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Correlation between Macrophage Activation and Bactericidal Function and *Mycobacterium leprae* Antigen Presentation in Macrophages of Leprosy Patients and Normal Individuals

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The killing of *Mycobacterium leprae* by resting and gamma interferon (IFN-γ)-activated macrophages in normal subjects and leprosy patients was assessed. Resting macrophages from normal individuals demonstrated the ability to kill *M. leprae*. For macrophages from tuberculoid patients, killing of *M. leprae* was only achieved in the presence of IFN-γ, suggesting that initial T-cell activation occurs prior to the killing of *M. leprae*. In contrast, though activation with IFN-γ rendered the lepromatous macrophage microbicidal, it failed to induce lymphocyte proliferation, suggesting a defect at either the antigen-presenting cell or the lymphocyte level or both. The concept that T-cell anergy is primarily due to lack of lymphokine generation was ruled out by our results, since responsiveness was restored in only a small proportion of lepromatous patients after exogenous lymphokine addition. In conclusion, this study demonstrated that killing and antigen presentation are two independent events. It appears that the ability of the macrophages per se to kill *M. leprae* may be of greater importance than lymphocyte-mediated activation for protection against *M. leprae* infection.

Cellular immunity in chronic infections is determined primarily by the extent to which macrophages are activated. Some microbes are killed soon after phagocytosis, while others bypass the conventional microbicidal mechanisms and multiply in the permissive environment provided by macrophages (30).

In the case of lepromatous leprosy, macrophages have been highlighted as important suppressor cells in the chain of events occurring after the host-parasite interaction (8, 25, 26). Our earlier studies have shown a defective regulation of *M. leprae* phagocytosis (19) initiating a series of disturbances in the metabolism of macrophages (5, 7). This probably aids the survival of the parasite and its replication within the susceptible host cell.

Various studies indicate gamma interferon (IFN-γ) as the major macrophage-activating factor found in antigen- and mitogen-stimulated lymphocyte culture supernatants which augment the microbicidal capacity of phagocytes (30). In contrast to tuberculoid patients and normal individuals, lepromatous leprosy patients have been reported to lack circulating T lymphocytes capable of responding to *M. leprae* by proliferation (12) or by the release of IFN-γ (20), resulting in inadequate macrophage activation to eliminate the mycobacteria.

This study aims at assessing whether in vitro activation with IFN-γ can restore the ability of defective macrophages (i) to kill *M. leprae* through oxidative mechanisms and (ii) to process and present *M. leprae* antigens to T lymphocytes.

Due to the inability to directly measure *M. leprae* viability in vitro, workers have resorted to the measurement of killing by indirect methods (5, 10, 17, 23, 28, 29, 32). In the present study, we have assessed *M. leprae* viability by using the following two methods: (i) growth in the mouse footpad (28) and (ii) the down-regulation of the macrophage Fc receptor (4, 5).

Antigen processing and presentation have been assessed by human leukocyte antigen DR (HLA-DR) expression, antigen-specific monocyte-lymphocyte physical interaction, and lymphoproliferation.

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MATERIALS AND METHODS

Subjects. Leprosy patients were classified according to the Ridley and Jopling classification (24). The choice of lepromatous patients was restricted to long-term-treated lepromatous patients who were bacteriologically negative (BI-ve), i.e., who did not demonstrate acid-fast bacilli in smears from multiple sites. All tuberculoid patients were treated for durations varying from 1 to 6 years. Occupational contacts who had various degrees of exposure to leprosy patients were used as normal controls.

Antigens. (i) *M. leprae* was obtained from infected armadillo tissue stored at −80°C. The bacteria were harvested by homogenizing the armadillo tissue. The bacterial pellet obtained after differential centrifugation to minimize tissue contamination was suspended in saline, stored at 4°C, and used within a week.

(ii) Purified protein derivative. Purified protein derivative (PPD) was obtained from the Statens Serum Institute, Copenhagen, Denmark, and was used at an optimal concentration of 4 μg/ml.

Monocyte-macrophage cultures. Mononuclear cells were isolated from heparinized peripheral blood on a Lymphoprep gradient (Nyegaard, Oslo, Norway) and depleted of lymphocytes by adhering them to glass for 24 h. Monocytes were further maintained for 7 days by changing the culture medium every 48 h to obtain differentiated macrophage cultures. The cells were cultured in minimal essential medium (MEM) containing 30% human AB serum at a concentration of 3×10^6 cells per ml either in petri dishes (Falcon, 55 mm; Becton Dickinson Labware, Oxnard, Calif.) in 3 ml of medium or on glass cover slips in Leighton tubes in 0.7 ml of medium.

