidly growing mycobacteria were the most common mycobacteria identified, with 36 separate isolates in group 1 and 39 separate isolates in group 2. Slowly growing mycobacteria were isolated from 15 patients in group 1 and from 8 patients in group 2 (Table 2). Nearly all patients in group 2 (41 of 45) had nontuberculous mycobacterial infections along with other opportunistic infections. All isolates from patients in groups 1 and 2 are listed in Table S2 in the Supplementary Appendix.

Lymphocyte phenotyping was completed for 76 patients and 47 healthy controls (Table S3 in the Supplementary Appendix). The total CD4+ T-lymphocyte counts in patients in groups 1 and 2 were similar to those in patients in groups 4 and 5, although there were significantly fewer naive T lymphocytes and more natural killer cells in groups 1 and 2 than in the other groups (Tables S3 and S4 in the Supplementary Appendix). As reported previously, there were significantly fewer memory T lymphocytes and total CD4+ T lymphocytes in group 3.<sup>23</sup> There were fewer memory B cells in all disease groups

(groups 1 through 4) than in healthy controls. Normal levels of interferon- $\gamma$  receptor 1 were detected in all participants tested.

#### ANTICYTOKINE AUTOANTIBODIES

Plasma obtained from all participants was tested for 41 anticytokine autoantibodies. The distribution of anti-interferon- $\gamma$  autoantibodies differed markedly across the groups; 81% of patients in group 1 and 96% in group 2 (88% overall in groups 1 and 2 combined) had high-titer anti-interferon- $\gamma$  autoantibodies (Table 1), as compared with only one patient each from groups 3, 4, and 5 ( $P<0.001$ ) (Fig. 2). Patients with other opportunistic infections (group 2) were more likely to have anti-interferon- $\gamma$  autoantibodies than those with nontuberculous mycobacterial infection alone (group 1) ( $P=0.03$ ). Disease activity at baseline did not appear to greatly influence the likelihood of positivity for anti-interferon- $\gamma$  autoantibodies (Fig. S1 in the Supplementary Appendix).

Anti-interleukin-1 $\alpha$  autoantibodies were common and were evenly distributed across all five groups. Modest titers of anti-interleukin-27B and



**Table 2. Isolated Organisms in 97 Patients with Opportunistic Infections.**

| Variable                                   | Group 1<br>(N=52) | Group 2<br>(N=45) |
|--|-------------------|-------------------|
| Organisms isolated (no./patient)           |                   |                   |
| Median                                     | 1                 | 2                 |
| Range                                      | 1–4               | 1–5               |
| Mycobacteria (no. of patients)             |                   |                   |
| Rapidly growing                            | 36                | 39                |
| Slowly growing                             | 15                | 8                 |
| Nontuberculous mycobacteria, not specified | 5                 | 2                 |
| <i>Mycobacterium tuberculosis</i>          | 4*                | 10†               |
| Total                                      | 60                | 59                |
| Bacteria (no. of patients)                 |                   |                   |
| Salmonella species                         |                   | 25                |
| <i>Burkholderia pseudomallei</i>           |                   | 4                 |
| Other                                      |                   | 9                 |
| Fungi (no. of patients)                    |                   |                   |
| <i>Cryptococcus neoformans</i>             |                   | 10                |
| <i>Histoplasma capsulatum</i>              |                   | 7                 |
| <i>Penicillium marneffei</i>               |                   | 7                 |
| Varicella-zoster virus (no. of patients)   |                   |                   |
| Disseminated                               |                   | 3                 |
| Local                                      | 5                 | 10                |
| Parasites (no. of patients)                |                   |                   |
| <i>Strongyloides stercoralis</i>           |                   | 1                 |

\* Two patients had pulmonary tuberculosis, and two had disseminated tuberculosis.

† Three patients had pulmonary tuberculosis, and seven had disseminated tuberculosis.

anti-interleukin-33 autoantibodies were present in all plasma samples. Few persons had high-titer autoantibodies against interferon- $\alpha$ 1, interleukin-10, or GM-CSF (Fig. S2 in the Supplementary Appendix). With the use of the Bonferroni-adjusted P value for significance ( $P=0.0012$ ), the levels of 11 cytokines (interleukins 27B, 1Ra, 18, 15, 12p35, and 27p28; G-CSF; interleukins 32 and 21; CD4; and interferon- $\lambda$ 1) differed significantly between the groups, but only anti-interferon- $\gamma$  autoantibodies significantly distinguished patients with opportunistic infections (groups 1 and 2) from patients with pulmonary tuberculosis and healthy controls (groups 4 and 5, respectively;  $P<0.001$ ) (Fig. S2 and Tables S5 and S6 in the Supplementary Appendix).

