Inhibition of Interferon Gamma Production by Peripheral Blood Mononuclear Leukocytes of Patients with Sarcoidosis*
Pathogenic Implications

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Interferon gamma (IFN-γ) production by stimulated peripheral mononuclear leukocytes of 30 patients with sarcoidosis was studied. A significant inhibition (64 percent, 5 to 330 IU/ml vs normal individuals = 1,000 ± 250 IU/ml) in the IFN-γ synthesis was found. The inhibition is due to a defect in the circulating monocytes and not in the peripheral T lymphocytes of these patients. This defect in the peripheral IFN-γ production could be involved in the pathogenesis of this disease. (Chest 1992; 101:996-99)

It is well known that interferon gamma (IFN-γ), besides its antiviral and antimicrobial actions, has many functions as an immunomodulator. Specifically, IFN-γ has been shown to modulate a variety of immune functions, including antibody production, natural killer (NK) cells activity, macrophage functions, and activation of T cells for the expression of interleukin 2 (IL-2) receptor. Many diseases present immunologic disorders associated with defects in the production of IFN-γ by peripheral blood mononuclear leukocytes (PBML), lepra, tuberculosis, rheumatoid arthritis. Sarcoidosis, a multisystem granulomatous disease of unknown etiology, presents as characteristic immunologic disorders. In the lung, the most commonly affected organ, the immune system is abnormally activated. The alveolar macrophages spontaneously release IFN-γ without stimulus, and these cells produce a high amount of interleukin 1 (IL-1). The number of T-helper (CD4) cells is augmented in the lung, and spontaneously release IL-2 and soluble IL-2 receptors. Alveolar macrophages are also involved in the migration of cells from the peripheral immune system. On the other hand, little is known about disorders of the peripheral immune system in this abnormality. The peripheral blood lymphocyte DNA synthesis is inhibited. However, it is not known if this inhibition is due to the migration of the peripheral cells, particularly CD4 T lymphocytes, to the lung or if it is due to an intrinsic defect of this cells. In this case, the cultured PBML lymphokine production, i.e., IFN-γ, in response to exogenous stimuli would be altered.

Thus, IFN-γ production by PBML of sarcoid patients was evaluated to establish the possible specific alteration in the peripheral cells in this pathologic condition.

Materials and Methods

Patients with Sarcoidosis

The diagnosis of pulmonary sarcoidosis was established in 30 patients according to previously described criteria, including analysis of a lung biopsy specimen. No patient had systemic manifestations of sarcoidosis or involvement of other major organs. None was receiving therapy.

Cell Cultures

The PBMLs were obtained by density-gradient centrifugation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Sweden) by the standard procedure employing 30 ml of venous blood from patients with sarcoidosis or normal donors. Cells from a single patient or donor were used in each experiment and each study was performed with separate cells of at least six different donors. At each independent experiment each experimental condition was replicated in at least four wells.

Cells were immediately plated or subsequently treated to obtain either monocyte-depleted populations or T cell-depleted populations. Purification was achieved by conventional techniques involving cell adhesion to plastic, phagocytosis of carbonyl iron (Sigma Chemical), and subsequent separation on a Ficoll-Hypaque gradient, as described elsewhere. Briefly, 10 ml of PBML was plated in 75 cm² plastic bottles (Corning, Corning, NY), at a cell density of 7 × 10⁶ cells per milliliter in RPMI 1640 culture medium (MA Bioproducts) supplemented with 5 percent fetal bovine serum (FBS) (Gibco, Grand Island, NY). Incubation lasted 3.5 h at 37°C in a wet atmosphere of 5 percent CO₂ and 95 percent O₂. Then, two fractions were obtained. (1) Adherent cells (monocytes + B lymphocytes) were extensively washed with Hank’s balanced salt solution and then detached from plastic by incubation with phosphate buffer, containing 0.1 percent edetic acid (EDTA), for 30 min at 4°C. This fraction was highly enriched in adherent cells, as