Determination of *M. leprae* viability. (i) Infection of monocyte-macrophage monolayers. The cultures were infected with 5×10^6 *M. leprae* cells per ml for 24 h before the uningested *M. leprae* were washed off.

(ii) Activation with IFN-γ. IFN-γ was added at an optimal concentration of 10 U/ml either simultaneously with the bacilli or 24 h postinfection.

(iii) Treatment with superoxide dismutase (SOD) from canine blood. SOD (Sigma Chemical Co., St. Louis, Mo.) was added at a concentration of 10 μg/ml simultaneously with IFN-γ and *M. leprae*.

(iv) Viability assays. Viability of *M. leprae* was assessed by determination of the footpad growth curve (28) and by the Fc receptor assay (4, 5).

Footpad growth curve. Macrophage cultures maintained in petri dishes were scraped off with a rubber policeman, suspended in saline, and exposed to 10 cycles of freeze-thawing. The released bacilli were suspended in a known volume of saline and counted by the method of Shepard and McRae (28). A total of 10 mice were inoculated with 10^4 organisms per footpad. The footpads were harvested at 6, 7, 8, 10, and 12 months postinoculation. A sample of the *M. leprae* suspension derived from the armadillo tissue and used for infecting the macrophage cultures in the assay system was used as a positive control.

Fc receptor assay. This assay has been used as a measure of viability since our earlier studies showed that erythrocyte rosetting (EA rosetting) levels are reduced in bacteriologically negative lepromatous patients only in the presence of viable *M. leprae* and not after the addition of autoclaved or rifampin-treated *M. leprae* (5). Macrophage cultures were maintained on glass cover slips for 72 h after infection before EA rosetting was carried out. This duration of incubation was essential to distinguish between the direct action of IFN-γ on the macrophages per se (since IFN-γ is known to transiently increase the Fc receptor levels in macrophages) and its effect on the intracellular *M. leprae*. A similar protocol has been used earlier to distinguish between bacteriostatic and bactericidal drugs (2, 4).

Sheep erythrocytes (SRBCs) in a 2% suspension in MEM were sensitized with an equal volume of goat anti-SRBC antibody (1:900 dilution; Wellcome Research Laboratories, Beckenham, England). A suspension of 1% sensitized SRBCs was overlaid on the macrophage monolayer and allowed to rosette for 30 min at 37°C in an atmosphere of 5% CO_2. Nonrosetted SRBCs were removed by washing, and the monolayers were fixed in 2.5% glutaraldehyde and stained by the Ziehl-Neelsen acid-fast staining method. A total of 200 cells were counted, and the percentage of cells with three or more SRBCs attached was determined.

Antigen-specific monocyte-lymphocyte physical interaction. Mononuclear cells from peripheral blood were isolated on a Lymphoprep gradient. The cells so obtained consisted of 80 to 90% lymphocytes and 10 to 20% monocytes. They were suspended in MEM containing 20% inactivated human AB serum in a concentration of 4×10^6 cells per ml and distributed into Leighton tubes containing cover slips. *M. leprae* was added (3×10^6 per tube) in the presence or absence of activating agents (IFN-γ [10 U/ml] and recombinant interleukin-2 [20 U/ml]), and the cells were incubated at 37°C for 18 h in an atmosphere of 5% CO_2.

The nonrosetted lymphocytes were then washed off, and the cells were fixed in 2.5% glutaraldehyde and stained by the Ziehl-Neelsen acid-fast staining method. The percentage of monocytes with two or more lymphocytes adhering to them was determined and expressed as the percent interaction. (IFN-γ and recombinant interleukin-2 were generous gifts from, respectively, M. Harboe, I.G.R.I., Oslo, Norway, and Francis Singaglia, Hoffman-La Roche, Basel, Switzerland.)

HLA-DR antigen expression on macrophages. Macrophage cultures derived from peripheral blood of bacteriologically negative patients and normal individuals were incubated in the presence of media containing IFN-γ or *M. leprae* (or both) for 24 h at concentrations similar to those used for the Fc assay. Before being stained, the cultures were washed and incubated with freshly collected human serum for 1 h. The cover slips were washed with saline, flooded with anti-HLA-DR (Biotin conjugate, $1:10^3$ dilution; Becton Dickinson and Co., Paramus, N.J.), and incubated in a moist chamber for 30 min. Unbound antibody was removed by washing the cultures three times with phosphate-buffered saline. The cover slips were fixed in cold absolute alcohol for 30 min, washed, and then flooded with anti-immunoglobulin G (anti-IgG) (Avidin-rhodamine conjugate, $1:10^3$ dilution; Becton Dickinson) and incubated in a moist chamber for 30 min. The cover slips were washed, mounted in phosphate-buffered saline-glycerol, and examined under a fluorescent microscope under epi-illumination. The percentage of cells stained positive in IFN-γ-treated cultures in the presence or absence of *M. leprae* was compared with that in control cultures from the same individual.

