Immunotherapy with interferon-\(\gamma\) and multidrug therapy for multibacillary leprosy

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Abstract

Treatment for multibacillary leprosy is presently performed with a multidrug therapy (MDT) scheme maintained for 2 years. Leprosy treatment however can benefit from the reduction of length. The lack of interferon-\(\gamma\) (IFN-\(\gamma\)) production by lepromatous leprosy (LL) patients’ lymphocytes lead us to use this cytokine in the treatment of multibacillary leprosy associated with MDT in the treatment of multibacillary leprosy, and monitor several clinical and immunological parameters during the course of treatment. A total of 20 multibacillary leprosy patients were evaluated, 10 treated with MDT alone, and 10 treated with MDT + 10 daily doses of \(2 \times 10^6\) international units (IU) of recombinant human IFN-\(\gamma\):\(m^2\) followed by 10 daily doses of \(10^7\) IU IFN-\(\gamma\):\(m^2\), intramuscularly, during the first 20 days of MDT. IFN-\(\gamma\) was well tolerated and did not cause any increase in the rate of leprosy reactions development during treatment. Decrease of bacillary load, fall of anti-\(Mycobacterium leprae\) IgG serum antibodies, changes of histological pattern, as well as changes in lymphocyte proliferation assay in response to mitogens (PHA or PWM), \(M. leprae\) antigen or PPD was similar in both groups of patients. Among several soluble immunological markers measured before and 30 days after beginning of treatment, levels of soluble IL-2R receptor increased in patients treated with MDT plus IFN-\(\gamma\) whereas decreased in patients

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treated with MDT alone. Soluble ICAM-1 levels decreased in the MDT group but did not change in the MDT + IFN-γ treated patients. Soluble CD4 and soluble CD8 markers did not change significantly in either group of patients. Neopterin, a marker of macrophage activation, increased in all but one patient treated with MDT + IFN-γ but in none treated with MDT alone, indicating that IFN-γ was active in vivo. Our findings indicate that despite being able to promote macrophage activation in multibacillary leprosy patients a short course of systemically administered IFN-γ is not able to change the clinical course of a long standing disease such as leprosy. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Leprosy; Multibacillary leprosy; Interferon-γ; Multi-drug therapy; Immunochemotherapy

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### 1. Introduction

Leprosy is well known as a long standing disease that has a wide range of clinical manifestations, varying from a localized and limited form (the tuberculoid pole) to a generalized, intense and diffuse form (the lepromatous pole). All these spectral clinical manifestations have a close relationship with bacillary load and with the immunological response of the host. Since *Mycobacterium leprae* is an intracellular bacterium, cellular immune reactions resulting in macrophage activation are considered to be essential for protective immunity and resistance. Interferon-gamma (IFN-γ), is known to activate macrophages to kill intracellular microorganisms by an enhanced production of toxic reactive oxygen intermediates, and other substances (Nathan et al., 1983). IFN-γ also influences the production of interleukin (IL)-12 and IL-10 by macrophages (Libraty et al., 1997), which may be important for a sustained maintenance of a Th1-type of environment.

In lepromatous leprosy (LL) there is a well documented selective anergy of the T-cell response to *M. leprae* antigens. The T cells do not proliferate and have decreased cytokine production in response to mycobacterial antigens (Kaplan et al., 1985). Additionally, while mRNAs encoding for IL-2 and IFN-γ are most evident in the tuberculoid granulomas, configuring a Th1 cytokine profile, in lepromatous granulomas mRNAs for IL-4, IL-5 and IL-10 predominate (Yamamura et al., 1991).