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contamination with T lymphocytes was less than 0.5 percent. (2) Nonadherent cells were transferred to a petri dish containing carbonyl iron (12.5 mg/ml) in a 1:1 mixture of RPMI:plasma obtained from the same patient or donor and incubated for 30 min at 37°C. Later, this whole fraction was centrifuged on a Ficoll-Hypaque gradient and a band of monocyte-depleted cells (<2 percent monocytes) was recovered. Purity of cell populations was assayed by classic immunofluorescence and cytotoxicity tests using IOM and IOT monoclonal antibodies (Immunotech, France). The PBML and monocyte or T cell-depleted cultures were plated (0.5 ml per well) in multiwell plates (Nunc, Denmark) in the RPMI 1640 culture medium supplemented with 5 percent FBS and maintained at 37°C in a wet atmosphere of 5 percent CO2 and 95 percent O2, for the indicated time. Flat-bottom multiwell dishes plated at a density of 5 x 10^6 cells per milliliter were used for IFN-γ assays. In some experiments, monocyte or T cell-depleted cultures were cocultured or the supernatant of the macrophages (SM) was added at 24 h to the enriched T cells. The SM was obtained stimulating the macrophages at 0 h with the mitogen phytohemagglutinin (PHA). After plating cells were incubated as described above for 30 min and different wells were stimulated with PHA obtained from Phaseolus vulgaris as previously described. All experiments were performed with the same batch of PHA at the equivalent of 40 mg/L standardized with PHA-M (Difco, Detroit), or anti-CD3 monoclonal antibody (Immunotech, France) at 0.1 mg/L.

**IFN Assay**

Interferon activity was assayed by the biologic method, as described elsewhere. Briefly, monolayers of a continuous line of human amnion (WISH) cells were incubated in 96-well microtiter plates for 24 h at 37°C in a CO2 incubator with serial twofold dilutions of test samples in a volume of 100 μl. After incubation, samples were removed and the monolayers were challenged with 100 to 200 pfu of vesicular stomatitis virus (VSV), Indian strain, and incubated again at 37°C in a 5 percent CO2 atmosphere. About 20 h later, when a complete cytopathic effect of VSV was observed in control monolayers, plates were stained with 0.5 percent gentian violet in 70 percent methanol. Antiviral activity was always calculated in international units of IFN-γ per milliliter (IU/ml) by the use of an IFN-γ standard (4,000 IU/ml) obtained from the Research Reagent Branch of the National Institute of Allergy and Infectious Diseases (Bethesda, Md) included in all tests assayed.

**IFN Characterization**

Samples were tested by (1) pH 2 treatment and (2) neutralization with anti-human IFN-γ antiserum (provided by Dr E. Falcoff, Paris, France). In the pH assay, samples of about 1,000 IU/ml were incubated at pH 2 during 24 h (4°C). Titration to pH 2 was done by stepwise addition of 0.1 N HCl. Then, samples were returned to pH 7 by stepwise addition of 0.1 N NaOH. In (2) mixtures of serial dilutions of positive IFN test samples and a constant dilution of anti-human IFN-γ serum were incubated for 1 h at 37°C. In both cases, after the incubation period, the residual IFN was titrated in the IFN assay previously described. The IFN titration without antiserum or pH treatment was done in parallel.

**Statistical Analysis**

Results are expressed as the mean±SE. Student’s t test was applied to data to evaluate the significance of differences found. A threefold difference in IFN-γ biologic assay is significant at p<0.05.

**RESULTS**

**Inhibition of IFN-γ by Peripheral Mononuclear Cells of Sarcoidotic Patients**

The IFN-γ production by the peripheral blood mononuclear cells was evaluated in 24 patients with diagnosis of sarcoidosis confirmed by biopsy specimen. In all of these patients, the synthesis of IFN-γ, in response to the inductor stimulus (PHA), was inhibited with respect to the values obtained for the normal control (Table 1).