Lymphocyte proliferation assay. Mononuclear cells were separated on a Lymphoprep gradient, and the cell count was adjusted to 10^6 cells per ml. Cells were suspended in MEM supplemented with 20% human AB serum and antibiotics. A 100-μl portion of this cell suspension was added to each well of a flat-bottomed 96-well microdilution plate.

Viable *M. leprae* at a concentration of 3×10^6 bacilli per ml was added in the presence or absence of concanavalin A (ConA)-derived lymphokines from mononuclear cells. Cultures were maintained in a 5% CO_2 atmosphere at 37°C for 5 days. Eighteen hours before termination 0.5 μCi of tritiated thymidine was added (specific activity, 26,000 mCi/mmol) to each well. Cultures were harvested onto glass microfiber filters. Sample disks were dried and counted in a liquid scintillation counter (Kontron). Results were expressed in terms of stimulation index, which was calculated as the ratio of the mean counts per minute of cultures stimulated simultaneously with *M. leprae* and lymphokines to the mean counts per minute of the cultures stimulated with lymphokines alone.

Preparation of ConA supernatants. ConA supernatants were prepared by incubating 3×10^6 mononuclear cells from normal individuals in 2 ml of MEM containing 25% human AB serum with 20 μg of ConA (Sigma) per ml for 2 days at 37°C in a 5% CO_2 atmosphere. At the end of the incubation period, the cells were sedimented at 2,000 rpm (IEC CENTRA-7R) for 10 min at 4°C. Supernatants were removed, filtered through a 0.2-μm filter (Millipore Corp., Bedford, Mass.) and stored at −90°C until further use. ConA supernatant controls were prepared in the same way as described above except that addition of ConA was made at the end of the 2-day incubation period. ConA supernatants and supernatants from control cultures were used at an optimal concentration of 50 μl per well.

Modified lymphoproliferative assay. Monocytes were enriched on a Nycodenz-monocyte gradient (Nyegaard) from peripheral blood mononuclear cells. Monocytes were added (10^4 per well) to a flat-bottomed 96-well microdilution plate. T lymphocytes were primarily obtained on a Lymphoprep gradient and further enriched on a nylon-wool column. The T lymphocytes were used at a concentration of 10^5 per 100 μl. Viable *M. leprae* at a concentration of 3×10^6/ml or 10 U of IFN-γ per ml (or both) was added for 24 h before the addition of T cells. Cultures were maintained for 5 days and

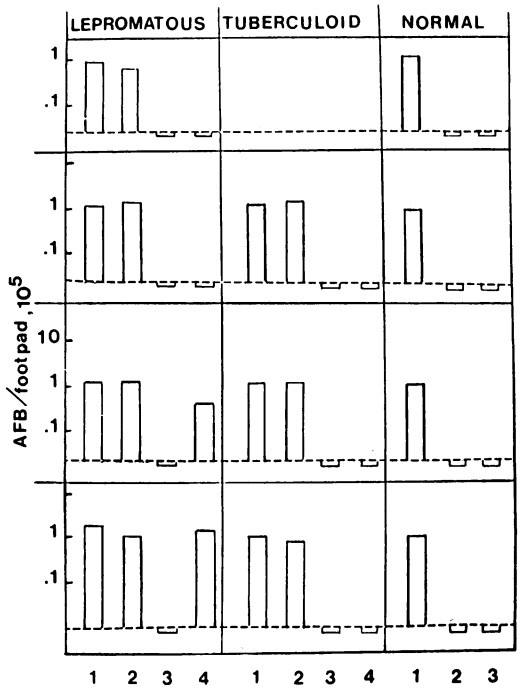

FIG. 1. *M. leprae* viability within resting and activated macrophages from normal individuals and from tuberculoid and lepromatous leprosy patients as assessed by the mouse footpad technique. Each set of bars represents results obtained from a single individual and depicts growth of bacilli obtained at 12 months postinoculation. Resting macrophages from normal individuals were able to kill *M. leprae*. However, macrophages from leprosy patients were able to kill *M. leprae* only after activation with IFN-γ. AFB, Acid-fast bacilli. Lanes: 1, original *M. leprae* from armadillo tissue used for infecting macrophages; 2, *M. leprae* harvested from infected macrophage cultures; 3, *M. leprae* harvested from infected macrophages stimulated simultaneously with IFN-γ; 4, *M. leprae* harvested from macrophages activated with IFN-γ 24 h after *M. leprae* infection.

