DEFECTIVE PRODUCTION OF MONOCYTE-ACTIVATING CYTOKINES IN LEPROMATOUS LEPROSY

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Leprosy is a chronic inflammatory disease caused by an obligate intracellular bacterial pathogen, *Mycobacterium leprae*, that multiplies within mononuclear phagocytes. The disease exhibits a broad clinical spectrum (1) reflecting gradations in patient capacity to develop a specific cell-mediated immune response. In lepromatous leprosy, patients fail to mount an effective cell-mediated immune response and they are anergic to *M. leprae* antigens. The disease is widespread, and infiltrates are poorly organized and contain extraordinarily high numbers of leprosy bacilli in foamy macrophages. In tuberculoid leprosy, patients mount a vigorous cell-mediated immune response, and they exhibit cutaneous delayed-type hypersensitivity to *M. leprae* antigens. In this form, the disease is localized, and well-organized granulomatous infiltrates are found that contain few bacilli.

The immunological defect underlying the lack of an effective cell-mediated immune response in lepromatous leprosy is not understood. The failure of macrophages to control the intracellular multiplication of leprosy bacilli may be secondary to an inability of these macrophages to be activated or to a lack of necessary cytokines. The latter possibility is suggested by the failure of lymphocytes from patients with lepromatous leprosy to proliferate in response to *M. leprae* antigens (2–7). The defect is essentially specific to *M. leprae* antigens. Patients with lepromatous leprosy commonly display cutaneous delayed-typed hypersensitivity (2, 8–10) as well as, in vitro, a lymphocyte blastogenic response to antigens other than *M. leprae* (2–6), although sometimes responses to one or another antigen are impaired to varying degrees (2, 3, 7, 9–11).

In this paper, we studied the monocytes and mononuclear cells of patients with lepromatous and tuberculoid leprosy to learn about the capacity of their monocytes to undergo activation and the capacity of their mononuclear (lymphoid) cells to produce cytokines able to activate monocytes. We assayed monocyte activation by measuring the capacity of monocytes to inhibit the intracellular multiplication of *Legionella pneumophila*, the agent of Legionnaires' disease (12–13). This assay is highly relevant to leprosy since the agent of Legionnaires' disease, like the agent of leprosy, is an intracellular bacterial pathogen for human

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[1] *Abbreviations used in this paper:* CFU, colony-forming units; Con A, concanavalin A; IFA, indirect fluorescent antibody assay.

mononuclear phagocytes (14, 15).

We found that monocytes from patients with either form of leprosy undergo activation in response to lymphokines and strongly inhibit *L. pneumophila* multiplication. However, specifically in response to *M. leprae* antigens, mononuclear cells from patients with lepromatous leprosy fail to release monocyte-activating cytokines. In contrast, mononuclear cells from patients with tuberculoid leprosy secrete such cytokines, which activate monocytes to inhibit the intracellular multiplication of *L. pneumophila*.

Materials and Methods

Patients. All patients were adults and were seen at the Rockefeller University Hospital (Table I). The diagnosis of leprosy was supported in all cases by a skin biopsy examined by pathologists at the U. S. Public Health Service National Hansen's Disease Center, Carville, LA. Clinical diagnosis was based on the Ridley-Jopling classification scheme (1). Informed written consent was obtained from all patients.

Media. Egg yolk buffer, with or without 1% bovine serum albumin, and RPMI 1640 medium were prepared or obtained as described previously (10). No antibiotics were added to any medium in any of the experiments.

Reagents. Concanavalin A (Con A), three times crystallized and lyophilized (Miles-Yeda Ltd., Kankakee, IL); [^{3}H]thymidine (Schwarz/Mann Division, Becton, Dickinson & Co., Orangeburg, NY).

Agar. Modified charcoal yeast extract agar was prepared in 100 × 15-mm bacteriologic petri dishes as described (14).

Serum. Venous blood was obtained, clotted, and serum separated and stored at −70°C until used as described (16). Normal (nonimmune) human serum (type AB) with an indirect fluorescent antibody (IFA) anti-*L. pneumophila* titer (17) of <1:64 was obtained from an adult donor not known to have ever had Legionnaires' disease.

Bacteria. L. pneumophila, Philadelphia 1 strain, was grown in embryonated hens' eggs, harvested, tested for viability and for the presence of contaminating bacteria, stored at −70°C, and partially purified by differential centrifugation just before use, as described (14).

Human Blood Mononuclear Cells. Heparinized venous blood was obtained from patients and the blood mononuclear cell fraction was separated by centrifugation over a Ficoll-sodium diatrizoate solution as previously described (14).

