

found that once the blood sample was dried on filter paper and kept dry, it remained preserved (even up to 1 year) until it was reconstituted, thus averting the common problem of contamination. We have also found that the extreme storage temperature of 40°C for 2 weeks did not affect the antibody levels of the samples. It appears, therefore, that this is a reliable method which can be used despite seasonal variations of temperature.

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REFERENCES

1. BURGESS, P. J., FINE, P. E. M., PONNIGHAUS, J. M. and DRAPER, C. C. Serological tests in leprosy. The sensitivity, specificity and predictive value of ELISA tests based on phenolic glycolipid antigens, and the implications for their use in epidemiological studies. *Epidemiol. Infect.* **101** (1988) 159–171.
2. FRAKER, P. J. and SPECK, J. C., JR. Protein and cell membrane iodination with a sparingly soluble chloramide 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochem. Biophys. Res. Commun.* **80** (1978) 849–857.
3. IVANYI, J., SINHA, S., ASTON, R., CUSSELL, D., KEEN, M. and SENGUPTA, U. Definition of species specific and cross-reactive antigenic determinants of *Mycobacterium leprae* using monoclonal antibodies. *Clin. Exp. Immunol.* **52** (1983) 528–536.
4. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity; a five-group system. *Int. J. Lepr.* **34** (1966) 255–273.
5. SINHA, S., SENGUPTA, U., RAMU, G. and IVANYI, J. Serological survey of leprosy and control subjects by a monoclonal antibody based immunoassay. *Int. J. Lepr.* **53** (1985) 33–38.

Detection of Interleukin-2 Receptor (IL-2r) by Indirect Immunofluorescence with Anti-Tac Monoclonal Antibody on the Surface of T Lymphocytes from Patients with Lepromatous Leprosy

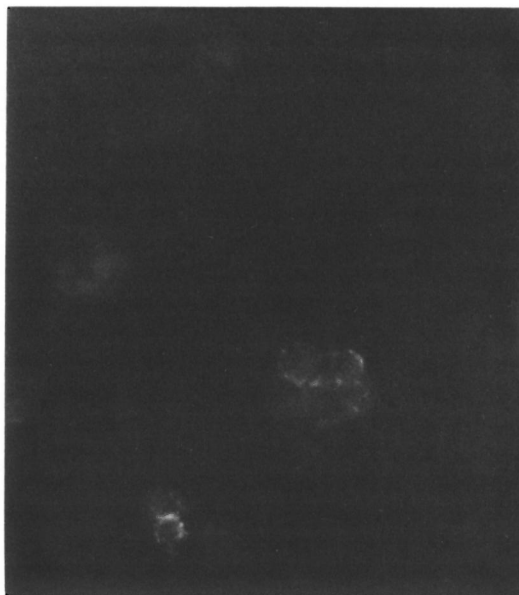
TO THE EDITOR:

We have recently published that lymphocytes from patients with lepromatous leprosy (LL) stimulated with concanavalin A (ConA) are deficient in the synthesis of interleukin-2 (IL-2); however, the cells possess receptors for IL-2 (IL-2r) in such a manner that when activated lymphocytes are cultured *in vitro* and exogenous IL-2 is added, they recover their capacity to proliferate as demonstrated by the incorpora-

tion of tritiated thymidine (³). A demonstration of the physical presence of IL-2r on the surface of T lymphocytes activated with phytohemagglutinin (PHA) by means of indirect immunofluorescence (IIF) using an anti-Tac monoclonal antibody (^{4, 8, 9, 11}) is reported here. (The anti-Tac monoclonal antibody was kindly provided by Dr. T. A. Waldmann, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A., and we thank him for his courtesy.)

Five women and eight men between the ages of 20 and 60 years old were studied at the Instituto Dermatologico de Guadalajara, Jalisco, Mexico. All of them had nodular or diffuse LL, a positive bacilloscopy, and had been receiving irregular treatment with 100 mg of dapsone daily for from 2 to 15 years. The control group consisted of eight healthy persons, of similar sex and age, not related to the patients.

The mononuclear cells from heparinized venous blood were obtained by means of Ficoll-Hypaque and resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum (3). The IL-2r was studied and detected by IIF (1) on the membrane of the lymphocytes using anti-Tac monoclonal antibody; for this purpose two groups of cells were studied. The first group (To) corresponds to lymphocytes without any stimulation; the second group (T₇₂) consisted of lymphocytes previously activated with PHA for 72 hr. Cells (1 × 10⁶) obtained from experiments To and T₇₂ were incubated for 30 min at 4°C with anti-Tac monoclonal antibody diluted 1:500. The cells were then washed and again incubated for 30 min at 4°C with anti-mouse IgG antibody labeled with fluorescein isothiocyanate (FITC) (Sigma Chemical Co., St. Louis, Missouri, U.S.A.), and were analyzed by fluorescence microscopy. Data were obtained by count-



THE FIGURE. Tac+ cells revealed by indirect immunofluorescence. Anti-Tac monoclonal antibodies are bound to IL-2r and demonstrated by fluorescent anti-mouse IgG antibodies.

ing 300 cells and the percent of Tac+ cells was calculated for each of the studied groups.

