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Anti-IFN-γ Autoantibodies in Disseminated Nontuberculous Mycobacterial Infections

Smita Y. Patel,* Li Ding,* Margaret R. Brown,† Larry Lantz,‡ Ted Gay,§ Stuart Cohen,¶ Lenna A. Martyak,‖ Bernard Kubak,‖ and Steven M. Holland¶

Although many patients with disseminated nontuberculous mycobacterial disease have molecular defects in the IFN-γ/IL-12 axis, recent case reports have shown autoantibodies against IFN-γ associated with severe nontuberculous mycobacterial infections. To check this finding in an independent population, we screened 35 patients with either disseminated or pulmonary nontuberculous mycobacterial infections for whom no molecular defect was known. We identified high-titer-neutralizing anti-IFN-γ IgG in the plasma of six patients. All six patients were female, parous, of East Asian descent, and had disseminated infection, predominantly with rapidly growing mycobacteria. The anti-IFN-γ IgG had in vitro biological activity on the IFN-γ-dependent phosphorylation of STAT-1 as well as on the IFN-γ-dependent up-regulation of TNF-α and IL-12. In contrast, this anti-IFN-γ Ab had no effect on IFN-α-dependent STAT-1 phosphorylation. These patients confirm a novel syndrome linking autoimmunity and immunodeficiency.

Mycobacterium tuberculosis infects ~30% of the world’s population, but does not often lead to active infection (8.8 million cases per year, ~0.5%), and death is a smaller number than those with active disease (~3 million per year, or 0.15% overall) (1). Therefore, for tuberculous infections, host factors, both genetic and environmental, are likely critical in determining whether exposure leads to infection or disease. However these factors have not yet been clearly identified.

In contrast, the nontuberculous mycobacteria (NTM) have yielded a trove of information over the last decade on the genetic causes of susceptibility to mycobacterial infection, with numerous defects involving the type 1 cytokine pathway having been identified (reviewed in Refs. 2–5). However, these genetic defects have been identified predominantly in the setting of disseminated nontuberculous mycobacterial infection and account for only ~50% of patients studied. In the remainder, only syndromic clinical phenotypes have been identified. Because the exposure to NTM is essentially universal, infection likely reflects underlying host susceptibility factors. Whether those NTM susceptibility factors will identify or predict host factors relevant to susceptibility to tuberculosis remains to be determined.

Anti-IFN-γ autoantibodies have been described previously in patients with tuberculosis, HIV, and African trypanosomiasis (6, 7). However, the pathophysiologic significance of these Abs has been unclear, without clear links between Ab activity and the course of infection. Recently, patients have been identified with anti-IFN-γ IgG autoantibodies associated with disseminated mycobacterial and other infections (8, 9). In both cases, high-affinity inhibitory anti-IFN-γ IgG autoantibodies were demonstrated. Doffinger et al. (9) went on to prove biological activity of these autoantibodies, but could not determine their causal role in the disease of their patient.

These reports prompted us to examine a cohort of patients with disseminated and pulmonary nontuberculous mycobacterial infections followed at or referred to the National Institutes of Health. We found high-titer-neutralizing IgG autoantibodies to IFN-γ only in patients with disseminated nontuberculous mycobacterial infection. All of those who had these autoantibodies were parous women of East Asian descent, none of whom had been treated with exogenous IFN-γ.

The patients clinical characteristics and laboratory investigations are presented in Tables I and II, respectively.

**Materials and Methods**

*Subjects*

Patients were referred to the National Institutes of Health on the basis of mycobacterial disease, without regard to race, ethnicity, or national origin. Only patients with mycobacterial disease without a known underlying cause were included. These were composed of 23 patients with disseminated nontuberculous mycobacterial disease (19 females) and 12 patients with isolated pulmonary nontuberculous mycobacterial disease (9 females). All patients gave informed consent under approved National Institute of Allergy and Infectious Diseases protocols (93-I-0119 or 02-I-0202). Normal samples were obtained through the National Institutes of Health Blood Bank, under appropriate protocols.

*Plasma and PBMC*

Plasma was obtained from patients and normal donors by centrifugation of heparinized blood, and was frozen at −80°C.

PBMC from normal donors were obtained by density gradient centrifugation of heparinized whole blood through lymphocyte separation medium as described (10). Mononuclear cells were prepared at 1 × 10⁶ cells/ml in complete medium consisting of RPMI 1640, 2 mM glutamine, 20 mM HEPES, and 0.01 mg/ml penicillin and streptomycin with 10% FCS. Samples were left unstimulated or stimulated with PHA (1%) plus IL-12 (1 ng/ml), or LPS (200 ng/ml) plus IFN-γ (1000 IU/ml) for 48 h at 37°C in 5% CO₂. Supernatants were harvested after 48 h and stored at −20°C until cytokine determination.