Preparation of Mononuclear Cell Supernatants. A single large batch of Con A-induced mononuclear cell supernatant (Con A supernatant) and of supernatant control (Con A supernatant control) was prepared from the mononuclear cells of a single normal donor who did not have leprosy (13). Con A supernatant was prepared by incubating donor mononuclear cells (3×10^6/ml) in 2 ml RPMI medium containing 25% fresh normal human serum in plastic petri dishes with 15 μg/ml Con A for 2 d at 37°C in 5% CO_2-95% air. This concentration of Con A and this incubation period yield a maximally potent Con A supernatant (12). At the end of the incubation, the cultures were transferred to conical tubes and the cells were sedimented at 200 *g* for 10 min at 4°C. The supernatant was removed, filtered through 0.2 μm Millipore filters (Millipore Corp. Bedford, MA), and stored at −70°C. Con A supernatant control was prepared in the same way except that Con A was added at the end rather than at the beginning of the 2-d incubation period.

Con A supernatants and supernatant controls were prepared from patients with leprosy in the same way except that cells were cultured in 16-mm tissue culture wells (Linbro, 24-well plates; Flow Laboratories, Inc., McLean, VA) containing 500 μl of medium; cell and reagent concentrations were the same as in the above paragraph.

M. leprae supernatants were prepared from patients in the same way and at the same time as Con A supernatants except that 100 μl of partially purified, armadillo-derived, freeze-dried *M. leprae* was used instead of Con A, and mononuclear cells were cultured

TABLE I

Data on Patients with Leprosy

Patient	Age (years)	Sex	National origin	Type of leprosy*	Duration of leprosy	Antibiotic therapy	
						Drug	Duration
A	44	M	Italy‡	LL	11 yr	Dapsone	8 mo
						Rifampin	8 mo
B	41	M	Puerto Rico	BT	9 yr	Dapsone	5 yr
						Rifampin	1 yr
C	36	F	Antigua	BL	3 yr	Dapsone	3 yr
						Rifampin	3 yr
						Clofazimine	3 mo
D	35	M	Nigeria	BT	5 yr	Dapsone	5 yr
E	41	F	Puerto Rico	LL	7 yr	Dapsone	5 yr
						Rifampin	5 yr
F	45	M	China	LL	28 yr	Dapsone	14 yr
						Rifampin	6 yr
G	48	F	Puerto Rico	TT	2 yr	None	
H	55	F	Cambodia	BT	3 mo	None	
I	53	M	Trinidad	LL	35 yr	Dapsone	25 yr
						Rifampin	2 yr
J	23	M	Guyana	BL	6 mo	Dapsone	3 wk
						Rifampin	3 wk
K	36	M	Haiti	TT	6 mo	None	
L	57	F	Dominican Republic	BT	3 yr	Dapsone	3 yr
						Rifampin	3 yr
M	31	F	Equador	LL	3 yr	Dapsone	3 yr
						Rifampin	2 yr
						Clofazimine	6 mo
N	25	M	Vietnam	BT	2 yr	Dapsone	2 yr
						Rifampin	4 mo

* Based on Ridley-Jopling classification: LL, lepromatous leprosy; BL, borderline lepromatous leprosy; BT, borderline tuberculoid leprosy; TT, tuberculoid leprosy. Two patients (C and M) had erythema nodosum leprosum at the time of the study. One patient (C) was on steroids at the time of the study. None of the patients were lymphopenic at the time of the study.

‡ Lived in endemic area (Brazil) prior to illness.

with the *M. leprae* antigen for 5 d instead of 2 d. *M. leprae* supernatant controls were prepared in the same way except that *M. leprae* antigen was added at the end rather than at the beginning of the 5-d incubation period. In another study (13), patient mononuclear cell responses to this *M. leprae* antigen were shown to be relatively specific in both of the assays used in this study. In both the mononuclear cell thymidine incorporation assay and the assay for monocyte-activating cytokines, mononuclear cells from patients recovered from Legionnaires' disease responded more strongly to *L. pneumophila* antigens than to

M. leprae antigens whereas mononuclear cells from patients with tuberculoid leprosy responded more strongly to *M. leprae* than to *L. pneumophila* antigens (13). The *M. leprae* antigen was kindly provided by the Immunology of Leprosy Unit (IMMLEP), World Health Organization, Geneva, Switzerland.

The sediments remaining after the removal of supernatants were all tested for the presence of contaminating microorganisms by resuspending them in RPMI medium and culturing aliquots of the suspension on 5% sheep blood tryptic soy broth agar; none of the preparations used in this study were contaminated.

