Published in final edited form as:

N Engl J Med. 2024 March 21; 390(12): 1105–1117. doi:10.1056/NEJMoa2210665.

Anti–Interleukin-23 Autoantibodies in Adult-Onset Immunodeficiency

Aristine Cheng, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Division of Infectious Diseases, Department of Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

Anuj Kashyap, B.A.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Helene Salvator, M.D., Ph.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Department of Respiratory Medicine, Hôpital Foch, Unité Mixte de Recherche 0892, Virology and Molecular Immunology Laboratory, Suresnes Paris–Saclay University, Suresnes, France

Lindsey B. Rosen, Ph.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Devon Colby, B.S.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Fatemeh Ardeshir-Larijani, M.D.,
Indiana University Melvin and Bren Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis

Patrick J. Loehrer, M.D.,
Indiana University Melvin and Bren Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis

Li Ding, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Saul O. Lugo Reyes, M.D.,

Dr. Holland can be contacted at smh@nih.gov or at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg. 10, Rm. 11N248A, MSC 1960, 9000 Rockville Pike, Bethesda, MD 20892.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

Immune Deficiencies Laboratory, National Institute of Pediatrics, Mexico City

Sean Riminton, M.D., Ph.D.,
Department of Immunology, Repatriation General Hospital Concord, University of Sydney, Concord, NSW, Australia

Madison Ballman, B.A.,
Thoracic and GI Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Joseph M. Rocco, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Beatriz E. Marciano, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Alexandra F. Freeman, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Sarah K. Browne, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Amy P. Hsu, Ph.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Adrian Zelazny, Ph.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Arun Rajan, M.D.,
Thoracic and GI Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Irini Sereti, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Christa S. Zerbe, M.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Michail S. Lionakis, M.D., Sc.D.,
Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Steven M. Holland, M.D.

Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Abstract

BACKGROUND: Autoantibodies against interleukin-12 (anti–interleukin-12) are often identified in patients with thymoma, but opportunistic infections develop in only some of these patients. Interleukin-12 (with subunits p40 and p35) shares a common subunit with interleukin-23 (subunits p40 and p19). In a patient with disseminated *Burkholderia gladioli* infection, the identification of both anti–interleukin-23 and anti–interleukin-12 prompted further investigation.

METHODS: Among the patients (most of whom had thymoma) who were known to have anti–interleukin-12, we screened for autoantibodies against interleukin-23 (anti–interleukin-23). To validate the potential role of anti–interleukin-23 with respect to opportunistic infection, we tested a second cohort of patients with thymoma as well as patients without either thymoma or known anti–interleukin-12 who had unusual infections.

RESULTS: Among 30 patients with anti–interleukin-12 who had severe mycobacterial, bacterial, or fungal infections, 15 (50%) also had autoantibodies that neutralized interleukin-23. The potency of such neutralization was correlated with the severity of these infections. The neutralizing activity of anti–interleukin-12 alone was not associated with infection. In the validation cohort of 91 patients with thymoma, the presence of anti–interleukin-23 was associated with infection status in 74 patients (81%). Overall, neutralizing anti–interleukin-23 was detected in 30 of 116 patients (26%) with thymoma and in 30 of 36 patients (83%) with disseminated, cerebral, or pulmonary infections. Anti–interleukin-23 was present in 6 of 32 patients (19%) with severe intracellular infections and in 2 of 16 patients (12%) with unusual intracranial infections, including *Cladophialophora bantiana* and *Mycobacterium avium* complex.

CONCLUSIONS: Among patients with a variety of mycobacterial, bacterial, or fungal infections, the presence of neutralizing anti–interleukin-23 was associated with severe, persistent opportunistic infections. (Funded by the National Institute of Allergy and Infectious Diseases and others.)

ANTICYTOKINE AUTOANTIBODIES ARE increasingly recognized as causes of susceptibility to severe infections. These autoantibodies include anti–interferon-γ in mycobacterial disease, anti–interleukin-6 in staphylococcal disease, anti–interleukin-17 in chronic mucocutaneous candidiasis, anti–granulocyte–macrophage colony-stimulating factor (GM-CSF) in cryptococcosis or nocardiosis, and anti–type I interferons in severe coronavirus disease 2019 (Covid-19), varicella-zoster virus, and disease associated with yellow fever vaccine.[1–4] Autoantibodies against interleukin-23 (anti–interleukin-23) have been described as cross-reactive phenomena in patients with thymoma who have autoantibodies against interleukin-12 (anti–interleukin-12).[5]

Interleukin-23 consists of two protein subunits, p40 and p19, whereas interleukin-12 consists of the p40 subunit bound to a different protein, p35. Both interleukin-23 and interleukin-12 engage the common interleukin-12Rβ1 receptor.[6,7] Despite the similarity of interleukin-23 to interleukin-12 in both structure and the ability of innate lymphoid and memory T cells to increase interferon-γ production, the ability of interleukin-23 to

induce the production of interleukin-17 distinguishes it from interleukin-12 in both the development and the maintenance of chronic inflammation.[8–10] Interleukin-23, but not interleukin-12, is a dominant cytokine in controlling inflammation in tissues such as the skin, lung, gastrointestinal tract, joints, and brain and is preferentially secreted by dendritic cells and resident macrophages in response to glucans and lipid components of the cell walls of mycobacteria and fungi.[11–13] Cases of mycobacterial infection have been reported in patients with inflammatory bowel disease or psoriasis who were receiving monoclonal therapy that blocked interleukin-23 and interleukin-12 through subunit p40.[14–17] However, only one study has linked spontaneous anti–interleukin-12 with severe intracellular infection.[18] This finding is surprising, given the common occurrence of anti–interleukin-12 in patients with thymoma.[18–21] This dichotomy between the frequency of anti–interleukin-12 in patients with thymoma and the rarity of opportunistic infections associated with their presence prompted our search for neutralizing anti–interleukin-23.

METHODS

STUDY OVERSIGHT

From 2007 through 2021, all the study patients were enrolled under protocols of the institutional review boards at the study sites and underwent blood collection at the National Institutes of Health (NIH) or had samples sent from collaborating sites. Healthy controls underwent blood collection through the NIH Blood Bank. Written informed consent was provided by all the participants or their guardians.

STUDY DESIGN

We evaluated multiple groups of patients for anti–interleukin-23 in the discovery, validation, and expansion cohorts of the study (Fig. 1). In the discovery cohort, we tested 30 patients with known anti–interleukin-12 and 30 healthy controls for anti–interleukin-23. We compared the frequency and neutralizing potency of anti–interleukin-23 in 17 patients with anti–interleukin-12 who had opportunistic infections with that in 13 such patients without opportunistic infections. In these analyses, the effective 50% concentration (EC_{50}) was the level at which half the maximal phosphorylation of signal transducer and activator of transcription (STAT) was observed by serial dilutions of the cytokines over the range 0.01 to 100 ng per milliliter. We compared the EC_{50} in patient plasma divided by the EC_{50} in healthy control plasma to determine the EC_{50} ratio ($EC_{50}R$). Opportunistic infection was defined as invasive bacterial, fungal, or mycobacterial infection, with the exclusion of chronic mucocutaneous candidiasis and viral infections, particularly those caused by Herpesviridae infections.