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Table I. Clinical features of patients with IFN-γ autoantibodies and DMAC infections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex/Ethnicity</th>
<th>Parity</th>
<th>Infecting Organism</th>
<th>Organs Affected</th>
<th>Other Pathology</th>
<th>Duration of Antimicrobial Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43/Female/Taiwanese Well until age 35</td>
<td>2</td>
<td>MAC</td>
<td>Bone, skin, and soft tissue</td>
<td>Pneumonia</td>
<td>Five years; now on secondary prophylaxis</td>
<td>Complete resolution of lesions</td>
</tr>
<tr>
<td>2</td>
<td>45/Female/Filipino Well until age 40</td>
<td>2</td>
<td>MAC, M. chelonae</td>
<td>Cervical LN, lungs, and skin</td>
<td>Meningoencephalitis</td>
<td>More than 5 years on active therapy</td>
<td>Persistent infection</td>
</tr>
<tr>
<td>3</td>
<td>52/Female/Chinese-Vietnamese Well until age 39</td>
<td>2</td>
<td>M. tuberculosis, Mycobacterium szulgaii, Mycobacterium kansasi, Mycobacterium scrofulaceum</td>
<td>Cervical LN, bone, lung, and skin</td>
<td>Meningoencephalitis</td>
<td>More than 5 years on active therapy</td>
<td>Persistent infection</td>
</tr>
<tr>
<td>4</td>
<td>40/Female/Filipino Well until age 34</td>
<td>2</td>
<td>Mycobacterium abscessus, Mycobacterium fortuitum, MAC</td>
<td>Cervical LN and lung</td>
<td>Hepatitis, C, Pseudomonas aeruginosa bacteremia, Enterococcus cloacae pneumonia, Achromobacter xylosidans bacteremia</td>
<td>More than 5 years on active therapy</td>
<td>Persistent infection</td>
</tr>
<tr>
<td>5</td>
<td>66/Female/Filipino Well until age 61</td>
<td>2</td>
<td>M. abscessus, M. avium (pulmonary)</td>
<td>Cervical LN and lung</td>
<td>Cerebellar venous angioma</td>
<td>On active therapy</td>
<td>Persistent infection</td>
</tr>
<tr>
<td>6</td>
<td>31/Female/Filipino Well until age 30</td>
<td>3</td>
<td>MAC</td>
<td>Appendix, bone, cutaneous soft tissue, retropharyngeal, parapharyngeal, prevertebral (cervical)</td>
<td>Varicella zoster (multiple dermatomes)</td>
<td>More than 2.5 years on active therapy</td>
<td>Persistent infection, resolution of retropharyngeal, parapharyngeal, and prevertebral abscesses (surgical debridement)</td>
</tr>
</tbody>
</table>

a LN, Lymph node.

Cytokine determination

The Bio-plex cytokine assays (Bio-Rad) were used in a modified competitive inhibition assay by incubating supernatant from normal stimulated PBMCs with 1/10 v/v patient plasma at 37°C for 1 h (2 µl of patient plasma and 18 µl of PBMC culture supernatant). Samples were read as percent inhibition of cytokine binding to beads as determined by the Bio-Rad 17-plex assay: IFN-γ, TNF-α, GM-CSF, G-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IFN-β, PDGF, VEGF, GM-CSF, and G-CSF.

Table II. Laboratory features of patients with IFN-γ autoantibodies and DMAC infections