Activation of Patient Monocytes. Patient monocytes were tested for their capacity to be activated by Con A-induced cytokines generated by normal mononuclear cells. Activation was assayed by measuring the capacity of the cytokine-treated monocytes to inhibit the intracellular multiplication of *L. pneumophila* (12, 13). Freshly explanted patient mononuclear cells (1.5×10^6) were incubated in 16-mm tissue culture wells in 500 μl RPMI 1640 medium containing 10% fresh normal human serum for 1.5 h at 37°C in 5% CO_2-95% air to allow monocytes to adhere. The culture wells were then vigorously washed to remove the nonadherent leukocytes. The monocyte monolayers were then incubated for 24 h in 500 μl RPMI medium containing 20% fresh serum and 30% (vol/vol) Con A supernatant or supernatant control prepared as described above. After 24 h, virulent egg yolk-grown *L. pneumophila* (5×10^3 colony-forming units (CFU)/ml) were added to the cultures. The cultures were incubated at 37°C in 5% CO_2-95% air on a gyratory shaker for 1 h and under stationary conditions thereafter. CFU of *L. pneumophila* in each culture were determined daily on charcoal yeast extract agar as described (14). All cultures were prepared and tested in triplicate.

Activation of Normal Monocytes by Supernatants Derived from Patients with Leprosy. Mononuclear cells from a single adult donor were used in all experiments; the donor had no history of Legionnaires' disease and had a serum IFA anti-*L. pneumophila* titer of <1:64. Monocyte monolayers were prepared in 16-mm tissue culture wells as described in the previous assay and incubated for 24 h in 500 μl RPMI 1640 medium containing 20% fresh normal human serum and 30% (vol/vol) Con A supernatant, Con A supernatant control, *M. leprae* supernatant, or *M. leprae* supernatant control generated from the mononuclear cells of patients, as described above. After 24 h, the cultures were infected with *L. pneumophila* and cultured daily for CFU/ml as described in the previous assay. All cultures were prepared and tested in duplicate. All supernatants from a given patient were tested at the same time. Two patients with lepromatous leprosy were tested at the same time as two patients with tuberculoid leprosy.

Thymidine Incorporation by Mononuclear Cells. The mononuclear cells remaining after the generation of Con A or *M. leprae* supernatants or supernatant controls were resuspended in 600 μl RPMI medium ($\sim 1 \times 10^7$ of the originally cultured mononuclear cells/ml) as described (13). Triplicate 50-μl aliquots of this cell suspension were added to microtest wells (Falcon, 96-well tissue culture plate; Becton, Dickinson & Co., Oxnard, CA) and mixed with an equal volume of RPMI containing 2% serum and 5.0 μCi [^{3}H]-thymidine/ml. The cells were incubated for 2 h at 37°C in 5% CO_2-95% air, and harvested on glass fiber filter paper (Whatman, Inc., Clifton, NJ) with a cell harvester. The filters were dried, placed in glass vials containing liquid scintillation-counting solution (Hydrofluor; National Diagnostics, Inc., Somerville, NJ), incubated overnight at 4°C, and counts per minute registered with a liquid scintillation counter (Nuclear Chicago Corp., Subsidiary of G. D. Searle and Co., Des Plaines, IL).

Statistics. Data were analyzed by the two sample *t* test, two-tailed (18).

Results

Monocytes from Patients with Lepromatous and Tuberculoid Leprosy Can Both Be Activated to an Enhanced Antimicrobial State. We first examined the activation of monocytes from patients with lepromatous or tuberculoid leprosy, as measured by the capacity of their monocytes to inhibit the intracellular multiplication of *L. pneumophila*. Monocytes of patients with either form of leprosy were activated

by Con A supernatant (Fig. 1 and Table II) and they strongly inhibited *L. pneumophila* multiplication in comparison to monocytes pretreated with Con A supernatant control. The degree of inhibition was comparable in both groups. *L. pneumophila* multiplication was decreased by a mean of 2.03 logs by activated monocytes from patients with lepromatous leprosy and by a mean of 1.81 logs by activated monocytes from patients with tuberculoid leprosy (Table II) (difference not significant). The degree of inhibition by activated monocytes from either group of patients was not significantly different from that of activated monocytes from normal persons (mean 2.04 logs; Table II).

