Inhaled IFN-γ for persistent nontuberculous mycobacterial pulmonary disease due to functional IFN-γ deficiency

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Inhaled IFN-γ for persistent nontuberculous mycobacterial pulmonary disease due to functional IFN-γ deficiency. T.S. Hallstrand, H.D. Ochs, Q. Zhu, W.C. Liles. ©ERS Journals Ltd 2004.

ABSTRACT: Pulmonary infection with nontuberculous mycobacteria (NTM) in previously healthy human immunodeficiency virus-seronegative individuals is difficult to treat. Recently, functional interferon (IFN)-γ deficiency has been identified in individuals susceptible to this disease. Treatment with inhaled IFN-γ for NTM pulmonary disease associated with functional IFN-γ deficiency has not been previously described.

In this study, the IFN-γ pathway was characterised in an individual who had progressive NTM pulmonary infection, despite appropriate multidrug antibiotic therapy, and 10 healthy controls. Levels of IFN-γ and tumour necrosis factor-α in whole blood were assessed before and after incubation with lipopolysaccharide, heat-killed Escherichia coli, heat-killed Staphylococcus aureus and phorbol myristate acetate/fomycin. The coding regions of interleukin (IL)-12, IL-18 and the IL-12 receptor were sequenced using nested primers. IFN-γRIb (100 µg-dose) was administered to the affected individual by ultrasonic nebuliser 3 days/week for 3 months.

In conclusion, inhaled interferon-γ can be effective for the treatment of nontuberculous mycobacteria pulmonary disease associated with functional interferon-γ deficiency.

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Progressive pulmonary disease caused by infection with nontuberculous mycobacteria (NTM) in human immunodeficiency virus (HIV)-seronegative patients without pre-existing structural lung disease is a well-described entity [1]. Infection with NTM, usually Mycobacterium avium complex, occurs predominantly in the right middle lobe and lingual of nonsmoking, middle-aged Caucasian females [1]. Bronchiectasis and obstructive lung disease occur as sequelae of persistent infection. The occurrence of this disorder in a well-defined population suggests an acquired or genetic deficiency in host immunity; however, the underlying molecular mechanism has not been fully delineated [2].

Interferon (IFN)-γ plays a key role in host defence against mycobacterial disease via macrophage activation [3]. Complete absence of the IFN-γ receptor impairs the ability to form mature granulomas and leads to severe disseminated mycobacterial infections in infancy [4, 5]. Low in vitro production of IFN-γ has been described in HIV-seronegative patients with NTM pulmonary disease [6, 7]. The cause of inadequate IFN-γ production in this patient population is unknown. Deficiency of the interleukin (IL)-12 receptor leads to decreased IFN-γ production by natural killer (NK) and T-cells, and mutation of this receptor has been described in systemic mycobacterial disease, with preservation of the ability to form granulomas [8]. Production of IFN-γ is also dependent on IL-18, and deficiency of IL-18 in a murine model leads to reduced granuloma formation in response to mycobacterial infection [9]. Recently, two patients were described with NTM pulmonary infection and abnormally low in vitro IFN-γ production, but normal-to-high IFN-γ staining in CD3+ T-cells, implicating defective IFN-γ secretion [10]. Since the IFN-γ receptor is unaffected in these instances in which IFN-γ production is reduced, NTM pulmonary disease may be amenable to IFN-γ replacement therapy.

In this report, the authors describe an individual who presented in late adulthood with progressive bronchiectasis and recurrent pulmonary infections caused by NTM, initially M. avium complex, followed by M. abscessus. Despite aggressive, appropriate multidrug antimicrobial treatment, the patient deteriorated clinically and continued to have high concentrations of mycobacteria in her sputum. In vitro production of IFN-γ at baseline and in response to endotoxin and bacterial antigens was reduced. Treatment of this individual with inhaled IFN-γ resulted in clinical stabilisation and clearance of mycobacteria from sputum.

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Case presentation

A 51-yr-old Caucasian female developed cough, sputum production and low-grade fevers. Six years previously, she had been treated for *M. avium* complex infection with ciprofloxacin, rifampin and ethambutol for 12 months. Prior to *M. avium* complex infection, she had no history of chronic lung disease or recurrent infections. She had a remote 10-pack-yr smoking history. A chest radiograph revealed multiple nodules throughout both lung fields, and consolidation of the left lower lobe and lingula. A computed tomography scan demonstrated peribronchial or "tree-in-bud" pattern infiltrates and widespread bronchectasis. A sputum sample revealed 3+ mucoid *Pseudomonas aeruginosa* and 4+ *M. abscessus*. After successful treatment of *P. aeruginosa* with tobramycin and ceftazidime, treatment for *M. abscessus* was initiated with cefoxitin, clarithromycin and amikacin, based on drug-sensitivity testing. Despite various three-drug treatment regimens, she continued to have high concentrations of *M. abscessus* in her sputum over 19 months of treatment and developed worsening obstructive lung disease.

