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Role of Macrophages in Defective Cell Mediated Immunity in Lepromatous Leprosy. I. Factor(s) from Macrophages Affecting Protein Synthesis and Lymphocyte Transformation[1]

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The clinical manifestations of leprosy are heterogenous, ranging between two polar forms—tuberculoid and lepromatous. Enough evidence has accumulated both *in vivo* and *in vitro* to demonstrate a defect in cell mediated immunity in lepromatous leprosy. However, the mechanisms underlying the cell mediated immunological defects in lepromatous leprosy patients continue to be a debatable topic.

We had earlier observed, following a study using the mouse model, that susceptible mice (Swiss white) had a population of macrophages which incorporated a lesser amount of protein precursor, leucine, in the presence of *M. leprae* as compared to the macrophage population from a comparatively resistant group (C57BL). Initial work with patients also showed a similar trend in the results. Leucine uptake, an indicator of protein synthesis, in macrophages from lepromatous leprosy patients was depressed in the presence of viable *M. leprae* as compared to the macrophages from normal subjects and tuberculoid leprosy patients ([2]).

In this study we have extended our initial observations with several additional experiments to further analyze the role of macrophages in the cell mediated immunity of leprosy.

MATERIALS AND METHODS

Leprosy patients were classified according to the Ridley and Jopling classification ([8]). *M. leprae* were harvested from freshly collected biopsies of untreated lepromatous leprosy patients according to the technique developed earlier in our laboratory ([1]).

Macrophage culture technique. This was as described in the accompanying paper ([6]).

^{3}H-leucine incorporation by macrophages. After 5 days in culture, 5×10^6 bacilli (*M. leprae*) were added to 2 cultures and incubated for 24 hr after which the excess *M. leprae* were removed by washing. Two other cultures were maintained as controls. Twenty-four hr after *M. leprae* addition, the macrophage monolayer was washed, and fresh medium, consisting of leucine-free Eagle's Minimal Medium containing 10% human AB serum and 1 μCi ^{3}H-leucine (Isotope Division, BARC, Bombay, sp act 7.7 mCi/mMol) was added. After 3 hr incubation at 37°C in 5% CO_2, the cells were gently scraped off the Leighton tubes with a rubber policeman. A cell count was taken after which 0.05 ml of suspension was spotted onto preacidified Whatman No. 3 filter paper discs. The discs were allowed to dry and then washed twice each in cold 5% trichloroacetic acid, cold methanol, and cold diethyl ether. The dried discs were then put into vials along with cocktail for liquid scintillation counting. The total incorporation was considered as an index of protein synthesis.

Exposure of macrophages to various other antigens was carried out essentially in the same manner described above. Two tubes without any antigen and 2 tubes with *M. leprae* were always maintained as controls in these sets of experiments.

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Preparation of lysate. Macrophage cultures were set up from the blood of highly bacteriologically positive lepromatous patients. After 5 days in culture, the cells were scraped off the Leighton tubes with a rubber policeman. The cells were concentrated in a known volume, and the cell count was determined. Six cycles of freeze-thawing were carried out, and the lysate thus obtained was passed through a sterile Millipore filter (1.25 μ pore size) to remove cell debris and particles. Lysate from normal and tuberculoid macrophages was prepared as described above.

Effect of lysate on ^{3}H-leucine uptake. Six Leighton tube cultures of macrophages from normal individuals were maintained for 5 days after which lysate from 0.5×10^6 macrophages was added to 2 tubes. To 2 other tubes only *M. leprae* were added, and 2 tubes were maintained as controls. After 24 hr of incubation, the ^{3}H-leucine incorporation was studied.

Effect of lysate on lymphocyte transformation. Leukocytes were separated as described above from the blood of normal individuals and resuspended in Eagle's Minimal Medium containing 20% human AB serum. Cultures in 2 ml aliquots containing 4×10^6 cells were placed in test tubes. The cultures were set up in duplicate, and a total of 8 tubes was used for each experiment. The leukocytes were exposed to *M. leprae* (5×10^6), lysate (equivalent of 0.5×10^6 cells), and a combination of lysate and *M. leprae*.

