Clinical and Immunologic Features of an Atypical Intracranial Mycobacterium avium Complex (MAC) Infection Compared with Those of Pulmonary MAC Infections

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Members of the Mycobacterium avium complex (MAC) may cause chronic pulmonary infections in otherwise healthy elderly persons but rarely invade parts of the body outside of the lungs in immunocompetent hosts. We present a case of an isolated intracranial MAC infection in an apparently immunocompetent individual and review previous reports. We studied the T-cell and monocyte responses in healthy volunteers, individuals with a pulmonary MAC infection, and one individual with an isolated intracranial MAC infection. Genomic DNA from the individual with the brain MAC infection was studied for gamma interferon (IFN-γ) receptor mutations. Individuals with localized pulmonary MAC infections showed increased activation of monocytes and enhanced monocyte and T-cell tumor necrosis factor alpha (TNF-α) production in response to lipopolysaccharide and MAC antigens but defects in T-cell IFN-γ secretion. The individual with an intracranial MAC infection showed a lack of monocyte activation and deficiencies in both monocyte and T-cell TNF-α production and monocyte interleukin-12 (IL-12) production but had preserved T-cell IFN-γ production. Mutations or deletions in the IFN-γ receptor were not detected in the individual with the intracranial MAC infection. Our data suggest that distinct immune defects characterize two different manifestations of MAC infection. A relative defect in IFN-γ production in response to MAC may predispose an individual to localized but partially controlled lung disease, whereas defects leading to reduced IL-12 and TNF-α production may allow the dissemination of MAC. Further studies delineating the potential role of TNF-α in limiting the spread of MAC outside the lung are warranted.

Members of the Mycobacterium avium complex (MAC) are ubiquitous nontuberculous mycobacteria that seldom cause disease in immunocompetent hosts. In human immunodeficiency virus (HIV)-infected individuals who have severe CD4 T-cell depletion, MAC causes disseminated infections. However, MAC can also cause chronic pulmonary infections in elderly persons with apparently normal immune function (34). Characteristically, some older nonsmoking women, generally those with bronchiectasis, may develop right middle lobe infiltrates and a nonproductive cough, termed “Lady Windermere syndrome.” The coordinated function of T cells and monocytes is generally required to resolve mycobacterial infections (10). Few case reports have demonstrated that isolated intracranial MAC infections in the absence of an underlying medical condition account for immunosuppression (7, 8, 32, 40). However, immune defects or genetic predispositions were not adequately described in those reports. Immune defects have previously been reported in elderly persons with pulmonary MAC infections. These have specifically included a level of higher production of interleukin-10 (IL-10) but lower concentrations of gamma interferon (IFN-γ), IL-12, and tumor necrosis factor alpha (TNF-α) in peripheral blood mononuclear cells (PBMCs) in response to heat-killed MAC (17, 35, 36, 41).

Granuloma formation, which is essential for mycobacterial control, consists of a complex interplay between activated αβ-T-cell-receptor-expressing cells that have been primed to respond to mycobacterial antigens and macrophages that have engulfed organisms. Their overall effect is to contain the bacilli and prevent further spread. Signals which aid macrophages in granuloma formation and the clearance of mycobacterial infections are complex and are incompletely understood. These include IFN-γ and TNF-α. TNF-α is secreted by macrophages and T cells and is able to induce mycobacteriostatic and mycobactericidal activity in both cultured mouse and human macrophages (3, 14). Recently, TNF-α has been demonstrated to recruit T cells to the granuloma site, thus maintaining the granuloma (12). IFN-γ is a macrophage-activating factor secreted by T-helper cells that leads to multiple cellular pathways within the macrophage, including the production of superoxide, TNF-α, IL-12, IL-1, and IL-6 (4). IL-12 is thought to potentiate IFN-γ secretion by T cells via a positive-feedback mechanism and thus contributes to the control of mycobacterial infections (2, 15). Defects in either IFN-γ or TNF-α signaling are associated with poorly defined granuloma formation and the escape of the mycobacteria (37). Granulomas also induce intense inflammatory responses in the presence of persisting mycobacteria, which can lead to tissue damage and clinical manifestations of mycobacterial disease. In this regard, IL-10, a counterregulatory cytokine, prevents T-cell prolifera-
tion and inhibits T-cell responses (19), thus alleviating excess inflammation. It is also, however, responsible for the induction of anergy and suppression of the immune response to mycobacteria (5), likely by inhibition of TNF-α expression (9).

