AN ANALYSIS OF IN VITRO T CELL RESPONSIVENESS IN LEPROMATOUS LEPROSY

By GILLA KAPLAN,* DAVID E. WEINSTEIN,* RALPH M. STEINMAN,* WILLIAM R. LEVIS,* ULRIKE ELVERS,‡ MANUEL E. PATARROYO,§ AND ZANVIL A. COHN*

*From *The Rockefeller University and The Irvington House Institute, New York 10021; ‡Frederico Lleras Acosta, Hospital de Dermatologia and §Departamento de Imunologia, Universidade National, Bogota, Colombia*

Antimicrobial defense mechanisms are enhanced by antigen-specific T cell differentiation followed by activation of mononuclear phagocytes (1, 2). γ Interferon (IFN-γ)[1] is an important mediator of both antimicrobial activity and hydrogen peroxide secretion in mononuclear phagocytes (3). A number of observations suggest that an immunological defect exists in lepromatous leprosy which allows the extensive replication of *M. leprae* within dermal macrophages (4). The skin lesions are relatively deprived of helper T cells (OKT4), which are an important source of IFN-γ (5–7). The exposure of peripheral blood T cells to *M. leprae* fails to result in appreciable T cell proliferation and synthesis of lymphokine, including IFN-γ (8–10). In contrast, the lymphocytes of patients with lepromatous leprosy can respond to lectins and other antigens to which they are sensitized (4, 8–13). This had lead to the concept of selective anergy in which, either due to the absence of *M. leprae*–reactive T cells and/or the activity of suppressor cells or factors, macrophage activation does not occur and microbial dissemination ensues (11–23).

In the course of studies on the reversal of T cell nonresponsiveness with purified IL-2 in lepromatous leprosy patients (8), we were struck by the heterogeneous response to this T cell growth factor. We have therefore reexamined the responsiveness of T cells to *M. leprae* and other antigens, and evaluated their stimulation by recombinant IL-2 and their possible inhibition by suppressor cell populations.

Materials and Methods

Patients. Patients (see Table I) were seen in New York at the regional Hansen's Disease Center, Baley Seton Hospital, Staten Island, or at the Frederico Lleras Acosta Hospital de Dermatologia, Bogota, Colombia. The clinical diagnosis of leprosy was supported in

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[1] *Abbreviations used in this paper:* BCG, bacillus Calmette Guerin; C*, rabbit complement; Con A, concanavalin A; IFN-γ, immune (gamma) interferon; IL-2, interleukin 2; mAb, monoclonal antibodies.

all patients by a skin biopsy which was examined by Dr. Job at the U. S. Public Health Service, National Hansen's Disease Center, Carville, LA, and diagnosed by the Ridley Jopling classification (24).

Mononuclear cells. Cell-mediated immunity in vitro was assessed in bulk blood mononuclear cells (9), or in populations that were depleted of monocytes, T cells, and B lymphocytes. Monoclonal antibodies (mAb) against monocytes (3C10), B cells (BA-1), T helper cells (OKT4), T suppressor/cytotoxic cells (OKT8), and rabbit complement (C*) were used to deplete defined subpopulations of leukocytes (25–27). Controls were treated with C* only. Monocyte elimination was monitored by cytology, staining for nonspecific esterase, or immunofluorescent staining for residual 3C10; T cell subset elimination by staining for residual OKT4 or OKT8 antigen; B cell depletion by staining with fluorescent goat anti-human Ig.

Antigen Stimulation of Lymphocytes. Mononuclear cells were plated in triplicate at 1.5×10^5 cells per well in 96-well, round-bottom plates in 200 μl of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% human AB+ serum, 100 U/ml penicillin, 1 μg/ml streptomycin, and 2 mM glutamine. Armadillo-derived, freeze-dried *M. leprae* antigen was obtained from Dr. R. J. W. Rees (IMMLEP Bank), The National Institute of Medical Research, Mill Hill, England, and was added to the cultures at 8 μg/well. Heat-killed bacillus Calmette-Guerin (BCG) (The Trudeau Institute, Saranac Lake, NY) was added at 2×10^4 bacteria per well. At the end of 5 d, 75–100 μl of supernatant was removed from each well to quantitate IFN-γ levels, and 1 μCi [^{3}H]thymidine (Schwarz Mann Div., Becton, Dickinson & Co., Orangeburg, NY) was added (5 μCi/ml). After an additional 16 h, [^{3}H]thymidine uptake was assessed. To evaluate the statistical significance of changes in cell proliferation, the paired comparisons *t* test was used.