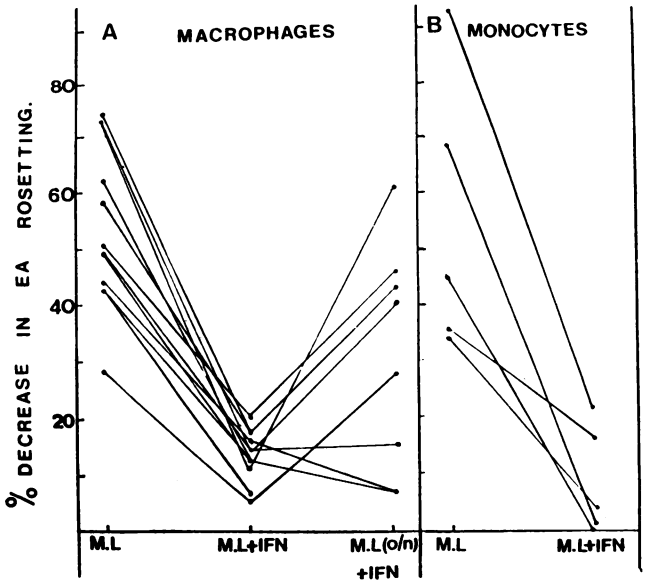

FIG. 2. Effect of IFN-γ activation on the decrease in EA rosetting induced by viable *M. leprae* in monocyte (B) and macrophage (A) cultures. Reduction in EA rosetting induced by viable *M. leprae* (M.L) was abrogated by simultaneous addition of IFN-γ (M.L + IFN) ($P < 0.001$, Student's *t* test). However, it was found to be less efficacious when administered 24 h after *M. leprae* infection [M.L (o/n) + IFN]. Each line represents results obtained from a single lepromatous patient. The percent decrease in EA rosetting in lepromatous macrophages was calculated as follows: [(control values − experimental values)/(control values)] × 100.

processed routinely as described above. Results were expressed as counts per minute plus or minus the standard error of the mean.

Statistical analysis. All cultures were set up and read at least in duplicate and occasionally in triplicate. The statistical significance of the difference between each test and the respective control in the assays was determined by Student's *t* test.

RESULTS

***M. leprae* viability within resting or activated human macrophages from normal individuals and leprosy patients. (i) Mouse footpad assay.** The intracellular bacilli were liberated from the infected macrophages by 10 cycles of freeze-thawing. To determine whether this method of extraction affected the viability of *M. leprae* per se, a suspension of the bacilli was subjected to freeze-thawing and compared with an untreated suspension. No loss of viability of the bacilli was noted, as comparable growth was observed with both preparations (data not shown).

M. leprae derived from 7-day-old resting macrophage cultures from four normal individuals did not show any growth in the mouse footpad (Fig. 1). This indicated that macrophages of normal individuals had the ability to kill *M. leprae*. In contrast, *M. leprae* derived from macrophages from three tuberculoid and four lepromatous cases displayed the characteristic growth pattern in the mouse footpad, indicating the absence of detectable antimycobacterial activity. However, activation with IFN-γ rendered the macrophages microbicidal in both groups of patients, provided they were added along with the *M. leprae*. When IFN-γ was administered 24 h after infection with *M. leprae*, its efficacy appeared to be reduced because growth could be observed in two of four lepromatous cases.

(ii) Fc receptor assay. Our earlier studies have shown that EA rosetting levels are reduced in bacteriologically negative lepromatous patients only in the presence of viable *M. leprae* and not after the addition of autoclaved or rifampin-treated *M. leprae* (5). Figure 2A summarizes data from experiments with macrophages from 12 lepromatous leprosy patients. The percent decrease in EA rosetting caused by in vitro infection of *M. leprae* was completely negated by simultaneous addition of IFN-γ ($P < 0.001$, Student's *t* test). However, in five of the eight lepromatous cases tested no significant difference was seen if activation with IFN-γ was attempted 24 h after *M. leprae* infection.

No significant difference was noted in the killing ability of monocytes and differentiated macrophage cultures (Fig. 2B).