The aims of this study were to (i) examine in depth the immune response of an index patient with an intracranial MAC infection who had an apparently normal immune function and (ii) compare the cytokine profiles of the index case to those of individuals with pulmonary MAC infections and healthy controls. Our findings have elucidated potential immune mechanisms important against atypical mycobacterial organisms in humans.

MATERIALS AND METHODS

Study population. Written informed consent was obtained from the participants in accordance with the guidelines for the conduct of clinical research at the University of Toronto and St. Michael’s Hospital, and the protocol was approved by the review board of St. Michael’s Hospital.

(i) Patients. Blood was obtained from four patients (age range, 60 to 63 years) with a diagnosis of pulmonary or intracranial disease due to MAC. A diagnosis was made on the basis of the guidelines recommended by the American Thoracic Society (18). In all cases of pulmonary MAC, sputum culture results were positive, chest radiographs were abnormal, the disease was confined to the lung, and the patients were symptomatic. They were excluded if they had (i) evidence of immunological compromise (e.g., HIV infection) or (ii) if they were taking immunosuppressive medication.

(ii) Healthy control subjects. Blood was obtained from four healthy control subjects (age range, 28 to 46 years). Volunteers were excluded if they had a history of respiratory illness or were taking any immunosuppressive medication.

Isolation of PBMCs. PBMCs were isolated from blood at the time of diagnosis by differential centrifugation over Ficoll-Paque (Amersham Pharmacia Biotech), and the samples were stored in a nitrogen freezer (~150°C) for future use.

Antigens used. MAC antigen was produced as an aqueous solution of a sonicated heat-killed culture of an M. avium intracellulare isolate derived from the patient from the same geographical area as the study subjects and was obtained from the provincial public health laboratory (Ontario Public Health Laboratories). Various dilutions of MAC antigen (1/10, 1/50, 1/100, 1/200, 1/500, and 1/1,000) were tested in PBMC cultures, and the levels of production of cytokines from monocytes was found to be the highest at the 1/500 dilution, which was set as our standard. Lipopolysaccharide (LPS; Sigma, Oakville, Ontario, Canada) was used at 1 µg/ml to stimulate monocytes, and anti-CD3 (BD Biosciences) was used at 0.1 µg/ml to stimulate T cells.

Flow cytometry and intracellular cytokine determination. To assess the monoocytes ex vivo, freshly isolated PBMCs were stained with CD14 ex vivo and run on a FACSCalibur flow cytometer. Monocytes were gated according to their forward and side scatter and assessed for CD14 expression. In addition, the PBMCs were cultured in the presence of medium or antigen (LPS or MAC antigen) for 6 h and then assessed for CD14 expression by flow cytometry. The procedure for intracellular staining of cytokines in T cells and monocytes from PBMC samples was performed according to the protocols of BD Biosciences (San Diego, CA). Briefly, PBMCs were incubated with one of four antigens, (i) medium alone, (ii) MAC antigen, (iii) LPS, and (iv) anti-CD3, for 6 h in the presence of 10 µg/ml brefeldin A and 1 µg/ml anti-CD49d and anti-CD28 antibodies for costimulation (BD Biosciences). Staining was performed with a panel of conjugated antibodies after they were permeabilized (BD Biosciences). The following antibodies were used: IFN-γ-fluorescein isothiocyanate, IL-10-phycocerythrin, CD3-peridinin chlorophyll protein complex, CD14-peridinin chlorophyll protein complex, and TNF-α-antigen-presenting cells. The cells were then washed and resuspended in 1% paraformaldehyde–phosphate-buffered saline and were then analyzed on the following day on a FACSCalibur flow cytometer (BD Biosciences). Data were acquired by the use of CellQuest software (BD Biosciences) and were analyzed with the FlowJo program (Tree Star, San Carlos, CA). A total of 100,000 events were acquired per sample.