Mitogen Stimulation. Cells were plated as above except that 96-well flat-bottom plates were used, proliferation was monitored at 72 h, and concanavalin A (Con A) was added at 2 μg/ml.

Effects of Exogenous IL-2. Recombinant IL-2 (rIL-2) (the generous gift of Dr. Seth Rudnick; sp act, 8.5×10^5 U/mg; Biogen Research Corp., Cambridge, MA) was added at the onset at 3 U/well with and without *M. leprae* or BCG. T cell proliferation was measured at day 6. In other studies, 1.5 U was added every 24 h (5 times) and the proliferative responses were evaluated daily. The dose of IL-2 corresponded to a dose that moderately enhanced background T cell proliferation in the absence of mycobacteria (see Results), so that synergy between IL-2 and antigen could be detected.

Radioimmunoassay for IFN-γ. The amount of IFN-γ in the culture medium was determined with an assay kit (IMRX Corp., Centocor Malvern, PA). Samples were incubated with polystyrene beads coated with a mouse monoclonal anti-IFN-γ antibody, washed, and incubated with a ^{125}I-labeled second mouse anti-IFN-γ mAb directed to an epitope distinct from that recognized by the first mAb. The amount of IFN-γ in the supernatant was estimated by comparison with a standard curve generated with purified IFN-γ. The limit of detection was 0.1–0.2 U/ml.

Results

The pertinent information including diagnosis, bacterial loads, country of origin, and therapy of 32 unselected patients is summarized in Table I. In subsequent tables, only the corresponding patient number will be used.

Proliferative Responses to Antigen and to Mitogen. Mononuclear leukocytes from patients were cultured in the presence or absence of antigen (*M. leprae*, BCG) or mitogen (Con A), and their proliferative response evaluated (Table II). The ability to respond to *M. leprae* varied widely. In addition to the previously reported differences between the lepromatous and tuberculoid forms of leprosy (4, 9, 11), the lepromatous leprosy and (LL and BL) patients fell into two groups. 14 of 26 were nonresponders to *M. leprae* (group A, Table I); i.e., the mean stimulation index was 1.5 relative to the no antigen control and the absolute

TABLE I

Pertinent Demographic Diagnostic and Therapeutic Information of Leprosy Patients

Patient No.	Age (years)	Sex	National origin	Diagnosis	Bacterial index	Treatment (time, drugs)
1	55	F	Colombia	LL	4+	None
2	32	M	Colombia	LL	3–6+	None
3	59	M	Caribbean	LL	1+	5 yr (DDS100; B663200)
4	36	M	Caribbean	LL	5+	1 yr (DDS100; RIF600)
5	30	M	Guyana	LL	6+	1.5 yr (DDS100; RIF600)
6	68	M	Venezuela	LL	4+**	15 yr (DDS irregular)
7	55	F	Colombia	LL	6+	4 yr (DDS irregular)
8	64	M	Colombia	BL	3+	3 yr (DDS100; RIF600; Thal100)
9	30	M	Grenada	LL (ENL)	5+	2 yr (DDS100; RIF600; Thal100)
10	36	M	Mexico	LL	4+	4 yr (DDS100; RIF600)
11	41	M	Colombia	LL	3–6+	3 mo (DDS100; RIF600)
12	31	M	Colombia	LL	4+	1 mo (DDS100; RIF600; LAMP600)
13	32	M	Greece	LL	1+	8 yr (DDS100)
14	27	F	Santo Domingo	LL	6+	None
15	30	F	Caribbean	LL (ENL)	—	5 yr (B663100; Thal100)
16	49	M	Vietnam	LL	3+	3 yr (B663100; Thal200)
17	35	F	Vietnam	BL/BB	4+	None
18	27	M	Colombia	LL	6+	3 yr (DDS100; RIF600)
19	33	M	China	LL	—	5 yr (DDS100; RIF600)
20	32	M	Puerto Rico	LL (inact.)	—	7 yr (DDS100)
21	38	F	Trinidad	BL	2+	4 yr (DDS100)
22	37	M	Santo Domingo	LL	6+	10 yr (DDS100)
23	78	M	Caribbean	LL (inact.)	—	6 yr (DDS100; RIF600)
24	41	F	Trinidad	LL	1+	14 yr (DDS100)
25	51	M	Caribbean	LL	—	19 yr (DDS100; RIF600)
26	33	M	Venezuela	BL	5+	2 mo (DDS100; RIF600)
27	44	M	Colombia	BT	—	None
28	8	M	Colombia	BL/BB	1+	None
29	60	F	Caribbean	BT	—	1 yr (DDS100)
30	50	M	China	BT (N)	—	>5 yr (DDS100; RIF600)
31	35	M	Ghana	BT (N)	—	10 yr (DDS100)
32	23	F	Philippines	BT	—	3 yr (DDS100)

