

Deficiency in the Biosynthesis of Interleukin 2 (IL-2) and Functional Presence of the IL-2 Receptor in Lepromatous Leprosy

TO THE EDITOR:

Patients with lepromatous leprosy (LL) have a depressed cellular immune capacity to respond to antigens from *Mycobacterium leprae*, a reduction in effector and helper T cells, and an increase in suppressor T cells (2, 4, 11, 16). A reduction in the synthesis of interleukins has been postulated in some murine models (9). In man, it has been shown that upon the addition of interleukin 2 (IL-2) to cultures of lymphocytes from patients with LL, these lymphocytes recover their capacity to proliferate (7, 8). However, in another paper (12) it has been pointed out that this phenomenon does not occur.

We report here the results of a study looking for the production of IL-2 by lymphocytes from patients with LL, and the func-

tional presence of IL-2 receptors on their lymphocytes.

Study subjects. Twenty patients, 11 men and 9 women, from the Instituto Dermatológico at Guadalajara, Jalisco, México, were diagnosed as having nodular LL according to international criteria (14). All presented positive bacilloscopy, and all received an irregular treatment of 100 mg of dapsone (diaminodiphenyl sulfone) per day. The length of treatment ranged from 2 to 15 years. Fourteen patients were studied for the biosynthesis of IL-2 by T lymphocytes obtained from peripheral blood, and the functional presence of the IL-2 receptor on T lymphocytes was studied in 6 patients. Controls consisted of 14 unrelated healthy subjects for the first study and 6 apparently

normal subjects for the second, all matched for sex and age as much as possible.

Mononuclear cells. Heparinized blood (20 IU/ml) was obtained from each subject by venipuncture. After the blood had been centrifuged on a Ficoll-Hypaque gradient^(3,10) at $400 \times g$, the mononuclear cells were recovered and washed three times with Hanks' balanced salt solution (HBSS). The cells were then resuspended in RPMI 1640 culture medium (GIBCO, Grand Island, New York, U.S.A.) supplemented with 5% heat-inactivated fetal calf serum, 2 mM glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, and penicillin 100 U/ml and streptomycin 100 $\mu\text{g/ml}$.

Biosynthesis of IL-2 by T lymphocytes. To 2×10^5 mononuclear cells for culture (which included T lymphocytes), 10 $\mu\text{g/ml}$ of concanavalin A (ConA) was added. The cultures were incubated for 48 hr at 37°C in a mixture of 95% air and 5% CO₂. Thereafter, each supernatant was assayed for IL-2 activity⁽⁵⁾.

IL-2 activity assay. The assay method used was that described by Gillis^(5,6). In brief, a cytotoxic T-cell line (CTL-2) obtained from C57BL/6 mouse spleen was maintained in culture for long periods through the use of T-cell growth factor (IL-2)⁽⁵⁾. These cells were washed in HBSS, resuspended in 0.1 ml of culture medium in a concentration of 1×10^4 cells, and added to each well of a 96-well microplate (Costar 3596, Cambridge, Massachusetts, U.S.A.). Serial dilutions of the supernatants (0.1 ml) from the ConA-stimulated T-lymphocyte cultures were added to these cells with alpha methyl mannoside at a final concentration of 0.1 M, and incubated for 24 hr under culture conditions. Thereafter, each well was pulsed with 1 μCi of ³H-thymidine (specific activity 6.7 Ci/ μmole ; New England Nuclear, Boston, Massachusetts, U.S.A.). After 18 hr of incubation, the cells were harvested and the incorporation of ³H-thymidine was measured in a beta counter (Packard).

Functional presence of IL-2 receptors on the surface of T cells. To test the presence of IL-2 receptors on the surface of T cells from LL patients and controls which had been previously stimulated by ConA for 72 hr (activated T cells)⁽¹⁾, these cells were

harvested, washed twice in HBSS, and placed in a 96-well microplate at a concentration of 1×10^4 cells (0.1 ml) per well and at a final concentration of 0.1 M alpha methyl mannoside. Serial dilutions of commercial purified IL-2 (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) or of IL-2-enriched supernatants from cultures of Wistar rat spleen cells which had been stimulated with ConA were added to the microplate (0.1 ml per well). After 24 hr of incubation under culture conditions, each well was pulsed with ³H-thymidine as described above. The cells were harvested 18 hr later, and the incorporation of ³H-thymidine was measured^(5,15). The data obtained were analyzed by the Student's *t* test.

Results from the 14 LL patients in whom biosynthesis of IL-2 was looked for are expressed in Table 1. It can be seen that the production of IL-2 by previously ConA-stimulated T lymphocytes from LL patients was significantly diminished compared with those from a healthy subject control group ($p < 0.001$).