Although IgG usually comprises the IgG1 and

IgG3 subclasses, autoantibodies do not always follow the same distribution. Sixteen randomly selected participants (nine from groups 1 and 2 and seven from groups 3, 4, and 5) were evaluated for total IgG subclasses, anti-interferon- $\gamma$  IgG subclasses, and anti-interferon- $\gamma$  IgM and IgA. The level of anti-interferon- $\gamma$  IgG<sub>4</sub> was disproportionately high in patients in groups 1 and 2, as compared with their total IgG subclass distribution (Fig. S3A and S3B in the Supplementary Appendix). Anti-interferon- $\gamma$  IgM binding activity was similar in all five groups (Fig. S3C in the Supplementary Appendix) but was not neutralizing (Fig. S4 in the Supplementary Appendix); anti-interferon- $\gamma$  IgA was not detected.

#### PBMC FUNCTION

We tested PBMCs that were free of autologous plasma to determine cell-intrinsic functions. Interferon- $\gamma$  augmentation of lipopolysaccharide-induced TNF- $\alpha$  was intact and slightly increased in PBMCs from patients who were positive for blocking anti-interferon- $\gamma$  autoantibodies as compared with PBMCs from patients who were negative for such autoantibodies ( $P<0.001$ ) (Fig. S5 in the Supplementary Appendix). These findings largely ruled out the major mendelian defects associated with disseminated mycobacterial and opportunistic infections.

#### PLASMA ACTIVITY

To assess the effect of the patients' plasma on interferon- $\gamma$  signaling, normal PBMCs were incubated in 10% plasma. Interferon- $\gamma$  augmentation of lipopolysaccharide-induced TNF- $\alpha$  production was absent in cells incubated with antibody-positive plasma specimens ( $P<0.001$ ) (Fig. S5 in the Supplementary Appendix). Interferon- $\gamma$ -induced STAT1 phosphorylation was also abrogated by antibody-positive plasma specimens from patients in groups 1 and 2 ( $P<0.001$ ) (Fig. 3A).

Almost all plasma specimens containing anti-interferon- $\gamma$  autoantibodies inhibited interferon- $\gamma$ -induced STAT1 phosphorylation while permitting interferon- $\alpha$ -induced STAT1 phosphorylation (Fig. S6 in the Supplementary Appendix). These findings confirmed the specificity of interferon- $\gamma$  inhibition. Assessment of plasma fractions purified from specimens obtained from patients showed that IgG-depleted (but IgM- and IgA-containing) fractions and all fractions from patients without anti-interferon- $\gamma$  autoantibodies permitted in-

interferon- $\gamma$ -induced STAT1 phosphorylation (Fig. S4 in the Supplementary Appendix). In addition, only IgG that was specific for anti-interferon- $\gamma$ , not IgG depleted of anti-interferon- $\gamma$ -specific autoantibodies, prevented interferon- $\gamma$ -induced STAT1 phosphorylation (Fig. 3B). Notably, plasma specimens containing the anti-interferon- $\gamma$  autoantibodies from one patient each in groups 3, 4, and 5 (Fig. 2) did not block STAT1 phosphorylation (Fig. 3A). Plasma specimens with anti-interleukin-1 $\alpha$  autoantibodies did not inhibit interleukin-1 $\alpha$  activity.<sup>4</sup>