<table>
<thead>
<tr>
<th>Patient</th>
<th>IgG/A/M</th>
<th>CRP</th>
<th>Autoantibodies</th>
<th>CD4/CD8/CD20</th>
<th>Virology</th>
<th>Pathology</th>
<th>Radiology</th>
<th>Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1770/590/361</td>
<td>3.6</td>
<td>ND</td>
<td>222/264/127</td>
<td>ND</td>
<td>Left knee/chronic inflammation with ulcer and granulation tissue</td>
<td>RUL infiltrate, bronchiectasis. Multiple lytic bone lesions. Destruction of left knee joint.</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>2070/655/62</td>
<td>ANA 1:5120 speckled RNP SSA positive, ENA positive, anti-Smith positive,</td>
<td>544/1065/370</td>
<td>EBV 180 copies</td>
<td>L. benign follicular hyperplasia</td>
<td>Axillary, cervical and pretracheal lymphadenopathy. Partially collapsed LUL with atelectasis. Cerebellar venous angioma.</td>
<td>Hypercellular marrow with &lt;5% blasts and increased erythrophagocytosis.</td>
<td>Hypercellular marrow with a slight reticulohistiocytic and plasmacytosis.</td>
</tr>
<tr>
<td>6</td>
<td>1960/434/156</td>
<td>18.4</td>
<td>ANA negative, SM Ab negative, RNP Ab negative, SSA negative, SSb negative</td>
<td>250/516/57</td>
<td>Parapharyngeal and retropharyngeal abscesses: granulomatous and necrotizing abscess contents.</td>
<td>MRI: Soft tissue abscesses with mild peripheral enhancement; MRI neck 9 × 13 × 25 mm enhancing lesion seen at prevertebral and retropharyngeal space (C2–C5 levels); enhancing lesion in the right parapharyngeal space (6 × 14 mm). T2 hypointensity and T1 hyperintensity at the C2 vertebral body. MRI chest: osteomyelitis, clavicle, scapula, humeral head.</td>
<td>Hypercellular marrow with multinodular maturation and mucoid preponderance.</td>
<td></td>
</tr>
</tbody>
</table>

a CT, Computed tomography; LN, lymph node; MRI, magnetic resonance imaging; ANA, anti-nuclear Ab; ENA, extractable nuclear Ag; RNP, ribonucleoprotein; RUL, right upper lobe; RLL, right lower lobe; LUL, left upper lobe; HCV, hepatitis C virus; SM, synovial membrane.

b Normal up to 200 copies/million genomes.
MIP1α, MCP1, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17.

Immunoblotting
Recombinant IFN-γ (R&D Systems; 500 ng) was subjected to SDS-PAGE using a gradient gel (4–12%) (Invitrogen Life Technologies) under reducing conditions at 200 V for 60 min. The protein was transferred electrophoretically to a polyvinylidene fluoride membrane (Invitrogen Life Technologies) at 20 V constant voltage for 75 min. The membrane was blocked in 5% BSA with 0.1% Tween 20 overnight at 4°C. The membrane was incubated with a 1/50 dilution of patient or control plasma for 1 hour at room temperature. After three washes, the membrane was incubated in a 1/10,000 dilution of sheep anti-human IgG conjugated to HRP (Amersham) for 30 min at room temperature. After three washes, the membrane was developed with ECL plus (Amersham). As a positive control, blots were incubated with rabbit anti-IFN-γ primary and mouse anti-rabbit IgG HRP secondary Abs (Amersham).

Titration of IFN-γ autoantibody
Serial dilutions of patient and normal plasma were incubated 1/10 v/v with a fixed concentration (736 pg/ml) of recombinant IFN-γ for 1 h at 37°C, 5% CO₂ in a total volume of 60 µl. IFN-γ was detected using a standard IFN-γ ELISA following the manufacturer’s instructions (Endogen).

Ig depletion
Plasma was subjected to standard affinity chromatography purification of IgG. Briefly, each sample was diluted in column equilibration/wash buffer (10 mM NaPO₄, 150 mM NaCl, pH 7.0). The Ig fraction was isolated on protein G columns (Amersham/Pharmacia-HiTrap) and eluted with 100 mM glycine, pH 2.5. Purified Abs were neutralized to pH 7.4 with 4.0 M Tris, pH 8.0. Samples were concentrated using a Microcon 10 column (Millipore) and retested in the Bio-plex assay.

Biological function of the autoantibodies: phospho-STAT-1 detection by flow cytometry
A total of 10⁶ PBMCs in 1 ml of PBS were incubated with 100 µl of normal plasma or patient plasma for 10 min at room temperature. Cells were then stimulated with PHA and IL-12 in the presence of complete media with fetal calf serum. The level of cytokine detected in the absence of patient plasma was taken as 100%. The level of cytokine detected in the presence of patient plasma was compared to the level detected in the absence of patient plasma. The results were analyzed using the Bio-plex assay.
were then stimulated with 1000 IU IFN-γ or 1000 IU IFN-α for 10 min at 37°C in a shaking water bath (11). After incubation, the cells were fixed and permeabilized by adding 400 μl of fixation reagent (Reagent A, Fix and Perm; Caltag Laboratories) to 1 ml of cell suspension and incubated at room temperature for 2–3 min. Five-hundred-microliter aliquots were made of each sample, and 3 ml of ice-cold absolute methanol was added to each tube while vortexing, followed by two wash steps with PBS. Next, 100 μl of permeabilization medium (Reagent B, Fix and Perm; Caltag Laboratories) together with a specific primary Ab was added to each cell pellet followed by a 30-min incubation at room temperature. Primary Abs were as follows: 4 μl of phospho-STAT-1 (New England Biolabs), 20 μl of MOPC141, 4 μl of intracellular-STAT-1 (BD Transduction Laboratories).