Measurement of extracellular cytokine concentration. PBMCs (1 × 10^6) were cultured in triplicate in 48-well plates in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 25 mM HEPES, and antibiotics (Invitrogen Life Tech, Carlsbad, CA) under the following conditions: (i) with medium alone, (ii) with MAC antigen, (iii) with LPS, or (iv) with antibodies to CD3 and CD28 (0.1 µg/ml and 1 µg/ml, respectively; BD Biosciences) at 37°C under a 5% CO2 atmosphere for 5 days. The supernatants were collected and stored at ~20°C. The concentrations of IL-12 (after 24 h) and IFN-γ (after 5 days) in the supernatants were measured by enzyme-linked immunosorbent assay, according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

Sequenceing of IFN-γR1 and IFN-γR2 genes. DNA was extracted from PBMCs from the index patient (Puregene kit; Qiagen). For the detection of partial IFN-γ receptor 1 (IFN-γR1) deficiency, exon 3 and exon 6 of the IFN-γR1 gene were amplified by PCR and sequenced as described previously (26). For the detection of mutations in IFN-γR2, exon 3 of IFN-γR2 was amplified and sequenced as described previously (26).

Statistical analysis. Data were compared by Student’s t test (two tailed) with SPSS software (Chicago, IL).

RESULTS

Case report and previous literature. A 63-year-old man presented with a history of 4 months of headaches and word-finding difficulties but no constitutional symptoms. His medical history was significant for a history of pulmonary sarcoidosis, of which he had no respiratory symptoms at the time of presentation. He was not on any oral medications, had not recently traveled, and had no infectious exposures. The physical examination was unremarkable. His total white cell count was 9,000 cells/mm3, and his lymphocyte count was 1,170 cells/mm3 (CD4 count, 220 cells/mm3; CD8 count, 157 cells/mm3). A test for HIV and the Mantoux skin test were negative.

A contrast head computed tomography revealed a ring enhancing lesion in the left frontal lobe (Fig. 1a). A left frontal craniotomy was performed and revealed purulent-looking necrotic tissue. Microscopic examination revealed multiple foci of spindle cell pseudotumor formation (Fig. 1b). Ziehl-Neelsen staining demonstrated a large number of acid-fast bacilli (Fig. 1c). There was no evidence of an intrapulmonary MAC infection either clinically or radiologically. He was started on clarithromycin and ethambutol. After approximately 1 year of treatment, he showed resolution of all symptoms, and imaging demonstrated abscess resolution with residual scarring.

The clinical characteristics of previously reported cases and our case of isolated intracranial MAC infection in the absence of HIV infection are depicted in Table 1. Of note is that three of five cases had a history of sarcoidosis. Three of the four previously reported cases had a history of intermittent steroid treatment, and the current case had a history of local inhaled steroid use but had not used inhaled steroids during at least the 6 months prior to presentation. At least three of the five cases had preexisting CD4 lymphocytopenia. Treatment included ethambutol and a macrolide for most cases, and the outcomes were variable, with poor responses reported in two of the five cases. Detailed immune studies were reported for only one case, who showed a reduced ability to produce TNF-α and IFN-γ in response to mitogen stimulation.

Study population. In order to identify the immune defects that might be associated with the extrapoluminal dissemination of MAC, as observed in our index patient, we studied three individuals with localized pulmonary MAC infection and four healthy volunteers.

Monocyte activation pattern. The activation of CD14 monocytes has previously been shown to be associated with down-regulation of the CD14 receptor, which produces a CD14dim phenotype. CD14dim monocytes are thought to be major pro-
Producers of cytokines, and the levels of CD14dim monocytes have previously been shown to be elevated in septic patients (13). In addition, the percentage of CD14dim (activated) monocytes has been shown to increase after stimulation with sepsis-specific antigens, such as LPS, staphylococcal enterotoxin B, peptidoglycan, and even TNF-α (38). Ex vivo PBMCs were isolated, and the percentage of CD14 dim monocytes was measured as a marker of baseline inflammatory responses. Patients with localized pulmonary MAC infections had a higher percentage of CD14dim monocytes circulating in their peripheral blood than the controls and the individual with a brain MAC infection, even after stimulation with MAC antigen in culture (Table 2). LPS and MAC antigens activated monocytes in all subjects with pulmonary MAC and all controls, as assessed by CD14 downregulation, but this effect was less pronounced in our index patient with an intracranial MAC infection (Table 2).