However, two groups are observed. The first one (70 percent of the patients) presents total or almost total inhibition (98.5±1.1 percent of inhibition); meanwhile, the other group (30 percent of the patients) presents a partial inhibition (66.9±8.87 percent of inhibition) (Table 1, Fig 1).

**Characterization of the Cellular Population Affected**

For the production of IFN-γ by PHA induction, the interrelationship between two cellular populations is necessary (cooperation of macrophages and production of T lymphocytes). In view that this production is inhibited in peripheral blood mononuclear cells of patients with sarcoidosis, we evaluated which of this cells are altered in these patients.

Macrophages and T cells obtained from peripheral

<table>
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<th>IFN-γ (IU/ml)*</th>
<th>Basal</th>
<th>PHA 40 mg/L</th>
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<tbody>
<tr>
<td>Normal healthy donors (10)†</td>
<td>&lt;5</td>
<td>1000±250</td>
</tr>
<tr>
<td>Sarcoidotic patients with total inhibition (17)</td>
<td>&lt;5</td>
<td>15±10</td>
</tr>
<tr>
<td>Sarcoidotic patients with partial inhibition (7)</td>
<td>&lt;5</td>
<td>330±250</td>
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*IFN-γ was measured, as indicated in "Materials and Methods," at 48 h. Threefold difference in IFN-γ titer is significant at p<0.05.
†Number of patients or donors indicated in parentheses.

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**Figure 1.** Distribution and percentage of interferon-γ production of the data from Table 1. A, Distribution of the 24 sarcoidotic patients according to the type of interferon-γ synthesis inhibition. B, Percentage of interferon-γ synthesis inhibition of both groups of the 24 sarcoidotic patients. Open bars = partial inhibition; dashed bars = total or almost total inhibition.
Table 2 — IFN-γ Production by Purified T Lymphocytes and Macrophages from Sarcoidotic Patients and Normal Healthy Donors

<table>
<thead>
<tr>
<th>Experimental Condition*</th>
<th>IFN-γ (IU/ml)#</th>
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<tbody>
<tr>
<td>Ly Tn basal</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Ly Ts basal</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Ly Tn + PHA</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Ly Ts + PHA</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Ly Tn + PHA + SMn</td>
<td>1000 ± 150</td>
</tr>
<tr>
<td>Ly Ts + PHA + SMn</td>
<td>190 ± 90</td>
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<td>Ly Tn + PHA + SMs</td>
<td>400 ± 100</td>
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<tr>
<td>Ly Ts + PHA + SMs</td>
<td>300 ± 100</td>
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<tr>
<td>Ly Tn + Ms + PHA</td>
<td>900 ± 70</td>
</tr>
<tr>
<td>Ly Ts + Ms + PHA</td>
<td>150 ± 50</td>
</tr>
<tr>
<td>Ly Tn + anti-CD3</td>
<td>140 ± 20</td>
</tr>
<tr>
<td>Ly Ts + anti-CD3</td>
<td>140 ± 20</td>
</tr>
</tbody>
</table>

*Experimental conditions: Ly Tn = T lymphocytes from normal healthy donors; Ly Ts = T lymphocytes from sarcoidotic patients; PHA = phytohemagglutinin, 40 mg/L; SMn = normal macrophages supernatant; SMs = sarcoidotic macrophages supernatant; Ms = macrophages from sarcoidotic patients; and Mn = macrophages from normal healthy donors.

†Six patients and six normal donors were studied.

§Anti-CD3 = anti-CD3 monoclonal antibody 0.1 mg/L.

#IFN-γ was measured, as indicated in Materials and Methods, at 48 h. Three-fold difference in IFN-γ titer is significant at p<0.05.

blood from six patients with sarcoidosis, which presented IFN-γ production by PBML decreased with respect to normal controls, were evaluated. The T cells from these patients with sarcoidosis as well as T cells from normal donors do not produce IFN-γ with the stimulus of PHA alone, but they do so in the presence of a macrophage supernatant obtained by stimulating normal macrophages with PHA for 24 h. The T cells from patients with sarcoidosis with supernatant of normal macrophages produced levels of IFN-γ similar to those observed for the normal donors (Table 2). But, when both normal and sarcoid T cells were stimulated with supernatant obtained from sarcoid macrophages, the synthesis of IFN-γ was decreased showing inhibition similar to that observed for the PBML sarcoma responses (Table 2).