The cells were maintained for 5 days in culture in a 5% CO_2 atmosphere at 37°C. On the 5th day, 2 μCi/ml ^{3}H-thymidine was added to each tube. After 18 hr the cells were harvested, washed twice each in cold saline, followed by 5% cold trichloroacetic acid and cold methanol. Hyamine hydroxide (0.1 ml) was added to each tube and incubated at 60°C for 45 min to 1 hr until the cells were solubilized. This was then transferred to scintillation vials for counting.

The effects of normal, tuberculoid, and heated lysates were also tested in the lymphocyte transformation test system. The lysate was heated at 56°C for 15 min.

The results were expressed as stimulation ratios between stimulated and unstimulated leukocytes.

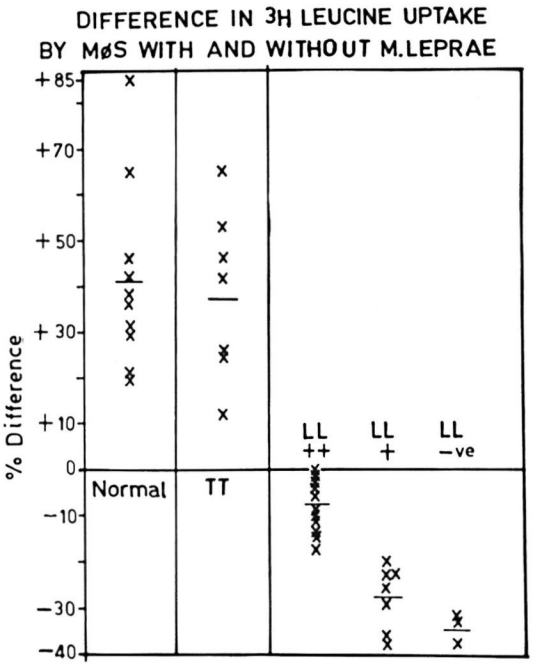

FIG. 1. The scatter plot demonstrates the mean and % difference in ^{3}H-leucine uptake of macrophages before and after *M. leprae* infection *in vitro*. See footnote to Fig. 5 for explanation of symbols.

RESULTS

Leucine uptake in normal subjects and in lepromatous and tuberculoid leprosy patients. Macrophages from normal subjects and tuberculoid leprosy patients showed an increase in leucine incorporation on addition of *M. leprae* in culture. The increase observed in macrophages from normal subjects was higher than that observed in tuberculoid macrophages. Macrophages from lepromatous leprosy patients showed a depression in leucine uptake even in control cultures not exposed to *M. leprae*. On addition of *M. leprae*, further depression was observed depending on the patients' bacteriologic index at the time of blood collection. It was observed that there was a significant depression in leucine uptake in macrophages collected from bacteriologically negative lepromatous leprosy patients exposed to *M. leprae in vitro* (Fig. 1, Fig. 5, Table).

To further study the specificity of the antigen causing depression in leucine uptake of lepromatous macrophages, various other *in vitro* cultivated mycobacteria and auto-

TABLE. *Statistical analysis of data of leucine uptake studies with* M. leprae.

	Macrophage groups to be compared			
	1	vs	2	P values calculated using Student's t test
1	Normal control		Normal with *M. leprae*	$p < 0.0005$
2	LL (bacillary negative) control		LL (bacillary negative) with *M. leprae*	$p < 0.0005$
3	LL (bacillary positive, +) control		LL (bacillary positive, +) with *M. leprae*	$p < 0.0005$
4	LL (bacillary positive, ++) control		LL (bacillary positive, ++) with *M. leprae*	$p < 0.0500$
5	TT control		TT with *M. leprae*	$p < 0.0025$
6	Normal control		LL (bacillary positive, ++) control	$p < 0.0005$
7	Normal with *M. leprae*		LL (bacillary positive, ++) with *M. leprae*	$p < 0.0005$
8	Normal control		LL (bacillary positive, +) control	$p < 0.0005$
9	Normal with *M. leprae*		LL (bacillary positive, +) with *M. leprae*	$p < 0.0005$
10	Normal control		LL (bacillary negative) control	$p < 0.0025$
11	Normal with *M. leprae*		LL (bacillary negative) with *M. leprae*	$p < 0.0005$
12	Normal control		TT control	$p < 0.0050$
13	Normal with *M. leprae*		TT with *M. leprae*	$p < 0.0125$