The data obtained by IIF show that both the cells from the group of LL patients and those of the control group not activated with PHA (To) had practically no Tac+ cells (The

THE TABLE. Presence of IL-2r on the surface of T lymphocytes by IIF with anti-Tac antibody.

Case	LL cases (N = 13)		Control group (N = 8)	
	To ^a	T ₇₂ ^b	To ^a	T ₇₂ ^b
1	1.0	72.0	0.0	70.3
2	1.0	63.1	0.0	60.0
3	1.5	51.1	3.0	80.0
4	4.7	57.9	0.0	64.4
5	6.4	22.2	2.0	54.7
6	0.6	69.3	1.0	51.4
7	0.0	70.2	0.3	76.8
8	1.0	64.8	2.0	65.0
9	0.9	80.1		
10	0.0	63.0		
11	1.0	62.0		
12	0.0	69.3		
13	0.0	53.0		
Mean ± S.D.	1.3 ± 1.8	60.0 ± 13.4 ^c	1.04 ± 1.08	65.3 ± 9.4 ^c

^a Mononuclear cells from peripheral blood taken from LL patients and controls without stimuli.

^b Mononuclear cells cultured 72 hr with PHA (10 µg/ml).

^c Difference not statistically significant; Student's *t* test.

Table). However, in both groups after activation with PHA for 72 hr (T_{72}) the percentages were 60.8 ± 13.4 for cells from LL subjects and 65.3 ± 9.4 for the control group. These results were not different statistically when an independent Student's *t* test was performed.

This study demonstrates the physical presence of the IL-2r by the specific union of anti-Tac monoclonal antibodies to the 55-kDa chain of the high-affinity IL-2r⁽⁶⁻⁹⁾, and confirms that T cells of LL patients truly possess IL-2r but are incapable of proliferating adequately due to the deficiency of IL-2 biosynthesis⁽²⁻⁴⁾. It should be mentioned that Mohaghehpour, *et al.* found similar results using ConA⁽⁵⁾, but the percentages of T cells expressing IL-2r (Tac+) were lower than those found by us; this difference is probably due to the fact that PHA is a better inducer of IL-2r⁽¹¹⁾.

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REFERENCES

1. COONS, A. H. Histochemistry with labeled antibody. *Int. Rev. Cytol.* **5** (1956) 1-4.
2. HAREGEWOIN, A., MUSTAFA, A. S., HELLE, I., WATERS, M. F., 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) 76-86.
3. ISLAS, R. A., MORALES, O. R., FAFUTIS, M. M., GONZALEZ, M. A. and ORTIZ, O. L. Deficiency in the biosynthesis of interleukin-2 (IL-2) and functional presence of the IL-2 receptor in lepromatous leprosy. *Int. J. Lepr.* **55** (1987) 566-569.
4. LEONARD, W. J., DEPPER, J. M., UCHIYAMA, T., SMITH, A. and WALDMANN, T. A. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. *Nature* **300** (1982) 267-269.
5. MOHAGHEHPOUR, N., GELBER, R. H., LARRICK, J. W., SASAKI, D. T., BRENNAN, P. J. and ENGLEMAN, E. G. The effective cell-mediated immunity in leprosy: failure of T cells from lepromatous leprosy patients to respond to *Mycobacterium leprae* is associated with the defective expression of interleukin-2 receptors and is not reconstituted by interleukin-2. *J. Immunol.* **135** (1985) 1433-1449.
6. NOGUEIRA, N., KAPLAN, G., LEVY, E., SARNO, E. N., KUSHNER, P., GRANELLI-PIPERNO, A., VIEIRA, L., GOULD, V. C., 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.
7. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity; a five-group system. *Int. J. Lepr.* **34** (1966) 255-273.
8. ROBB, R. J., GREENE, N. C. and RUSJ, C. M. Low and high affinity cellular receptors for interleukin-2; implications for the level of Tac antigen. *J. Exp. Med.* **160** (1983) 1126-1146.
9. ROBB, R. J., MUNCK, A. and SMITH, K. A. T cell growth factor receptors; quantification, specificity, and biological relevance. *J. Exp. Med.* **154** (1982) 1455-1474.
10. SMITH, K. A. The 2-chain structure of high-affinity IL-2 receptors. *Immunol. Today* **8** (1987) 11-14.
11. UCHIYAMA, T., BRODER, S. and WALDMANN, T. A. I. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* **126** (1981) 1393-1397.