Table 2 shows the result of adding IL-2 supernatants from rat spleenocytes or commercial purified IL-2 to previously ConA-stimulated T lymphocytes from LL patients in order to show the receptor for IL-2. Such T lymphocytes showed a lower incorporation of ³H-thymidine when they were previously stimulated with ConA (mean 24,871) compared with a healthy subject group (mean 75,686) ($p < 0.01$). Although both groups of T cells produced IL-2, the amount produced by those from LL patients was significantly lower than the amount produced by those from normal subjects. However, when these T-activated lymphocytes in both groups (LL patients and controls) were supplemented with exogenous IL-2 added to the cell cultures, the cells from LL patients showed normal activity (mean 10,352) when compared with controls (mean 9198).

It is important to point out that the number of cells tested in the second part of this experiment was lower (1×10^4 cells) than in the first part (2×10^5 cells); this fact explains why the counts per minute (cpm) were lower in this second part.

The group of 20 LL patients represents a heterogeneous group. However, when these

TABLE 1. Stimulation of CTLL-2^a cells by interleukin-2 present in the culture supernatants of T lymphocytes activated with ConA from patients with lepromatous leprosy and healthy subjects.

Experiment ^b	Counts per minute from		
	Cells plus medium (background)	Cells stimulated with IL-2 supernatants	
		LL patients ^c	Healthy subjects
1	3,966	4,488	31,336
2	3,498	4,336	21,663
3	2,230	4,745	14,106
4	1,786	3,794	20,576
5	3,699	3,184	12,180
6	3,894	3,254	9,168
7	5,870	27,406	37,565
8	5,491	11,261	38,430
9	4,543	2,031	16,293
10	4,220	3,505	16,200
11	4,512	5,014	11,485
12	5,543	6,505	20,530
13	5,810	5,045	21,570
14	5,540	5,306	22,333

^a Cytotoxic T-lymphocyte line-2 (CTLL-2) from spleen of C57BL/6 mice 10,000 per culture in 0.2 ml medium.

^b The dilution in which the maximum IL-2 activity was present was 1:256 for all the experiments.

^c Significantly less than healthy subjects, $p < 0.001$, Student's *t* test.

patients were diagnosed all had a positive bacilloscopy and a biopsy with a heavy load of bacilli. Treatment with dapsone was started once they were diagnosed but, unfortunately, as frequently happens, the treatment was not followed in a regular manner. For this reason, bacilloscopy remained positive in all of them for a period of treatment as long as 15 years. Also for this reason, it is difficult to determine the action dapsone could have played (if any) in modifying the immune response of the host.

In the 14 cases studied, the T lymphocytes of LL patients exhibited reduced levels of IL-2 activity. These data suggest that the T cells of the patients may have an alteration in the biosynthesis of IL-2, confirming previous results from Nogueira, *et al.* (13). This may explain the inability of these cells to adequately proliferate after stimulation with mitogens and their inability to help in the control of *M. leprae* infection.

It has been reported in specific systems (7, 8) that when IL-2 is added to T-cell cultures stimulated with *M. leprae* the cells regain their full capacity to proliferate. On the other hand, Mohaghehpour, *et al.* (12) reported that when exogenous IL-2 is added

TABLE 2. Presence of IL-2 receptor on previously ConA-stimulated T lymphocytes obtained from LL patients.

Diagnosis	Counts per minute of			
	Medium (unstimulated)	ConA-stimulated T lymphocytes ^a	Medium (not supplemented)	ConA-activated T lymphocytes ^b supplemented with exogenous IL-2
LL	826 ± 65	32,100 ± 3,440	664 ± 120	16,035 ± 552
LL	1,447 ± 325	13,504 ± 2,444	238 ± 53	14,386 ± 254
LL	1,357 ± 231	22,959 ± 5,002	617 ± 32	6,176 ± 580
LL	1,531 ± 514	39,878 ± 3,590	410 ± 206	6,374 ± 750
LL	1,594 ± 252	28,801 ± 1,825	356 ± 91	8,071 ± 1,040
LL	1,569 ± 205	11,987 ± 958	535 ± 132	10,876 ± 474
Mean ± S.D.	1,387 ± 288	24,871 ^c ± 10,879	470 ± 163	10,352 ^d ± 4,139
Healthy	1,273 ± 44	74,396 ± 5,904	1,064 ± 154	15,376 ± 340
Healthy	924 ± 219	65,730 ± 3,504	864 ± 207	8,573 ± 349
Healthy	2,246 ± 1,651	56,041 ± 7,044	550 ± 118	7,155 ± 900
Healthy	927 ± 118	71,042 ± 2,511	421 ± 122	15,079 ± 278
Healthy	519 ± 74	58,584 ± 3,001	1,228 ± 108	7,042 ± 278
Healthy	643 ± 12	128,291 ± 5,544	909 ± 63	1,964 ± 105
Mean ± S.D.	1,089 ± 780	75,680 ± 26,713	839.5 ± 305	9,198 ± 5,182

^a $2 \times 10^5/0.2$ ml lymphocytes were used for these experiments.

^b $1 \times 10^4/0.2$ ml lymphocytes were used for these experiments.