On the other hand, T cells from patients with sarcoidosis stimulated with the macrophage independent stimulus27,28 monoclonal antibody anti-CD3 produced IFN-γ levels similar to those produced for the anti-CD3 stimulation by normal T cells (Table 2).

Finally, normal T cells cocultured with macrophages of patients with sarcoidosis do not produce IFN-γ; meanwhile, production is observed in the inverse situation (sarcoid T cells cocultured with normal monocytes) (Table 2).

These results clearly show that the defect in the production of IFN-γ is due to the peripheral sarcoid macrophages.

DISCUSSION

Our results show the existence of an inhibition in the IFN-γ production by the peripheral cells of patients with sarcoidosis. The fact that T cells from normal healthy donors do not produce IFN-γ in the presence of sarcoid macrophage supernatant or cocultured with these cells, and conversely the fact that T cells from sarcoid patients reestablish the production by the macrophage independent stimulus, anti-CD3, or in the presence of supernatants from normal healthy donor macrophages or cocultured with these cells, clearly demonstrate that the peripheral sarcoid defect is in macrophages. The lung sarcoid macrophages produce abnormally spontaneous IFN-γ7,8 in contrast to normal macrophages which do not produce IFN-γ even when stimulated. The inverted activation stage among the peripheral monocyte and the lung macrophage is also observed in sarcoid patients for the expression of chemotactic factor.16 The differential behavior in both lung and peripheral macrophages may be attributed to the fact that the lung cells are innervated, and innervation has been shown to play an important role in immune regulation.29-31 The inhibition in the production of IFN-γ shows that the peripheral cells of sarcoid patients have an intrinsic defect. Peripheral IFN-γ produced may be involved in the feedback regulation of the lung immune cells and the defect in its synthesis could be involved in the pathogenesis of the disease, contributing to the lung immune cells' abnormal activation.

In some diseases that show IFN-γ synthesis inhibition, like rheumatoid arthritis, the treatment with very low, immunomodulatory doses of IFN-γ has been proved to reduce the symptoms.32

The cellular defect in the monocytes of sarcoid patients and the consequent inhibition of IFN-γ production could be related to a possible increase of a specific inhibitor33 or an interference in the normal activity of IL-1 on peripheral lymphocytes.

The lack of control of sarcoid lung macrophages, expressed by the overstimulation of this cell, could be a consequence of the peripheral alteration. Otherwise, the lung lesion and the defect in the lung macrophage could generate a circuitry in which the immunomodulation that the peripheral IFN-γ exerts could be damaged.

REFERENCES

3 Pace J, Russell S, Torres B, Johnson H, Gray P. Recombinant mouse gamma-interferon induces the priming step in macrophage activation for tumor cell killing. J Immunol 1983; 130:
17 Interleukin-2
16 Lymphokine
15 Host
14 Lymphocytes
13 Spontaneously
12 Release
11 Patients
10 Invest
9 Compartmentalized
8 Proliferation
7 Alveolar
6 Berlin
[abstract].
5 Immunologic
4 Disease
3 Sarcoidosis.
2 In vitro
1 Observations

2011-13
6 Murray H. Interferon-gamma, the activated macrophage and host defense against microbial challenge. Ann Intern Med 1989; 100:595-608
16 Hamblin A, Barbosa I, Gant V. Expression of CD11/CD18 on mononuclear phagocytes and role in mediating T-cell adhesion in sarcoidosis [abstract]. Seventh International Congress of Immunology, 1989, Berlin
32 Lemmel E, Brackertz D, Franke M. Results of a multicenter placebo-controlled double-blind randomized phase, III: clinical study of treatment of rheumatoid arthritis with recombinant IFN-gamma. Rheumahol Int 1986; 8:87-93