

Interleukin-2-induced T-cell Response to *M. leprae* in Lepromatous Leprosy: Reversion of a Suppressor Mechanism or Expansion of a Small *M. leprae*-reactive T-cell Pool?

TO THE EDITOR:

A number of recent studies indicate that a fraction of lepromatous leprosy patients harbor circulating *Mycobacterium leprae*-reactive T cells, despite their well-documented anergy to *M. leprae* antigens⁽³⁾. Indeed, a *M. leprae*-triggered, *in vitro* T-cell response has been evidenced by different procedures. We previously reported that *in vitro* incubation at 37°C of peripheral blood mononuclear cells (PBMC) of some lepromatous patients could restore their proliferative response to *M. leprae*⁽¹⁾, a phenomenon which was recently attributed to the elimination of suppressor macrophages or of macrophage-released suppressor factors^(7,8). Experiments conducted by Mehra, *et al.*⁽⁹⁾ indicated that depletion of suppressor OKT8+ T cells could also restore T-cell responsiveness to *M. leprae* antigens in BL patients. Finally, Haregewoin, *et al.*^(4,5) observed that the addition of exogenous interleukin-2 (IL-2) to PBMC from lepromatous patients restored *M. leprae*-triggered T-cell proliferation. This finding attributed to the reversion of a suppressor mechanism was confirmed by Nath, *et al.*⁽⁸⁾, but was contradicted by Ottenhoff, *et al.*⁽⁹⁾.

We have undertaken a similar study in a group of 18 lepromatous leprosy patients of varied geographical origins (9 from the West Indies, 5 from Africa, 2 from Europe, and 2 from Asia), all living in Paris and followed at Saint-Louis Hospital (Dermatology Department, Service of Professor Cottenot). At variance with the above-cited report, we have also conducted similar studies in 13 tuberculoid patients and 5 healthy subjects, studying the responsiveness to PPD in parallel to that of *M. leprae* in all groups of subjects. We found an IL-2-induced increase of *M. leprae* responsiveness in 10 out of 18 lepromatous patients, but also in 3 out of 4 tuberculoid patients who happened to be nonresponsive *in vitro* to *M. leprae*, and in 2 out of 3 similarly *M. leprae*-unreactive healthy subjects. The ability of PBMC from *M. leprae* nonresponders to become reac-

tive in the presence of IL-2 was closely linked to their responsiveness to PPD.

PBMC were cultured for 6 days in RPMI 1640 medium supplemented with 20% human AB serum, with either *M. leprae* (WHO batch CD 40; 1 µg/ml), BCG-derived PPD (IP71, a gift of Dr. J. Augier, Pasteur Institute, Paris; 10 µg/ml) or no antigen, in the presence or absence of IL-2 (Lymphocult T lectin-free, Biotest; 5%). ³H-Thymidine incorporation was measured after an 18-hr pulse. Results from antigen-stimulated cultures were expressed as Δcpm = cpm with antigen - cpm without antigen. Background proliferation without antigen was low in the absence of IL-2 (around 1000 cpm in all 3 groups) but increased significantly in the presence of IL-2, to the same extent in lepromatous patients (mean ± S.E.M. = 12,078 ± 2700), tuberculoid patients (16,175 ± 3502), and healthy subjects (18,678 ± 8811). Such an effect of IL-2 on spontaneous PBMC proliferation, already noted by Haregewoin, *et al.*⁽⁴⁾ and Nath, *et al.*⁽⁸⁾, might result from the proliferation of IL-2-dependent, NK-like, large granular lymphocytes and/or of autoreactive T cells directed at MHC class II auto-antigens.