^c Significantly less than healthy subjects, $p < 0.001$, Student's *t* test.

^d No significant difference from healthy subjects.

to T-cell cultures stimulated with *M. leprae*, they do not regain their capacity to proliferate because they lack the IL-2 receptor. Here, in another system, we have studied the functional presence of the IL-2 receptor on ConA-activated T cells from LL patients, and we have demonstrated that the addition of exogenous IL-2 to cell cultures did, indeed, restore adequate proliferation of ConA-stimulated T cells, thereby implying that the IL-2 receptor was present and functioning on the surface of the LL patients' cells.

Finally, in 3 LL patients with type 2 reaction, in which all of the above-mentioned experiments were done, their ConA-stimulated T cells were incapable of responding to the addition of exogenous IL-2, suggesting that in this particular case (leprosy reaction) the receptor for IL-2 was absent or not functioning. More work is in progress, however, in order to test if this last statement is true or not.

—Alfonso Enrique Islas-Rodríguez, M.Sc.
 —Roberto Morales-Ortiz, M.D.
 —Mary Fafutis-Morris, M.T.
 —Amado González-Mendoza, M.D.
 —Liberado Ortiz-Ortiz, Ph.D.

*Instituto de Patología
 Infecciosa y Experimental
 de la Universidad de Guadalajara
 Calle Hospital 278
 Sector Hidalgo
 44280 Guadalajara, Jalisco, México*

REFERENCES

1. ASHWELL, J. D., ROBB, R. J. and MALEK, T. R. Proliferation of T lymphocytes in response to interleukin 2 varies with their state of activation. *J. Immunol.* **137** (1986) 2572–2578.
2. BLOOM, B. R. and MEHRA, V. Immunological unresponsiveness in leprosy. *Immunol. Rev.* **80** (1984) 5–28.
3. BOYUM, A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation, and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest.* **21** Suppl. 97 (1968) 77–89.
4. CASTELLANOS, C., ISLAS, A. E., GONZÁLES, A., ZAMBRANA, S. and ORTIZ-ORTIZ, L. Lepromatous leprosy; study of some subpopulations of lymphocytes and its functional analysis. *Arch. Invest. Méd. (Méx.)* **16** (1985) 217–224.
5. GILLIS, S., FERM, M. M., OU, W. and SMITH, K. A. Cell growth factor: parameters of production and quantitative microassay for activity. *J. Immunol.* **120** (1978) 2027–2032.
6. GILLIS, S. and SMITH, K. A. Long term culture of tumor-specific cytotoxic T cells. *Nature* **268** (1977) 154–156.
7. HAREGEWOIN, A., GODAL, T., MUSTAFA, A. S., BELEHU, A. and YEMANEBERHAN, T. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. *Nature* **303** (1983) 342–344.
8. HAREGEWOIN, A., MUSTAFA, A. S., HELLE, I., WATERS, M. F. R., LEIKER, D. L. and GODAL, T. Reversal by interleukin-2 of the T-cell unresponsiveness of lepromatous leprosy to *Mycobacterium lepraemurium*. *Immunol. Rev.* **80** (1984) 77–86.
9. HOFFENBACH, A., LAGRANGE, P. H. and BACH, M.-A. Deficit of interleukin 2 production associated with T cell proliferative response in *Mycobacterium lepraemurium* infection. *Infect. Immun.* **39** (1983) 109–116.
10. LIM, S.-D., KISZKISS, D. F., JACOBSON, R. R., CHOI, Y. S. and GOOD, R. A. Thymus-dependent lymphocytes of peripheral blood in leprosy patients. *Infect. Immun.* **9** (1974) 394–399.
11. MEHRA, V., MASON, L. H., ROTHMAN, W., REINHARZ, W., SCHLOSSMAN, E. and BLOOM, B. R. Delineation of a human T cell subset responsible for lepromin-induced suppression in leprosy patients. *J. Immunol.* **125** (1980) 1183–1188.
12. 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.
13. NOGUEIRA, N., KAPLAN, G., LEVY, E., SARNO, E. N., KUSHNER, P., GRANELLI-PIPERNO, A., VIERIRA, L., COLOMER GOULD, V., LEVIS, W., STEINMAN, R., YIP, Y. K. and COHN, Z. A. Defective γ interferon production in leprosy; reversal with antigen and interleukin 2. *J. Exp. Med.* **158** (1983) 2165–2170.
14. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity; a five-group system. *Int. J. Lepr.* **34** (1966) 255–273.
15. ROSENBERG, S. A., GRIMM, E. A., MCGROGAN, M., DOYLE, M., KAWASAKI, E., KOTH, K. and MARK, D. F. Biological activity of recombinant human interleukin-2 produced in *Escherichia coli*. *Science* **223** (1984) 1412–1415.
16. STRICKLAND, N. H. The influence of immunosuppression and immunodeficiency on infections with leprosy and tuberculosis. *Int. J. Lepr.* **53** (1985) 86–100.