Pertinent demographic and diagnostic information based on the Ridley-Jopling classification (22). LL, lepromatous leprosy; BL, borderline lepromatous; BB, mid-borderline; BT, borderline tuberculoid. Two patients had erythema nodosum leprosum (ENL) (Nos. 9, 15) at the time of the study. Patients 20 and 23 were inactive. Two patients had neural symptoms (N) (Nos. 30, 31). DDS, dapsone; RIF, rifampin; B663, clofazimine; Thal, thaliodmide; LAMP, lamprin. The number following the drug denotes the dose in mg/d.

* DDS resistant.

proliferation was <1,500 cpm. The other 12 patients exhibited low levels of proliferation (group B, mean stimulation index of 6.1). A third group of patients (mid-borderline leprosy [BB] and borderline tuberculoid [BT]) responded vigorously (group C, Table I). The nonresponder and low responder patients with lepromatous leprosy could not otherwise be distinguished on the basis of histopathologic classification, bacterial indices, or the duration and type of treatment (Table I). When cells from lepromatous patients were tested for BCG-induced proliferation, 5 of 11 nonresponders (Nos. 6, 11, 12, 13, and 14) and 4 of 7 low responders (Nos. 18, 19, 22, 25) reacted. This confirms previous reports (11–13) on the presence of a BCG-reactive T cell population in patients unable to respond to *M. leprae*. T cells from all patients proliferated in response to Con A as reported (9). Therefore, the T cell defect in lepromatous disease is specific for *M. leprae* but is manifested as both nonresponsive and hyporesponsive states.

Release of IFN-γ in Response to Antigen or Mitogen. The release of IFN-γ was used as an additional measure of T cell function. Patients classified as *M. leprae* nonresponders, based on antigen-induced proliferation, also failed to release

TABLE II

Proliferative Response to M. leprae, BCG, and Con A: Effect of Exogenous IL-2

Response to *M. leprae*	Patient No.	Proliferative response (cpm × 10^{-3} [^{3}H]thymidine) to:							
		Mitogen		Antigen			IL-2 plus:		
		Con A	None	*M. leprae*	BCG	None	*M. leprae*	BCG	No Ag
Nonresponders	1	27.1	0.4	0.6	0.8	0.5	5.4	3.8	4.4
	2	37.1	1.5	1.3	2.2	0.6	3.0	2.9	1.7
	3	77.9	0.2	1.1	3.4	0.4	4.3	5.4	2.5
	4	18.9	1.8	0.8	NT	0.9	NT	NT	NT
	5	81.8	0.3	0.9	3.3	0.9	5.2	5.7	7.3
	6	46.5	0.9	0.6	10.0*	0.9	1.8	1.3*	5.7
	7	36.0	0.5	3.0	5.2	1.5	20.6	28.0	15.0
	8	11.0	1.7	2.1	NT	1.9	14.5	NT	6.4
	9	13.6	1.7	2.5	NT	1.9	12.5	NT	6.0
	10	49.9	0.9	1.4	2.9	1.1	15.9	17.6	3.2
	11	89.6	0.4	1.5	8.4*	0.7	12.0	21.3*	10.7
	12	75.7	1.1	2.9	33.9*	2.1	10.7	36.1*	5.5
	13	50.7	0.5	2.0	21.3*	0.8	15.4	NT	8.4
	14	85.7	1.1	0.7	5.8*	0.7	7.5	NT	6.2
	Mean SI (SD)	105 (108)	—	1.5 (0.6)	3.9 (2.4) 14.4* (6.4)*	—	—	—	—
Low responders	15	11.2	1.0	3.5	NT	0.3	NT	NT	NT
	16	48.2	2.1	4.7	NT	2.4	NT	NT	NT
	17	8.1	1.0	4.4	4.6	0.7	4.4	NT	1.7
	18	99.2	1.0	5.0	9.1*	0.9	13.6	15.5*	7.7
	19	75.5	1.7	6.5	30.0*	2.0	15.5	28.3*	15.9
	20	41.0	0.5	5.1	4.3	1.5	6.3	NT	3.4
	21	NT	NT	6.1	NT	0.7	11.5	NT	2.5
	22	106.6	0.8	5.1	70.1*	1.2	7.1	NT	4.4
	23	41.1	0.5	5.1	4.3	1.5	6.3	NT	3.4
	24	NT	NT	6.1	NT	0.7	11.6	NT	2.5
	25	118.8	0.9	6.8	18.1*	0.7	10.2	NT	4.3
	26	47.7	0.5	8.5	6.0*	1.2	16.3	11.4*	7.2
	Mean SI (SD)	71.1 (44.3)	—	6.1 (2.9)	6.6 (5.2) 28.8* (20.9)*	—	—	—	—
Responders	27	36.5	0.8	15.9	NT	6.3	NT	NT	NT
	28	25.1	0.6	24.6	15.4*	1.0	32.2	28.2*	16.2
	29	24.5	1.8	26.4	NT	0.5	NT	NT	NT
	30	42.5	1.2	27.7	13.8*	1.1	31.4	NT	2.6
	31	53.9	1.3	49.7	27.3*	1.3	56.6	NT	4.3
	32	13.6	2.0	29.2	NT	0.9	NT	NT	NT
	Mean SI (SD)	30.8 (14.9)	—	29.3 (15.2)	16.3* (3.5)*	—	—	—	—