Monocyte responses. Monocytes were examined for their ability to produce cytokines in response to LPS (a Toll-like receptor 4 [TLR4] agonist) and to MAC antigens (a TLR2 and TLR4 agonist) (Fig. 2a and b). Monocytes from the individuals with pulmonary MAC infections produced significantly more TNF-α and significantly less IL-10 in response to LPS or MAC antigen than the healthy controls (for LPS, 86.9 ± 14.5% TNF-α and 9.3 ± 5.6% IL-10 versus 37.6 ± 15.3% TNF-α and 39.6 ± 1.9% IL-10, respectively [P < 0.01]; for MAC antigen, 75.3 ± 15.5% TNF-α and 8.2 ± 4.6% IL-10 versus 40.1 ± 14.0% TNF-α and 24.5 ± 4.2% IL-10, respectively [P < 0.05]).

Monocytes from our patient with intracranial MAC infection showed levels of TNF-α expression similar to those for the healthy controls in response to LPS but reduced levels of TNF-α production in response to MAC antigen. In addition, monocytes from this individual produced small amounts of...
<table>
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<tr>
<th>Authors</th>
<th>Year</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Presentation</th>
<th>Symptom duration (yr)</th>
<th>CSF examination (described in Table 1)</th>
<th>Diagnostic method (CSF)</th>
<th>Underlying disease</th>
<th>Immunologic study (CD4 lymphocytopenia)</th>
<th>Antibiotic treatment (1106 cells/liter)</th>
<th>Treatment duration (mo)</th>
<th>Response to antimycobacterial therapy</th>
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<tr>
<td>Uldry et al. (40)</td>
<td>1985</td>
<td>31</td>
<td>F</td>
<td>Headaches, nausea, vomiting, ataxia</td>
<td>4</td>
<td>Not done</td>
<td>Not done</td>
<td>3</td>
<td>Not done</td>
<td>Not done</td>
<td>11</td>
<td>Poor response, death</td>
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<td>Dickerman et al. (7)</td>
<td>1996</td>
<td>38</td>
<td>M</td>
<td>Seizure, ataxia</td>
<td>Not done</td>
<td>Brain biopsy</td>
<td>Sarcoidosis (treated with steroids)</td>
<td>CD4 lymphocytopenia</td>
<td>Low extracellular INF and TNF levels</td>
<td>Rifampin, ethambutol, clarithromycin</td>
<td>6</td>
<td>Poor response; required repeat surgical debridement, with good outcome</td>
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<td>Morrison et al. (32)</td>
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<td>38</td>
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<td>Headaches, slurred speech, nystagmus, ataxia</td>
<td>2</td>
<td>Not done</td>
<td>Brain biopsy</td>
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<td>CD4 lymphocytopenia</td>
<td>Not available</td>
<td>Not available</td>
<td>Not available</td>
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<td>Di Patre et al. (8)</td>
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<td>50</td>
<td>F</td>
<td>Enlarging scalp mass</td>
<td>Not done</td>
<td>Brain biopsy</td>
<td>SLE (treated with prednisone and azathioprine)</td>
<td>Not done</td>
<td>Not done</td>
<td>Ethambutol, clarithromycin</td>
<td>Not available</td>
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<td>Index patient (this study)</td>
<td>2005</td>
<td>63</td>
<td>M</td>
<td>Headaches, word-finding difficulties</td>
<td>4</td>
<td>Not done</td>
<td>Brain biopsy</td>
<td>Sarcoidosis</td>
<td>See Results</td>
<td>Ethambutol, clarithromycin</td>
<td>11</td>
<td>Resolution of neurological symptoms</td>
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Abbreviations: CSF, cerebrospinal fluid; F, female; M, male; SLE, systemic lupus erythematosus.
IL-10 to LPS and MAC compared to the amounts produced by the monocytes from the healthy controls.