Treatment for multibacillary leprosy is presently conveniently performed with a multidrug therapy (MDT) scheme maintained for two years. Leprosy treatment however can benefit from the reduction of length, and ideally by the possibility of killing ‘persistent’ organisms. The lack of IFN-γ production by LL patients’ lymphocytes prompted investigators to use this cytokine in the treatment of multibacillary leprosy (Nathan et al., 1986; Kaplan et al., 1989; Nathan et al., 1990; Botasso et al., 1992; Damasco et al., 1992; Sampaio et al., 1992; SivaSai et al., 1993; Sampaio et al., 1996). In this study we evaluated the use of IFN-γ, associated with MDT, in the treatment of multibacillary leprosy, and monitored several clinical and immunological parameters during the course of treatment.
2. Material and methods

2.1. Patient selection

A total of 21 newly-diagnosed leprosy patients were enrolled in the study, and they were randomly assigned to either treatment with standard MDT therapy, or to treatment with a combination of MDT plus IFN-\(\gamma\).

2.1.1. Inclusion criteria

Clinical diagnosis of LL or BB, with histopathological confirmation; negative DTH for \textit{M. leprae} (Mitsuda), normal hematological and biochemical blood parameters; no previous treatment for leprosy.

2.1.2. Exclusion criteria

Patients exhibiting leprosy reactions at the time of enrollment, pregnant women, presence of other associated disease, unwillingness to participate. All patients signed an informed consent before enrollment in the study. This study has been approved by the Ethics Committee of the University of Bahia Hospital.

2.2. Treatment

MDT: rifampicin 600 mg once a month under supervision, together with auto-administered Dapsone 100 mg/day and Clofazimine 50 mg/day; MDT + rhIFN-\(\gamma\): MDT as above plus 10 daily doses of \(2 \times 10^6\) international units (IU) recombinant human (rh) IFN-\(\gamma\)/m\(^2\) of body surface followed by 10 daily doses of \(10 \times 10^6\) IU rhIFN-\(\gamma\)/m\(^2\), given intra-muscularly, during the first 20 days of MDT. rhIFN-\(\gamma\) was a generous gift of Institute Roussel Uclaf (Paris, France).

2.3. Follow-up

Pre-treatment examination comprised hematological (Ht, Hb and platelet count) and biochemical profile, lesion biopsy, bacillary index, serum antibody levels and peripheral blood mononuclear cell reactivity (proliferation and IFN-\(\gamma\) production) as well as several serum soluble markers of immune response, as well as neopterin levels were measured. On day 10 of treatment hematological and biochemical profiles were evaluated; on day 30 of treatment hematological and biochemical profiles, biopsy, bacillary index, antibody and cellular reactivity evaluation were performed, and on day 60 the biopsy, bacillary index, antibody and cell mediated-immunity evaluation were repeated. Hematological and biochemical profiles, biopsy, bacillary index, antibody and cell mediated-immunity evaluation were also performed at days 90, and 180 of treatment. Patients remained under clinical observation at monthly visits, during their period of treatment. A biopsy was performed 1 year after the beginning of treatment.
2.4. Bacillary index

Material was obtained from six skin lesion sites (including ear lobes) previous to treatment and at days 30, 60, 90 and 180 following therapy. The smear was stained by modified Ziehl-Neelsen and scored blinded from 0 to 6+ in a log scale (Ridley and Jopling, 1966).

2.5. Antigens

Soluble and whole \textit{M. leprae} antigen were kindly provided by Dr R.J.W. Rees (under the TDR/IMMLEP programme). Antigen solution was prepared in RPMI 1640 (GIBCO, Grand island, NY).