In the validation cohort, we collected banked plasma or serum samples from 91 patients with thymoma who were being followed at Indiana University and the National Cancer Institute. The samples were collected in a blinded manner with respect to clinical data. We determined the anticytokine autoantibody status alone in blinded comparisons and then performed unblinded comparisons to calculate the prognostic accuracy of the presence of neutralizing anti–interleukin-23.

In the expansion cohort, we systematically performed screening for anti–interleukin-23 in patients with infections that were similar to those seen in patients with thymoma, such as disseminated intramacrophagic infections and cerebral or sinopulmonary fungal infections. We also screened patients with unrelated infections, such as Covid-19 and chronic mucocutaneous candidiasis, to establish the specificity of the presence of anti–interleukin-23. The detection and functional characterization of anticytokine autoantibodies are detailed in the Supplementary Appendix, available with the full text of this article at NEJM.org.[22,23]

STATISTICAL ANALYSIS

We compared differences in the binding intensities and neutralizing titers of anti–interleukin-12 and anti–interleukin-23 between infected and non-infected patients using the Mann–Whitney test. To trigger the innate immune system, the presence of both interleukin-23 and interleukin-18 is necessary for the marked up-regulation of interferon-γ production, which is necessary for host defense. We used the paired t-test to evaluate differences in interferon-γ generation at rest as compared with measurements obtained at least 24 hours after stimulation with interleukin-23 and interleukin-18 in mucosal-associated invariant T (MAIT) cells. We used analysis of variance for continuous variables with Tukey's multiple comparisons test to measure differences in interferon-γ induction after stimulation with interleukin-23 and after stimulation with interleukin-18; this difference was then compared between patients with opportunistic infections and those without such infections. We also used analysis of variance to measure differences in interferon-γ expression or colony-forming units under the following conditions: first, at rest; second, after *Mycobacterium avium* infection; third, after *M. avium* infection with neutralization of interleukin-12; fourth, after *M. avium* infection with neutralization of both interleukin-12 and interleukin-23; and fifth, after *M. avium* infection with neutralization of both interleukin-12 and interleukin-23 but compensated by interferon-γ supplementation.

A two-sided P value of 0.05 was considered to indicate statistical significance. All statistical analyses were performed with the use of PRISM software, version 9.0.0 (GraphPad).

RESULTS

DISCOVERY COHORT

We first reported the presence of anti–interleukin-12 (subunits p40 and p35) in a Cambodian woman with caseating lymphadenitis and persistent bacteremia caused by *Burkholderia gladioli*.[18] We subsequently found that her autoantibodies also bound heterodimeric interleukin-23 (subunits p40 and p19) and inhibited interleukin-23–induced STAT3 phosphorylation and interferon-γ generation (Fig. 2). This finding led to screening for anti–interleukin-23 in other patients who were found to have anti–interleukin-12.

Of the 30 patients with anti–interleukin-12, 25 (83%) had thymoma, and 20 (67%) had persistent or recurrent invasive infections with fungal, mycobacterial, or bacterial pathogens (Table S1 in the Supplementary Appendix). As previously observed,[5,19,20] the binding titers of anti–interleukin-12 were similar among the patients with infections (Group 1) and those

without infections (Group 2). In addition, the inhibitory potency of anti–interleukin-12 on STAT4 phosphorylation was also similar in the two groups, which indicated that the presence of anti–interleukin-12 per se was neither necessary nor sufficient for the development of severe infection (Figs. S1 and S2).

Anti–interleukin-23 binding was identified in 23 patients (77%), but not all the autoantibodies were neutralizing. Patients with invasive infections had slightly more intense binding of interleukin-23 (subunits p40 and p19) and p40 IgG than those without invasive infections (Fig. 3A and Fig. S3). The neutralizing activity against interleukin-23–induced STAT3 phosphorylation had the highest correlation with the occurrence of invasive opportunistic infections ($P<0.001$) (Fig. 3B). Among the 15 patients (50%) with neutralizing activity against interleukin-23–induced STAT3 phosphorylation, the degree of inhibition correlated with the severity of the infection (Table 1). Patients in Group 1 who had disseminated mycobacterial, fungal, or bacterial disease (Group 1A) had the most potent anti–interleukin-23 activity, with an $EC_{50}R$ of more than 50. Patients in Group 1 who had severe but localized mycobacterial, fungal, or bacterial sinopulmonary disease (Group 1B) had intermediately potent anti–interleukin-23, with an $EC_{50}R$ of 50 or less (Table 1 and Fig. S4). Patients in Group 2 had no invasive infections except for chronic mucocutaneous candidiasis or viral infections and did not have neutralizing anti–interleukin-23 (average $EC_{50}R$, <4). Autoantibodies against interferon-α, interleukin-17, interleukin-22, and interleukin-28 were common among patients with thymoma, regardless of infection status, but no patient in this cohort had autoantibodies against GM-CSF or interferon-γ (Fig. S5).

Anti–interleukin-23 effectively inhibited the synergistic induction of interferon-γ by interleukin-23 plus interleukin-18 in MAIT cells, whereas the presence of binding but nonneutralizing anti–interleukin-23 in patients without infections did not have this effect (Fig. 3C and 3D). The degree of inhibition of interferon-γ production that was mediated by anti–interleukin-23 also tracked with infection severity (Figs. S6 and S7). Patients in Group 1A with persistent disseminated infections had completely blocked interferon-γ responses to interleukin-23 and interleukin-18, with no differences over the unstimulated state. Patients in Group 1B with localized infections had significantly lower responses than healthy controls, whereas patients in Group 2 with no infections except for chronic mucocutaneous candidiasis or viral infections had responses similar to those in healthy controls (Fig. S7). The inhibitory potency of anti–interleukin-23 on MAIT activation, as evidenced by CD69 up-regulation, tracked with the degree of interferon-γ abrogation (Fig. S8). The neutralizing activities of patient plasma on interleukin-23–induced STAT3 phosphorylation and interferon-γ in MAIT cells were lost after IgG depletion and after anti–interleukin-23–specific IgG depletion (Figs. S9, S10, and S11).

VALIDATION COHORT

To confirm the relevance of anti–interleukin-23 to infection susceptibility, we sought a cohort of patients with thymoma, in whom the likelihood of the presence of autoantibodies against both interleukin-12 and interleukin-23 was high. Of 91 patients with thymoma, 24 (26%) had interleukin-23 binding activity, and 17 (19%) had

inhibition of interleukin-23–induced STAT3 phosphorylation (Table S2). Ten patients with neutralizing anti–interleukin-23 activity had opportunistic infections, including disseminated histoplasmosis (in 1 patient), cerebral toxoplasmosis (in 1 patient), pulmonary nontuberculous mycobacteria (in 1 patient), sinopulmonary aspergillosis (in 2 patients), and recurrent bacterial pneumonias leading to hospitalization (in 9 patients) (Table S3). The 4 patients who had nonneutralizing anti–interleukin-23 IgG but autoantibodies against downstream effectors of interleukin-23 (namely, interleukin-17A, interleukin-22, and interleukin-28) had sinusitis (in 1 patient), cutaneous blastomycosis (in 1 patient), and pneumonias (in 3 patients). In contrast, 64 patients without neutralizing anti–interleukin-23 activity did not have infections. The serum immunoglobulin levels and B and T lymphocyte counts at baseline were similar in those with infections and in those without infections in the discovery and validation cohorts, regardless of whether the patients had Good's syndrome (thymoma with immunodeficiency) (Fig. S12).

