CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

Effect of Interleukin-1 (IL-1) and IL-2 on Lymphocytes from Patients with Leprosy

TO THE EDITOR:

Leprosy is a disease with a wide clinical, histological, and immunological spectrum. The benign form, tuberculoid leprosy, is characterized by a good response measured *in vitro* by T-cell proliferation assay. In the multibacillary malignant form, lepromatous leprosy, T cells do not proliferate in the presence of specific and crossreacting antigens of *Mycobacterium leprae* ([3]).

In the development of T-cell-mediated immune responses to an antigen, lymphokines are required in a given sequence, and it has been postulated that the amount of interleukin-2 (IL-2) produced might determine the balance between immunity and unresponsiveness ([1]). To test if the lack of *in vitro* responsiveness to *M. leprae* antigens by T cells from lepromatous leprosy (LL) patients is due to a deficit in IL-1 or IL-2 production, the response of lymphocytes from LL patients to *M. leprae* in the presence of exogenously added IL-1 or IL-2 has been studied, but the data reported are contradictory ([4, 8]).

The purpose of this study is to evaluate the role of IL-1 and IL-2 in the *in vitro* T-cell proliferation of a group of leprosy patients characterized clinically and histopathologically.

Isolation of mononuclear cells. Mononuclear cells were isolated from heparinized peripheral blood by flotation over Ficoll-Hypaque gradients ([2]) and cultivated at a density of 2×10^5 viable cells/0.2 ml in microtiter plates. The cells were cultured in RPMI 1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated, pooled normal human AB serum.

Antigen. The antigen used was 20 μl purified *M. leprae* $6 \times 10 \times 5$ bac/ml. The antigen was used with or without IL-2 (Lymphocult T; Biosoft) 100 U/ml diluted 1:50 (100 μl) and IL-1 produced by stimulating human macrophage cultures with silica ([10]).

For assaying antigen stimulation, the culture plates were incubated for 6 days; 18 hr before harvesting, 1 μCi of ^{3}H-thymidine (specific activity 1 Ci/mmole) was added, and the cells were processed for liquid scintillation.

Patients. The patients were seen in the Instituto de Biomedicina, Caracas, Venezuela. All patients (17 lepromatous, LL) were skin biopsied and classified following the Ridley-Jopling criteria ([9]). Lymphocytes from three Mitsuda-positive contacts were also studied.

Chemotherapy. All patients were receiving treatment with sulfone, rifampin, and clofazimine.

As can be seen in Table 1, the increase in counts per minute (cpm) obtained when lymphocytes were incubated from *M. leprae* antigen with IL-2 was not significantly different from the cpm obtained by incubating lymphocytes with IL-2 alone.

The nonspecific mitogenic effect on the response of lymphocytes incubated with IL-1 was similar to that observed with IL-2 alone, but of lower intensity (Table 2). However, when the cells were cultured in the

TABLE 1. *Effects of adding IL-2 to lymphocyte proliferation to* M. leprae *antigens.*

Group	IL-2	Antigen	
		None	*M. leprae*
LL[a]	−	219.5 ± 19.4[b]	213 ± 41.01
(N = 12)	+	259.8 ± 9.68	237.07 ± 23.9
LL	−	238.7 ± 127.1	265.54 ± 241.3
(N = 5)	+	1183.2 ± 867	1322.06 ± 1051.2
Contacts			
Mitsuda-positive	−	312.7 ± 234.9	1427.16 ± 791
(N = 3)	+	1671.3 ± 212.9	5192.02 ± 1489.4

[a] LL patients are divided in two groups on the basis of their lymphocyte response to IL-2 in the absence of antigen.
[b] Counts per minute ± standard deviation.

presence of *M. leprae* antigens and IL-1, the cpm were lower than those observed with IL-1 alone, suggesting that IL-1 in the presence of antigen might have a down-regulatory action.

The objective of this study was to evaluate whether IL-1 and IL-2 could restore the *in vitro* immune response of lymphocytes obtained from LL patients. Most (70%) did not show an increase in the incorporation of ^{3}H-thymidine in the presence of IL-2 and *M. leprae*; whereas the rest of the LL patients (30%) did show increased responses (Table 1). However, the observed increases were not statistically significant, since the lymphocytes in the presence of IL-2 without *M. leprae* showed an increase in cpm that accounted practically for the increase observed in the presence of *M. leprae* and IL-2, indicating that the increment in cpm was not specific. Similar results were obtained using IL-1 (Table 2). It is noteworthy that the mitogenic effect of IL-2 with the antigens was greater in Mitsuda-positive contacts.

Kaplan, *et al.*, using recombinant IL-2, postulated the presence of a group of "low responder" patients, whose lymphocytes give a better response to *M. leprae* antigens in the presence of IL-2, and that the addition of exogenous IL-2 to leukocyte cultures does not appear to restore responsiveness to *M. leprae* in cells from nonresponder patients ([5, 6]). Haregewoin, *et al.* ([4]) have reported that IL-2 can reverse *in vitro* the anergy of LL patients to *M. leprae* antigens. However, these results have not been reproduced ([8]). Our data indicate that the observed increase in cpm after the addition of IL-2 in some patients is due to a nonspecific effect.

These results and those obtained with IL-1 allow us to conclude that in our LL patients anergy is not directly related to a defect in the production of either one of these interleukins.