2.6. Lymphocyte proliferation test

Peripheral blood mononuclear cells (PBMC) were obtained by gradient centrifugation (lymphocyte separation medium, Bionetics Laboratories, Kensington, MD) and resuspended in RPMI 1640 supplemented with HEPES (10 mM), L-glutamine (2 mM), penicillin (200 IU/ml), streptomycin (100 µg/ml) and 10% normal human AB serum (RPMI-10H). Cells were seeded in triplicate in flat-bottom 96-well plates (Falcon, Lincoln Park, NJ) at $10^5$ (200 µl)/well, and kept unstimulated or stimulated with Concanavalin A (Con A, Sigma, St. Louis, MO) at 10 µg/ml, Phytohemagglutinin (PHA, GIBCO) at 1:100, pokeweed mitogen (PWM, GIBCO), or with the following antigens PPD (Squibb-Connaught, ONT, Canada) at 2 µg/ml, tetanus toxoid (Wyeth-Ayerst, Marietta, PA) at 1 Lf or sonicated \textit{M. leprae} antigen at 20 µg/ml. After stimulation cells were kept at 37°C in a humid atmosphere with 5% CO$_2$, for 4 days when stimulated with Con A or PHA, and for 6 days when stimulated with PWM or antigens. $^3$H-methyl-thymidine ($^3$H-TdR, 6.7 Ci/mmol, DuPont, NEN Research Products, Boston, MA) at 1 µCi/well was added in the last 6 h of culture, and cells harvested and processed for $^3$H-TdR incorporation by liquid scintillation.

2.7. Cytokine production

For the evaluation of cytokine production, PBMC were adjusted to $3 \times 10^6$ cells/ml in RPMI-10H and stimulated with Con A (10 µg/ml), \textit{M. leprae} antigen (20 µg/ml) or kept without stimulation for a period of 48 h, at 37°C in a humid atmosphere 95% oxygen and 5% CO$_2$. After incubation the cell-free supernatant was obtained, filtered and stored at $-20°C$ for future determinations. Production of supernatants was performed at day 180 of therapy.

2.8. Interferon-$\gamma$ determination

IFN-$\gamma$ was evaluated by ELISA, using microtiter wells (Nunc-Immuno module, Kamstrup, Denmark) coated with a monoclonal antibody anti-human IFN-$\gamma$
blocking of free sites was performed by incubation with 5% free-fat milk in PBS containing 0.01% Tween-20 (incubation buffer). After extensive washings, IFN-γ standards and samples were incubated for 1 h at 37°C under agitation. Following another washing cycle, a rabbit anti-human IFN-γ antibody (diluted 1:2000 in PBS-Tween with 5% low fat milk) was incubated for 1 h at 37°C. After further washing, the wells received alkaline phosphatase-conjugated goat anti-rabbit IgG (Fisher Biotech, Pittsburgh, PA), at 1:1000 in incubation buffer, for 1 h at room temperature (RT). Substrate consisted of tablets of p-nitrophenylphosphate (Sigma) at 1 tablet per 5 ml of 0.06 M carbonate buffer pH 9.6 plus MgCl₂, and was incubated for 45 min at RT. Optical reading was performed using a 405 nm filter. Values of unknown samples were interpolated from the standard curve obtained with recombinant IFN-γ standards ranging from 20 pg to 2.5 ng/ml.

2.9. Determination of serum soluble markers

Evaluation of levels of soluble CD4 (sCD4), soluble CD8 (sCD8), soluble ICAM-1 (sICAM) and soluble IL-2 receptor (sIL-2R) was performed in sera obtained at days 0 and 30 of treatment using ELISA kits (Cellfree, T Cell Diagnostics, Cambridge, MA). sCD4 had a minimal detection level of 12 U/ml and the mean for controls is 33 U/ml. sCD8 had minimal detection threshold of 50 U/ml and normal mean of 336 U/ml. For sICAM and sIL-2R detection limits were 0.3 ng/ml and 50 U/ml, with mean for controls of 3.04 ng/ml and 573 U/ml, respectively.

2.10. Determination of neopterin levels

Neopterin was evaluated in the sera of patients at days 0 and 30 of treatment, using a commercial radioimmunoassay (Henning Berlin GMBH, IMMUtest Neopterin, Berlin, Germany). Standard curve was performed in the range of 0–729 nmol/l.