EXPANSION COHORT

To determine whether anti–interleukin-23 may be contributing to unusual infection presentations outside of thymoma, we obtained plasma or serum samples from 128 patients with other severe infections, primarily with intracellular organisms. Anti–interleukin-23 binding was also found in 6 of 32 patients (19%) with severe intracellular infections, in 2 of 16 patients (12%) with unusual intracranial infections, and in 3 of 30 patients (10%) with invasive mold infections; such binding was found in 7 of 753 patients (1%) with Covid-19, in 1 of 65 patients (2%) with chronic mucocutaneous candidiasis with APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy), and in none of 25 patients with chronic mucocutaneous candidiasis without APECED (Fig. 4A).

We found neutralizing anti–interleukin-23 in the absence of anti–interleukin-12 in 2 of 16 patients with unusual intracranial infections. The first patient was a 31-year-old previously healthy Filipino woman who had meningoencephalitis and obstructive hydrocephalus with intraventricular hemorrhage caused by *Cladophialophora bantiana* (Fig. 4B). Her plasma was positive for anti–interleukin-23 IgM, IgG, and IgE (Fig. S13) but negative for autoantibodies against interleukin-12, p40, interferon-γ, GM-CSF, interleukin-17A, interleukin-22, and interferon-α, -β, and -γ. Potent interleukin-23–neutralizing activity was also shown (Fig. 4C and 4D).

The second patient was a 58-year-old man with human immunodeficiency virus (HIV) infection who had *Mycobacterium avium* complex brain disease while his CD4 lymphocyte count was more than 100 cells per microliter; blood mycobacterial cultures were negative, and he had no other systemic involvement (Fig. 4E). His plasma contained binding anti–interleukin-23 that inhibited interleukin-23 but not interleukin-12 activity (Fig. 4F and Fig. S14). Of the other 68 patients with HIV infection who were tested, no patients with typical opportunistic infections tested positive for binding anti–interleukin-23, which suggests that the presence of this autoantibody is not a common epiphenomenon of HIV infection (Fig. 4D).

LONGITUDINAL NEUTRALIZING TITERS OF ANTI–INTERLEUKIN-23 AFTER RITUXIMAB

Rituximab therapy depleted B cells and diminished neutralizing activity against interleukin-23 in our index patient, which led to initial remission. However, after discontinuation of rituximab, B-cell recovery was associated with a rebound in neutralizing activity against interleukin-23 and clinical relapse of infection (Fig. S19). At the time of this report, the patient was receiving 6 monthly doses of rituximab and had not had a relapse because the B cells remained depleted and the neutralizing activity of anti–interleukin-23 remained low.

DISCUSSION

The presence of autoantibodies against cytokines has emerged as a distinct cause of acquired immunomodulation and immunodeficiency.[24,25] Although the effect of exogenous supplementation or autoantibody depletion remains to be proved, the identification of uncommon but pathogenic autoantibodies against cytokines, such as anti–interleukin-23, represents the first step toward the development of actionable strategies for improving patient outcomes.

We and others have found that the presence of anti–interleukin-12 is relatively common among patients with thymoma, but the clinical effect, if any, of such autoantibodies has been unclear.[5,20] It now appears that these patients had both anti–interleukin-12 and anti–interleukin-23 and that the latter, less-common autoantibodies were seemingly more biologically important. Even within anti–interleukin-23, marked distinctions remain between binding and neutralizing capacities. Among patients with thymoma, the presence of neutralizing anti–interleukin-23 was a discriminating factor between patients who had invasive infections and those who did not. Beyond thymoma, neutralizing anti–interleukin-23 was found in the absence of anti–interleukin-12 in searches for a cause of disseminated or unusual infections. Since the populations that were studied were recruited on the basis of underlying conditions or infections, there are intrinsic biases that may limit the generalizability of these findings. Nevertheless, these and other data suggest that interleukin-23 is a protective factor against intracellular and extracellular pathogens.

Patients with anti–interleukin-23 have clinical presentations that differ substantially from those of patients with autoantibodies against interferon-γ, a finding that reconfirms the relatively distinct roles of interleukin-23 in the homeostatic maintenance of various mucocutaneous and blood–brain barriers as well as in the regulation of interleukin-17, interleukin-22, interleukin-28, GM-CSF, and interferon-γ.[26–29] In our cohort of patients with anti–interleukin-23, infections caused by candida, pneumocystis, pseudomonas, and klebsiella species were common, a finding that was largely consistent with the spectrum of infections seen in patients with IL12Rβ1 deficiency (Table S4).[9] Candida and pneumocystis species are typically not found in patients with interferon-γ deficiency. When such patients are found to have fungal infections, they are usually caused by facultative intracellular species (i.e., those that thrive inside host macrophages) such as coccidioides and histoplasma, along with *Talaromyces marneffei*.[30,31] In an animal model of systemic candidiasis, myeloid survival after infection depended on the presence of interleukin-23.[32] Moreover, β-glucan and glycosphingolipids obtained from fungal-cell walls triggered the

preferential secretion of interleukin-23 over interleukin-12 by alveolar macrophages and dendritic cells.[33–35] We previously found that the gene encoding interleukin-23 (*IL23A*) is within the top 30 up-regulated genes in human airway epithelial cells during challenge with nontuberculous mycobacteria.[36] Interleukin-23 and interleukin–22 also mediate mucosal defense against klebsiella pneumonia, which may help to explain the development of pulmonary nontuberculous mycobacteria and klebsiella infections in our cohort.[37,38] This broader spectrum of infections suggests that patients with neutralizing anti–interleukin-23 have an adult-onset immunodeficiency akin to HIV infection, with its deficiencies in immunity mediated by T helper 1 and 17 cells.[39,40]

The presence of neutralizing anti–interleukin-23 was uncommon in patients with APECED and in those with isolated chronic mucocutaneous candidiasis, which suggests that defects in interleukin-23 were neither necessary nor sufficient for the development of these infections. In patients with APECED, enhanced mucosal interferon-γ–mediated STAT1 signaling has been shown to underlie epithelial-barrier disruption, thereby promoting chronic mucocutaneous candidiasis even in patients with a T helper 17 mucosal response.[41] Antibody-mediated neutralization of interleukin-17A, interleukin-17F, and interleukin-22 further increased the mucosal fungal burden but did not appear to initiate chronic mucocutaneous candidiasis in the deficiency caused by mutations in the gene encoding autoimmune regulator (*AIRE*).[41] Since interleukin-23 also signals through STAT4 and enhances interferon-γ–mediated immunity, blockade of interleukin-23 is not synonymous with the blockade of interleukin-17 or interleukin-22. Instead, interleukin-23 blockade may also prevent the mucosal interferonopathy that drives excessive inflammation, similar to the way in which monoclonal antibodies against interleukin-23 are used to reduce inflammation in inflammatory colitis, psoriasis, and leukocyte adhesion deficiency.[26,42] Further mechanistic experiments are warranted to define the exact roles of interleukin-23, interleukin-17, interleukin-22, and interleukin-28 in mucosal immunity against fungal disease.