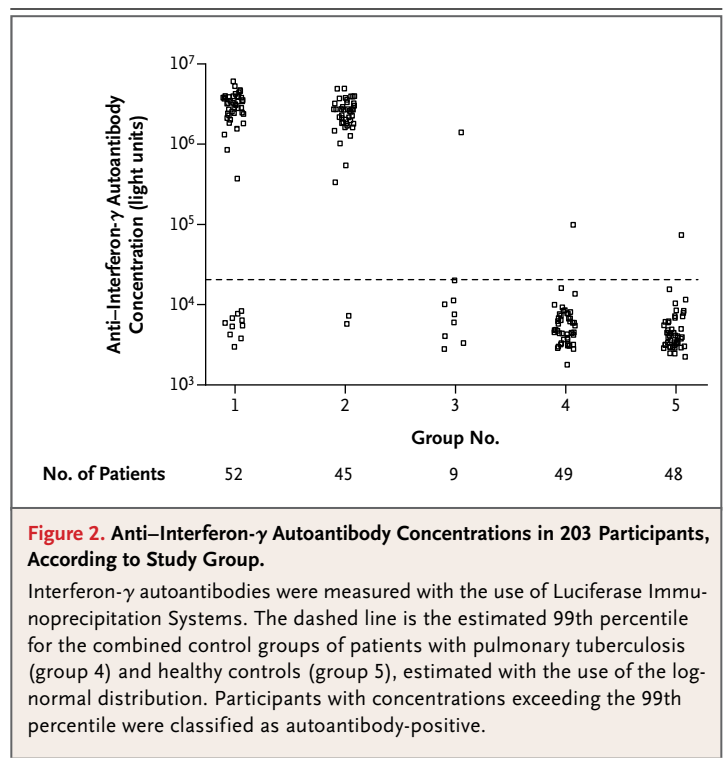
#### PATIENTS WITHOUT ANTI-INTERFERON- $\gamma$ AUTOANTIBODIES

A total of 12 patients did not have anti-interferon- $\gamma$  autoantibodies: 10 patients who had nontuberculous mycobacterial infection alone (in group 1) and 2 patients who had other opportunistic infections (in group 2). Of the 10 patients from group 1, 6 had disseminated mycobacterial disease limited to lymph nodes and 3 had disseminated disease limited to bone; in 5 of the 10 patients, the infection had been cured before enrollment. Of the 2 patients in group 2, 1 patient had neutralizing anti-GM-CSF autoantibodies with disseminated cryptococcosis and pulmonary tuberculosis; the other had cryptococcal meningitis alone (Table S7 in the Supplementary Appendix).

#### DISCUSSION

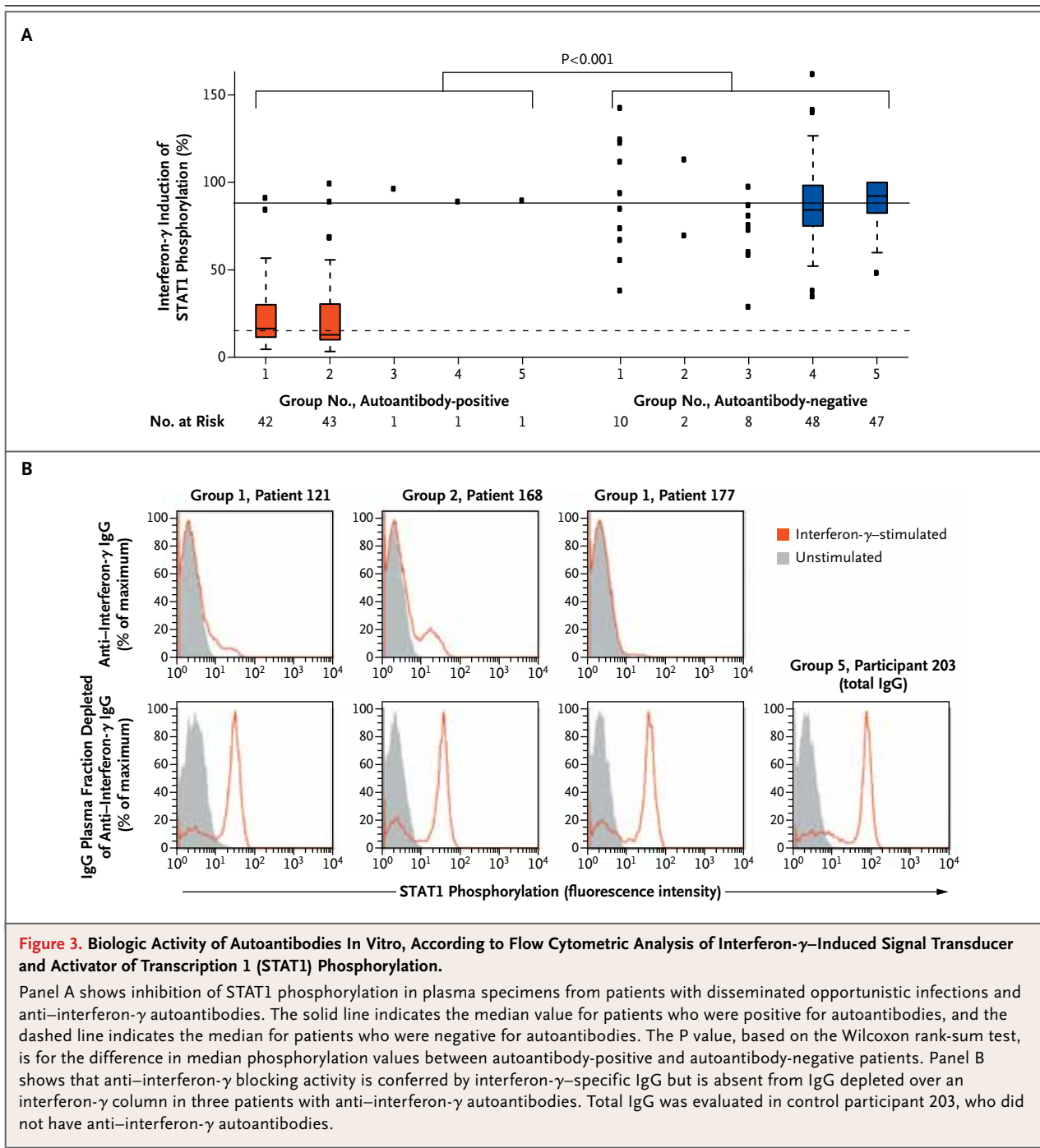
We performed an extensive study involving Thai and Taiwanese adults with opportunistic infections who were HIV-uninfected and who did not have previously identified immunodeficiency. The absence of familial clustering across cases and the late onset of disease argued against a monogenic cause. Lymphocytes (including CD4<sup>+</sup> T cells) and other hematopoietic elements were essentially normal in number and surface-receptor distribution, including the interferon- $\gamma$  receptor 1. Furthermore, all PBMCs obtained from the participants were fully capable of interferon- $\gamma$  synthesis and response when free of autologous serum, whereas serum specimens obtained from the participants transferred the defect to normal PBMCs. A screen for 41 anticytokine autoantibodies showed that only anti-interferon- $\gamma$  autoantibodies correlated with disseminated opportunistic infections.