2.11. Anti-Mycobacterium leprae IgG antibodies

An ELISA test was performed using microtiter plates (Nunc, Kamstrup, Denmark) coated with M. leprae soluble antigen (1 µg/ml in carbonate-bicarbonate buffer, pH 9.6) for 18 h at 4°C in a humid chamber. Serial dilutions of the tested sera (100 µl/well in incubation buffer) were incubated for 1 h at 37°C. This step was followed by incubation with alkaline phosphatase-conjugated anti human IgG (Sigma) at 1:1000 in incubation buffer, for 1 h at 37°C. Blocking of free sites, and washings, substrate incubation and reading was performed as described above. Titers were determined by the highest dilution above the mean plus two standard deviations of values obtained with normal sera.
2.12. Histological examination

Biopsies were obtained at days 0, 30, 60 and 360 of therapy, fixed in neutral formalin and processed for histological examination. Sections were stained by Hematoxylin-Eosin or by Fite-Faraco for bacilli staining. Evaluation of each biopsy took in account the presence and area of the lepromatous pattern (a monotonous infiltration of foamy macrophages full of acid-fast bacilli-AFB), the bacillary index and aspect of AFB (uniformly stained or granular), as well as the presence of inflammatory infiltrate of lymphocytes and plasma cells and the presence of epithelioid cell granulomas. Epithelioid granuloma is considered here as a chronic lesion containing epithelioid mononuclear cells and/or multinucleated giant cells. LL is diagnosed by the finding of the lepromatous pattern. For the diagnosis of borderline leprosy (BB) or mid-borderline leprosy the finding of foamy macrophages as well as epithelioid cells was required, with AFB in moderate numbers. In order to better evaluate the histological improvement, biopsies were always compared to the first biopsy of the same patient. Criteria for histological improvement were: disappearance or reduction of the lepromatous pattern; reduction in the bacillary index or appearance of granular AFB; increase of the infiltration of lymphocytes and plasma cells; appearance of epithelioid cell granulomatous reaction. In biopsies from BB cases, the presence of epithelioid granulomas were not considered as an aspect of improvement. Results are expressed as: no improvement (Without modification or ten times reduction of bacillary number); light improvement (≤ 50% reduction of the lepromatous pattern, with ≥ 50% of bacilli being uniformly stained); moderate improvement (> 50% reduction of the lepromatous pattern with > 50% of bacilli with a granular pattern); and marked improvement (disappearance of lepromatous pattern, and no uniformly stained bacilli or no visible bacilli and or appearance of granulomas). Histological grading of improvement was performed blindly by the histopathologist without knowledge of the clinical evolution of the patients.

2.13. Statistical treatment

Comparisons were always made using nonparametric tests. Wilcoxon’s test was used for comparison of levels of serum soluble markers in the same patients at different time points. Mann–Whitney tests were used for comparison between the two groups of patients. For evaluation of the evolution of antibody titers the slope of the curves corresponding to days 0, 30, 90 and 180 of treatment were obtained individually for each patient by the best fit approach. Medians of the two groups were compared by Mann–Whitney test.

3. Results

3.1. Clinical data

In one patient the study was interrupted due to severe leprosy reaction at the
beginning of the treatment. A total of 20 patients had a complete follow-up, and their characteristics are shown in Table 1. Distribution of LL and BL was similar in both groups, although the number of LL cases was greater in the group treated with MDT + IFN-γ. Clinical evolution was similar in both groups, with similar rates of clinical improvement.

Despite the high frequency of leprosy reactions in the study there was no particular relation of such finding with rhIFN-γ treatment. Frequency of reactions was similar in both groups (Table 1). Although with a high frequency reactions were clinically mild, except for two cases, [one who developed neuritis following reversal reaction (RR), and another who had a long Erythema Nodosum Leprosum (ENL)] both from the group treated with MDT alone.

3.2. Bacillary load

Rate of decrease of bacillary load was similar in the groups treated with MDT alone or MDT + IFN-γ (Table 2). After one year of treatment the mean bacillary index in the group treated with MDT was even lower than the mean index of the MDT + IFN-γ group. Such a difference however was due to a single patient and was not statistically significant.