Important phenotypic differences exist among patients with both anti–interleukin-12 and anti–interleukin-23, those with anti–interleukin-12 alone, and those with anti–interleukin-23 alone. Patients with neutralizing anti–interleukin-23 are at increased risk for infection, and the nature and severity of such infections are correlated with the degree of interleukin-23–neutralizing activity, which is not always reflected in the binding activity alone. In our study, we found that the presence of anti–interleukin-12 alone was poorly correlated with the development of opportunistic infections and was not necessarily of the same IgG subclass as anti–interleukin-23 (Fig. S15). This finding suggests that the major infection-predisposing neutralization of p40 is directed predominantly against interleukin-23 rather than against interleukin-12, as previously thought (Fig. S16).

Studies involving patients with mendelian susceptibility to mycobacterial disease suggest the essential contribution of human interleukin-23 rather than interleukin-12 to antimycobacterial immunity. Five unrelated kindreds with different types of deficiency caused by the gene encoding interleukin-23 receptor (*IL23R*) had complete penetrance of mycobacterial disease and incomplete penetrance of candidiasis (in 29%).[8,43,44] One patient with an *IL23R* C115Y mutation that caused a reduction in cell-surface IL23R

protein died from bacille Calmette–Guerin (BCG) disease after vaccination. In contrast, of the three patients with interleukin-12–specific deficiency (IL12Rβ2 p.Q138* mutation) that caused undetectable IL12Rβ2 on the cell surface, BCG disease developed in only one patient; moreover, one patient had a history of childhood pulmonary tuberculosis but not BCG disease despite vaccination, and one patient was asymptomatic.[8] Since exogenous interleukin-12 did not completely rescue the defective interferon-γ production of IL23R-deficient cells in that study, we used exogenous interferon-γ to restore control of intracellular mycobacterial growth in our experimental anti–p40 model (Fig. S17).

Collectively, these data help to explain the long-standing disconnect between the relatively common recognition of anti–interleukin-12 in patients with thymoma and the relative paucity of severe infections in these patients. These findings also explain why certain patients with thymoma have perplexingly persistent infections, such as *Burkholderia gladioli*, that are attributed to Good's syndrome or immunosuppression but are more typically associated with deficiencies in interleukin-23, interferon-γ, or both (Table S5).[45] Finally, the presence of neutralizing anti–interleukin-23 appears to be an independent or additive risk factor for intracranial infections. The more encompassing role of these autoantibodies beyond the effects of anti–interleukin-17 may help to explain the divergent effects of interleukin-23 and interleukin-17 blockade in inflammatory colitis.[46,47] A comprehensive approach to autoantibodies against cytokines is needed to understand their role in both the source and the severity of infections.[2]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). Dr. Cheng is supported by an honorary Fellowship Award from the Primary Immunodeficiency Treatment Consortium and by a grant (111–2314-B-002–173-MY3) from the National Science and Technology Council of Taiwan.

We thank Stephen K. Reddel and MaiAnh Nguyen at the Concord Hospital, Sydney, Australia, and Ana Paola Macia Robles of the Pediatric Hospital, Centro Medico Nacional de Occidente, Guadalajara, Mexico, for providing access to the patient clinical samples and for patient care; Luigi D. Notarangelo and Helen C. Su for providing access to patients' clinical samples and their suggestions for improving the clarity of descriptions; Ian A. Myles and Polly Matzinger for their questions and ideas that have critically improved this work; Debra Long Priel for managing, storing, and retrieving blood samples for this study; staff members at the NIH Tetramer Core Facility at Emory University for providing the MR1 tetramers; and Sally Hunsberger for review of our statistical analysis.

References

1. Barcenas-Morales G, Cortes-Acevedo P, Doffinger R. Anticytokine autoantibodies leading to infection: early recognition, diagnosis and treatment options. Curr Opin Infect Dis 2019;32:330–6. [PubMed: 31116133]
2. Cheng A, Holland SM. Anticytokine autoantibodies: autoimmunity trespassing on antimicrobial immunity. J Allergy Clin Immunol 2022;149:24–8. [PubMed: 34998474]
3. Ansari R, Rosen LB, Lisco A, et al. Primary and acquired immunodeficiencies associated with severe varicella-zoster virus infections. Clin Infect Dis 2021;73(9):e2705–e2712. [PubMed: 32856043]

4. Bastard P, Michailidis E, Hoffmann H-H, et al. Auto-antibodies to type I IFNs can underlie adverse reactions to yellow fever live attenuated vaccine. J Exp Med 2021;218(4):e20202486. [PubMed: 33544838]
5. Kärner J, Pihlap M, Ranki A, et al. IL-6-specific autoantibodies among APECED and thymoma patients. Immun Inflamm Dis 2016;4:235–43. [PubMed: 27957331]
6. Glassman CR, Mathiharan YK, Jude KM, et al. Structural basis for IL-12 and IL-23 receptor sharing reveals a gateway for shaping actions on T versus NK cells. Cell 2021;184(4):983–999.e24. [PubMed: 33606986]
7. Oppmann B, Lesley R, Blom B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13:715–25. [PubMed: 11114383]
8. Martínez-Barricarte R, Markle JG, Ma CS, et al. Human IFN-γ immunity to mycobacteria is governed by both IL-12 and IL-23. Sci Immunol 2018;3(30):eaau6759. [PubMed: 30578351]
9. Teng MWL, Bowman EP, McElwee JJ, et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med 2015;21:719–29. [PubMed: 26121196]
10. Aggarwal S, Ghilardi N, Xie M-H, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem 2003;278:1910–4. [PubMed: 12417590]
11. Maher CO, Dunne K, Comerford R, et al. *Candida albicans* stimulates IL-23 release by human dendritic cells and downstream IL-17 secretion by Vδ1 T cells. J Immunol 2015;194:5953–60. [PubMed: 25964489]
12. Kreymborg K, Böhlmann U, Becher B. IL-23: changing the verdict on IL-12 function in inflammation and autoimmunity. Expert Opin Ther Targets 2005;9:1123–36. [PubMed: 16300465]
13. Verreck FA, de Boer T, Langenberg DM, et al. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. Proc Natl Acad Sci U S A 2004;101:4560–5. [PubMed: 15070757]
14. Tsai T-F, Chiu H-Y, Song M, Chan D. A case of latent tuberculosis reactivation in a patient treated with ustekinumab without concomitant isoniazid chemoprophylaxis in the PEARL trial. Br J Dermatol 2013;168:444–6. [PubMed: 22816505]
15. Errichetti E, Piccirillo A. Latent tuberculosis reactivation in a patient with erythrodermic psoriasis under treatment with ustekinumab and a low dose steroid, despite isoniazid chemoprophylaxis. Eur J Dermatol 2014;24:508–9. [PubMed: 25120233]
16. Lynch M, Roche L, Horgan M, Ahmad K, Hackett C, Ramsay B. Peritoneal tuberculosis in the setting of ustekinumab treatment for psoriasis. JAAD Case Rep 2017;3:230–2. [PubMed: 28443318]
17. Shim HH, Cai SCS, Chan W, Low JGH, Tan HH, Ling KL. Mycobacterium abscessus infection during ustekinumab treatment in Crohn's disease: a case report and review of the literature. J Crohns Colitis 2018;12:1505–7. [PubMed: 30169620]
18. Sim B-T, Browne SK, Vigliani M, et al. Recurrent *Burkholderia gladioli* suppurative lymphadenitis associated with neutralizing anti-IL-12p70 autoantibodies. J Clin Immunol 2013;33:1057–61. [PubMed: 23708995]
19. Meager A, Vincent A, Newsom-Davis J, Willcox N. Spontaneous neutralising antibodies to interferon-α and interleukin-12 in thymoma-associated autoimmune disease. Lancet 1997;350:1596–7.
20. Meager A, Wadhwa M, Dilger P, et al. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. Clin Exp Immunol 2003;132:128–36. [PubMed: 12653847]
21. Burbelo PD, Browne SK, Sampaio EP, et al. Anti-cytokine autoantibodies are associated with opportunistic infection in patients with thymic neoplasia. Blood 2010;116:4848–58. [PubMed: 20716769]
22. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020;370(6515):eabd4585. [PubMed: 32972996]