claved *M. leprae* were used in the leucine uptake studies. As shown in Fig. 2, all the *in vitro* cultivated mycobacteria including ICRC did not cause any reduction in leucine uptake by lepromatous macrophages. Lepromatous macrophages exposed to autoclaved *M. leprae* showed only a marginal increase in leucine uptake.

Characterization of lysate. a) Normal macrophage cultures when exposed to *M. leprae* showed an increase in leucine uptake; however, when lepromatous lysate was added to normal macrophage cultures, there was a depression in leucine uptake (Fig. 3).

b) The lysate also interferred with the normal lymphocyte transformation as observed in Fig. 4. Lysate alone did not have any lymphoblastogenic effect, thus excluding the possibility of it stimulating the responder cells. Normal leukocytes, when exposed to *M. leprae* antigen, responded by showing increased ^{3}H-thymidine incorporation over the control value. However, when lysate was added to the cultures, the blastogenesis was much reduced as observed by the reduced thymidine uptake.

Lysate prepared from macrophages of normal individuals, which were exposed to *M. leprae*, and similarly lysate from tuberculoid leprosy patients, showed no inhibitory activity in leucine incorporation and lymphocyte transformation in normal cultures (Figs. 3 and 4). The lepromatous lysate is heat stable up to 56°C for 15 min, at least for its effect on lymphocyte transformation (Fig. 4).

DISCUSSION

The leucine incorporation studies show that only macrophages from lepromatous patients respond with a depression in leucine uptake when exposed to *M. leprae in vitro*. Control macrophages from these patients (without added *M. leprae*) also show a depressed value of leucine incorporation. This could be due to the already existing *M. leprae* in the monocytes of these patients. Long-term treated lepromatous leprosy patients, who were negative for acid-fast bacilli, showed a marked depression in leucine uptake by macrophages when exposed to *M. leprae in vitro*. The leucine uptake value in the control macrophages (where no *M. leprae* were added) of these patients showed a tendency of returning towards the normal values (Fig. 5). It is clear from these observations that unresponsiveness to *M. leprae* in the form of reduced protein synthesis is a long lasting and unchanging phenomenon in spite of the patient becoming bacteriologically negative due to therapy.

Autoclaved *M. leprae* and *in vitro* cultivated mycobacteria did not inhibit leucine uptake in lepromatous macrophages. Thus the phenomenon seems to be specific for viable *M. leprae* in the macrophages from lepromatous patients only.

Our results show that the macrophage

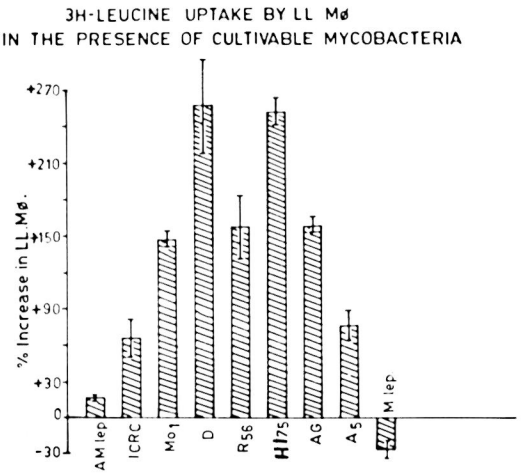

FIG. 2. Each of the cultivable mycobacteria has been tested twice, and the results demonstrate the mean and range of the percentage difference obtained.