Mohagheghpour, *et al.* ([7]) have suggested that the problem of LL patients lies in a defect in the expression of the IL-2 receptor. However, our results show a mitogenic ef-

TABLE 2. *Effects of adding IL-1 to lymphocyte proliferation to* M. leprae *antigens.*

Group	IL-1	Antigen	
		None	*M. leprae*
LL[a]	−	219.5 ± 19.4[b]	213 ± 41.01
(N = 12)	+	214.3 ± 77	271.2 ± 143.6
LL	−	238.7 ± 127.1	265.5 ± 241.3
(N = 5)	+	671.4 ± 417	474.7 ± 232.6
Contacts			
Mitsuda-positive	−	312.7 ± 234.9	1427.2 ± 791.0
(N = 3)	+	2101.5 ± 1134	1336.3 ± 95.25

[a] LL patients are divided in two groups on the basis of their lymphocyte response to IL-2 in the absence of antigen.
[b] Counts per minute ± standard deviation.

fect of IL-2 in lymphocytes from a group of LL patients, demonstrating the presence of receptors for IL-2 in a portion of peripheral blood lymphocytes.

The results obtained in LL patients show that IL-1 and IL-2 cannot restore, *in vitro,* the immune response against *M. leprae* antigens. In a group of the patients, IL-2 had a nonspecific mitogenic effect which is now being evaluated.

—Elsa M. Rada S., Lic. Biol.
—Walter Mosco, M.D.
—Nacarid Aranzazu, M.D.
—Jacinto Convit, M.D.

Instituto de Biomedicina
Apartado 4043
Caracas 1010A, Venezuela

Acknowledgments. We wish to thank Dr. F. Tapia for his helpfulness in the preparation of this manuscript, and Dr. Ulrich for advice and careful review of the same.

REFERENCES

1. Asherson, G. L., Colizzi, V., Malkovsky, M., Colonna-Ramano, G. and Zembala, M. The role of interleukin-2 as one of the determinants of the balance between immunity and unresponsiveness. Folia Biol. (Praha) **31** (1985) 387–395.
2. Boyum, A. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. **21** Suppl. 97 (1968) 77.
3. Godal, T., Myklestad, B., Samuel, D. R. and Myrvang, B. Characterization of the cellular immune defect in lepromatous leprosy: a specific lack of circulating *Mycobacterium leprae*-reactive lymphocytes. Clin. Exp. Immunol. **9** (1971) 821–831.
4. Haregewoin, A., Godal, T., Mustafa, A. S., Belehu, O. and Yemaneberhan, T. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. Nature **303** (1983) 342–344.
5. Kaplan, G. and Cohn, Z. A. Cellular immunity in lepromatous and tuberculoid leprosy. Immunol. Lett. **11** (1985) 205–209.
6. Kaplan, G., Weinstein, D. E., Steinman, R. M., Levis, W. R., Elvers, U., Patarroyo, M. E. and Cohn, Z. A. An analysis of *in vitro* T-cell responsiveness in leprosy. J. Exp. Med. **162** (1985) 917–929.
7. Mohagheghpour, N., Gelber, R. H., Larrick, J. W., Sasaki, D. T., Brennan, P. J. and Engleman, E. G. Defective cell-mediated immunity in leprosy: failure of T cells from lepromatous leprosy patients to respond to *Mycobacterium leprae* is associated with defective expression of interleukin-2 receptors and is not reconstituted by interleukin-2. J. Immunol. **135** (1985) 1443–1449.
8. Ottenhoff, T. H. M., Elferink, D. G. and de Vries, R. R. P. Unresponsiveness to *Mycobacterium leprae* in lepromatous leprosy *in vitro*: reversible or not? Int. J. Lepr. **52** (1984) 419–422.
9. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity; a five-group system. Int. J. Lepr. **34** (1966) 255–273.
10. Schmidt, J. A., Oliver, C. N., Lepe-Zuniga, J. L., Green, I. and Gery, I. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin-1. A potential role for interleukin-1 in the pathogenesis of silicosis. J. Clin. Invest. **73** (1984) 1462–1472.

An Unusual Case of Untreated Lepromatous Leprosy with Rare Bacilli: An Immunologic Follow-up

To the Editor:

Previously one of us ([1]) reported in the Journal a most unusual case of untreated leprosy with clinical signs and symptoms of nodular lepromatous leprosy which was confirmed by characteristic dermal histopathology following routine hematoxylin and eosin staining. Surprisingly, acid-fast organisms were found only after extraordinary effort. Organisms from these lesions were, however, characteristic of *Mycobacterium leprae* in morphology, growth kinetics in mice, lack of growth on Löwenstein-Jensen medium, antimicrobial susceptibility, and were demonstrated to contain phenolic glycolipid-I. At the time this case was reported, the immunologic mechanisms of this unique host–parasite interaction were under investigation. Herein we wish to report studies whereby we examined the ability of peripheral blood mononuclear leukocytes (PBL) from this patient to respond to mycobacterial antigens. As shown in The Table, fresh PBL, studied repeatedly over an 18-month period, responded vigorously (stimulation index $\geq$ 10) to whole *M. leprae* as well as to purified protein derivative of tuberculin (PPD). Although, as can be seen, a minority (18%) of lepromatous patients' fresh peripheral blood leukocytes respond to *M. leprae* ([2]), this patient is remarkable even as compared to that