3.3. Anti-"Mycobacterium leprae" antibody titers

Since bacillary load determined in skin smears are subjected to variability due to site sampling, we serially evaluated anti-"M. leprae" IgG antibody levels, using the decline in titers as an indirect measure of bacillary load. Rate of decrease of anti-"M. leprae" IgG antibody titers was calculated by the slope of the line linking the titers obtained at days 0, 30, 90 and 180 of treatment. Median slope was $-0.195$ (range $0.624$ to $-1.101$) for the MDT group, and of $-0.416$ ($0.254$ to $-1.912$) for the MDT + IFN-γ group. The difference between the two groups was not statistically significant.

Table 1
Characteristics of patients at study entry, and development of leprosy reactions

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Sex</th>
<th>Age (years)*</th>
<th>Presentation</th>
<th>Reactions^</th>
<th>LL^</th>
<th>BL^</th>
<th>RR^</th>
<th>ENL^</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDT</td>
<td>10</td>
<td>8M:2F</td>
<td>23.9 (10–57)</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDT + IFN-γ</td>
<td>10</td>
<td>6M:4F</td>
<td>30.5 (13–57)</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean (range).
^ Number of patients who developed leprosy reactions during the first 6 months of therapy.
^ Lepromatous leprosy.
^ Borderline lepromatous.
^ Reversal reaction.
^ Erythema Nodosum Leprosum.
Table 2
Median and percentile scores of bacillary index in multibacillary leprosy patients treated with MDT alone or MDT combined with IFN-γ

<table>
<thead>
<tr>
<th>Days after Rx</th>
<th>MDT 25th percentile</th>
<th>MDT Median</th>
<th>MDT 75th percentile</th>
<th>MDT+IFN-γ 25th percentile</th>
<th>MDT+IFN-γ Median</th>
<th>MDT+IFN-γ 75th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>4.1</td>
<td>4.3</td>
<td>4</td>
<td>4.35</td>
<td>4.6</td>
</tr>
<tr>
<td>22</td>
<td>3.9</td>
<td>4</td>
<td>4.8</td>
<td>4.1</td>
<td>4.35</td>
<td>4.6</td>
</tr>
<tr>
<td>60</td>
<td>3.7</td>
<td>4</td>
<td>4.6</td>
<td>4</td>
<td>4.15</td>
<td>4.3</td>
</tr>
<tr>
<td>90</td>
<td>3</td>
<td>4.05</td>
<td>4.3</td>
<td>3.8</td>
<td>4.05</td>
<td>4.2</td>
</tr>
<tr>
<td>180</td>
<td>3.3</td>
<td>3.5</td>
<td>4.1</td>
<td>3.8</td>
<td>4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

3.4. Histological alterations

There was no histological difference between patients from MDT or MDT + IFN-γ groups of treatment. Rate and percentage of histological improvement was similar in both groups at all time periods it was evaluated (Table 3). At days 30 and 60 after beginning of therapy most patients were exhibiting no or a very modest improvement of their histological pattern, at day 360 however all but one patient from the MDT group had a moderate or marked histological improvement. The presence of granulomas was observed in few patients, and mononuclear cell inflammatory infiltration was observed at the beginning of treatment subsiding thereafter. No differences in these two indications of cell-mediated immunity were observed between the MDT and MDT + IFN-γ groups of patients.

3.5. Lymphocyte proliferation

Lymphoproliferative responses to mitogens (PHA or PWM) or to antigens (PPD or M. leprae antigen) of patients treated with MDT or MDT + IFN-γ at different time points during therapy are shown in Fig. 1. Proliferative responses to mitogens were preserved during the disease and did not change significantly with therapy (Fig. 1, upper panel). No response to M. leprae antigen was observed in any patient regardless of treatment group at any period of observation (Fig. 1, lower panel). In order to evaluate the integrity of the antigen it was tested in a few patients with tuberculoid leprosy and stimulated potent lymphocyte proliferative responses (14 730 ± 266 CPM when used at 4 μg/ml). Responses to PPD increased during the period of treatment in the MDT + IFN-γ group, and did not change significantly in the MDT group (Fig. 1, lower panel).

