Response to Phytohemagglutinin of LL Patients' Lymphocytes Preincubated in Culture Media

TO THE EDITOR:

Patients with leprosy present a well-known, wide spectrum of clinical manifestations ([1]). Recently, we have published that lymphocytes from lepromatous (LL) patients stimulated with T mitogens are deficient in the synthesis of interleukin-2 (IL-2). However, the cells possess receptors for IL-2 ([3, 4]). Mohagheghpour, *et al.* ([6]) have published that the failure of T lymphocytes from LL patients to respond to *Mycobacterium leprae* was associated with the defective expression of IL-2 receptors, and that this deficiency was not corrected by exogenous IL-2. Later, they reported ([5]) that when T lymphocytes from LL patients were preincubated in culture media for 48 hr and then stimulated with specific antigen, the cells recovered their ability to proliferate.

According to the above, we wondered if T cells from LL patients were also able to recover their capacity of proliferation when they were preincubated in culture media and then stimulated with a nonspecific T mitogen, such as phytohemagglutinin (PHA).

Study subjects. Based on Mohagheghpour, *et al.*'s patients' classifications ([5]) as "responders" and "nonresponders," we tested 23 patients (8 "responders" and 15 "nonresponders") from the Instituto Dermatologico at Guadalajara, Jalisco, Mexico, diagnosed as having LL according to international criteria ([7]).

Mononuclear cells. Heparinized blood (20 IU/ml) was obtained from each subject by venipuncture. After centrifugation on a Ficoll-hypaque gradient ([1]), the mononuclear cells were recovered and washed. The

THE TABLE. *Response to phytohemagglutinin of LL patients' lymphocytes preincubated in culture media.*

	Nonpreincubated lymphocytes		Preincubated lymphocytes	
	Λcpm[a] (mean ± S.D.)	SI[b]	Λcpm[a] (mean ± S.D.)	SI[b]
		Nonresponders		
	1,592 ± 97	1.0	1,553 ± 547	1.9
	6,409 ± 212	1.0	7,734 ± 349	1.2
	16,295 ± 2,400	3.4	18,606 ± 2,294	2.9
	14,193 ± 305	2.7	17,013 ± 4,512	2.9
	14,643 ± 644	3.2	9,485 ± 285	1.0
	4,912 ± 1,013	3.2	17,934 ± 1,742	10.3
	2,845 ± 324	2.7	1,780 ± 190	2.4
	1,417 ± 190	1.8	5,350 ± 110	3.7
	7,853 ± 58	1.1	15,641 ± 192	3.8
	7,475 ± 239	1.0	7,961 ± 452	1.2
	11,424 ± 1,654	4.3	16,145 ± 1,686	5.2
	10,404 ± 574	3.9	20,072 ± 2,093	6.3
	10,560 ± 789	4.2	5,971 ± 193	4.4
	1,361 ± 153	2.5	3,359 ± 341	5.6
	15,803 ± 1,059	5.7	2,850 ± 186	1.4
Mean ± S.D.	8,479 ± 647	2.8	10,096 ± 1,011	3.6
		Responders		
	25,296 ± 2,228	5.2	27,865 ± 228	4.8
	18,897 ± 162	14.5	26,899 ± 2,514	23.7
	36,860 ± 3,069	29.3	56,069 ± 3,245	35.4
	72,312 ± 3,051	35.7	84,721 ± 238	39.0
	30,288 ± 2,189	21.7	47,339 ± 3,609	36.3
	17,682 ± 519	7.0	26,921 ± 2,980	7.0
	39,286 ± 1,194	28.0	52,776 ± 2,180	33.8
	28,410 ± 1,798	27.3	7,667 ± 741	4.1
Mean ± S.D.	33,628 ± 1,776	21.0	41,282 ± 1,966	23.0

[a] Λcpm = Increase of counts per minute of nonstimulated cells to counts per minute of stimulated cells in culture.
[b] SI = Stimulation index (ratio between stimulated and nonstimulated cells in culture).
No significant differences between responses of nonpreincubated and preincubated lymphocytes were found.

cells were then resuspended in RPMI 1640 culture medium (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) and supplemented as previously reported ([4]).

Lymphoproliferation assay. To 2×10^5 mononuclear cells, 10 μg/ml PHA or supplemented RPMI 1640 medium was added. The cultures were incubated for 48 hr at 37°C in a mixture of 95% air–5% CO_2. Thereafter they were pulsed with 1 μCi ^{3}H-thymidine (specific activity 6.7 Ci/μmole; New England Nuclear, Boston, Massachusetts, U.S.A.). After 24-hr incubation, the cells were harvested, and the incorporation of ^{3}H-thymidine was measured in a Packard beta counter ([4]).

Preincubation. Simultaneously, 2×10^5 mononuclear cells were incubated for 48 hr at 37°C in a mixture of 95% air–5% CO_2. Thereafter they were assayed for lymphoproliferation as mentioned above.

The results from the eight patients of the "responder" group showed that in 2 (patients nos. 2 and 5) of the 8 patients the T cells increased their proliferation capacity (The Table). On the other hand, T lymphocytes from only 1 (patient no. 6) of the 15 LL patients of the "nonresponder" group did not recover that capacity.

Mohagheghpour, *et al.* ([5]) have shown that the inability of T lymphocytes from LL patients to proliferate in response to specific antigen was reversible if the cells were preincubated in culture medium alone for 48 hr. This fact suggests that in those conditions an excess of *M. leprae* antigen is released or that the cell surface receptors are re-expressed. Based on this study ([5]), we tried

to ascertain whether the inability of T lymphocytes from LL patients to proliferate in response to PHA stimulation was also reversible by preincubating those cells.

This hypothesis was not confirmed; our results showed that in only 3 of 23 cases did T lymphocytes from LL patients recover proliferation capacity. These results are not in contradiction with those obtained by Mohagheghpour, *et al.* who used specific antigen for proliferation of T cells. They only show that PHA cannot induce proliferation under the above-mentioned conditions, that perhaps the failure of T lymphocytes to respond to the mitogen stimulus is due to inadequate calcium metabolism, T-cell cycle and IL-2 synthesis ([8]) and not to the steric obstruction of the receptor to PHA with *M. leprae* antigen.

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