A. M. lep: Autoclaved *M. leprae*.
ICRC:[a] Cultivable acid fast bacilli isolated from lepromatous nodules.
MO1:[b] Mycobacteria isolated from a lepromatous patient.
D:[b] Mycobacteria isolated from a lepromatous patient from Dakar, Senegal.
R 56:[b] Mycobacteria isolated from a subcutaneous murine leproma.
HI-75:[b] Mycobacteria isolated by Dr. O. K. Skinsnes from a lepromatous patient.
AG:[b] Mycobacteria isolated from an armadillo infected with *M. leprae* isolated from a lepromatous patient.
A5:[b] Mycobacteria isolated from an armadillo infected with *M. leprae*.

[a] Strain originally obtained from Dr. C. V. Bapat, Biology Division, Cancer Research Institute, Dr. Earnest Borges Marg., Parel, Bombay-400 012 and maintained by us in Dubos' medium.

[b] Strains obtained from Professor L. Kato, Institute of Microbiology, University of Montreal, C.P. 100, Laval-des-Rapides, Quebec, Canada.

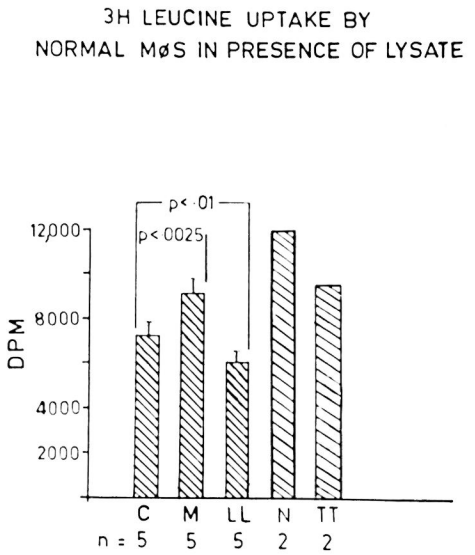

FIG. 3. C—Control macrophages to which no *M. leprae* have been added in culture.
M—Normal macrophages + *M. leprae* added *in vitro*.
LL—Normal macrophages + lepromatous lysate.
N—Normal macrophages + normal lysate.
TT—Normal macrophages + tuberculoid lysate.

The results of the effect of lepromatous lysate on ^{3}H-leucine uptake by normal macrophages have been expressed as mean ± S.E. Five experiments were carried out. Results of the experiments with tuberculoid and normal lysates have been expressed as the average of the two experiments.

population in lepromatous patients is different from that of normal individuals and tuberculoid patients, the characteristic feature being that this population in the presence of *M. leprae* appears to be altered in their biochemical function. They have reduced protein synthesis and also produce factor(s) that result in a reduction of protein synthesis in normal macrophages. The factor(s) are also capable of reducing lymphocyte stimulation in the presence of *M. leprae* as tested by the lymphocyte transformation test. The most striking observation, however, is that these defects are only present in macrophages from lepromatous patients and are specific to freshly isolated viable *M. leprae*.

It appears from our data that the macrophage is most likely to be the defective cell in lepromatous leprosy. Similar conclusions have been drawn from indirect experimentation by other workers ([4-7]). Direct evidence was obtained in our earlier experiments. The recent work by Hirschberg ([5]) on the role of macrophages in lymphoproliferative response to *M. leprae in vitro* also suggests that the lack of response in lepromatous leprosy is due to a failure of the macrophages to present *M. leprae* antigens in an immunogenic form.

The accompanying paper ([3]) on macrophage-lymphocyte interaction adds another dimension to the defective macrophage system in lepromatous leprosy patients and helps us to project a possible model for understanding the role of macrophages.

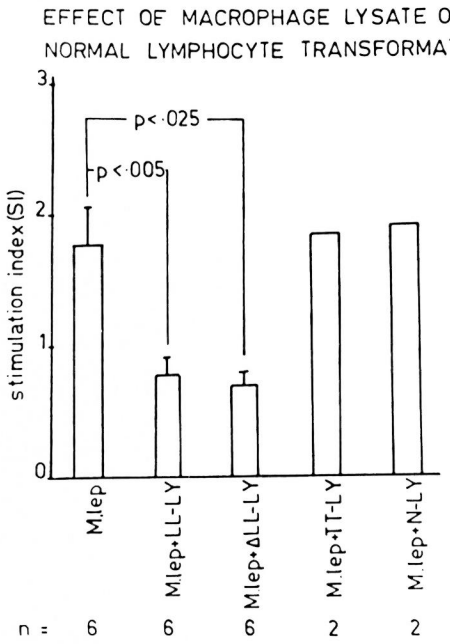

FIG. 4. *M. leprae*—Stimulation with *M. leprae* alone.