3.6. In vitro Interferon-γ production

PBMC from patients treated with MDT or MDT + IFN-γ were obtained 180 days after stimulated with Con A or M. leprae antigen, and supernatants were
collected at 48 h to evaluate production of IFN-γ by PBMC. IFN-γ production was higher in patients treated with MDT + IFN-γ than in patients treated with MDT alone (Fig. 2). Levels of IFN-γ following M. leprae antigen stimulation obtained from LL patients were lower than those obtained when cells from TT patients were stimulated (Fig. 2). IFN-γ production by cells from LL patients were compromised even in response to stimulation by Con A, since it was lower than the levels obtained from TT patients or normal volunteers cells.

3.7. Serum immunological markers

Soluble markers related to lymphocytes obtained at days 0 and 30 of treatment are shown in Fig. 3. Levels of soluble IL-2R (Fig. 3, upper left) slightly but significantly decreased in the group treated with MDT (Z = −2.014, P = 0.044), whereas increased in the same period in the group treated with MDT + IFN-γ (Z = 2.66, P = 0.0008). Levels of ICAM-1 (Fig. 3, upper right) decreased in the MDT group (Z = −2.52, P = 0.012) and did not change in the MDT + IFN-γ group (Z = −1.72, P = 0.086). As for soluble CD8 levels (Fig. 3, lower left) although there was no significant change in the MDT group (Z = −0.178, P = 0.0859) there was a significant increase in the MDT + IFN-γ group (Z = 2.5, P = 0.011). Soluble CD4 levels (Fig. 3, lower right) did not change significantly neither in the MDT (Z = −0.21, P = 0.83) nor in the MDT + IFN-γ group (Z = 0, P = 1.0).

Table 3
Histological improvement in the biopsies from multibacillary patients treated with MDT alone or combined with IFN-γ at 30, 60 or 360 days following beginning of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Histological improvement</th>
<th>Appearance of granuloma</th>
<th>Increase of inflam. inflt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDT + IFN</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDT</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDT + IFN</td>
<td>9</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDT</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>360 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDT + IFN</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>MDT</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Histological improvement always referred to the first biopsy, as per the following code: 0 = no improvement or very light improvement = 10× reduction of bacilli number; +, light improvement = ≤50% reduction of the lepromatous pattern (LP), ≥50% of bacilli being uniformly stained; ++, moderate improvement = >50% reduction of the LP with >50% of granular bacilli; ++++, marked improvement = disappearance of the LP, no uniformly stained bacilli, or no visible bacilli.
3.8. Neopterin levels

Neopterin, a marker of macrophage activation, increased in all but one patient treated with MDT + IFN-γ (Fig. 4), and this difference was statistically significant ($Z = 2.8$, $P = 0.005$). However, no increase was observed in the patients treated with MDT alone (Fig. 4, $Z = −1.1$, $P = 0.28$).

4. Discussion

In the present study the use of systemically administered IFN-γ in multibacillary leprosy patients, in addition to standard MDT, did not change significantly clinical
Fig. 2. In vitro IFN-γ production. Mean levels of IFN-γ (pg/ml) measured by ELISA in 48 h supernatants of PBMC from multibacillary leprosy patients treated with MDT alone or MDT + IFN-γ, upon stimulation with Con A at 10 μg/ml (dotted bars) or M. leprae antigen at 20 μg/ml (open bars). Levels obtained from normal volunteers or patients with tuberculoid leprosy under the same conditions are shown for comparison.

or bacteriological parameters. Patients treated with MDT alone or MDT + IFN-γ had similar improvement of their clinical characteristics. Alteration of the histological pictures of lesions was very similar in both groups of patients, evaluated by the reduction of the lepromatous pattern and number and staining characteristics of

Fig. 3. Serum lymphocyte soluble markers. Variation of levels of soluble IL-2 receptor (sIL-2R), soluble ICAM-1, soluble CD8 or soluble CD4 between days 0 and 30 of treatment are shown for multibacillary leprosy patients treated with MDT alone or MDT + IFN-γ. Levels were determined by ELISA. Each point represents a single patient and line links pre- to 30-day individual levels.
AFB. Reduction rate in the bacillary load as measured by evaluation of the skin slit smear was also similar in both groups of patients, a finding that was paralleled by a similar decrease in the IgG anti-\textit{M. leprae} antibody titers.