23. Gupta S, Tatouli IP, Rosen LB, et al. Distinct functions of autoantibodies against interferon in systemic lupus erythematosus: a comprehensive analysis of anticytokine autoantibodies in common rheumatic diseases. Arthritis Rheumatol 2016;68:1677–87. [PubMed: 26815287]
24. Ku C-L, Chi C-Y, von Bernuth H, Doffinger R. Autoantibodies against cytokines: phenocopies of primary immunodeficiencies? Hum Genet 2020;139:783–94. [PubMed: 32419033]
25. Knight V. Immunodeficiency and autoantibodies to cytokines. J Appl Lab Med 2022;7:151–64. [PubMed: 34996092]
26. Moutsopoulos NM, Zerbe CS, Wild T, et al. Interleukin-12 and interleukin-23 blockade in leukocyte adhesion deficiency type 1. N Engl J Med 2017;376:1141–6. [PubMed: 28328326]
27. Watford WT, O'Shea JJ. Autoimmunity: a case of mistaken identity. Nature 2003;421:706–8. [PubMed: 12610607]
28. Uhlig HH, McKenzie BS, Hue S, et al. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. Immunity 2006;25:309–18. [PubMed: 16919486]
29. Cella M, Fuchs A, Vermi W, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 2009;457:722–5. [PubMed: 18978771]
30. Pruetpongpun N, Khawcharoenporn T, Damronglerd P, et al. Disseminated Talaromyces marneffei and Mycobacterium abscessus in a patient with anti-interferon-γ autoantibodies. Open Forum Infect Dis 2016;3:ofw093. [PubMed: 27419165]
31. Guo J, Ning X-Q, Ding J-Y, et al. Anti-IFN-γ autoantibodies underlie disseminated *Talaromyces marneffei* infections. J Exp Med 2020;217(12):e20190502. [PubMed: 32880631]
32. Nur S, Sparber F, Lemberg C, et al. IL-23 supports host defense against systemic *Candida albicans* infection by ensuring myeloid cell survival. PLoS Pathog 2019;15(12):e1008115. [PubMed: 31887131]
33. Dennehy KM, Willment JA, Williams DL, Brown GD. Reciprocal regulation of IL-23 and IL-12 following co-activation of Dectin-1 and TLR signaling pathways. Eur J Immunol 2009;39:1379–86. [PubMed: 19291703]
34. Chamilos G, Ganguly D, Lande R, et al. Generation of IL-23 producing dendritic cells (DCs) by airborne fungi regulates fungal pathogenicity via the induction of T(H)-17 responses. PLoS One 2010;5(9):e12955. [PubMed: 20886035]
35. Carmona EM, Kottom TJ, Hebrink DM, et al. Glycosphingolipids mediate pneumocystis cell wall β-glucan activation of the IL-23/IL-17 axis in human dendritic cells. Am J Respir Cell Mol Biol 2012;47:50–9. [PubMed: 22343219]
36. Matsuyama M, Martins AJ, Shallom S, et al. Transcriptional response of respiratory epithelium to nontuberculous mycobacteria. Am J Respir Cell Mol Biol 2018;58:241–52. [PubMed: 28915071]
37. Happel KI, Dubin PJ, Zheng M, et al. Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. J Exp Med 2005;202:761–9. [PubMed: 16157683]
38. Happel KI, Lockhart EA, Mason CM, et al. Pulmonary interleukin-23 gene delivery increases local T-cell immunity and controls growth of *Mycobacterium tuberculosis* in the lungs. Infect Immun 2005;73:5782–8. [PubMed: 16113296]
39. Prendergast A, Prado JG, Kang Y-H, et al. HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells. AIDS 2010;24:491–502. [PubMed: 20071976]
40. Morou A, Brunet-Ratnasingham E, Dubé M, et al. Altered differentiation is central to HIV-specific $CD4^+$ T cell dysfunction in progressive disease. Nat Immunol 2019;20:1059–70. [PubMed: 31308541]
41. Break TJ, Oikonomou V, Dutzan N, et al. Aberrant type 1 immunity drives susceptibility to mucosal fungal infections. Science 2021;371(6526):eaay5731. [PubMed: 33446526]
42. Frieder J, Kivelevitch D, Haugh I, Watson I, Menter A. Anti-IL-23 and anti-IL-17 biologic agents for the treatment of immune-mediated inflammatory conditions. Clin Pharmacol Ther 2018;103:88–101. [PubMed: 28960267]
43. Philippot Q, Ogishi M, Bohlen J, et al. Human IL-23 is essential for IFN-gamma-dependent immunity to mycobacteria. Sci Immunol 2023;8(80):eabq5204. [PubMed: 36763636]

44. Staels F, Lorenzetti F, De Keukeleere K, et al. A novel homozygous stop mutation in IL23R causes mendelian susceptibility to mycobacterial disease. J Clin Immunol 2022;42:1638–52. [PubMed: 35829840]

45. Imataki O, Kita N, Nakayama-Imaohji H, Kida J-I, Kuwahara T, Uemura M. Bronchiolitis and bacteraemia caused by *Burkholderia gladioli* in a non-lung transplantation patient. New Microbes New Infect 2014;2:175–6. [PubMed: 25566397]

46. Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut 2012;61:1693–700. [PubMed: 22595313]

47. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as induction and maintenance therapy for Crohn's disease. N Engl J Med 2016;375:1946–60. [PubMed: 27959607]