Proliferative response to *M. leprae*, heat-killed BCG, and Con A. IL-2: 3 U rIL-2 were added to some cultures at the start of incubation.

* Patients that gave a proliferative response to BCG. NT, not tested. Mean stimulation index (SI) and standard deviation (SD) are shown for each group.

IFN-γ when presented with *M. leprae*. In contrast, 4 of 8 low responders (Nos. 18, 22, 25, 26) and all high responders (Nos. 28, 30, 31) released IFN-γ (Table III). Cells from patients that proliferated to BCG also released IFN-γ in response to this antigen. Con A induced high levels of IFN-γ release from most of the patients tested (Table III). Therefore, any proliferative response of the cells to antigen or mitogen was accompanied by the concomitant release of IFN-γ.

TABLE III

Effect of Antigen and Mitogen Stimulation on Release of IFN-γ

Response to *M. leprae*	Patient No.	Units IFN-γ produced in response to:			
		M. leprae	BCG	Con A	No antigen
Nonresponders	1	0.1	11.4	88.0	1.3
	2	1.8	2.4	39.2	1.8
	5	0.7	5.2	140.0	1.5
	6	0.2	7.2*	21.8	0.2
	7	2.0	7.1	80.1	1.5
	8	2.6	NT	5.2	1.4
	10	2.7	5.0	160.0	2.3
	11	0.1	11.4*	137.0	1.4
	12	3.0	58.0*	520.0	1.8
	14	0.8	8.0*	150.0	0.5
	Mean SI	1.1	4.1	11.4	—
	(SD)	(0.5)	(2.6)	(97.0)	
			23.0*		
			(11.4)*		
Low responders	17	0.1	4.6	2.2	2.2
	18	5.0	5.5	12.2	1.3
	19	2.7	NT	120.0	1.5
	22	29.0	150.0*	270.0	0.5
	23	0.2	NT	NT	1.0
	24	0.1	NT	NT	0.1
	25	24.0	97.0*	NT	0.5
	26	10.8	7.8*	167.0	1.2
	Mean SI	15.1	5.1	157.6	—
	(SD)	(22.2)	(0.5)	(223.0)	
			170*		
			(117.2)*		
Responders	28	15.0	10.4*	212.0	1.0
	30	31.0	48.8*	66.0	0.8
	31	175.0	182.0*	250.0	1.2
	Mean SI	66.5	74.3*	167.3	—
	(SD)	(56.9)	(58.5)*	(60.3)	

The results are expressed as mean units IFN-γ released (measured by a direct radioimmunoassay) and mean stimulation index (SI) and standard deviation (SD) for each group.

NT, not tested.

* Patients that respond to BCG (see Table II). The mean SI (SD) for all nonresponders to BCG was 5.9 (2.8) and, for all responders, 58.1 (64.5).

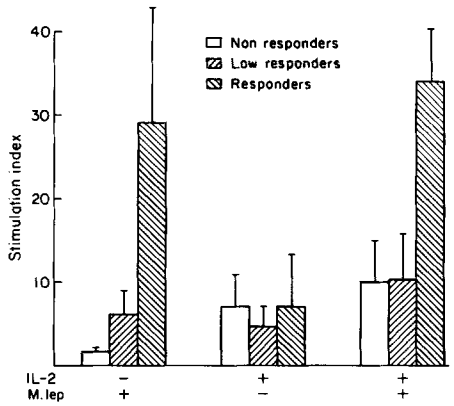

FIGURE 1. The effect of rIL-2 on proliferation in response to *M. leprae*, in nonresponder (□), low responder (▨), and responder (▧) patients. The results are expressed as mean stimulation index ± standard deviation (bar). 3 U IL-2 added at start of culture where indicated.