Mononuclear Cells from Patients with Lepromatous Leprosy Fail to Incorporate [^{3}H]-thymidine Avidly in Response to M. leprae. We assayed the mononuclear cells of patients with both forms of leprosy for their capacity to incorporate [^{3}H]-thymidine in response to *M. leprae* antigens. To determine the optimal concentration of *M. leprae* antigens, we incubated mononuclear cells of two patients with tuberculoid leprosy for 5 d with *M. leprae* at concentrations ranging from 10 to 250 μg/ml (Fig. 2). The mononuclear cells incorporated [^{3}H]thymidine in response to *M. leprae* and the amount of incorporation was proportional to the concentration of *M. leprae*. Maximal or near maximal responses were obtained with 50–100 μg/ml *M. leprae*. In all subsequent studies, we used *M. leprae* antigens at a concentration of 100 μg/ml.

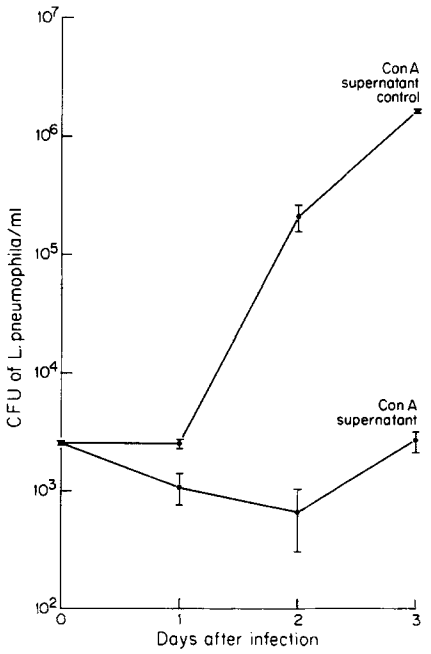

FIGURE 1. Activated monocytes from patients with leprosy inhibit *L. pneumophila* multiplication. Monocyte monolayers were prepared in tissue culture wells from freshly explanted mononuclear cells of patient A, as described in Materials and Methods. The monocytes were incubated for 24 h with 30% Con A-induced supernatant or supernatant control prepared from the mononuclear cells of a normal person. The monocytes were then infected with *L. pneumophila* (2.5×10^3 CFU/ml) and CFU/ml was determined daily. Each point represents the mean CFU/ml for three replicate tissue culture wells ± SEM.

TABLE II

CFU of L. pneumophila at Peak of Infection in Monocyte Cultures Preincubated with Con A Supernatant or Supernatant Control

Leprosy type	Patient	Supernatant control	Supernatant	Log inhibition*
		CFU/ml		
Lepromatous	A	$1.7 \pm 0.03 \times 10^6$	$2.8 \pm 0.6 \times 10^3$	2.78
	C	$9.1 \pm 1.5 \times 10^4$	$1.4 \pm 0.3 \times 10^3$	1.81
	E	$2.5 \pm 0.3 \times 10^6$	$3.8 \pm 0.3 \times 10^4$	1.82
	F	$8.1 \pm 2.7 \times 10^4$	$2.8 \pm 0.3 \times 10^3$	1.46
	I	$1.5 \pm 0.8 \times 10^4$	$4.7 \pm 1.8 \times 10^2$	1.51
	J	$4.5 \pm 0.5 \times 10^5$	$6.7 \pm 2.2 \times 10^2$	2.82
				Mean 2.03 ± 0.35‡
Tuberculoid	B	$3.9 \pm 0.8 \times 10^5$	$6.8 \pm 0.9 \times 10^3$	1.76
	D	$1.6 \pm 0.2 \times 10^5$	$3.1 \pm 0.4 \times 10^3$	1.71
	G	$9.8 \pm 5.2 \times 10^4$	$4.7 \pm 2.5 \times 10^3$	1.32
	H	$4.8 \pm 1.5 \times 10^4$	$3.2 \pm 1.6 \times 10^2$	2.25
	K	$1.2 \pm 0.1 \times 10^6$	$5.8 \pm 0.9 \times 10^3$	2.32
	L	$2.7 \pm 0.4 \times 10^5$	$8.8 \pm 0.9 \times 10^3$	1.49
				Mean 1.81 ± 0.23‡

Monocyte monolayers were prepared in tissue culture wells from freshly explanted mononuclear cells of patients A–L, incubated for 24 h with 30% Con A supernatant or supernatant control, and infected with *L. pneumophila* as in Fig. 1. CFU/ml were determined daily. Data presented are CFU/ml at the peak of infection, i.e., when CFU/ml in control cultures reached their highest level (2 or 3 d after infection). Data are the mean CFU/ml for three replicate tissue culture wells ± SEM. Monocytes from three normal persons activated by the same batch of Con A supernatant used throughout this study inhibited *L. pneumophila* multiplication by a mean of 2.04 ± 0.40 logs. This degree of inhibition was not significantly different from that of activated monocytes from either group of patients.