Laboratory evaluation for immunodeficiency was negative, including normal serum values for total immunoglobulin (IgG) and IgG subclasses, total IgE, CH50, C3 and C4, and normal neutrophil and lymphocyte counts in peripheral blood. Sweat chloride was 8 mEq L\(^{-1}\) (normal <60 mEq L\(^{-1}\)). Genetic analysis revealed that the patient was a carrier of the ΔF508 mutation of the cystic fibrosis transmembrane conductance regulator gene. Serological testing for HIV was negative. The neutrophil oxidative burst was normal, as was T-cell proliferation to tetanus, candida and phytohemagglutinin. Antibody titres to rubella and rubeola were normal, and intradermal skin testing showed anergy to candida, mumps and trichophyton. Additional laboratory investigations were conducted to characterise the IFN-γ pathway.

Methods

In vitro whole blood cytokine assays

Venous blood samples were obtained from the affected individual and 10 healthy volunteers (five males; five females; age range 28–58 yrs). Each participant gave written informed consent and the University of Washington Human Subjects Review Committee approved the study procedures. Heparinised venous blood was diluted 1:5 in RPMI 1640 (GIBCO-Invitrogen Corp., Grand Island, NY, USA) and incubated for 18 h at 37°C in a 5% CO\(_2\)-humidified cell culture incubator with lipopolysaccharide (LPS; 100 ng mL\(^{-1}\); from *E. coli* O111:B4 (Sigma, St Louis, MO, USA)), heat-killed *E. coli* (5×10\(^{6}\) cfu mL\(^{-1}\)), heat-killed *S. aureus* (5×10\(^{6}\) cfu mL\(^{-1}\)), phorbol myristate acetate (PMA; 10 ng mL\(^{-1}\); Sigma) and ionomycin (500 ng mL\(^{-1}\); Sigma). After incubation, culture supernatants were removed for determination of IFN-γ and tumour necrosis factor (TNF-α) concentrations by cytokine-specific ELISA (Duoset paired antibody reagents; R&D Systems, Minneapolis, MN, USA).

Sequence analysis of IL-12, IL-12β1 and IL-18

Total RNA was isolated from peripheral blood mononuclear cells with Trizol (Invitrogen, Carlsbad, CA, USA). The first strand cDNA was synthesised using the Omniscript Kit (Quiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The cDNAs of IL-12p35, IL-12p40, IL-12β1 and IL-18 were amplified by polymerase chain reaction (PCR) using the following primers: IL-12p35, 5′-AAAGCAAGAGACCAGGGTCC (forward) and 5′-CCC TTCCATGTTCCTATTCC (reverse); IL-12p40, 5′-GGTT CAGGGCCATTGGGACTC (forward) and 5′-TTGGCAAC TTTGAGAGCTGGA (reverse); IL-12β1, 5′-TGAACC TCGCAGGTGGCAGA (forward) and 5′-AGGCTCGGGC GAGTCACTCA (reverse); and IL-18, 5′-GCTCGGA CAGTCAGCAAGGA (forward) and 5′-CCAGGCTAG AGGCGAATGGT (reverse). Purified PCR products were sequenced with the BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer, Foster City, CA, USA) using sets of nested primers (available on request).

IFN-γ administration

IFN-γ1b (Actimmune®; Intermune, Brisbane, CA, USA) was administered 3 days week\(^{-1}\) at a dose of 100 µg (0.5 mL) diluted in 4 mL of sterile water by intranasal nebuliser (Pari LC Plus, Monterey, CA, USA). Spirometry was performed before and 15 min after the initial dose.

Results

Table 1.—In vitro production of interferon (IFN)-γ and tumour necrosis factor (TNF)-α from the affected individual and 10 healthy control volunteers

|                     | Control\(^b\) | LPS 100 ng mL\(^{-1}\) | E. coli 5×10\(^6\) mL\(^{-1}\) | S. aureus 5×10\(^6\) mL\(^{-1}\) | PMA 10 ng mL\(^{-1}\) + ionomycin 500 ng mL\(^{-1}\) |
|---------------------|--------------|------------------------|-------------------------------|---------------------------------|-----------------------------------------------|
| IFN-γ production pg mL\(^{-1}\) |              |                        |                               |                                 |                                               |
| Controls            | 23±54        | 1938±1061              | 3401±2222                     | 2028±2056                       | 7712±2872                                     |
| (0–164)             |              | (324–3658)             | (253–7003)                    | (57–5458)                       | (183–9500)                                    |
| Patient             | ND\(^*\)     | ND                     | ND                            | ND                              | 6356                                          |
| TNF-α production pg mL\(^{-1}\) |              |                        |                               |                                 |                                               |
| Controls            | 122±178      | 5523±2749              | 12132±3798                    | 8116±3796                       | 11701±3702                                    |
| (0–444)             |              | (2043–11332)           | (3662–16179)                  | (1371–14172)                    | (5900–16631)                                  |
| Patient             | ND\(^*\)     | 5539                   | 11112                         | 2585                            | 3795                                          |