Effect of Exogenous IL-2 on Proliferation and IFN-γ Release. The effect of exogenous IL-2 on the proliferative response to antigen and mitogen was examined. The addition of a single dose of 3 U of rIL-2 at the beginning of the culture period resulted in a 2–15-fold increase in [^{3}H]thymidine incorporation in all patients, compared with the no antigen control (Table II and Fig. 1). In the majority of low or nonresponders tested (15/22), the addition of IL-2 and *M. leprae*, or IL-2 and BCG, gave an additive response. Only in 4 patients (Table II, Nos. 8–10, 13) was the response more than the sum of the individual *M. leprae* and IL-2 effects.

To ensure that functional IL-2 was available throughout the culture period, additional studies were performed in which IL-2 was added daily for 5 d. If the cells were from nonresponder patients, multiple additions of IL-2 did not enhance proliferation beyond the predicted additive responses to *M. leprae* and IL-2 only (not shown). However, low responder patients did exhibit an increase in the absolute response to *M. leprae*, measured by proliferation as well as IFN-γ release (Figs. 2, *A* and *B*). Under these conditions, the ratio of the response to *M. leprae* over background (4.2, 4.6, 5.1; mean, 4.6 ± 0.4) was the same as that observed from *M. leprae* and IL-2 over IL-2 alone (5.8, 3.8, 3.4, respectively; mean, 4.5 ± 0.9). These data indicate that additional IL-2 does not restore T cell function in nonresponders, but does drive further expansion of *M. leprae*–specific T cells in low responders.

Effects of Monocyte Depletion on Antigen and Mitogen Responses. We next examined if the blood of lepromatous patients had suppressor cells that dampened the *M. leprae* response. Suppressor monocytes were considered first using the mAb 3C10 and C*. This reagent specifically depletes monocytes, but dendritic cells, an accessory cell for many T-dependent responses, are unaffected (28). In most experiments, B cells were also depleted together with monocytes to ensure that we were evaluating a T cell–proliferative response. The mAb elimination method was evaluated with BCG as antigen. 7 of 8 BCG responders showed significantly enhanced proliferation after monocyte depletion (Table IV, footnote *; $0.025 > P > 0.01$). In contrast, responses to Con A were almost uniformly

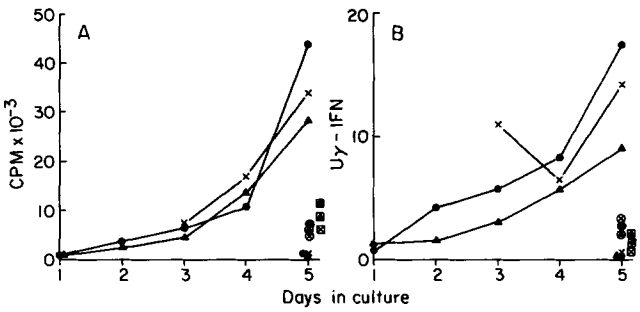

FIGURE 2. The effect of exogenous IL-2 on proliferation (*A*) and IFN-γ production (*B*). Results are for three low responder lepromatous patients (Nos. 22–24), and are expressed as cpm (*A*) and units IFN-γ (*B*). (▲——▲), (●——●), (×——×) Cells incubated with *M. leprae* and 1.5 U of rIL-2 added daily. ◬, ◉, ⊗, Cells incubated with *M. leprae* only. ▲, ●, ×, Cells incubated with 1.5 U IL-2 added daily but no antigen. ▲, ○, ×, Cells incubated without IL-2 or antigen. When 7.5 U were added at the start of culture, the results were similar to those with 1.5 U added daily (not shown).

reduced (Table IV). Therefore, it appears that monocytes are contributory accessory cells in mitogen responses, but are not required and may even be suppressive in the BCG system.

When responses to *M. leprae* were studied in 3C10 plus C*–treated leukocytes, only 1 of 8 nonresponders (Table IV, group A, No. 4) showed >50% enhancement over the C* control. In contrast, 4 of 5 low responders and all responders to *M. leprae* exhibited significantly increased proliferation when monocytes were depleted ($0.05 > P > 0.025$ and $0.025 > P > 0.01$, respectively) (Table IV). These results indicate that monocyte depletion can enhance preexisting responses but seldom reverses nonresponsiveness.

Monocyte depletion also enhanced IFN-γ production in response to antigens, including BCG in BCG-reactive patients (Table V, Nos. 2, 6, 11, 26, 28, 30, 31) and *M. leprae* in two low responders (Table V, Nos. 18, 26). No enhancement of IFN-γ release was observed when monocytes were eliminated from nonresponsive patients.