* Log inhibition = (log CFU/ml in monocyte cultures treated with supernatant control) − (log CFU/ml in monocyte cultures treated with supernatant).

‡ $P > 0.5$.

We next studied [^{3}H]thymidine incorporation in response to *M. leprae* antigens and Con A of mononuclear cells from patients with lepromatous and tuberculoid leprosy (Table III). Mononuclear cells from both types of patients showed strong [^{3}H]thymidine incorporation in response to Con A. Mononuclear cells from patients with tuberculoid leprosy also showed strong [^{3}H]thymidine incorporation in response to *M. leprae* antigens. In contrast, patients with lepromatous leprosy showed weak [^{3}H]thymidine incorporation in response to *M. leprae* antigens. Similar results have been reported by others (2–7).

Mononuclear Cells from Patients with Lepromatous Leprosy Fail to Generate Monocyte-activating Cytokines in Response to M. leprae. We assayed the mononuclear cells of patients with lepromatous and tuberculoid leprosy for their capacity to generate monocyte-activating cytokines in response to Con A and *M. leprae*. To do this, we prepared Con A and *M. leprae* supernatants and supernatant controls from the mononuclear cells of patients with leprosy as described in Materials and Methods. We then tested the capacity of these preparations to activate monocytes by treating normal monocytes with each preparation and measuring monocyte capacity to inhibit the intracellular multiplication of *L. pneumophila* (Table IV). The various supernatant preparations were obtained from the same mononuclear

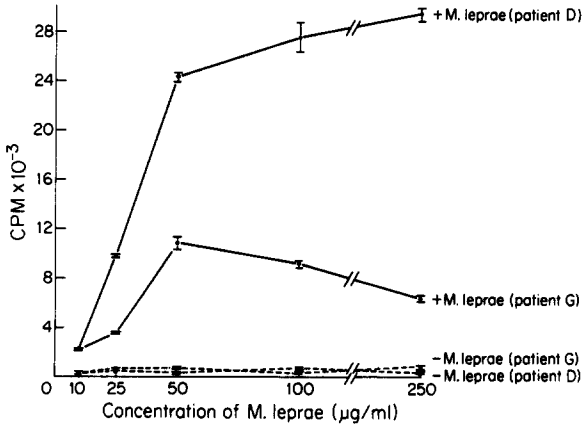

FIGURE 2. [^{3}H]thymidine incorporation by mononuclear cells from patients with tuberculoid leprosy in response to *M. leprae*. Mononuclear cells (3×10^6/ml) were incubated in tissue culture wells at 37°C for 5 d with *M. leprae* (+*M. leprae*) at concentrations ranging from 10 to 250 μg/ml, as indicated, or without *M. leprae* (−*M. leprae*). At the end of the incubation period, *M. leprae*, at the concentration indicated, was added to control cultures that had been incubated without it. The mononuclear cells from two replicate tissue culture wells were combined, distributed into three microtest wells, and assayed for capacity to incorporate [^{3}H]thymidine, as described in Materials and Methods. Each point represents the mean cpm/microtest well for three replicate microtest wells ± SEM.

cell cultures used to study proliferative responses to Con A and *M. leprae* in Table III.

Mononuclear cells from patients with both lepromatous and tuberculoid leprosy responded to Con A with the generation of monocyte-activating cytokines, although Con A-induced supernatants from patients with lepromatous leprosy were less potent than Con A-induced supernatants from the patients with tuberculoid leprosy. Supernatants from patients with lepromatous leprosy resulted in a mean inhibition of 0.87 logs in *L. pneumophila* multiplication while supernatants from patients with tuberculoid leprosy resulted in a mean inhibition of 1.61 logs.

Mononuclear cells from patients with tuberculoid leprosy, with one exception, also responded to *M. leprae* antigens with the production of monocyte-activating cytokines. *M. leprae* supernatants generated from the mononuclear cells of patients with tuberculoid leprosy resulted in a mean inhibition of 0.69 logs. This degree of inhibition is comparable to that resulting from treatment of normal monocytes with *L. pneumophila*-induced supernatants generated from the mononuclear cells of patients recovered from Legionnaires' disease (13). In contrast to mononuclear cells from patients with tuberculoid leprosy, mononuclear cells from patients with lepromatous leprosy failed to generate monocyte-activating cytokines in response to *M. leprae*. In fact, in three of five cases, monocytes treated with *M. leprae* supernatants showed somewhat enhanced multiplication of *L. pneumophila*. On the average, *M. leprae* supernatants from patients with lepromatous leprosy resulted in an enhancement of 0.13 logs.