IFN-\gamma has been used together with MDT in the treatment of lepromatous leprosy. Several studies have used intradermal applications of the product, and evaluated short term histological (Nathan et al., 1986; Botasso et al., 1992), immunological (Nathan et al., 1986; SivaSai et al., 1993) or microbial (Damasco et al., 1992) changes, mostly the in very same or in a lesion close to the point of application. Ten times reduction of bacilli and the presence of granulomas 6 months after 4–6 intradermal injections of IFN-\gamma has been described (Kaplan et al., 1989). Such reductions were observed in 8 out of 9 patients comparing 3 weeks to 6 month biopsies from the site of IFN-\gamma injection. This beneficial effect seemed to be localized since biopsies taken at random sites did not show similar aspects, exhibiting typical features of a LL lesion. In one study which used intramuscular administration of IFN-\gamma the accumulation of lymphocytes and monocytes was observed at lesions distant to the site of application and persisted for 8 weeks (Nathan et al., 1990). In this study, however the evaluation was restricted to the aspects of the biopsies and no information is given on the clinical improvement of the patients. In the present study we have also observed a 10 times reduction in bacilli number in skin smears in a period of 6 months in patients treated with MDT + IFN-\gamma, but a similar decrease was also observed in the patients treated with MDT alone. Marked improvement, characterized by extensive reduction of the lepromatous pattern and reduction of bacilli number, has also been observed in the biopsies in the 6 months biopsies of both groups of patients. Additionally, granulomas and mononuclear cell infiltration, markers of cell-mediated immunity,
appeared in some patients in both groups. The appearance of granulomas in patients treated with MDT alone shows the importance of a comparative study in the evaluation of the effects of IFN-\(\gamma\) in the treatment of lepromatous leprosy.

Elevation in the frequency of appearance of ENL has been associated to prolonged treatment with IFN-\(\gamma\), with a frequency of 60% in the group treated with IFN-\(\gamma\) as compared to 15% in patients treated with MDT (Sampaio et al., 1992). In the present study the rate of ENL was not above the level observed in multibacillary leprosy patients undergoing MDT therapy, but there were a very large number of patients developing reversal reaction. This phenomenon is clearly unrelated to IFN-\(\gamma\) therapy, since five cases were observed in the group treated with MDT alone and two in the group with combined therapy. Such a frequency of reversal reaction is well above the rate observed among the multibacillary leprosy patients under MDT therapy in our outpatient leprosy clinic. The association of thalidomide to a combined IFN-\(\gamma\) + MDT therapy led to a reduced frequency of IFN-\(\gamma\)-induced ENL, but also abrogated the observed effect of reducing bacterial load (Sampaio et al., 1996).

The in vivo activity of the IFN-\(\gamma\) used in our patients is confirmed by the increased serum levels of Neopterin in the absence of a similar increase in the group treated with MDT alone. Further evidence of the in vivo activity of the IFN-\(\gamma\) preparation is the alteration of the serum levels of immune markers. Patients treated with MDT + IFN-\(\gamma\) exhibited increased levels of soluble IL-2 receptor and soluble ICAM-1 levels. Neopterin is a pteridine produced almost exclusively by monocytes/macrophages in response to stimulation by IFN-\(\gamma\) (Fuchs et al., 1991; Henderson et al., 1991; Landmann et al., 1992). Determination of neopterin levels has been used as a marker of active cell-mediated immunity (CMI) in several diseases (Brown et al., 1990; Magalini et al., 1991; Fuchs et al., 1992; Tilz et al., 1992; Ayehunie et al., 1993; Zerlotti et al., 1994). Despite this indication, patients treated with IFN-\(\gamma\) did not sustain a CMI response against \(M.\ leprae\). They did not exhibit a lymphocyte proliferative response against \(M.\ leprae\) antigen, nor their lymphocytes produced IFN-\(\gamma\) upon mycobacterial antigen stimulation.