Cells were washed and incubated with either 1 μg of FITC-conjugated goat anti-rabbit or goat anti-mouse FITC (Caltag Laboratories) for 30 min at room temperature in the dark. Cells were again washed with 2 ml of PBS before resuspension in 100 μl of PBS. Data were collected with a FACScan flow cytometer (BD Biosciences) and analyzed using FlowJo software (TreeStar).

PBMC cytokine production

A total of 10⁶ PBMCs in a total volume of 1 ml of complete medium (RPMI 1640 and 10% FCS) were stimulated with LPS (200 ng) and IFN-γ (1000 IU/ml) in the presence of 100 μl of normal plasma or patient plasma, (final volume 1.1 ml) for 48 h at 37°C in 5% CO₂. Supernatants were harvested and frozen at −20°C until tested for TNF-α, IL-12, IL-1β, and IL-10 in the Bio-plex assay.

Results

Inhibition of IFN-γ detection

The effect of added plasma on the amount of native IFN-γ detected in the Bio-plex assay is shown in Fig. 1. A total of 48 plasma samples were tested, including 23 from patients with disseminated M. avium complex (DMAC), 12 from patients with pulmonary M. avium complex (PMAC), and 13 normals. Among the DMAC cohort, six samples had significantly stronger inhibition of IFN-γ detection than all others, causing a >90% reduction in the detection of IFN-γ. In contrast, plasma from all other patients and normals also inhibited IFN-γ detection, but to a lesser extent. This precise pattern of inhibition was specific for IFN-γ, and was not seen in any of the other cytokines measured in the 17-plex assay. Data for IL-10, IL-1β, IL-12, and TNF-α are shown. (Fig. 2). None of the other cytokines gave a discriminatory pattern between patients and normals.

Demonstration of an anti-IFN-γ IgG

The six plasma that showed >10-fold reduction in IFN-γ detection in the Bio-plex assay (designated high in Fig. 1) were used in immunoblotting, to verify the exact nature of the binding activity. (Fig. 3). Plasma was incubated with human recombinant IFN-γ (500 ng per lane) and developed with a sheep anti-human IgG. Some patients with M. avium complex (MAC) and normals had faint bands upon immunoblotting, consistent with the low level inhibition of IFN-γ detection in normals seen Fig. 1. However, the six plasma that showed the highest levels of inhibition of IFN-γ detection also had the most intense staining for IgG, indicating that the anti-IFN-γ activity detected in the plasma was due to IgG. Therefore, these patients had a high-titer-neutralizing anti-IFN-γ IgG autoantibody.

Having determined that IgG was readily detected as an IFN-γ binding moiety in immunoblotted patient plasma, we sought to determine whether it was the sole anti-IFN-γ activity. To do this we depleted patient sera of Igs (IgG), by running the samples over a protein G column. Adequate samples for purification were available only from patients 1, 2, and 6. Ig depletion entirely removed the IFN-γ inhibitory activity, leaving all the anti-IFN-γ activity detected in the plasma was due to IgG. Therefore, these patients had a high-titer-neutralizing anti-IFN-γ IgG autoantibody.

We used a standard IFN-γ ELISA to titrate the anti-IFN-γ autoantibodies. The six high-titer patient plasma samples inhibited detection of a fixed concentration of IFN-γ at dilutions out to 1/5000 and above. The dilution at which there was first detection of >10% of the input IFN-γ was considered to represent the inflection point and denoted as the titer (Fig. 5).
Inhibition of IFN-γ mediated STAT-1 phosphorylation