Thus, the capacity of mononuclear cells from patients with tuberculoid or

TABLE III

[^{3}H]Thymidine Incorporation by Mononuclear Cells of Patients with Leprosy in Response to Con A and M. leprae

Leprosy type	Patient	[^{3}H]thymidine incorporation (cpm/microtest well) by mononuclear cells incubated with (+) or without (−):			
		Con A		*M. leprae*	
		+	−	+	−
Lepromatous	A	12,486 ± 527	220 ± 43	450 ± 24	160 ± 4
	C	12,119 ± 87	357 ± 42	682 ± 177	851 ± 311
	E	7,811 ± 173	207 ± 10	1,112 ± 38	207 ± 39
	F	5,197 ± 98	217 ± 24	3,116 ± 135	415 ± 30
	I	28,887 ± 719	192 ± 8	588 ± 30	196 ± 35
	J	25,363 ± 299	195 ± 4	991 ± 34	226 ± 14
	M	5,271 ± 714	166 ± 13	1,303 ± 29	211 ± 9
		Mean 13,876 ± 5520*		Mean 1,177 ± 524‡	
Tuberculoid	D	12,437 ± 50	486 ± 153	27,588 ± 1203	640 ± 67
	G	12,497 ± 308	203 ± 22	9,071 ± 297	314 ± 27
	H	11,683 ± 556	259 ± 4	4,636 ± 137	134 ± 6
	K	15,971 ± 18	191 ± 6	20,443 ± 896	195 ± 29
	L	21,188 ± 863	202 ± 4	12,796 ± 69	315 ± 13
	N	16,082 ± 157	—	12,402 ± 672	190 ± 14
		Mean 14,976 ± 2069*		Mean 14,489 ± 4766‡	

Mononuclear cells (3×10^6/ml) were incubated in tissue culture wells at 37°C for 2 d with (+) or without (−) Con A (15 μg/ml) or for 5 d with or without *M. leprae* (100 μg/ml). At the end of the 2 or 5 d incubation period, Con A or *M. leprae* was added to the appropriate control cultures that had been incubated without them. The monuclear cells from two tissue culture wells were combined, distributed into three microtest wells, and assayed for capacity to incorporate [^{3}H]thymidine as described in Materials and Methods. Each pair of tissue culture wells was assayed in triplicate microtest wells and the mean cpm/microtest well determined. Each datum on the table for patients A–J is the mean cpm/microtest well for the three microtest wells generated by one pair of tissue culture wells ± SEM. Each datum on the table for patients K–N is the average of the mean cpm/microtest well for three pairs of tissue culture wells (three microtest wells/pair) ± SEM. Patients were studied in six separate experiments as follows: Experiment 1, patient A; experiment 2, patients C and D; experiment 3, patients E and F; experiment 4, patients G, H, I, and J; experiment 5, patient K; experiment 6, patients L, M, and N.

In separate experiments, mean [^{3}H]thymidine incorporation in response to Con A by mononuclear cells from seven normal persons was 11,939 ± 2,356, an amount not significantly different from that of mononuclear cells from patients with either form of leprosy. In experiment 1, [^{3}H]-thymidine incorporation in response to *M. leprae* by mononuclear cells from a normal person with no history of mycobacterial disease was 196 ± 44; in the same experiment, [^{3}H]thymidine incorporation in response to *M. leprae* by a patient with lepromatous leprosy (patient A) was 450 ± 24 and by a patient with tuberculoid leprosy (patient D) was 24,139 ± 552.

* $P > 0.5$.

‡ $P < 0.001$.

lepromatous leprosy to generate monocyte-activating cytokines generally parallels their capacity to incorporate thymidine in response to Con A and *M. leprae*.