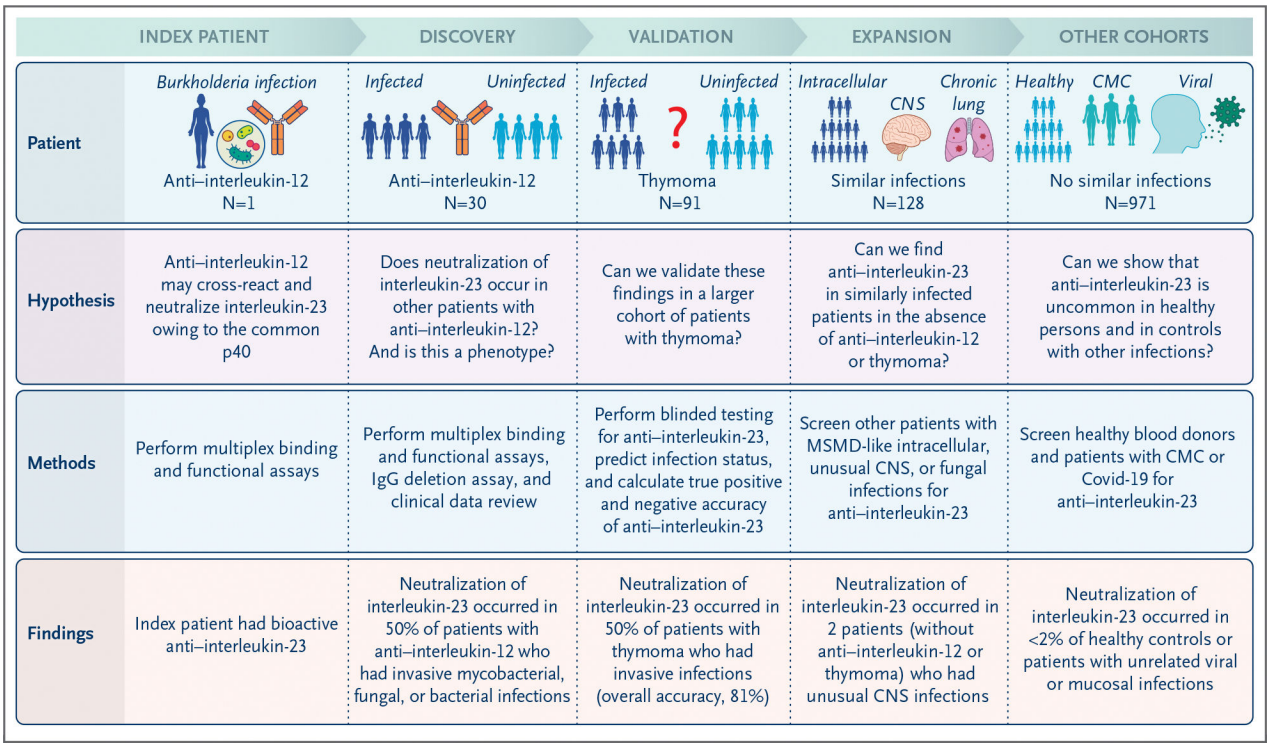

Figure 1. Discovery, Validation, and Expansion Cohorts in the Study.

Three cohorts of patients were evaluated for the presence of autoantibodies against interleukin-23 (anti–interleukin-23). In the discovery cohort, 30 patients with known autoantibodies against interleukin-12 (anti–interleukin-12) and 30 healthy controls were tested for the presence of anti–interleukin-23. In 17 patients with anti–interleukin-12 who had opportunistic infections, the activity of anti–interleukin-23 was compared with that in 13 such patients without infections. In the validation cohort, banked plasma or serum samples were collected from 91 patients with thymoma, in whom anti–interleukin-12 is often identified. Status with respect to anticytokine autoantibodies was determined to calculate the prognostic accuracy of the presence of neutralizing anti–interleukin-23. In the expansion cohort, screening for anti–interleukin-23 was performed in 128 patients with infections that were similar to those seen in patients with thymoma. Screening was also performed in 971 patients who were healthy or had unrelated infections to establish the specificity of anti–interleukin-23. CMC denotes chronic mucocutaneous candidiasis, CNS central nervous system, Covid-19 coronavirus disease 2019, and MSMD mendelian susceptibility to mycobacterial diseases.

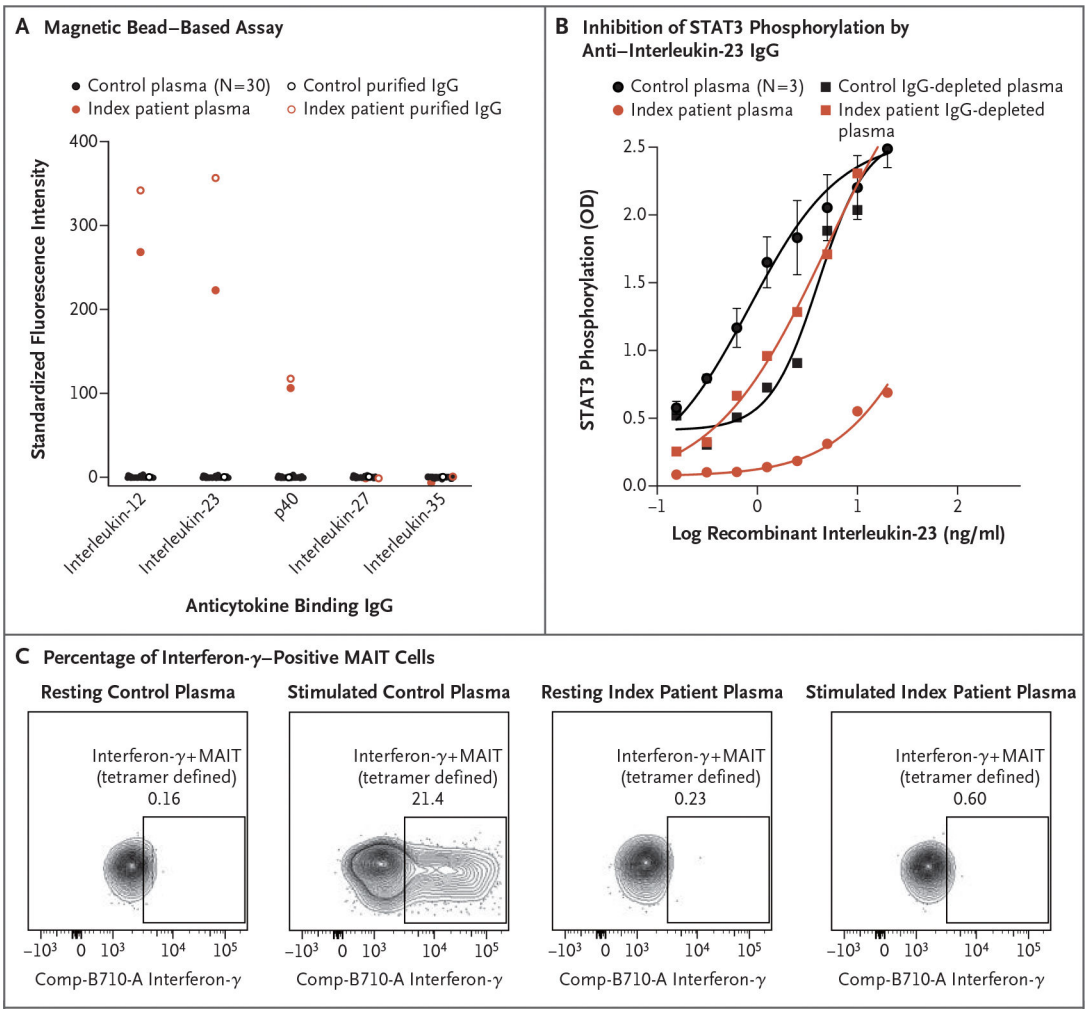

Figure 2. Activity of Interleukin-12, Interleukin-23, and p40 in the Index Patient and in Healthy Controls.

