Research Article

Treatment of Disseminated Mycobacterial Infection with High-Dose IFN-γ in a Patient with IL-12RIβ1 Deficiency

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IFN-γ has been used in the treatment of IL-12RIβ1 deficiency patients with disseminated BCG infection (BCGosis), but the optimal dose to reach efficacy is not clear. We used IFN-γ in the treatment of a 2.7-year-old patient with IL-12RIβ1 deficiency and refractory BCG-osis. IFNγ was started at a dose of 50 μg/m² 3 times per week. The dose was upgraded to 100 mcg/m² after 3 months, then to 200 mcg/m² 6 months afterwards. Serum mycobactericidal activity and lymphocytes number and function were evaluated throughout the study. There was no clinical response to IFN-γ with 50 or 100 μg/m² doses. However, there was some response to the 200 μg/m² dose with no additional adverse effects. The serum mycobactericidal activity was not significantly different during the whole treatment period. Lymphocytes proliferation in response to PHA was significantly higher after 3 months of using the highest dose as compared to the lowest dose. The tuberculin skin test reaction remained persistently negative. We conclude that in a patient with IL-12RIβ1 deficiency, IFN-γ at a dose of 200 μg/m², but not at lower dosages, was found to have a noticeable clinical effect with no additional adverse effects.

1. Introduction

Investigation of a human syndrome known as Mendelian susceptibility to mycobacterial diseases (MSMD) (OMIM 209950) has, in the past 15 years, led to the identification of a series of genetic defects in the IL-12/IFN-γ axis. These include defects in three autosomal genes controlling the response to IFN-γ: IFNGR1, encoding the ligand binding, first chain of the IFN-γ receptor; IFNGR2, encoding the signaling, second chain of the IFN-γ receptor; STAT1, encoding the signal transducer and activator of transcription 1 downstream from the IFN-γ receptor. They also include defects in two other autosomal genes controlling the production of IFN-γ: IL12B, encoding IL-12p40 shared by IL-12 and IL-23; IL12RB1, encoding the first chain of the IL-12 and IL-23 receptor (IL-12RIβ1). In addition, there is one X-linked gene that encodes nuclear factor-kB essential modulator (NEMO) [1–3].

IL-12RIβ1 defect was first described in 1998 [4] and is the most common among the known genetic disorders that predispose to mycobacterial infections. The spectrum of infections reported in these patients is, however, surprisingly narrow. These patients display selective susceptibility to weakly virulent Mycobacteria, such as environmental mycobacteria (EM) and live bacille Calmette-Guérin (BCG) vaccine (an attenuated substrain of Mycobacterium bovis), and Salmonella. They showed almost no increased susceptibility to other pathogens including other bacteria
and ubiquitously distributed viruses and fungi [5]. Evidence suggests that the immune system is redundant in its response to many intracellular microorganisms. For other organisms like varicella zoster virus, Th1 activation and IFN-γ production was found to be stimulated by IFN-α not IL-12 [6]. In a very recent report, that included the largest cohort of patients with IL-12Rβ1 deficiency (141 patients) [7], the mean age of onset of the first infection was 2.4 years. The severity of the disease varied significantly from subjects who were asymptomatic until adulthood to patients who died in early childhood from complications of the disease. The mortality rate was 32% among symptomatic subjects and the mean age of death was 7.5 years, mostly secondary to BC Go sis or EM disease. In patients with IL-12Rβ1 deficiency who do not respond to prolonged therapy with multiple anti-mycobacterial drugs, the mycobacteria frequently develop resistance to some of these medications. The management of those patients subsequently becomes very difficult and frequently they will not recover using antimycobacterials alone. Since the most important reason of those patients’ failure to control mycobacterial infections is the inability of their T cells and NK cells to produce IFN-γ in response to IL-12 stimulation, IFN-γ is an attractive candidate therapy that can be tried in those patients [8]. However, the optimal dose and duration of using IFN-γ are not clear.

2. Subject and Methods

2.1. Study Patient. Our patient was referred to us at 6 months of age with left axillary lymphadenitis that grew the vaccine strain of Mycobacterium bovis (BCG), a routine vaccine given in day one of life in Saudi Arabia. There was no granuloma formation on the lymph node biopsy. She was otherwise well apart from intermittent fever and night sweats. Her parents were not consanguineous but from the same tribe. She had a 5-years-old brother who had left axillary BCGitis in infancy that resolved promptly after treatment with isoniazid alone for 6 months. She also had a 7-years-old sister who has been completely healthy. She did not respond to isoniazide and rifampicin. Sensitivity results then showed that the organism was resistant to both, so she was started on ethambutol 300 mg OD, cycloserine 250 mg OD, and moxifloxacin 200 mg OD for which the organism was sensitive. Meanwhile, immunological investigations revealed that our patient’s lymphocytes have no IFN-γ production in response to IL-12+BCG as compared to controls (those investigations were performed in Dr. Casanova’s lab in Paris. Her parents were travel controls). Her brother had a similar cellular phenotype (i.e., no IFN-γ production after BCG and BCG+IL-12 stimulation in whole-blood assay) (Table 1). In addition, no cell surface-expressed IL12RB1 could be detected from their PHA-T cell blast (data not shown). Genetic testing confirmed that she and her brother have homozgyous 1336delC mutation in the IL12RB1 gene, leading to complete IL12Rβ1 deficiency. Her sister was functionally and genetically normal. To our knowledge this is the only family with this specific type of mutation to be reported [7].