Discussion

We have found that monocytes from patients with either lepromatous or tuberculoid leprosy can be activated normally, as measured by a highly relevant assay: the capacity of these monocytes to inhibit the multiplication of another

TABLE IV

Production of Monocyte-activating Cytokines by Mononuclear Cells of Patients with Leprosy in Response to Con A and M. leprae

Leprosy type	Patient	CFU of *L. pneumophila* 2 d after infection in monocyte cultures preincubated with:					
		Con A			M. leprae		
		Supernatant control	Supernatant	Log inhibition*	Supernatant control	Supernatant	Log inhibition*
		CFU/ml			*CFU/ml*		
Lepromatous	A	$5.6 \pm 0.1 \times 10^5$	$2.6 \pm 0.7 \times 10^5$	0.34	$5.2 \pm 1.1 \times 10^5$	$7.2 \pm 1.3 \times 10^5$	−0.14
	C	$3.9 \pm 0.7 \times 10^5$	$2.7 \pm 0.4 \times 10^4$	1.16	$1.0 \pm 0.6 \times 10^6$	$8.5 \pm 1.2 \times 10^5$	0.07
	I	$2.5 \pm 0.1 \times 10^6$	$1.1 \pm 0.5 \times 10^5$	1.33	$4.9 \pm 0.8 \times 10^6$	$1.5 \pm 0.3 \times 10^7$	−0.49
	J	$1.7 \pm 0.3 \times 10^6$	$1.8 \pm 0.5 \times 10^5$	0.97	$3.3 \pm 0.3 \times 10^6$	$1.9 \pm 0.4 \times 10^6$	0.23
	M	$8.4 \pm 1.1 \times 10^5$	$2.3 \pm 0.1 \times 10^5$	0.56	$4.2 \pm 0.6 \times 10^5$	$8.6 \pm 1.4 \times 10^5$	−0.31
				Mean 0.87 ± 0.24‡			Mean -0.13 ± 0.17§
Tuberculoid	D	$2.1 \pm 1.2 \times 10^6$	$2.0 \pm 0.1 \times 10^4$	2.01	$7.9 \pm 0.6 \times 10^5$	$9.6 \pm 2.4 \times 10^4$	0.92
	G	$2.6 \pm 0.1 \times 10^6$	$2.0 \pm 0.2 \times 10^5$	1.12	$2.2 \pm 0.3 \times 10^6$	$5.1 \pm 1.0 \times 10^5$	0.62
	H	$4.0 \pm 1.2 \times 10^6$	$2.4 \pm 0.4 \times 10^5$	1.22	$3.5 \pm 0.5 \times 10^6$	$3.5 \pm 1.5 \times 10^5$	0.99
	K	$1.2 \pm 0.1 \times 10^6$	$1.2 \pm 0.1 \times 10^4$	2.00	$3.1 \pm 0.6 \times 10^4$	$5.5 \pm 2.2 \times 10^4$	−0.25
	L	$6.9 \pm 2.3 \times 10^5$	$3.7 \pm 0.5 \times 10^3$	2.27	$8.5 \pm 2.2 \times 10^5$	$1.7 \pm 0.3 \times 10^5$	0.70
	N	$8.3 \pm 1.3 \times 10^5$	$8.2 \pm 0.3 \times 10^4$	1.01	$1.6 \pm 0.3 \times 10^5$	$1.1 \pm 0.06 \times 10^4$	1.16
				Mean 1.61 ± 0.32‡			Mean 0.69 ± 0.29§

Patient Con A-induced and *M. leprae*-induced supernatants and supernatant controls were prepared using 15 μg/ml Con A or 100 μg/ml *M. leprae* as described in Materials and Methods. Monocytes were incubated for 24 h with 30% supernatant or supernatant control as indicated and then infected with *L. pneumophila* (10^4 CFU/ml). CFU were determined 2 d after infection in replicate monocyte cultures. Data are the mean CFU/ml 2 d after infection for two replicate tissue culture wells ± SEM. Patients were studied in three separate experiments as follows: experiment 1, patients A, C, and D; experiment 2, patients G, H, I, and J; experiment 3, patients K, L, M, and N.

* Log inhibition is as defined in Table II.

‡ $P < 0.05$.

§ $P < 0.02$.

In separate experiments, supernatants generated by mononuclear cells from seven normal persons in response to Con A resulted in a mean inhibition of 1.77 ± 0.16 logs, an amount not significantly different from that of Con A-induced mononuclear cell supernatants of patients with tuberculoid leprosy but significantly different from that of Con A-induced mononuclear cell supernatants of patients with lepromatous leprosy ($P < 0.01$).

intracellular bacterial pathogen, *L. pneumophila*. Unless inhibition of *M. leprae* multiplication requires a qualitatively or quantitatively different form of activation, this suggests that mononuclear phagocytes of patients with lepromatous and tuberculoid leprosy have the capacity to control *M. leprae* infection, if activated. This finding therefore supports the hypothesis that the defect in cell-mediated immunity in lepromatous leprosy derives from a failure to activate mononuclear phagocytes rather than from an intrinsic inability of these cells to be activated. This hypothesis does not exclude the possibility that mononuclear phagocytes located at the site of infection are somehow inhibited from being activated.