To evaluate the possibility that monocytes could inhibit the response to IL-2, monocytes were depleted and then exogenous rIL-2 was added to cells from nonresponder patients. Exogenous rIL-2, in the presence or absence of monocytes, did not significantly enhance *M. leprae*–induced T cell proliferation (not shown).

Effects of Depletion of T Cell Subsets, in Combination with Monocyte Depletion and Addition of IL-2. T cell proliferation was next monitored in cultures depleted of suppressor T cells (OKT8), monocytes (3C10), helper T cells (OKT4), or monocytes and suppressor T cells. Depletion of $OKT8^+$ cells had no effect on proliferation to *M. leprae* or to BCG in nonresponder patients, but did reduce the Con A response (Table VI). Combined depletion of monocytes and suppressor T cells (3C10 plus OKT8 plus C*) also had no effect on the response to *M. leprae*. Depletion of $OKT4^+$ T cells ablated the proliferation induced by Con A or BCG.

We also added 7.5 U of exogenous rIL-2 to the OKT4 and OKT8 antibody and C*–treated leukocytes (data not shown). IL-2 did not restore responses to

TABLE IV

Effects of Monocyte Depletion on T Cell Proliferation in Leprosy

Responses to *M. leprae*	Patient No.	Percent monocyte depletion	Proliferative response (cpm[^{3}H]thymidine) to: *M. leprae* C*	*M. leprae* mAb + C*	BCG C*	BCG mAb + C*	Con A C*	Con A mAb + C*	No antigen C*	No antigen mAb + C*
			cpm	*% of C* control*	*cpm*	*% of C* control*	*cpm*	*% of C* control*	*cpm*	*% of C* control*
Nonresponders	1	81	1,056	132	567	52	26,955	45	1,528	125
	2	71	1,251	101	2,392	90	9,614	148	1,619	148
	3	96	1,090	140	1,437	249	59,920	59	1,154	109
	4	NT	1,200	425	NT	NT	24,726	80	2,450	78
	6	97	720	81	7,267*	257	36,966	52	2,747	179
	7	92	1,056	132	1,158	171	13,744	43	1,183	61
	11	91	1,548	141	9,135*	199	68,036	51	2,476	73
	13	NT	2,228	193	10,718*	256	NT	NT	1,585	305
	Mean SI		0.8	1.1	—	—	13.1	0.7	—	—
	(SD)		(0.3)	(0.8)			(7.7)	(0.3)		
	P value		$0.1 > P > 0.05$				$0.025 > P > 0.01$		$P > 0.1$	
Low responders	15	99	12,461	156	NT	NT	31,984	80	1,659	98
	16	NT	3,393	205	NT	NT	23,889	89	1,695	98
	17	99	2,980	67	2,772	36	15,407	23	1,435	128
	18	80	2,004	341	2,347*	940	56,477	59	973	59
	26	NT	6,784	431	3,997*	820	37,978	77	1,456	363
	Mean SI		3.6	9.5	—	—	25.6	0.6	—	—
	(SD)		(2.2)	(3.8)			(16.9)	(0.2)		
	P value		$0.05 > P > 0.025$				$0.05 > P > 0.025$		$P > 0.1$	
Responders	27	97	19,537	183	NT	NT	24,487	61	1,567	102
	28	94	28,863	102	17,351*	103	27,351	43	4,046	35
	29	NT	26,823	164	NT	NT	26,819	67	1,933	98
	30	95	26,272	138	3,508*	182	14,993	123	1,062	360
	31	96	46,651	137	14,793*	320	24,320	75	5,792	280
	Mean SI		13.2	17.5	1.2‡	1.4‡	13.8	0.7	—	—
	(SD)		(6.0)	(7.8)	(0.5)‡	(1.2)‡	(7.4)	(0.2)		
	P value		$0.025 > P > 0.01$		$P > 0.1$‡		$P < 0.005$		$P > 0.1$	
	Mean SI*				3.5*	10.6*				
	(SD)				(1.4)*	(11.9)*				
	P value				$0.025 > P > 0.01$*					

The data are actual counts per minute for the complement (C*) control, and percent of C* control when cells were treated with 3C10 and C*. The percent depletion was calculated as described in Materials and Methods. Mean stimulation index (SI) and standard deviation (SD) are shown for each group. NT, not tested. For significance testing, the paired comparisons *t* test was used.

* Patients that respond to BCG (see Table II).

‡ Data for *all* BCG nonresponders.