The concentrations of different soluble molecules have been employed as a safe and rapid means for the prediction and continuous assessment of therapeutic response in several disorders (Ho et al., 1989; Pui et al., 1989; Rubin and Nelson, 1990; Barral-Netto et al., 1991; Pui et al., 1991; Beckham et al., 1992; Cush et al., 1993; Sfikakis et al., 1993; Schriefer et al., 1995). Such markers are shed into the circulation during the pathogenic process of the disease (cell activation or cell lysis), and reflect the progress of the underlying condition and the effectiveness of the treatment without necessarily playing an active part in the pathogenic process.

sCD4 and sCD8 are natural candidates for evaluation considering the potential role of suppressor T cells in the leprosy antigen-specific immunosuppression observed in LL patients. Whereas sCD4 did not change in either group of patient, sCD8 increased in the group treated with IFN-\(\gamma\). Elevation of sCD8 has been
related to poor treatment outcome in Hodgkin’s disease. (Pui et al., 1989). Increased levels of sCD8 may be related to either suppression of CMI or to activation of cytotoxic cells (Hancock et al., 1989) which have been shown in leprosy. In either case this elevation did not lead to any measurable difference in the disease outcome.

ICAM-1 is an adhesion molecule present on the surface of endothelial cells and fibroblasts (Springer, 1990), serves as a homing receptor to lymphocytes and granulocytes (Seth et al., 1991), and its expression is induced by pro inflammatory lymphokines like IFN-γ and IL-1 (Dustin et al., 1986). sICAM-1 is a soluble form of ICAM-1 normally occurring in healthy subjects (Rothlein et al., 1991; Seth et al., 1991). Elevated levels of sICAM-1 have been reported for patients with rheumatoid arthritis (Cush et al., 1993) and for systemic sclerosis, a disease associated to increased expression of ICAM-1 in the skin of the affected individuals (Sfikakis et al., 1993). Elevated levels of sICAM-1 have been reported in LL patients with a significant decrease after one month of anti-mycobacterial treatment (Rieckmann et al., 1996). Elevation of sICAM-1 in the sera of patients treated with IFN-γ while such levels were decreasing in patients treated with MDT alone, observed in the present study, is also an indication of the in vivo activity of the IFN-γ treatment. Elevation of sICAM-1 in the present study, however, did not lead to increased lymphocyte influx in the dermis.

Activated T cells release sIL-2R in the circulation (Rubin et al., 1985) and elevated sIL-2R serum levels have been correlated to a better prognosis in hematopoietic diseases (Ho et al., 1989). sIL-2R and sICAM-1 serum levels are more elevated in the serum of visceral leishmaniasis patients that successfully respond than in those that did not respond to antimonial therapy (Schriefer et al., 1995). Elevation of sIL-2R serum levels in patients treated with MDT + IFN-γ goes along with the result of sICAM-1, and is also indicative of a positive immunological response, although not sufficient to change the course of disease.

Despite the statistically significant differences observed in the serum levels of sCD8, sIL-2R and sICAM-1 in the groups of patients treated with MDT alone or MDT combined to IFN-γ, it is not possible to define a threshold indicative of a positive response. Both pre- and post-treatment levels of all these markers were similar in both groups, the differences are observed in paired analysis and reflects the change in individual levels.

In summary, despite being able to promote macrophage activation in multibacillary leprosy patients a short course of systemically administered IFN-γ is not able to change the clinical course of a long standing disease such as leprosy. Since the effects of most cytokines have a bell-shaped curve, larger doses may lead to even lower results. Future use of IFN-γ in multibacillary leprosy must then consider the possibility of longer periods of treatment.

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**References**