2.2. Study Plan. After parental consent, baseline and follow-up investigations were obtained including CBC, ESR, liver enzymes, lymphocytes subsets, and tuberculin skin test (TST) every 3 months. Immunoglobulin levels, serum mycobactericidal activity, and lymphocytes proliferation in vitro were performed every 6 months.

The patient was seen in the clinic every 6 weeks. IFN-γ-1b (Imukin, Boehringer Ingelheim) was started at a dose of 50 μg/m2 subcutaneously 3 times/week. If there is no significant response within 3 months the dose will be doubled to 100 mcg/m2, at the same frequency.

2.3. Cellular Studies. Blood samples were taken from the patient before starting treatment with IFNγ (S1), 6 months (S2) and 12 months (S3) after starting treatment. PBMCs were isolated from whole blood by density centrifugation (Lymphoprep, Nycomed, Oslo, Norway) and assayed for in vitro proliferative responses by the thymidine incorporation method against phytohemagglutinin (PHA) (Sigma, St. Louis, Mo.) and IL2 (R&D System, Abington, UK).

The proliferation results are expressed as mean count per minute of triplicate cultures for the antigen concentration giving maximum response minus the mean count-per-minute values for 12 wells without antigen (medium only).

2.4. Serum Mycobactericidal Assay. Two specimens of the patient’s serum were collected at 6 months and 12 months of the study period, 2 hours after anti-mycobacterial drug administration, to determine the serum inhibitory and bactericidal titers against the patient’s mycobacterial isolate as well as mycobacterium bovis BCG vaccine strain (Statens Serum Institute, Copenhagen S, Denmark) and mycobacterium tuberculosis H37RV reference strain (ATCC, Atlanta, GA, USA), as previously described [9].

3. Results

Our patient was started on IFN-γ at a dose of 50 μg/m2 three times a week in addition to her anti-mycobacterial medications as above (Figure 1). Three months later, the patient’s condition was slightly worse, so ethionamide 250 mg OD was added to her drug regimen and IFN-γ dose was upgraded.
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Figure 1: Time line of the patient’s course. Above are the changes in medications and below are the changes in clinical condition from the beginning of IFN-γ introductions till the patients’ death 15 months later.

to 100 μg/m² three times a week. Six months from the beginning of the study her clinical condition continued to worsen and she developed a left chest wall abscess. At that time amikacin was added to her drug regimen for 6 weeks only. She was continued on the same dose of IFN-γ. Nine months from the beginning of the study there was no clinical improvement. She was on four anti-mycobacterial medications as mentioned above. At this point the dose was upgraded to 200 μg/m². One month later the enlarged lymph nodes and the chest wall abscess started to discharge pus that was positive for acid-fast bacilli on ZN stain and the culture grew *Mycobacterium bovis*. The patient felt better and the abscesses and the discharging lymph nodes healed. One month later she developed right pleural effusion, but remained clinically stable. Cultures from the effusion were negative. After 12 months from the beginning of the study we ran out of IFN-γ and the patient continued on her usual anti-mycobacterial medications. She then started to deteriorate gradually. Three months later she developed massive pneumonia and died. The only adverse effect from IFN-γ therapy that was noted throughout the study at the different doses was fever (up to 39°C) and lethargy up to 8 hours from giving the injection. Her CBC, liver enzymes, lymphocyte subsets, and immunoglobulin levels were not significantly changed and her ESR fluctuated between 80–107 mm/hr throughout the study period.

The spectrum of proliferative responses of T cell following stimulation with PHA and IL-2 is shown in Figure 2. The percent increase of T cell proliferation in response to PHA (1:10 dilution) rose from 17% at baseline to 26% at 6 months of the study, reaching 32% at 12 months (P = .02) after increasing the dose of IFN-γ to 200 μg/m². There was, however, no change after IL-2 stimulation, where percent increase of T cell proliferation was 20%, 18%, and 17% at baseline, 6 months, and 12 months, respectively.

There was no significant difference between the serum mycobactericidal activity at 6 months and at 12 months.

4. Discussion

Our patient had a severe phenotype of IL-12Rβ1 deficiency. In addition, the organism was resistant to anti-BCG antibiotics. Therefore, it was more stringent to try IFN-γ as a therapeutic agent. IFN-γ was tried initially at a dose of 50 μg/m² 3 times a week based on the experience of using it in other conditions like chronic granulomatous disease (CGD) [10]. However, it was not surprising that it did not work at this concentration with our patient since, unlike patients with CGD, she is not able to produce IFN-γ and the pathophysiology of the disease is completely different. The clinical effect noted after using the high-dose IFN-γ
In conclusion, in one patient with IL-12Rβ1 deficiency, IFN-γ at a dose of 200 μg/m², but not at lower dosages, was found to have a positive clinical effect with no additional adverse effects. This medication holds promise in the management of such patients especially if used early in the course of disease. Multicenter studies are needed to establish the effectiveness, dose, and duration of IFN-γ treatment on a large number of patients.

Conflict of Interest
The authors declare no conflict of interest.

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