Panel A shows the results of magnetic bead–based assays indicating human IgG binding of interleukin-12, interleukin-23, and p40 in the plasma of the index patient with disseminated *Burkholderia gladioli* infection in whom both anti–interleukin-12 and anti–interleukin-23 had been identified. These findings were absent in plasma samples obtained from 30 healthy controls. The fluorescence intensity of anti–interleukin-12 and anti–interleukin-23, along with p40 IgG (as measured by optical density [OD]), was increased in the index patient's IgG-purified fraction after total IgG had been captured and eluted from a protein G column (GE Healthcare). Panel B shows dose-inhibition curves representing substantial inhibition of phosphorylation of signal transducer and activator of transcription 3 (STAT3) by anti–interleukin-23 IgG in the index patient's plasma, which markedly inhibited STAT3 phosphorylation at 1 ng per milliliter of interleukin-23. The plasma IgG fraction contains all the interleukin-23–neutralizing activity. In contrast to the replete plasma, which inhibited 100% of STAT3 at 1 ng per milliliter of interleukin-23 after removal of total IgG by means of a protein G column, the index patient's plasma no longer inhibited interleukin-23 signaling and had a dose responsiveness to interleukin-23 similar to that in control plasma. The I bars indicate the standard deviation. Panel C shows representative flow cytometry

plots indicating the percentage of interferon-γ–positive mucosal associated invariant T (MAIT) cells at rest and after costimulation with interleukin-23 and interleukin-18 in the presence of healthy control plasma (two plots at left) as compared with the index patient's plasma (two plots at right). The synergistic induction of interferon-γ after costimulation with interleukin-23 and interleukin-18 is completely inhibited by the index patient's plasma containing anti–interleukin-23 IgG (far right). Comp-B710-A denotes compensation matrix for blue laser–excitable 710/40 nm bandpass filter acquisition.

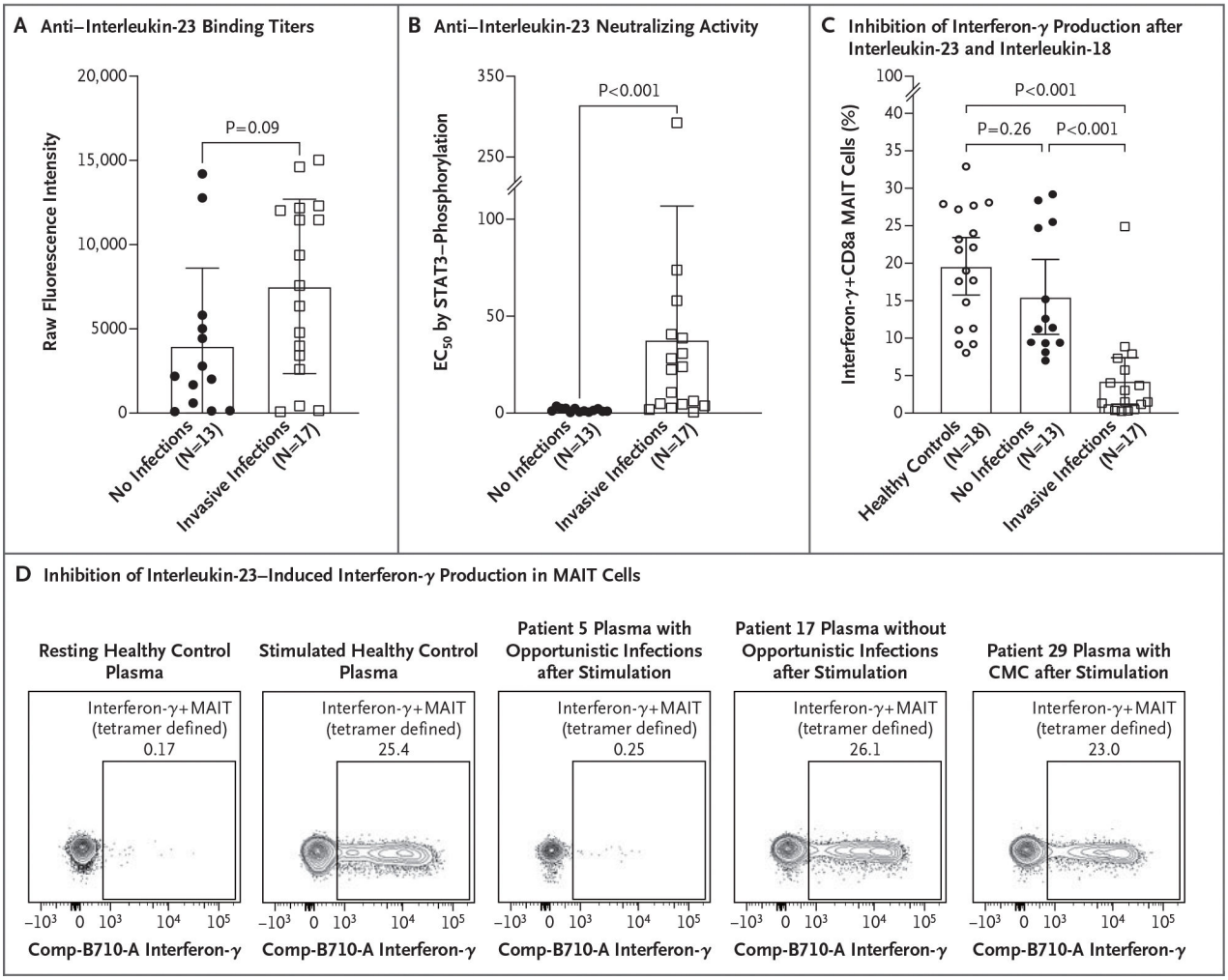

Figure 3. Effects of Binding and Neutralizing Anti–Interleukin-23 Activity.

In Panel A, among patients who tested positive for anti–interleukin-12, shown is the binding of anti–interleukin-23 (expressed as raw fluorescence intensity) in 17 patients with opportunistic infections as compared with 13 patients without infections, a difference that was not significant. In Panel B, anti–interleukin-23 neutralizing activity is expressed as the effective concentration of interleukin-23 that was required to induce 50% (EC_{50}) of the maximal STAT3 phosphorylation response. This response was significantly more potent in the patients with opportunistic infections than in those without infections. In Panel C, the inhibition of interferon-γ production by anti–interleukin-23 is shown after stimulation with interleukin-23 and interleukin-18. This inhibition correlates with the presence of opportunistic infections (in Group 1) or the absence of infections (in Group 2), according to the percentage of interferon-γ–positive MAIT cells with Live+/CD3+/CD8a+/CD161+/TCR Vα7.2+ or MR1–5-OP-RU tetramer-binding MAIT cells. In Panels A, B, and C, the I bars indicate the standard deviation. In Panel D, representative fluorescence-activated cell sorter (FACS) plots show the inhibition of interleukin-23–induced interferon-γ production in MAIT cells, which correlates with the presence of opportunistic infections. These values ranged from less than 1% of interferon-γ–positive MAIT cells that were associated with

the presence of anti–interleukin-23 IgG in plasma obtained from a patient with multiple opportunistic infections (including recurrent pulmonary nontuberculous mycobacterial disease, recurrent severe *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* sinopulmonary infections requiring hospitalization, and CMC to more than 20% MAIT cells in patients with only CMC infections or no infections and in healthy controls.