Ten out of 18 lepromatous patients (Table 1) exhibited after IL-2 addition a significant proliferative response to *M. leprae*, as defined by a Δcpm exceeding 5000. The proportion of patients whose *M. leprae* unresponsiveness was reversed by IL-2 in our study was closely similar to that reported by Haregewoin, *et al.*⁽⁵⁾ and by Nath, *et al.*⁽⁸⁾ but much higher than that reported by Ottenhoff, *et al.*⁽⁹⁾ (2 out of 12). The geographical distribution, that of BL vs LL forms, and of treatment duration was quite similar among the patients who did respond to *M. leprae* in the presence of IL-2 and among those who did not. The level of proliferative response to *M. leprae* observed in the presence of IL-2 among lepromatous patients, however, still remained lower than that seen in the absence of IL-2 among tuberculoid patients ($p < 0.05$). Four lepromatous patients, nos. 2 and 3 who did not

TABLE 1. Effect of IL-2 addition on *M. leprae* and PPD-stimulated proliferative response in lepromatous patients.

Patient no. and type of leprosy	Duration of treatment	<i>M. leprae</i>		PPD	
		Without IL-2	With IL-2	Without IL-2	With IL-2
1 (BL)	30 mos.	-45 ^a	-17	34	449
2 (BL)	5 mos.	36	-2,346	903	-2,233
3 (LL)	4 yrs.	163	-2,774	361	505
4 (LL)	6 yrs.	-1,327	1,504	-1,257	3,833
5 (BL)	13 yrs.	-80	-3,329	3,762	3,093
6 (LL)	33 yrs.	-96	2,545	-199	-2,066
7 (LL)	15 yrs.	-135	-5,622	2,872	4,515
8 (LL)	8 mos.	27	1,448	41,828	20,572
9 (LL)	2 yrs.	864	8,207	1,899	21,866
10 (BL) ^b	1 mo.	2,040	59,272	109	30,479
11 (LL)	0	-301	21,939	-1,756	13,495
12 (LL)	19 yrs.	678	14,576	1,731	10,848
13 (BL)	17 mos.	4,154	6,655	9,066	3,328
14 (BL)	0	1,673	8,495	15,601	30,271
15 (LL)	11 yrs.	220	14,020	11,848	33,253
16 (BL)	11 yrs.	5	6,402	17,980	22,893
17 (LL)	6 mos.	2,456	14,805	27,732	49,203
18 (LL)	10 yrs.	530	7,908	92,494	142,901
Mean \pm S.E.M.		679 \pm 482	8,538 ^c \pm 3,461	12,455 \pm 5,455	21,511 ^c \pm 7,932

^a Results of ³H-thymidine incorporation measurement are expressed as Δ cpm = cpm with antigen - cpm without antigen.

^b Patient showing reversal reaction at the time of study.

^c Significantly different from the results obtained without IL-2 addition (paired *t* test, *p* < 0.05).

respond to *M. leprae* in the presence of IL-2 and nos. 10 and 12 who did, were re-tested to compare the inductive effect of recombinant IL-2 (a gift of Sandoz Laboratories, Basel, Switzerland, used at concentrations of 0.02–0.1 μ g/ml) with that of the chromatography-purified, lectin-free IL-2 routinely used in our experiments. Indeed, Haregewoin, *et al.* (⁵) reported a higher frequency of IL-2-induced reversal of *M. leprae* unresponsiveness when recombinant IL-2 was used instead of a crude T-cell-conditioned medium. In the present study, no difference could be seen in any patient between the results obtained with recombinant IL-2 and those obtained with Lymphocult T lectin-free, which would indicate that crude, unpurified T-cell-conditioned medium is not optimal for inducing reversal of *M. leprae* unresponsiveness in lepromatous patients but that a partial purification may be sufficient to improve its efficiency.