Although the levels of several anticytokine autoantibodies differed significantly between



groups, only anti-interferon- $\gamma$  autoantibodies distinguished patients with opportunistic infections from the other groups (Fig. S2 and Tables S5 and S6 in the Supplementary Appendix), and only anti-interferon- $\gamma$  IgG inhibited interferon- $\gamma$ -dependent STAT1 phosphorylation. The IgG-depleted plasma fractions containing other plasma factors, including IgM (Fig. S4 in the Supplementary Appendix), and the IgG plasma fraction that was depleted of anti-interferon- $\gamma$ -specific IgG (but contained all other anticytokine autoantibodies) did not inhibit STAT1 phosphorylation (Fig. 3B, and Fig. S7 in the Supplementary Appendix). Only binding of anti-interferon- $\gamma$  antibodies to free interferon- $\gamma$  conferred the inhibitory effects, not any inhibitory property of anti-interferon- $\gamma$  antibodies (i.e., the complex containing interferon- $\gamma$  bound to anti-interferon- $\gamma$  autoantibody) (Fig. S8 in the Supplementary Appendix). The three participants without opportunistic infections in whom high-titer anti-interferon- $\gamma$  autoantibodies were detected had no interferon- $\gamma$ -blocking activity. Therefore, high-titer neutralizing anti-interferon- $\gamma$  autoantibodies explain these signal-transduction and cytokine-induction defects and account for these opportunistic infections.

Ninety-six percent of patients in groups 1 and 2



were infected with nontuberculous mycobacteria. In contrast, only 14% (14 of 97 patients) had *M. tuberculosis* disease (9 patients had disseminated infection and 5 had pulmonary infection), despite the virulence of tuberculosis and the high rate of infection in Thailand (210 cases per 100,000 population).<sup>20</sup> Cases of disseminated nontuberculous infection in the absence of tuber-

culosis, in a region where tuberculosis is endemic, may indicate distinctive roles for interferon- $\gamma$  in the control of different mycobacterial species.