We also measured the relative contributions of cytokine expression within the CD14\textsuperscript{bright} and CD14\textsuperscript{dim} fractions in each sample and found that TNF-\(\alpha\) was equally distributed in both fractions, whereas the majority of IL-10 produced in the healthy controls was found in the CD14\textsuperscript{dim} subset (data not shown) under both LPS and MAC antigen stimulation conditions.

Overall, monocytes isolated from the individuals with pulmonary MAC infections produced more TNF-\(\alpha\) than the healthy controls and the patient with intracranial MAC infection and less IL-10 than the healthy controls, whether it was in response to LPS or MAC antigen. Monocytes from the index patient with intracranial MAC infection appeared to have a relative defect in TNF-\(\alpha\) production in response to MAC antigen, even compared to that for the controls, and this defect was not associated with any increased production of IL-10. In addition, the CD14\textsuperscript{dim} monocytes from the healthy controls produced significant amounts of IL-10 compared to the amounts produced by the patients with pulmonary and intracranial MAC infections.

**T-cell response to CD3/CD28.** In order to test for general defects in T-cell function, the cytokine response to incubation with CD3/CD28 costimulation was measured (Fig. 3a). T cells from individuals with pulmonary MAC infection showed sig-

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<th>Infection group</th>
<th>% CD14\textsuperscript{dim} monocytes</th>
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<td></td>
<td>Ex vivo and unstimulated</td>
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<tr>
<td>Pulmonary MAC</td>
<td>55.1 ± 7.8(^a)</td>
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<tr>
<td>Brain MAC</td>
<td>20.1</td>
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<tr>
<td>Controls</td>
<td>25.8 ± 5.8</td>
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\(^a\) \(P < 0.01\) for patients with lung MAC infection compared with the results for the healthy controls.

\(^{b}\) \(P < 0.01\) compared with the results obtained in the unstimulated condition.
a) Anti-CD3/CD28

- Pulmonary MAC
- Brain MAC
- Controls

% Expression in T-Cells

- TNF-α
- IFN-γ
- IL-10

**
* b) Medium

- Pulmonary MAC
- Intracranial MAC
- Control

CD3

- TNF-α
- 0.14%
- 0.15%
- 0.11%
- 0.38%

MAC Ag

- 15%
- 5.36%

- Pulmonary MAC
- Intracranial MAC
- Control

- TNF-α
- IFN-γ
- IL-10

% Expression in T-Cells

- Pulmonary MAC
- Brain MAC
- Controls

*
significantly reduced IFN-γ responses compared to those for the healthy controls but showed TNF-α responses similar to those for the healthy controls (for TNF-α, 2.6 ± 0.8% and 2.3 ± 0.9%, respectively; for IFN-γ, 0.5 ± 0.2% and 3.0 ± 0.5%, respectively [P < 0.01]). T cells from our patient with intracranial MAC infection showed reduced IFN-γ and TNF-α responses compared to those of T cells from the healthy controls. The IL-10 responses were comparable between the MAC-infected individuals and the healthy controls.

Thus, compared to the healthy controls, T cells from individuals with localized MAC infections display a reduced capacity to produce IFN-γ in response to T-cell stimulation via the CD3 receptor, and T cells from the patient with the intracranial MAC infection showed a reduced capacity to produce both TNF-α and IFN-γ when they were stimulated with CD3.

**T-cell responses to MAC antigen.** In order to test for specific T-cell dysfunction in response to MAC infection, the response to incubation with isolated MAC antigen was measured. The results of a representative experiment are shown in Fig. 3b, and summary data are depicted in Fig. 3c. T cells from individuals with localized pulmonary MAC infections showed significantly enhanced TNF-α production compared to that for the healthy controls (15.2 ± 3.6% and 2.5 ± 3.1%, respectively [P < 0.01]) and a more modest but not significant (P = 0.14) increase in the level of IFN-γ production compared to that for the healthy controls. The individual with brain MAC infection showed TNF-α responses to MAC similar to those for the healthy controls, but the TNF-α levels were at least threefold lower than those seen in the patients with pulmonary MAC infection, that is, in the context of an infection. Conversely, the IFN-γ responses to MAC in this individual were greater than those of the healthy controls and the patients with pulmonary MAC infections (Fig. 3b). There was no evidence of enhanced IL-10 production in response to MAC antigen in any individual.

