Response to Letter of Dr. Ottenhoff, *et al.*

TO THE EDITOR:

We are grateful for your invitation to comment on the Letter to the Editor from Ottenhoff, *et al.* As discussed by Ottenhoff, *et al.*, the discrepancies between our published data (Haregewoin, *et al.*, Nature **303** [1983] 342–344) and theirs may be due to either methodological differences or differences in the patient groups.

With the kind assistance of Dr. Leiker, we have had the opportunity of studying several patients from Holland. Our results are shown in Table 1. As can be seen from Table 1, we have also found a poor response of such patients to T-cell conditioned medium (TCM) (Lymphocult T, Biotest). Only 2 out of 8 patients gave a significant response (>5000 Δ cpm). However, both of the responders also responded to *M. leprae* alone. Thus, our data with TCM are quite analogous to those of Ottenhoff, *et al.* The only notable difference between their data and ours is that, while in their assay the overall response to PPD was quite low, we had good responses to BCG in the three patients tested. This difference may indicate that significant methodological differences exist between our two laboratories, but their relevance to the interleukin 2 (IL2) effect in lepromatous leprosy remains unclear. In contrast to TCM, recombinant IL2 appears to have significant effects in 3 out of 4 tested patients. We hope that Ottenhoff, *et al.* will have the opportunity also to study recombinant IL2. We agree with Ottenhoff, *et al.* that the most likely explanation for the dis-

TABLE 1. *Response*[a] *of patients from Holland*[b] *to* M. leprae, M. leprae + *TCM,*[c] M. leprae + *HPLC + purified IL2,*[d] *and* M. leprae + *recombinant IL2 (REC IL2).*[e]

Patient	Cell viability	^{3}H-thymidine incorporation (cpm × 10^{-3})								
		Cells alone	Cells + *M. leprae*	Cells + 1/200 TCM	Cells + 1/200 TCM + *M. leprae*	Cells + HPLC IL2 80/ml	Cells + HPLC IL2 80/ml + *M. leprae*	Cells + REC IL2 6 U/ml	Cells + REC IL2 6 U/ml + *M. leprae*	Cells + BCG
W.	>95%	1.2	53.3[f]	25.1	115.1	15.1	68.4	—	—	131.3
D.H.	>95%	0.9	7.8	27.9	43.8	17.7	29.0	—	—	74.5
N.G.	80%	1.7	3.8	9.3	7.4	10.2	11.7	—	—	—
T.O.	>95%	13.9	11.8	23.6	26.8	14.9	24.6	—	—	44.4
L.I.	>95%	11.2	8.7	5.2	8.7	6.1	8.7	5.7	6.5	—
H.E.	>95%	1.3	4.0	13.7	14.4	2.2	3.8	13.6	22.2	—
M.A.	—[g]	0.04	0.3	4.1	2.9	—	—	0.08	41.2	—
S.Y.	—	0.06	0.7	13.7	12.8	—	—	0.1	37.0	—

[a] Peripheral blood leukocytes (PBLs) were separated within 12 hr after blood collection; 2×10^5 PBL in complete medium (RPMI 1640 + 15% AB serum + 1% penicillin and streptomycin) were added to each well of round-bottom microtiter plates. Armadillo-derived whole *M. leprae* were added at a final concentration of 5×10^7 bacilli/ml. IL2 preparations were used at indicated concentrations. Total culture volume was kept at 200 μl. Cultures were pulsed with 1 μCi ^{3}H-thymidine on day 5 and harvested 20 hr later. Incorporated radioactivity was determined by liquid scintillation counting. Median cpm from triplicate cultures were tabulated.

[b] The patient material was obtained from Royal Tropical Institute, Amsterdam, Holland. Patients were diagnosed and the blood was sent under the kind supervision of Dr. Derk L. Leiker.

[c] TCM was Lymphocult T purchased from Biotest.

[d] HPLC IL2 was high-performance liquid chromatography purified IL2, kindly gifted by Dr. B. R. Bloom, Albert Einstein College of Medicine, New York, U.S.A.

[e] Recombinant IL2 was a kind gift from Cetus Corporation, California, U.S.A.

[f] Δ cpm ≥ 5000 are boxed.

[g] — = not done.

TABLE 2. *Number of lepromatous patients from different countries showing positive response to* M. leprae *in the presence of TCM, HPLC-purified IL2, and recombinant IL2.*[a]

Country	*M. leprae* + TCM[b]	*M. leprae* + HPLC IL2[c]	*M. leprae* + REC IL2[d]
England	1/4[e]	—[f]	4/4
Ethiopia	3/4	—	—
Holland	0/6	1/4	3/4
Norway	2/4	2/3	3/4
Total	6/18	3/7	10/12

[a] The patient material was obtained from ALERT/AHRI, Addis Ababa, Ethiopia; Ullevål Hospital, Oslo, Norway; Royal Tropical Institute, Amsterdam, Holland, and Hospital for Tropical Diseases, London, England. The patients were diagnosed and the blood was sent under the kind supervisions of Dr. Abebe Haregewoin, Dr. Ivar Helle, Dr. Derk L. Leiker, and Dr. Michael F. R. Waters. PBLs were separated within 12–24 hr after blood collection. The experiments were done as described in Table 1.

[b] TCM was used at a final dilution of 1/200.

[c] HPLC IL2 was added at a concentration of 8 U/ml.

[d] Recombinant IL2 was a kind gift from Cetus Corporation, California, U.S.A., and was used at 6 U/ml.

[e] Positive/tested. Positive responders were sorted on the basis of Δ cpm of >5000, i.e., difference in cpm between cultures with *M. leprae* and IL2 preparations − *M. leprae* alone.

[f] — = not done.

crepancies observed may be related to the patient population. Indeed, when we analyze our results based on patients from different locations, we find differences particularly with TCM (Table 2). While the material is too limited to draw definite conclusions, the data suggest that the lepromatous group is heterogeneous with regard to their resonse to TCM. This heterogeneity appears to become reduced when using recombinant IL2.

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