The discrepancy noted between the two viability assays was not significant. The Fc receptor assay, besides being a shorter in vitro assay, is more sensitive since it is an indicator of metabolic inactivation and also has a built-in amplification via diffusible factors as a result of infection with viable bacilli (4).

***M. leprae* viability within lepromatous macrophages in the presence of SOD.** Earlier studies from our laboratory have determined that, in contrast to macrophages from normal subjects, macrophages from leprosy patients are incapable

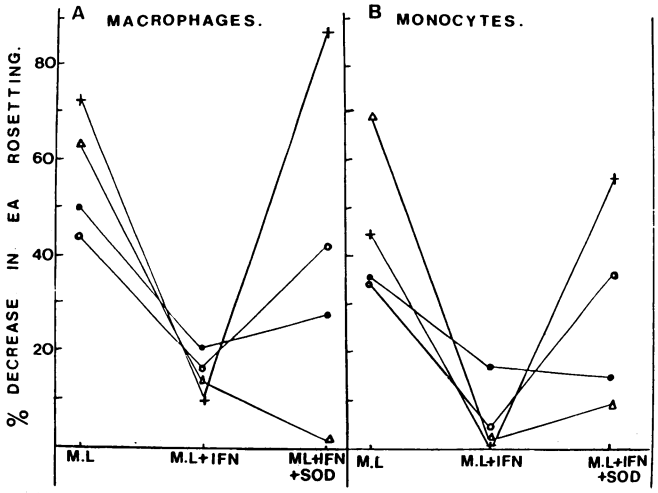

FIG. 3. The effect of SOD on EA rosetting of macrophages and monocytes induced by viable *M. leprae* (M.L) in the presence of IFN-γ. Superoxide was involved in the killing of *M. leprae* by macrophages and monocytes of the two patients denoted by + and ○ ($P < 0.001$, Student's *t* test). Each symbol represents results obtained from a single lepromatous patient. Results are expressed as the percent decrease in EA rosetting from uninfected cultures. M.L+IFN, *M. leprae* and IFN-γ added simultaneously; M.L+IFN+SOD, *M. leprae*, IFN-γ, and SOD added simultaneously.

of producing superoxide anions in response to viable *M. leprae* (18). To investigate whether the killing of *M. leprae* in IFN-γ-activated lepromatous macrophages was due to the production of superoxide anions, monocyte-macrophage cultures were treated simultaneously with *M. leprae* and IFN-γ in the presence of SOD.

In the present study, the decrease in EA rosetting was not abrogated in the presence of SOD in two of the cases studied. This suggests that superoxide anions were not involved in the killing of *M. leprae* in these cases (Fig. 3).

The effect of IFN-γ on HLA-DR antigen expression on macrophages from normal subjects and leprosy patients. Our earlier study (6) had established that *M. leprae* infection of macrophage cultures from bacteriologically negative lepromatous patients resulted in a significant decrease of HLA-DR-positive cells. In the present study, when IFN-γ was added simultaneously with *M. leprae*, a significant increase in the percentage of macrophages expressing HLA-DR antigen, which was comparable to that seen with activation with IFN-γ alone, was observed ($P < 0.005$, Student's *t* test) (Table 1). However, addition of IFN-γ 24 h postinfection also enhanced HLA-DR expression almost threefold over that seen with infected cells alone ($P < 0.001$).

In tuberculoid patients and normal individuals, the expected increase of HLA-DR-positive cells was observed after stimulation with IFN-γ and *M. leprae* (Table 1).

Monocyte-lymphocyte physical interaction in normal subjects and leprosy patients in response to *M. leprae* or activating stimuli or both. Monocyte-lymphocyte interaction constitutes the first step in the development of a specific immune response. Such a physical interaction is known to require functionally intact macrophages, while the role of the lymphocyte is considered passive because binding is not decreased by metabolic poisoning of lymphocytes (16, 31). Besides macrophages, such clusters are also known to occur between dendritic cells and lymphocytes (14).