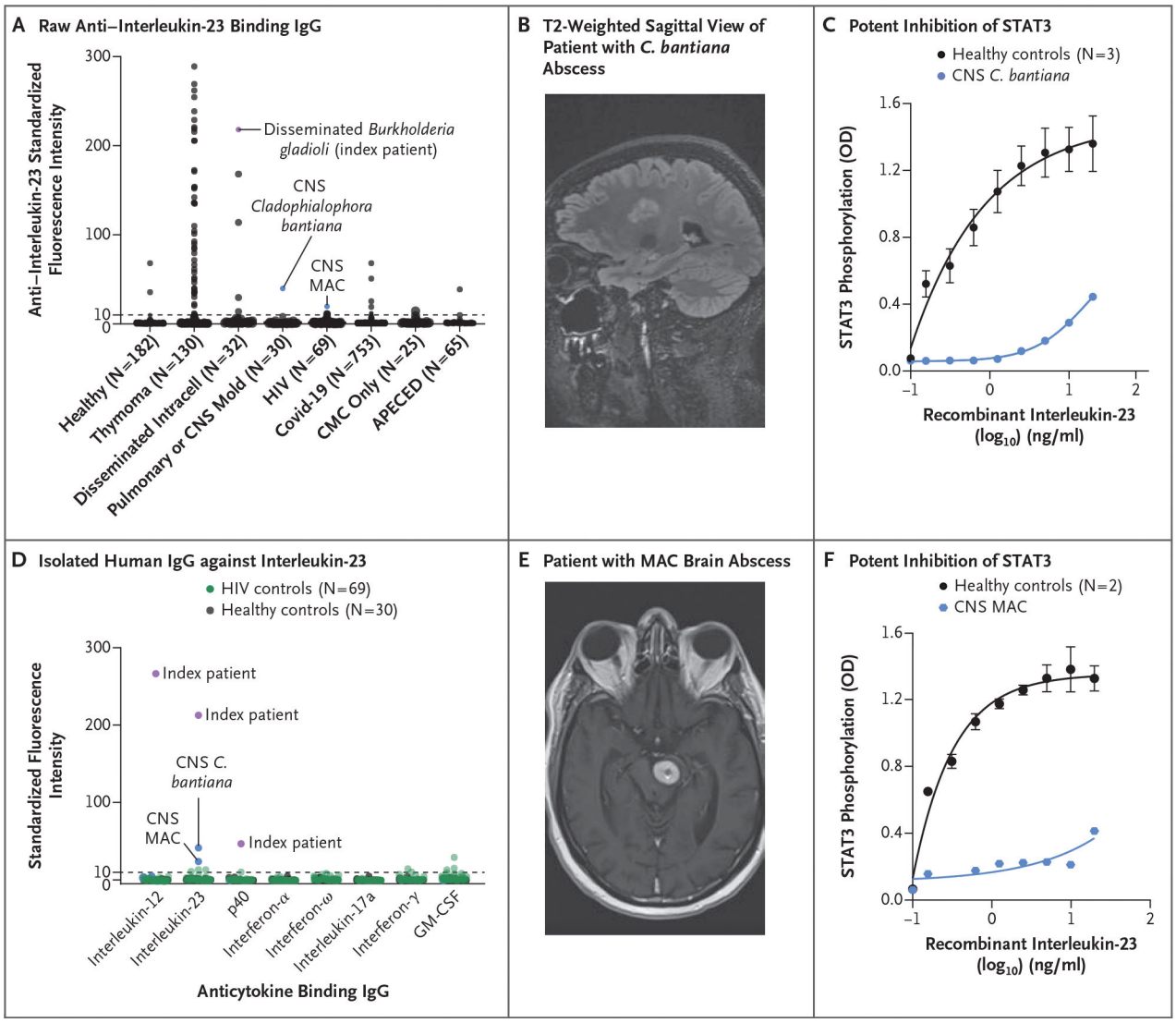

Figure 4. Detection of Anti–Interleukin-23 in Unusual Infection Presentations.

In Panel A, bead-based binding assays show the standardized fluorescence intensity of anti–interleukin-23 binding IgG in samples obtained from various patient cohorts along with healthy controls. The study patients included a mixture of those with similar opportunistic infections and those with nonsimilar infections. Highlighted are the findings in the index patient and in two other study patients with unusually severe opportunistic infections of the central nervous system (CNS). One of these patients had intracranial abscesses and obstructive ventriculitis caused by *Cladophialophora bantiana*, and the other patient had human immunodeficiency virus (HIV) infection with an abscess caused by *Mycobacterium avium* complex (MAC). In Panel B, magnetic resonance imaging (MRI) shows a T2-weighted sagittal view of the patient with *C. bantiana* infection. In Panel C, potent inhibition of STAT3 phosphorylation is shown in response to interleukin-23 stimulation in the presence of plasma from the study patient with severe *C. bantiana* CNS infection. In Panel D, multiplex autoantibody binding assays show isolated human IgG against interleukin-12 and interleukin-23 in the index patient and against interleukin-23 in the patients with

C. bantiana and MAC infections, as compared with 30 healthy controls and with 68 HIV controls with opportunistic infections. In Panel E, MRI shows a T1-gadolinium–enhanced axial view of the midbrain of the patient with MAC infection. In Panel F, potent inhibition of STAT3 phosphorylation in response to recombinant interleukin-23 stimulation is shown in the presence of plasma from the patient with MAC infection. APECED denotes autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, and GM-CSF granulocyte–macrophage colony-stimulating factor.

Table 1.

Anticytokine Autoantibodies in the Study Patients with or without Opportunistic Infections in the Discovery Cohort.*

Group and Infection Type	No. of Patients	Interleukin-12		Interleukin-23†		Interleukin-17	Interleukin-22	Interleukin-28	Interferon-α
		bAB‡	nAB§	bAB‡	nAB§	bAB‡	bAB‡	bAB‡	bAB‡
		percentage of patients with infection							
Group 1A: systemic infection¶	7	100	86	86	100	14	43	29	43
Disseminated infection									
Gram-negative bacteria	4								
Mycobacterial	2								
Fungal	3								
Pulmonary									
Gram-negative bacteria	3								
Mycobacterial	1								
Mold	1								
Peritoneal nocardia	1								
Chronic mucocutaneous candidiasis	2								
Viral	1								
Group 1B: localized infection¶	10	100	100	80	80	80	80	50	100
Pulmonary									
Gram-negative bacteria	7								
Mycobacterial	3								
Mold	4								
Nocardia	2								
Pneumocystis	2								
Cryptococcal	1								
Chronic mucocutaneous candidiasis	6								
Viral	6								
Group 2: no invasive infection¶	13	100	92	69	0	15	15	23	69
Chronic mucocutaneous candidiasis	3								
Viral	7								

[*] Listed are data for the 30 study patients with severe mycobacterial, bacterial, or fungal infections who had known anti–interleukin-12. Additional details regarding these analyses are provided in Table S6 in the Supplementary Appendix.

[†] In this analysis, the effective 50% concentration (EC_{50}) was defined as the level at which half the maximal phosphorylation of signal transducer and activator of transcription (STAT) was observed by serial dilutions of the cytokines over the range 0.01 to 100 ng per milliliter. For interleukin-23, the mean $EC_{50}R$ (the ratio of the EC_{50} in patient plasma divided by the EC_{50} in healthy control plasma) was 105.0 in Group 1A, 43.2 in Group 1B, and 2.2 in Group 2.

[‡] Listed is the percentage of patients with binding autoantibodies (bAB) that were positive for IgG that binds the cytokine with a fluorescence intensity of more than 10 standard deviations above the mean of healthy controls for all cytokines.

[§] Listed is the percentage of patients with neutralizing antibodies (nAB) that were positive for an $EC_{50}R$ value of more than 4.

[¶] The median number of infections per patient was three in Group 1A, four in Group 1B, and one in Group 2.