M. leprae in OKT8$^-$ cultures, but did restore proliferation in response to BCG in cultures depleted of OKT4$^+$ (helper) cells. This would suggest that the OKT4$^+$ cells are the main source of endogenous IL-2 and that the remaining OKT4$^-$ OKT8$^+$ T cells can proliferate if IL-2 is provided. We conclude that T cell anergy in lepromatous disease cannot be reversed by removing the OKT8$^+$ suppressor subset.

Discussion

Specific hyporesponsiveness of T cells to *M. leprae* antigens is well documented in lepromatous leprosy. It is manifested as diminished skin test responses to

TABLE V

Effect of Monocyte Depletion on the Production of IFN-γ in Response to Antigens

Patient number	Units IFN-γ produced in response to:					
	C* control			3C10 + C*		
	M. leprae	BCG	No Ag	*M. leprae*	BCG	No Ag
Nonresponders						
1	3.0	NT	2.0	3.0	NT	0.2
2	3.8	3.5	3.5	5.0	15.0*	5.0
6	0.3	5.6	0.3	3.8	74.5*	4.0
7	3.0	3.0	2.8	7.5	1.5	4.0
11	0.4	1.8	0.2	2.0	75.1*	0.1
12	2.4	NT	2.4	3.5	NT	6.0
Low responders						
18	2.4	NT	3.0	21.0	NT	1.8
26	2.8	3.2	0.8	9.6	36.6*	2.0
Responders						
28	5.2	3.0	0.8	9.0	4.0*	0.8
30	15.2	4.8	0.2	20.0	35.8*	2.3
31	68.5	19.7	0.2	68.4	180.0*	26.7

Results are expressed as units IFN-γ produced. Cells were treated with complement only (C* control) or antimonocyte mAb and C* (3C10 + C*). NT, not tested. No Ag, no antigen control.
* Patients that respond to BCG (See Table II).

TABLE VI

Effect of T Cell and Monocyte Depletion on the Proliferative Response to Antigen and Mitogen

Treatment of cells	Proliferative response (SI ± SD) to:		
	M. leprae (n = 9)	BCG (n = 2)	Con A (n = 7)
C* control	1.3 ± 0.5	14.3 ± 7.6	20.0 ± 13.7
3C10 + C*	1.4 ± 1.1	13.9 ± 8.2	9.4 ± 5.6
OKT8 + C*	1.4 ± 1.4	6.6 ± 4.2	10.5 ± 4.8
OKT4 + C*	2.0 ± 3.0	2.4 ± 1.1	0.7 ± 0.1
3C10 + OKT8 + C*	1.3 ± 0.8	NT	17.3 ± 11.8

Results are expressed as mean stimulation index ± standard deviation. Cells were treated with complement only (C* control), antimonocyte mAb and C* (3C10 + C*), antisuppressor T cell mAb and C* (OKT8 + C*), anti-helper T cell mAb and C* (OKT4 + C*), and antimonocyte and suppressor T cell mAb and C* (3C10 + OKT8 + C*). NT, not tested. The range of cpm incorporated in response to *M. leprae* was 500–4,800. The patients tested (not included in Table I) were all nonresponder LL with 0–14 yr of treatment. Results for BCG include only the two patients that responded to the antigen. Two patients were not tested for Con A.

lepromin antigens as well as an absence of T cell proliferation and lymphokine release in vitro (8, 9, 11, 12). However, patients exhibit responsiveness to mitogenic lectins and to the *M. leprae*–related organism, BCG, irrespective of their ability to respond to *M. leprae*. Recent observations have raised the possibility that *M. leprae*–specific sensitized T cells are present in lepromatous disease,

but that their function is compromised by inadequate release of IL-2 (10) and/or suppressor T lymphocytes (15, 16, 18) or macrophages (19–21).

Our findings in a substantial number of lepromatous patients from Bogota, Colombia and New York help clarify the deficiency in the cell-mediated immune response. Peripheral blood T cells from patients with lepromatous leprosy proliferate and release IFN-γ to varying degrees in response to *M. leprae* antigens in vitro (Tables II and III). We noted that patients who were judged to be at the lepromatous pole by histopathologic and clinical criteria, actually fell into two groups. Approximately half were unable to generate a detectable response (nonresponders) and half were hyporesponders. T cells from the latter group exhibited a 6.1 ± 2.9–fold boost in proliferation in response to *M. leprae* challenge and, in some cases, in IFN-γ release. It is this latter group in which some success has been achieved in enhancing the response to *M. leprae* in vitro.