In our earlier study (6), we had reported a positive antigen-dependent interaction in tuberculoid patients and normal individuals, while in lepromatous patients the interaction remained below the base line (<10%). The base-line value of interaction was established in normal individuals in the absence of any antigen. *M. leprae* viability was not required for the antigen-dependent interaction. Thus, in the present set of experiments, the inability of ConA-derived lymphokines and recombinant interleukin-2 to increase the

TABLE 1. Effect of IFN-γ on HLA-DR antigen expression on macrophages from normal subjects and leprosy patients

Patient type and no.	% HLA-DR-positive cells with:				
	Control	*M. leprae*	IFN-γ	IFN-γ + *M. leprae*[a]	*M. leprae* + IFN-γ[b]
Normal					
1	20	20	52	75	
2	18	20	48	38	
3	17	18	32	41	
Mean ± SEM	18 ± 1	19 ± 1	44 ± 6	51 ± 12	
P[c]		NS[d]	<0.01	<0.025	
Tuberculoid					
1	25	35	70	72	
2	19	22	50	46	
3	15	26	31	34	
Mean ± SEM	20 ± 3	28 ± 4	50 ± 11	50 ± 11	
P		NS	<0.05	<0.05	
Lepromatous					
1	26	10	58	53	30
2	20	9	72	53	22
3	16	7	35	55	25
Mean ± SEM	21 ± 3	9 ± 1	55 ± 11	54 ± 1	25 ± 3
P		<0.025	<0.005	<0.005	NS[e]

[a] *M. leprae* and IFN-γ added simultaneously.
[b] IFN-γ added 24 h after *M. leprae* infection.
[c] *P* is calculated relative to control.
[d] NS, Not significant.
[e] Not *significant* compared with control but significantly higher ($P < 0.001$) than infection with *M. leprae* alone.

degree of interaction in response to *M. leprae* in lepromatous cases (data not shown) is not surprising, even though killing of *M. leprae* was demonstrated.

Modulation by IFN-γ of the antigen-presenting capacity of lepromatous macrophages to autologous T lymphocytes. To check whether decreased IFN-γ production could be directly linked to aberrant macrophage function in lepromatous patients, a coculture system of IFN-γ-activated macrophages and autologous T cells was used. It was essential to separate the cells and treat the macrophages independently with IFN-γ because it is known to have an antimitotic effect on lymphocytes. (See section on the lymphoproliferative assay in Materials and Methods.)

No response was noted with *M. leprae* when 10^5 resting, autologous, nylon-wool-separated T lymphocytes were added to macrophages stimulated with *M. leprae* and IFN-γ for 24 h (Fig. 4A). Significant T-cell proliferation was observed by using an identical protocol with PPD as depicted in the two representative cases (Fig. 4B) ($P < 0.001$, Student's *t* test).

***M. leprae* responsiveness in lepromatous leprosy patients after exogenous addition of ConA-derived lymphokines.** To determine whether this lack of T-cell proliferation in response to *M. leprae* antigens was due to limiting concentrations of lymphokines, exogenous ConA-derived supernatants were used in the lymphoproliferative assay. Only 6 of 43 lepromatous leprosy patients exhibited a significant (i.e., stimulation index exceeding twofold) proliferative response to integral *M. leprae* after lymphokine addition (Fig. 5), indicating that the anergy is not due to limiting lymphokine concentrations. These results are similar to those reported by Ottenhoff et al. (21). In contrast to an earlier report that there was a higher reversion rate if the population tested consisted of low responders to *M. leprae* rather than nonresponders (16), we found restoration of *M. leprae* reactivity in the presence of ConA-derived lymphokines, independent of their basal response to *M. leprae*, disease status, and treatment duration. In a majority of cases, nonspecific activation was observed, since addition of lymphokines alone resulted in T-cell proliferation. No further enhancement was seen with the simultaneous addition of *M. leprae* and lymphokines. Similar results were obtained with recombinant interleukin-2 (data not shown).