**PBMC extracellular cytokine production in response to MAC.** It has previously been reported that individuals with pulmonary MAC infection may have T cells capable of intracellular IFN-γ production but have an inability to secrete them (35). In order to test for a relative deficiency in the ability to secrete cytokines in response to MAC antigen, PMBCs were incubated with MAC antigen and an enzyme-linked immunosorbent assay was performed to measure IFN-γ and IL-12 levels (Fig. 4a and b, respectively). For IFN-γ, PMBCs from the individual with brain MAC infections produced large amounts in response to MAC antigen, whereas the group with localized pulmonary MAC did not show any enhanced IFN-γ secretion compared to that for the healthy controls. Conversely, the index patient with brain MAC infection had a deficiency in IL-12 production in response to MAC antigen and LPS compared to the responses of the healthy controls, whereas individuals with pulmonary MAC infections produced larger amounts of IL-12 than the healthy controls.

**Sequencing of exons 3 and 6 of IFN-γR1 and exon 3 of IFN-γR2.** Given that the index patient secreted high levels of IFN-γ in response to MAC antigen, we hypothesized that a defect in the receptors for IFN-γ, i.e., IFN-γR1 and IFN-γR2, could explain defective immune control of mycobacteria. Partial IFN-γR1 and IFN-γR2 deficiency has been associated with late-onset mycobacterial infections (23). The most frequently described mutations have included nucleotide substitutions at positions 187 (C to T) and 260 (T to C) in exon 3 or deletions in exon 6 (position 818) for IFN-γR2 deficiency and mutations at position 114 (C to T) of exon 3 for IFN-γR1 deficiency (23, 29, 30). Sequencing of exons 3 and 6 of IFN-γR1 and exon 3 of IFN-γR2 in the index patient revealed none of these previously described mutations or deletions.

**DISCUSSION**

Isolated intracranial MAC infections are rare in the absence of obvious immunosuppression, such as HIV infection (20). Evaluation of our case with those reported previously revealed a number of features associated with intracranial MAC infections. These include the presence of sarcoidosis, intermittent steroid use, and CD4 lymphocytopenia. We noted a striking association between the reported cases of intracranial MAC and sarcoidosis. Whether some cases of sarcoidosis represent occult MAC pulmonary infections prior to the development of intracranial infection will require further study.

We contrasted the immunologic features of our case with those of a more common manifestation of MAC infection, that is, localized pulmonary MAC infection. Adequate monocyte/macrophage function is critical for the containment of mycobacterial infections. Patients with localized pulmonary MAC infections have a higher percentage of CD14<sup>dim</sup> monocytes, reflecting a greater degree of monocyte activation in vivo. This suggests an activated immune response at the baseline, which may be due to the chronic infectious process. We did not see this baseline level of activation in healthy controls, and the lack of monocyte activation in our index patient with an intracranial MAC infection suggests a defect in monocyte activation in the presence of MAC antigen. Despite the presence of ongoing monocyte activation in patients with localized pulmonary MAC infections, these individuals are unable to clear the infection from their lungs.

We show that localized pulmonary MAC infections are associated with the production of high levels of TNF-α in response to MAC but low levels of production of IFN-γ relative to those in healthy controls. There have been conflicting reports about the pathophysiology of localized pul-