The paucity of anti-interferon- $\gamma$  autoantibodies in patients with tuberculosis alone was unexpected and shows that mycobacterial infection itself does not lead to the development of anti-interferon- $\gamma$  autoantibodies. The higher frequency of nontu-



berculous mycobacteria relative to *M. tuberculosis* (Table 2, and Table S2 in the Supplementary Appendix) suggests distinct, albeit overlapping, pathways of susceptibility. Similarly, patients with isolated pulmonary nontuberculous mycobacteria do not have anti-interferon- $\gamma$  autoantibodies,<sup>11</sup> suggesting that mycobacterial defense is not only species-specific but also organ-specific.<sup>24</sup>

Many of the typical infections of advanced HIV developed in patients in groups 1 and 2, including disseminated *M. avium*, *P. marneffei*, *C. neoformans*, *H. capsulatum*, and varicella-zoster virus infections, despite essentially normal numbers of CD4+ T cells and other lymphocytes. Similarly, defects in antigen-specific interferon- $\gamma$  responses have been detected in patients with HIV infection.<sup>25,26</sup> In contrast to patients with advanced HIV infection, our patients were highly susceptible to rapidly growing mycobacteria.<sup>27</sup> Patients with complete interferon- $\gamma$ -receptor deficiency are susceptible to rapidly growing mycobacteria, whereas those with partial receptor defects are not.<sup>28</sup>

It is unlikely that one single mechanism underlies all cases of adult-onset immunodeficiency across two nations in Asia. Of the 12 patients in groups 1 and 2 who did not have anti-interferon- $\gamma$  autoantibodies, 5 patients had recent and severe opportunistic infection with no mechanistic explanation, 6 patients had previous disease that had apparently resolved, and 1 patient, with cryptococcosis and pulmonary tuberculosis, had high-titer, neutralizing anti-GM-CSF autoantibodies.<sup>29</sup> All had normal cellular responses to and production of cytokines, making genetic mutations within the interferon- $\gamma$ -interleukin-12 axis unlikely. Plasma from the patients who did not have anti-interferon- $\gamma$  autoantibodies permitted interferon- $\gamma$ -induced signaling and cytokine production, and washed PBMCs from these patients had intact responses to interferon- $\gamma$ . Although the majority of our patients had active disease, most of those with inactive disease remained positive for anti-interferon- $\gamma$  autoantibodies. Although antibody levels may decrease with disease quiescence, they can persist for years.<sup>30</sup> Therefore, it remains unknown whether the autoantibody-negative patients

with disseminated opportunistic infections that had resolved had another underlying disorder or whether anti-interferon- $\gamma$  autoantibodies can resolve completely.

We conducted this study in an effort to identify the cause of a discrete syndrome of acquired immunodeficiency in HIV-uninfected Asian adults. An extensive evaluation of cellular and humoral immunologic variables showed that anti-interferon- $\gamma$  autoantibodies were unique in providing a biologically plausible explanation, statistical separation between cases and controls, and functionality in vitro. Similar patterns of opportunistic infection are seen with iatrogenic TNF- $\alpha$  blockade, a pathway that mechanistically overlaps with that of interferon- $\gamma$ <sup>2</sup>; accordingly, anti-interferon- $\gamma$ -containing plasma inhibited TNF- $\alpha$  (Fig. S5 in the Supplementary Appendix). Although isolated cases and small series have been reported since 2004, the relative contribution of anti-interferon- $\gamma$  autoantibodies in large patient groups with opportunistic infections was unknown. The trigger for the production of anti-interferon- $\gamma$  autoantibodies remains elusive. The fact that nearly all the patients identified to date have been Asian-born Asians implicates host genetic factors, environmental exposure, or more likely, both. It will be important to understand the epidemiology of this disease across East Asia and also to determine whether similar rates of anti-interferon- $\gamma$  autoantibodies occur in members of the Asian diaspora born outside of Asia.

In conclusion, our study showed that this adult-onset immunodeficiency syndrome is strongly associated with high-titer neutralizing antibodies to interferon- $\gamma$ , supporting the central role of interferon- $\gamma$  in the control of numerous pathogens. Since many patients with anti-interferon- $\gamma$  autoantibodies remain actively infected despite antimicrobial therapy, this observation suggests that therapeutic targeting of anti-interferon- $\gamma$  autoantibodies may warrant investigation.<sup>5,30</sup>

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