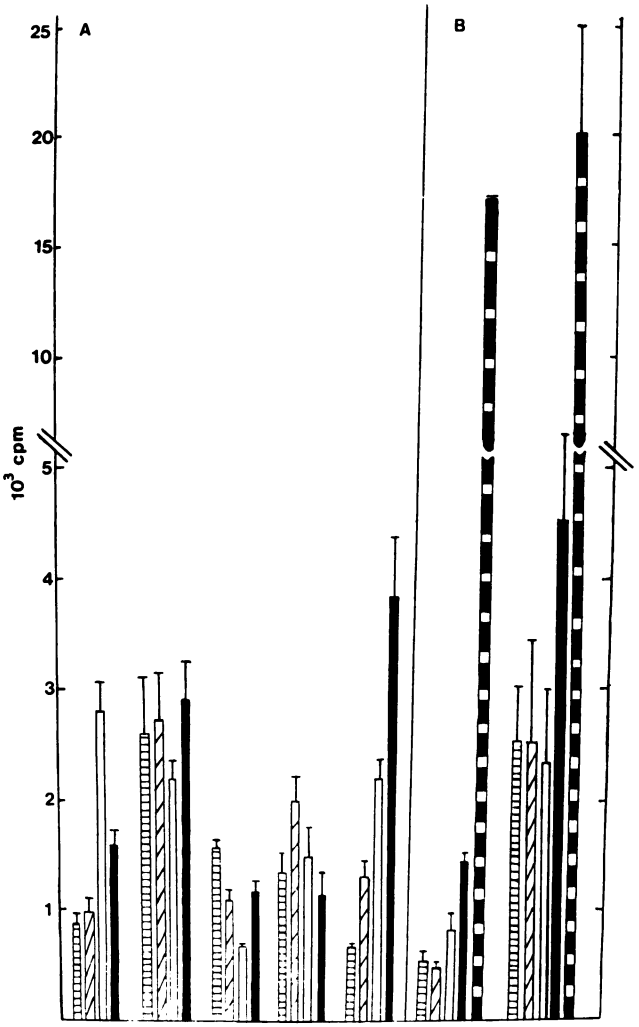

FIG. 4. Modified lymphocyte stimulation assay. Monocytes were stimulated with IFN-γ in isolation in the presence or absence of *M. leprae* or PPD or both and subsequently reconstituted with T lymphocytes. Each set of bars represents results from a single lepromatous patient and depicts the mean counts per minute of triplicate cultures. No response was noted with *M. leprae* in the presence of IFN-γ (A). However, significant T-cell proliferation was observed with PPD ($P < 0.001$) (B). ▭, Uninfected control cultures; ▨, *M. leprae*; ▭, IFN-γ; ■, *M. leprae* plus IFN-γ; ▬, PPD.

DISCUSSION

It has been generally assumed that the predominant cause underlying the failure in the immune response in lepromatous leprosy is a lack of T-cell activation and lymphokine secretion (12, 13, 15). Our results ruled out T-cell anergy as being the primary defect, since responsiveness to *M. leprae* could be restored by exogenous addition of lymphokines in only a fraction of the patients. This confirms the studies of Ottenhoff et al. (21) and Barnass et al. (3) and suggests that the reactivity may be due to an expansion of a few clones, which may be cross-reactive with environmental bacteria.

The lack of T-cell activation and lymphokine production could also be directly linked to aberrant macrophage function in the lepromatous patients. Thus, the concept that the lepromatous macrophages are normal in their function of antigen presentation needs reappraisal.

Our experiments demonstrated that macrophages from normal individuals are capable of killing *M. leprae*. In contrast, macrophages from both tuberculoid and lepromatous patients failed to bring about the destruction of the leprosy bacillus, denoting a defective macrophage effector function in leprosy patients.

In a majority of the lepromatous cases studied, killing of *M. leprae* was best observed only when IFN-γ activation was initiated simultaneously with *M. leprae* infection and not if the host cell was infected prior to activation. This may be because the intracellular presence of viable *M. leprae* or its suppressor products or both (8, 25, 26) compromises the functional capacity of the lepromatous macrophages rendering them incapable of responding to macrophage-activating factors such as IFN-γ (1, 9). In the tuberculoid macrophage, in which suppressor factors do not play any role, activation by IFN-γ to kill *M. leprae* is achieved even when the activating agent is added 24 h postinfection.

To date, the mechanisms responsible for the intracellular killing of *M. leprae* have yet to be unraveled. It has been postulated that reactive oxygen intermediates play an important role in the killing of *M. leprae*. Our results indicate the