Eleven out of 18 lepromatous patients had no PPD-induced proliferation of PBMC (vs 3 out of 13 tuberculoid patients), but 4 of them became PPD-responsive when IL-2

was added, and a significant increase (*p* < 0.05) of the mean response to PPD plus IL-2 was observed in this group when compared to the mean response to PPD alone. Interestingly, all patients who displayed a significant proliferative response to *M. leprae* plus IL-2 also showed a significant response to PPD, either spontaneous or IL-2 induced; whereas only 1 of the *M. leprae*-non-reactive patients did so. It is striking that 90% of the patients studied by Haregewoin, *et al.* (⁴), among whom a majority showed IL-2-inducible, T-cell responsiveness to *M. leprae*, also presented with a significant proliferative response to PPD. Conversely, patients investigated by Ottenhoff, *et al.* (⁹), whose T cells remained in most cases unresponsive to *M. leprae* in spite of IL-2 addition, exhibited insignificant or low responses to PPD with or without IL-2. Moreover, 1 of the 2 patients who did respond to *M. leprae* plus IL-2 actually responded to PPD plus IL-2 as well.

Among 13 tuberculoid patients (Table 2) investigated in this study, 4 (nos. 1–4, all BT) showed no proliferative response of PBMC to *M. leprae*, but only 1 of them (no.

TABLE 2. Effect of IL-2 addition on *M. leprae* and PPD-stimulated proliferative responses in tuberculoid patients and healthy controls.

Subjects No. and type of leprosy	Dura- tion of treat- ment	<i>M. leprae</i>		PPD	
		Without IL-2	With IL-2	Without IL-2	With IL-2
BT/TT patients					
1 (BT)	2 yrs.	-307 ^a	4,220	-366	1,040
2 (BT)	2 yrs.	357	5,784	3,539	10,818
3 (BT)	0	298	14,857	ND ^b	ND
4 (BT)	0	-355	6,370	821	14,847
5 (BT)	6 yrs.	5,004	18,471	10,503	34,865
6 (BT)	0	5,403	57,202	15,534	56,914
7 (BT)	0	8,268	13,104	14,418	16,631
8 (BT)	4 mos.	23,698	45,997	147,744	121,260
9 (BT)	0	34,542	24,739	40,436	26,552
10 (TT)	8 yrs.	38,336	26,454	114,336	57,454
11 (BT)	1 mo.	54,314	90,293	158,351	137,293
12 (BT)	7 yrs.	80,315	51,596	46,699	121,280
13 (BT)	6 mos.	113,288	119,744	165,562	125,707
Mean ± S.E.M.		27,588 ± 10,020	36,833 ± 9,819	57,798 ± 19,240	60,388 ± 14,919
Healthy subjects					
1		55	-662	-17	10,886
2		893	5,102	314	18,483
3		43	22,511	12,107	24,431
4		13,572	25,229	160,094	140,256
5		39,830	22,266	179,660	128,988
Mean ± S.E.M.		10,893 ± 7,691	14,889 ± 5,377	70,432 ± 40,775	64,609 ± 28,719

^a Results of ³H-thymidine incorporation are expressed as Δ cpm = cpm with antigen - cpm without antigen.

^b ND = not done.

1) remained unresponsive to *M. leprae* plus IL-2. It is noteworthy that this patient did not show any proliferative response to PPD, either with or without IL-2; whereas all other patients did.

Five healthy subjects (Table 2) previously investigated for other purposes were selected to represent varying intensities of PBMC proliferative response to PPD. In the absence of IL-2, only those subjects (nos. 4 and 5) who were strongly reactive to PPD also reacted, to a lesser extent, to *M. leprae*. Among the 3 other subjects, 2 (nos. 1 and 2) were also unresponsive to PPD (although they had been vaccinated with BCG); when the proliferation assay was performed in the presence of IL-2, both exhibited a significant proliferation to PPD but only 1 displayed a marginal response to *M. leprae*. The last *M. leprae* unreactive subject (no. 3), BCG vaccinated, exhibited in the absence of IL-2 a significant but low proliferative response to PPD (increasing upon IL-2 addition), and presented with a strong, *M. leprae*-triggered PBMC proliferative re-

sponse after IL-2 addition. These results are different from those of Haregewoin, *et al.* (⁴) who failed to induce a significant response to *M. leprae* with IL-2 in 5 not exposed and not responsive healthy controls, but did not provide information about their response to PPD.