Data are presented as mean±SD (range) and n. LPS: lipopolysaccharide; *E. coli*: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*; PMA: phorbol myristate acetate; ND: none detected. \(^*\): no stimulus; \(^\ddagger\): <15 pg mL\(^{-1}\); \(^\ddagger\): <8 pg mL\(^{-1}\).
individual; inducible expression of TNF-α was similar to that of normal volunteers. Production of IFN-γ and TNF-α in response to nonspecific stimulation with PMA/ionomycin was comparable between the affected individual and normal volunteers. Sequence analysis of the coding regions of IL-12 receptor 1 was identified in a study of patients with NTM pulmonary disease [12]. Evidence of low IFN-γ production, in response to endotoxin, antigen and mitogen in patients with NTM pulmonary disease, suggests that the defect in host defence is in the pathway leading to IFN-γ production and secretion [6, 7]. The current authors characterised the receptors for IL-12 and IL-18 revealed no evidence of mutation in the affected individual.

Prior to initiation of inhaled interferon-γ, sputum cultures were persistently positive for *M. abscessus* despite appropriate multidrug antmycobacterial treatment, and lung function progressively deteriorated over a period of 20 months (fig. 1; table 2). Following initiation of inhaled IFN-γ therapy, there was rapid clearance of the organism from the airways and stabilisation of lung function. After 3 months of inhaled IFN-γ, antmycobacterial treatment was continued for an additional 9 months.

**Discussion**

A well-defined group of patients, predominantly middle-aged females without pre-existing structural lung disease or immunodeficiency, may account for the majority of pulmonary infections caused by NTM [2, 11]. Recent evidence shows that reduced production of IFN-γ by T- and NK cells may predispose these patients to mycobacterial infection [6, 7]. In this report, it is shown that pulmonary NTM infection associated with impaired *in vitro* IFN-γ production can be successfully treated with inhaled IFN-γ.

Susceptibility to NTM pulmonary disease in a well-defined group of patients suggests an innate or acquired defect in host defence. Major defects in the IFN-γ pathway, including abnormalities of the receptors for IFN-γ and IL-12, have been associated with disseminated infection caused by weakly pathogenic mycobacteria, such as bacille Calmette-Guerin and NTM [4, 5, 8]. Neither of two known mutations of the IFN-γ receptor 1 was identified in a study of patients with NTM pulmonary disease [12]. Evidence of low IFN-γ production, in response to endotoxin, antigen and mitogen in patients with NTM pulmonary disease, suggests that the defect in host defence is in the pathway leading to IFN-γ production and secretion [6, 7]. The current authors characterised the receptors for IL-12 and IL-18, which play a major role in the activation of immune cells to produce IFN-γ, and found normal sequences of these receptors, despite undetectable levels of IFN-γ in vitro levels of IFN-γ at baseline, and following stimulation with endotoxin and bacterial antigens. These results are consistent with the observation that, despite low extracellular levels of IFN-γ, intracellular levels of IFN-γ in T-cells were normal to high, suggesting an abnormality in the transport or secretion mechanism for IFN-γ [10].

Treatment of NTM pulmonary disease with multidrug antmycobacterial therapy frequently fails. In one case study, negative sputum cultures were achieved in only seven out of 24 patients over a 1-yr follow-up period, as a result of either drug intolerance or development of drug resistance [2]. Evidence of low IFN-γ production in this disease process suggests a rational role for IFN-γ replacement therapy. IFN-γ has been approved by the US Food and Drug Administration for treatment of chronic granulomatous disease and malignant osteopetrosis. Previous reports have shown that subcutaneous IFN-γ was effective in reducing or eliminating disseminated *M. avium* complex infections in both HIV- and non-HIV-infected patients [13]. Treatment of multidrug-resistant tuberculosis with aerosolised IFN-γ (500 µg 3 times-week−1) temporarily reduced mycobacterial burden [15]. Aerosolised IFN-γ (500 µg 3 times-week−1) reduced the burden of *M. avium* in a patient with pre-existing cavitary lung disease from advanced silicosis [16].

This is the first study to report the use of inhaled interferon-γ for persistent nontuberculous mycobacteria pulmonary disease in an individual with low *in vitro* production of interferon-γ. Treatment with inhaled interferon-γ was well-tolerated, and resulted in microbiological cure and clinical resolution of disease. This finding suggests that inhaled interferon-γ therapy should be considered in individuals...
with nontuberculous mycobacteria infection that progresses despite conventional treatment. Additionally, when appropriate resources are available, laboratory evaluation should be performed to identify functional interferon-γ deficiency, and interferon-γ therapy should be considered early in the treatment of affected individuals. Further studies are warranted to determine if functional interferon-γ deficiency is a common feature of individuals with progressive nontuberculous mycobacteria disease. The favourable therapeutic results of interferon-γ treatment presented in the current report should be confirmed in controlled trials of groups of patients with nontuberculous mycobacteria pulmonary disease, with and without underlying functional interferon-γ deficiency.

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