Drutz et al. (19) previously reported that nonactivated human monocytes from patients with lepromatous and tuberculoid leprosy have comparable capacities to kill a variety of microorganisms (19) Although mononuclear phatocytes must generally be activated to control infection by intracellular pathogens, this study by Drutz et al. further supports the hypothesis that circulating mononuclear phagocytes from patients with lepromatous leprosy have a normal antimicrobial capacity.

We have also found that mononuclear cells from patients with lepromatous and tuberculoid leprosy respond to Con A with [^{3}H]thymidine incorporation and with the production of monocyte-activating cytokines whereas only mononuclear cells from patients with tuberculoid leprosy respond in these ways to *M. leprae*. These findings suggest that mononuclear cells of patients with lepromatous leprosy have a normal capacity to generate monocyte-activating cytokines but that they fail to do so in response to *M. leprae*. Alternatively, it is possible that mononuclear cells of patients with lepromatous leprosy, in response to *M. leprae*, generate substances inhibitory to monocyte activation at the same time as they generate monocyte-activating cytokines. However, this seems less likely in view of the fact that these mononuclear cells also fail to proliferate in response to *M. leprae*. These findings thus support the hypothesis that the defect in cell-mediated immunity in patients with lepromatous leprosy derives from a failure to generate, in response to *M. leprae* but not other antigens, cytokines necessary to activate mononuclear phagocytes. If this is so, then the underlying basis for this profound defect in cytokine production is obscure, although suppressor cells or factors have been suggested as playing a role (20, 21). Consistent with a suppressor mechanism, Van Voorhis et al. (22) have recently reported from this laboratory that the T cell population in skin lesions of patients with lepromatous leprosy is devoid of OKT4/Leu 3a-positive (helper) cells and is comprised almost exclusively of OKT8/Leu 2a-positive (suppressor) cells. In contrast, the T cell population in skin lesions of patients with tuberculoid leprosy is comprised predominantly of OKT4/Leu 3a-positive cells (22). Similarly, Modlin et al. (23) have reported that the T cell helper/suppressor ratio in two patients with uncomplicated lepromatous leprosy was lower than in four patients with tuberculoid leprosy. More recent studies indicate that peripheral blood mononuclear cells from patients with lepromatous leprosy fail to produce interleukin 2 or gamma interferon in response to *M. leprae*.

Summary

We have examined the capacity of monocytes from patients with leprosy to undergo activation and the capacity of mononuclear cells from these patients to incorporate [^{3}H]thymidine and produce monocyte-activating cytokines.

Monocytes from patients with either lepromatous or tuberculoid leprosy were activated by concanavalin A (Con A)-induced mononuclear cell supernatants generated from the leukocytes of a normal person. Monocytes activated by these supernatants strongly inhibited *L. pneumophila* multiplication, and the degree of inhibition was comparable in both groups of patients.

Mononuclear cells from patients with either form of leprosy responded comparably to Con A with vigorous [^{3}H]thymidine incorporation. Mononuclear cells from patients with tuberculoid leprosy also vigorously incorporated [^{3}H]thymidine in response to *M. leprae* antigens. In contrast, mononuclear cells from patients with lepromatous leprosy did not exhibit significant [^{3}H]thymidine incorporation in response to *M. leprae* antigens.

The capacity of mononuclear cells to generate monocyte-activating cytokines generally paralleled their capacity to incorporate [^{3}H]thymidine in response to Con A and *M. leprae*. Mononuclear cells from patients with either form of leprosy responded to Con A with the production of cytokines (supernatants) able to activate normal monocytes, expressed by inhibition of *L. pneumophila* multiplication. However Con A-induced supernatants from patients with lepromatous leprosy were less potent than Con A-induced supernatants from patients with tuberculoid leprosy. Mononuclear cells from patients with tuberculoid leprosy responded to *M. leprae* antigens with the production of potent monocyte-activating supernatants. In contrast, mononuclear cells from patients with lepromatous leprosy did not produce monocyte-activating cytokines in response to *M. leprae* antigens.

These studies support the hypothesis that the immunological defect in lepromatous leprosy results from a failure to activate mononuclear phagocytes rather than from an intrinsic inability of these cells to be activated. We suggest that the failure to activate mononuclear phagocytes stems from defective production of monocyte-activating cytokines in response to *M. leprae* antigens.

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