The binding of the IFN-γ homodimer to the IFN-γR starts a signaling cascade involving the autophosphorylation of Jak2, followed by the transphosphorylation of Jak1. This leads to the creation of a STAT-1 docking site on the IFN-γR1 intracellular domain, which allows STAT-1 to be phosphorylated. Phospho-STAT-1 forms a homodimer that translocates to the nucleus to activate IFN-γ-responsive genes, including TNF-α and IL-12. Therefore, the detection of phospho-STAT-1, TNF-α, and IL-12 represent early and late elements of IFN-γ stimulation, respectively.
Normal donor PBMCs readily phosphorylated STAT-1 after IFN-\(\gamma\) or IFN-\(\alpha\) stimulation in PBS alone and in the presence of normal alloserum, (Fig. 6). In the presence of 10% v/v autoanti-body patient plasma, formation of phospho-STAT-1 by normal PBMC was inhibited in response to IFN-\(\gamma\), but not in response to IFN-\(\alpha\). Therefore, patient plasma was not toxic to cells and did not inhibit the STAT-1 pathway directly, but specifically blocked IFN-\(\gamma\)-induced STAT-1 phosphorylation. It is also possible that Abs directed against the IFN-\(\gamma\)-R could lead to similar results. Against this possibility is that immunoblots using PBMC whole cell lysates probed with plasma from autoantibody-positive patients did not detect any specific bands, (data not shown).

**Anti-IFN-\(\gamma\) autoantibody inhibition of downstream cytokine induction**

Patient plasma significantly inhibited normal PBMC production of TNF-\(\alpha\) and IL-12 after LPS and IFN-\(\gamma\) stimulation for 48 h (Fig. 7). This inhibition was significant when compared with both the low-titer MAC group and the normal controls. However, the production of IL-1\(\beta\) and IL-10, two cytokines whose induction is not IFN-\(\gamma\)-dependent, was not significantly affected (Fig. 7). Therefore, this inhibitory autoantibody affected IFN-\(\gamma\) signaling selectively without disrupting other pathways, including other cytokines that are potently expressed in the setting of active mycobacterial infection.

**Discussion**

We have identified functional anti-IFN-\(\gamma\) autoantibodies in a group of East Asian women with disseminated mycobacterial infection, predominantly due to rapid growing mycobacteria. These autoantibodies were high titer, able to block binding of native human IFN-\(\gamma\), inhibitory to early aspects of IFN-\(\gamma\) signal transduction, (STAT-1 phosphorylation), and inhibitory to at least some of the downstream biological consequences of IFN-\(\gamma\)-binding (IFN-\(\gamma\)-dependent up-regulation of TNF-\(\alpha\) and IL-12). Plasma from normal controls, other patients with disseminated MAC, and patients with PMAC did not block IFN-\(\gamma\) detection or biological function to the same extent. Interestingly, similar to what has been reported before, low levels of IFN-\(\gamma\) autoantibodies were found in many healthy controls and patients with other cases of MAC, as demonstrated by immunoblotting. However, these low-titer anti-IFN-\(\gamma\) autoantibodies were not biologically active in terms of inhibiting STAT-1 phosphorylation or downstream cytokine induction. Therefore, these patients with high-titer anti-IFN-\(\gamma\) autoantibodies differ in both quality and quantity from those found in other MAC patients.

Over the last decade there have been recognized an increasing number of Abs directed against cytokines, both natural and recombinant (12, 13). However, the binding characteristics of most identified autoantibodies have not been determined, and antagonistic
functions of these Abs are largely inferred, rather than demonstrated. The majority of the autoantibodies are polyclonal and do not show affinity maturation. It has been proposed that naturally occurring anti-cytokine autoantibodies may be part of an immune regulatory response to inflammation and infection (14). This hypothesis is partially supported by the identification of low levels of anti-IFN-γ autoantibodies in our control population. However, in the environment of infection or inflammation these titers may increase. Bakheit et al. (15) proposed the induction of anti-cytokine autoantibodies to TNF-α and IFN-γ as mechanisms for cytokine regulation in experimental bacterial meningitis in rats. Dysregulation of this proposed normal mechanism, either leading to too much or too little autoantibody formation, might result in a pathogenic effect. However, it is not clear what the triggers for dysregulation of autoantibody formation are. It is possible that high levels of circulating cytokines may overcome peripheral T cell tolerance, allowing autoreactive T cells to provide help to autoreactive B cells. Another possibility is molecular mimicry between pathogens and host cytokines or other moieties. Pathogens could also secrete proteins that act as haptons for host proteins.