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**FIG. 3.** Production of TNF-α, IFN-γ, and IL-10 by antigen-stimulated T cells. T cells from patients with pulmonary MAC infections (n = 3), an intracranial MAC infection (n = 1), and healthy controls (n = 4) were incubated with antibodies to CD3 and CD28 (a) or heat-killed MAC antigen (b and c) for 6 h in the presence of brefeldin A. The cells were harvested and stained, and cytokine expression on gated CD3-positive cells was subsequently measured by flow cytometry, as described in the text. Representative data from the index patient, a healthy volunteer, and an individual with a localized pulmonary MAC infection are shown in panel b. In panel b, the values above the boxes indicate the percentage of CD3 staining cells expressing TNF-α. In panels a and c, mean values ± standards deviation are shown. *, P < 0.01 for patients with pulmonary MAC infections compared with the results for the healthy controls; ***, P < 0.05 for patients with pulmonary MAC infections compared with the results for the healthy controls.
monary MAC infections. Previous reports support structural abnormalities of the thorax that predispose elderly patients to such infections (27, 28). However, more recent data have documented IFN-γ deficiency as an underlying etiology (17, 36). Other reports have documented a defect in IFN-γ secretion as the main factor (35, 41). IFN-γ is important in antimycobacterial host defense via macrophage activation and granuloma formation. A defect in the synthesis and, possibly, secretion of IFN-γ predisposes individuals to persistent nontuberculous mycobacterial infections and may explain the poor response to treatment and frequent relapses in these patients (25, 34). Our data also show that in response to MAC antigens, patients with pulmonary MAC infections displayed a relative IFN-γ deficiency. Comparison of the intracellular levels to the extracellular levels of IFN-γ in patients with pulmonary MAC infections also suggests that a secretory defect leading to relatively low levels of extracellular IFN-γ may also be a contributing factor. Studies have shown clinical improvement with the administration of IFN-γ in patients with pulmonary MAC infections (21), and this supports a secretory dysfunction rather than a receptor abnormality. The mechanism of this IFN-γ secretion defect has yet to be characterized. The presence however, of a potent TNF-α response suggests that TNF-α may be important in limiting the spread of a localized infection as well as contributing to overall macrophage activation.

Our patient with an intracranial MAC infection showed a selective TNF-α and IL-12 defect of T cells and monocytes in response to MAC antigen but high levels of IFN-γ. This cytokine profile is suggestive of a partial IFN-γ receptor deficiency state. The lack of IL-12 production in response to LPS or MAC antigen, despite the induction of high levels of IFN-γ, is in keeping with a failure of signaling through the IFN-γ receptor, since IFN-γ primes monocytes/macrophages to produce IL-12 (39). Previous work with patients with IFN-γ receptor deficiency showed that such mutations lead to the loss of augmentation of TNF-α production by PBMCs (24), with the result being disseminated MAC infections (11, 22). We did not, however, detect the commonly described mutations in the IFN-γR1 and IFN-γR2 genes in this individual. Further studies examining mutations elsewhere in these genes or in other genes such as stat-5 (downstream from IFN-γ signaling) or IL-12 will likely be required to further elucidate a genetic basis for the index patient’s immune abnormalities. Of note is a
recent report which also could not find any evidence of partial deficiencies of IFN-γR1 and IFN-γR2 in association with localized nontuberculous mycobacterial lung disease (26).

TNF-α deficiency leads to susceptibility to infections by M. tuberculosis and the observed lack of granuloma formation (1), the latter of which was reflected in our index patient. The lack of TNF-α induction also suggests that this cytokine plays a central role in macrophage activation and the prevention of the spread of MAC to other tissues in humans. In this regard, previous reports have indicated that humans with tuberculosis were treated with TNF-α-blocking agents, such as etanercept or infliximab (16). It is thus quite possible that the increasing use of such agents will lead to more cases involving extrapulmonary MAC infections.

Boussirotas et al. previously showed that in patients with Mycobacterium tuberculosis infections, the IL-10 production induced by M. tuberculosis induces a relative immune suppression that prevents control of the organism (5). We did not find support for enhanced IL-10 expression as a mechanism for mycobacterial persistence either in patients with localized pulmonary MAC or in the patient with the intracranial MAC infection. Of interest, however, was that in the healthy controls, activated monocytes produced large amounts of IL-10, suggesting that monocytes may have a regulatory function in the absence of an active infectious process.

The association of sarcoidosis with our case and those reported previously is interesting and requires further study. The immune profile of our case differed from that usually described (15), supporting for enhanced IL-10 expression as a mechanism for aerosol Mycobacterium tuberculosis infection, which is not compensated for by lymphotixin. J. Immunol. 162:3504–3511.


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