Experiments in which specific cell populations were depleted indicated that monocytes or their products, but not $OKT8^+$ T cells, could depress the thymidine incorporation of T cells in response to *M. leprae* (Tables IV, V, and VI). This, however, was obtained only in responsive populations (hypo- and high responders), whereas cells from unresponsive patients were unaffected. Therefore, monocyte suppressor activity could not alone explain the anergic state. In addition, monocyte suppression was not limited to *M. leprae*, as it was observed in the response to BCG.

Studies from our laboratory (8) and those of others (10, 27, 28) have attempted to elucidate the capacity of IL-2 to reverse the unresponsiveness of T cells from patients with lepromatous leprosy. In the present study this parameter has been studied with an appreciation of the variable responses of lepromatous leprosy patients to *M. leprae*. The presence of exogenous IL-2 in cultures of cells from hyporesponsive patients enhances the *M. leprae* proliferation and IFN-γ release by these cells. Since IL-2 did not induce specific T cell function in the majority of *M. leprae* nonresponders, and because the stimulation of hyporesponders by *M. leprae* in the presence of IL-2 and in its absence were similar (about fourfold, Fig. 2), we conclude that the IL-2 supplement did not lead to de novo sensitization, but instead appeared to expand already sensitized cells. These facts suggest that the absence of IL-2 production per se is not responsible for the anergic state. Our results conflict with the reports that responsiveness is restored after exposure of the cells to exogenous IL-2 (10, 28). The discrepancy between our results and those of Haregewoin et al. (10) might be attributed to the heterogeneity of responsiveness of the lepromatous patients. Attempts to restore responsiveness with exogenous IL-2 would appear to be successful if many of the patients tested are low responders rather than nonresponders.

Although *M. leprae* are sequestered within phagolysosomes of dermal macrophages and Schwann cells, it is evident that antigen is gaining access to the immune system. Specifically, there are high levels of anti–*M. leprae* antibodies in lepromatous disease (31). The immunoglobulins are primarily of the IgM subclass and therefore are likely to be to T cell–independent antigenic determinants. It is not known if the *M. leprae* determinants that are required for effective T cell stimulation are also accessible to the immune system. Both specific and nonspecific suppressor mechanisms could be operative within the infected dermis that

might block sensitization. For example, recent evidence suggests a *M. leprae* antigen, the phenolic glycolipid I, specifically suppresses T cell proliferation in response to Con A in lepromatous patients (16). Unfortunately, current in vitro methodology monitors previously sensitized T cell function rather than the priming stage of cell-mediated immunity. These experiments do not provide information on the capacity to prime cells in nonresponder patients relative to responders. There is intriguing data that sensitization to antigens other than *M. leprae* is not effective in lepromatous disease (32, 33), and we are pursuing this in current clinical trials. There are also genetic studies indicating that certain HLA phenotypes (DQ1) predispose individuals to the nonresponsiveness observed in lepromatous disease (34). Many of these considerations can now be approached experimentally, and their elucidation should enrich our knowledge of cell-mediated immunity in man.

Summary

In lepromatous leprosy, there is extensive replication of *Mycobacterium leprae* (*M. leprae*) within dermal macrophages. This lack of microbial resistance has been attributed to a defective cell-mediated immune response to *M. leprae* antigens. We have examined the in vitro response of T cells to *M. leprae* to determine if hyporesponsiveness could be reversed. The study included 40 unselected patients from New York and from Colombia, most with the severe lepromatous form of the disease.

We first noted that lepromatous leprosy patients were of two types: those unable to respond, as assessed by T cell proliferation and immune (gamma) interferon (IFN-γ) release, and a second group, exhibiting low but detectable responses relative to tuberculoid controls. When the effect of exogenous recombinant interleukin-2 (IL-2) on the response to *M. leprae* antigens was compared in the two groups, many of the low responders, but not the nonresponders, showed enhanced proliferation and IFN-γ release. To evaluate a possible suppressive effect of monocytes, these cells were eliminated with a cell-specific monoclonal antibody and complement. Depletion of monocytes often expanded preexisting weak responses but did not reverse the anergy of the *M. leprae* nonresponders. The enhancement was not *M. leprae*–specific, since it was also observed when bacillus Calmette-Guerin was the antigenic stimulus for proliferation and IFN-γ production. Removal of the suppressor T cell subset, with OKT8 antibody and complement, also did not restore responses in nonresponder patients. We conclude that a sizable number of lepromatous leprosy patients exhibit a low degree of responsiveness to *M. leprae* and that the responses can be enhanced in vitro with IL-2 or with monocyte depletion. Nonresponsiveness, however, cannot be reversed. Since currently available assays measure the function of previously sensitized T cells, suppressor mechanisms may yet contribute to defective cell-mediated immunity by impairing the initial sensitization to *M. leprae* antigens.

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