In conclusion, our data indicate that a majority of *M. leprae* nonresponders, including all lepromatous patients, a few borderline tuberculoid patients, and some healthy subjects not exposed to *M. leprae*, may acquire an *in vitro* proliferative response to *M. leprae* upon IL-2 addition. This capacity seems to closely correlate with the ability of these individuals to develop a proliferative response to PPD, strongly suggesting that the IL-2-induced proliferative response to *M. leprae* is directed against epitopes shared by *M. bovis* and *M. leprae*. As suggested by Ottenhoff, *et al.* (⁹), this may explain the differences observed in the frequency of IL-2-induced reversal of *M. leprae* unresponsiveness among different populations. Thus, it would be very interesting

to investigate among lepromatous patients and *M. leprae*-exposed anergic contacts whether or not such an *in vitro* reversion would correlate with the ability to develop T-cell-mediated immunity to *M. leprae* after BCG vaccination, or after combined immunotherapy with BCG and killed *M. leprae*, as proposed by Convit, *et al.* (2).

Our observation on the IL-2 induction of *M. leprae* responsiveness in some healthy subjects raises a significant query on the mechanism of such a "reversal," both in these subjects and in lepromatous patients. The formerly proposed hypothesis of a suppression of IL-2 production in lepromatous patients, which could be bypassed by the addition of exogenous IL-2 (4, 5, 7, 8), might apply to the healthy subjects presented in this study as well, since they were unable to develop a significant T-cell response to PPD despite BCG vaccination. Alternatively, IL-2 may just amplify the proliferation of a low number of *M. leprae*-reactive, precursor T cells, allowing their proliferative response to reach the threshold of detection. The IL-2-induced proliferation to *M. leprae* in lepromatous patients did not reach the level of that attained in the absence of IL-2 by tuberculoid patients, which may be interpreted as a partial failure of IL-2 to overcome suppression but also could result from a simple quantitative difference of *M. leprae*-reactive cells between lepromatous and tuberculoid patients. The evaluation of the frequency of such precursors by the limiting dilution technique would, perhaps, provide a decisive answer.

—Premlata Shankar, M.D.

—Daniel Wallach, M.D.

—Marie-Ann Bach, M.D., Ph.D.

Unité de Pathologie de l'Immunité

Institut Pasteur

and

Service de Dermatologie du Pr. Cottenot

Hôpital St.-Louis

Paris, France

REFERENCES

1. BACH, M.-A., WALLACH, D., FLAGEUL, B. and COTTENOT, F. *In vitro* proliferative response to *M. leprae* and PPD of isolated T-cell subsets from leprosy patients. *Clin. Exp. Immunol.* **52** (1983) 107–114.
2. CONVIT, J., ARANZAZU, N., PINARDI, M. and ULRICH, M. Immunological changes observed in indeterminate and lepromatous leprosy patients and Mitsuda-negative contacts after the inoculation of a mixture of *Mycobacterium leprae* and BCG. *Clin. Exp. Immunol.* **36** (1979) 214–220.
3. GODAL, T. Immunological aspects of leprosy. Present status. *Prog. Allergy* **25** (1978) 211–242.
4. 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.
5. 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 leprae*. *Immunol. Rev.* **80** (1984) 77–86.
6. MEHRA, V., CONVIT, J., RUBINSTEIN, A. and BLOOM, B. Activated suppressor T-cells in leprosy. *J. Immunol.* **129** (1982) 1946–1951.
7. NATH, I., JAYARAMAN, J., SATHISH, M., BHUTANI, L. K. and SHARMA, A. K. Inhibition of interleukin-2 production by adherent cell factors from lepromatous leprosy patients. *Clin. Exp. Immunol.* **58** (1984) 531–538.
8. NATH, I., SATHISH, M., JAYARAMAN, T., BHUTANI, L. K. and SHARMA, A. K. Evidence for the presence of *M. leprae* reactive T lymphocytes in patients with lepromatous leprosy. *Clin. Exp. Immunol.* **58** (1984) 522–530.
9. 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.