Caruso et al. (7) described naturally occurring autoantibodies to IFN-γ in patients with acute viral infections (HSV, EBV, CMV, measles, mumps, rubella, HIV, and adenovirus) and in healthy controls. They posited that these autoantibodies were in response to high circulating levels of IFN-γ present during the acute phase of illness. The Abs they reported did not affect the antiviral effects of IFN-γ, but did alter the immunomodulatory effects of IFN-γ on MHC class II up-regulation in U937 cells. Madariaga et al. (6) demonstrated anti-IFN-γ autoantibodies in the sera of patients with \textit{M. tuberculosis}. They hypothesized that the level of autoantibody production increased as the disease progressed, and that inhibiting the Th1 type immune response contributed to the progression of disease. More recently, Hoflich et al. (8) and Doffinger et al. (9) described patients with high-titer anti-IFN-γ autoantibodies. Hoflich et al. (8) described disseminated \textit{Mycobacterium chelonea} infection complicated by fatal disseminated \textit{Burkholderia coccovenan} infection in a 27-year-old Thai woman; Doffinger et al. (9) described late onset disseminated \textit{M. tuberculosis} and \textit{M. chelonea} in a 47-year-old Filipino man with autoimmune polyclonocinopathy and recurrent candidiasis. Both patients had high-titer anti-IFN-γ autoantibodies. Because the phenotype of IFN-γ deficiency does not include candida, polyclonocinopathy, or \textit{Burkholderia} infection (16), anti-IFN-γ autoantibodies were not likely to have been responsible for the entire clinical phenotype reported in those cases. It is of particular interest that all patients identified in this study and previous reports are of Asian descent. This may reflect a shared HLA allele, which will need to be further explored in the setting of ethnically matched control populations.

The majority of anti-cytokine Abs described to date have occurred in autoimmune diseases, such as multiple sclerosis (17, 18), rheumatoid arthritis (19, 20), idiopathic pulmonary alveolar proteinosis (21), and myasthenia gravis (22). Among patients with myasthenia gravis and thymoma, anti-IL-12 and anti-IFN-α autoantibodies, in addition to those developed against the acetylcholine receptor, are common. Patients treated with recombinant erythropoietin have been shown to develop neutralizing anti-erythropoietin Abs, some of which led to pure red cell aplasia (23). However, in the vast majority of cases in which cytokines have been used therapeutically, patients have not been tested for the induction of autoantibodies.

Determining the biological activity of anticytokine autoantibodies has been problematic. ELISA, RIA assays, and recombinant cytokines themselves may produce false positives, due to nonspecific interactions of Igs with cytokines (12). The assay we have used is based upon competitive inhibition of detection of native IFN-γ produced by normal PBMC. Therefore, the autoantibody activity we detected represents an activity directed against an authentic human cytokine, not a recombinant commercial preparation that may have been produced in bacteria, yeast, or nonhuman cells. Furthermore, the inhibitory activity observed in the STAT-1, TNF-α, and IL-12 assays was biologically robust. TNF-α and IL-12 up-regulation in response to LPS plus IFN-γ in the absence of patient plasma proved the integrity of the IFN-γ response pathway. However, the inhibition of TNF-α and IL-12 production by normal cells in the presence of 10% patient plasma strongly suggests that these properties are likely to be active and relevant in vivo.

Autoimmunity in itself is generally not considered a major risk factor for infection, rather it is the iatrogenic immune suppression. Low-titer, low-avidity Abs exist in normals, presumably as part of a normal immune regulation network. In our patients, in response to an unknown trigger or triggers (a combination of environmental and genetic), B cells appear to have undergone affinity maturation to produce high-titer, high-avidity autoantibodies. These Abs recognize functional epitopes of the cytokine IFN-γ, leading to inhibition of IFN-γ activity and exacerbation of mycobacterial disease. We cannot yet discern whether the autoantibodies are cause or effect of the disseminated MAC infection in our patient population. It may be that infection triggers the initial production of high-level IFN-γ leading to autoantibody production, and further exposure leads to affinity maturation and increased specificity and activity, making the individual more susceptible. Apparently, in these patients, once present, these Abs are potent and neutralizing. It is critical to determine the epitope or epitopes to which the autoantibodies in our patients are directed. The determination of whether these autoantibodies are causal in our patients will guide potential therapeutic interventions, such as IFN-γ therapy, anti-CD20 Ab therapy, i.v. Ig, or plasmapheresis. The identification of a series of patients who are clinically, genetically, and pathophysiologically connected through the development of anti-IFN-γ autoantibodies, reconfirms the central role of IFN-γ in the control of mycobacterial infections. Long-term follow up will be required to see whether these autoantibodies or their titers change with treatment or resolution of infection. However, we should expect to identify other anti-cytokine or anti-receptor autoantibodies in this and other syndromes in the future.

Disclosures

The authors have no financial conflict of interest.

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