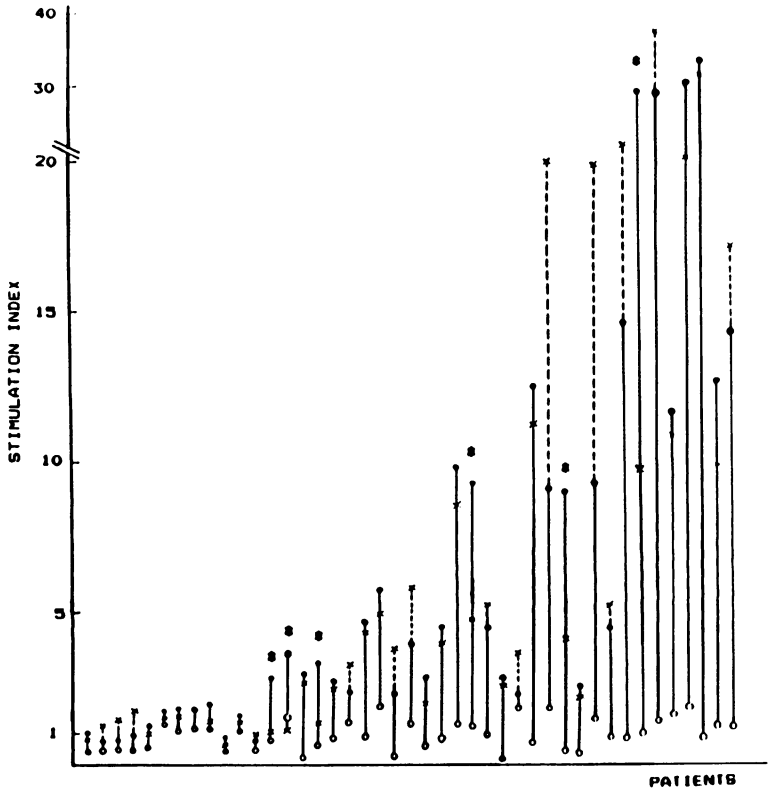

FIG. 5. Lymphocyte stimulation test response to *M. leprae* in lepromatous patients in the presence of ConA-derived lymphokines. Results are expressed as stimulation index. ○, *M. leprae*; ●, *M. leprae* plus lymphokines; ×, lymphokines. The majority of the cases showed nonspecific activation after addition of lymphokines alone. Enhancement (*) was seen only in 6 of 43 patients. Significance was calculated between *M. leprae* plus lymphokines and lymphokines alone ($P < 0.02$, Student's t test).

involvement of superoxide anions, since killing of *M. leprae* was blocked in the presence of SOD. However, this mechanism does not seem to be operative in all lepromatous patients, as killing was not prevented in the presence of SOD in all the cases studied. Thus, the ability to kill *M. leprae* via the superoxide pathway appears to be an individual trait and was not dependent on macrophage differentiation, since monocytes and macrophages of the same patient behaved similarly. In cases for which SOD was not effective or only partially effective, it is possible that other oxidative-nonoxidative mechanisms of killing may be operative, such as the lysosomal pathway. Flesch et al. (11) reported similar results on the ability of IFN-γ-activated macrophages to kill *Mycobacterium bovis*. Thus, death of the leprosy bacillus probably results from the cumulative effect of multiple injuries caused by the different bactericidal mechanisms operative in the host cell.

Nevertheless, it remains to be established whether resting and activated macrophages from normal individuals and patients use similar microbicidal pathways or whether the two types of cells are qualitatively or merely quantitatively different.

The next question which arises is whether the killing of *M. leprae* is the only prerequisite for the macrophages to be able to present processed *M. leprae* antigen(s) to the appropriate T lymphocytes. This was assessed by HLA-DR expression, antigen-specific monocyte-lymphocyte physical interaction, and lymphoproliferation.

Our observations indicated that the defective lepromatous macrophages can be activated by IFN-γ to kill *M. leprae*, as seen by the viability assays, but it did not result in T-cell proliferation. It is possible that the native antigen is ineffective in stimulating the T cells due to blockade at events, such as antigen degradation and processing, which occur prior to antigen presentation (22).

Moreover, restoration of HLA-DR levels on the *M. leprae*-infected lepromatous macrophages after IFN-γ activation indicated that although it fulfilled one of the requirements essential for antigen presentation, other components may be necessary to ultimately bring about T-cell activation and lymphokine secretion (22).

In contrast, in the tuberculoid cases significant lymphoproliferation in response to *M. leprae* is seen (27; P. R. Salgame, Ph.D. thesis, University of Bombay, 1984). Since killing of *M. leprae* by tuberculoid macrophages requires the presence of lymphokines, it signifies that killing and degradation of *M. leprae* may not be necessary for the initial stimulation of lymphocytes. Once T-cell activation has occurred, presumably by stimulation through secretory antigens (33), lymphokines are secreted which in turn activate the macrophages to kill the intracellular bacilli. Preliminary evidence shows that in contrast to those from lepromatous patients, tuberculoid patients respond to *M. bovis* BCG secretory antigens in lymphoproliferative assays (N. F. Mistry and M. Harboe, personal communication).

Since macrophages of normal individuals are able to kill *M. leprae* without the requirement of IFN-γ activation, the positive lymphoproliferation seen in these individuals (27; Salgame, Ph.D. thesis) may be in response to a different set of antigens than those to which tuberculoid patients respond.

In conclusion, this study demonstrates that killing and antigen presentation are two independent events. It appears

that the ability of the macrophage per se to kill *M. leprae* may be of greater importance than lymphocyte-mediated activation for protection against *M. leprae* infection.

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