M. leprae + LL-LY—Stimulation with *M. leprae* in the presence of lepromatous lysate.

M. leprae + ΔLL-LY—Stimulation with *M. leprae* in the presence of heated lepromatous lysate (56°C, 15 min).

M. leprae + TT-LY—Stimulation with *M. leprae* in the presence of tuberculoid lysate.

M. leprae + N-LY—Stimulation with *M. leprae* in the presence of normal lysate.

The results of the effect of lepromatous macrophage lysate on normal lymphoctyte transformation have been expressed as mean ± S.E. Six experiments were carried out. The results of the effect of tuberculoid and normal lysate have been expressed as the average of the 2 experiments.

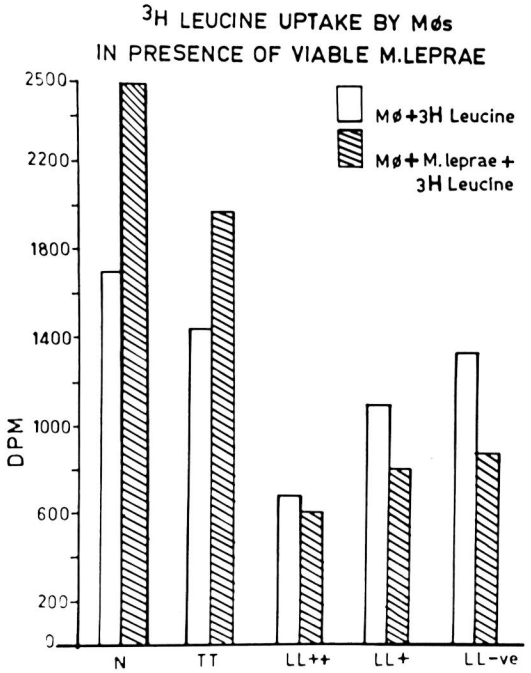

FIG. 5. N—Macrophages from normal healthy donors.

TT—Macrophages from tuberculoid leprosy patients.

LL++—Macrophages from lepromatous leprosy patients (bacteriologically positive, BI 3+ and above).

LL+—Macrophages from lepromatous leprosy patients (bacteriologically positive, BI 1+ and 2+).

LL-ve—Macrophages from long-term treated lepromatous leprosy patients (bacteriologically negative).

The diagram depicts results of a representative experiment. The results of the series of experiments have been shown in Fig. 1.

SUMMARY

Macrophages from lepromatous leprosy patients specifically show reduced protein synthesis in the presence of *M. leprae*. They also produce, as a result of interaction with *M. leprae*, factor(s) that reduce protein synthesis in normal macrophages as well as block lymphocyte transformation in normal leukocyte cultures in the presence of *M. leprae* as the antigen. These observations implicate a defective macrophage system in lepromatous leprosy patients.

RESÚMEN

Los macrófagos de los pacientes con lepra lepromatosa muestran una reducida capacidad de síntesis protéica en presencia del *M. leprae*. Dichos macrófagos también producen, como resultado de su interacción con el *M. leprae*, factores que reducen la síntesis de proteínas en los macrófagos normales y factores que bloquean la transformación de linfocitos en cultivos de leucocitos normales cuando se usa el *M. leprae* como antígeno. Estas observaciones implícan que los pacientes lepromatosos poseen un sistema macrofágico defectuoso.

RÉSUMÉ

En présence de *M. leprae*, des macrophages obtenus de malades atteints de lèpre lépromateuse montrent de manière spécifique une réduction dans la synthèse des protéines. Ces macrophages produisent également, suite à une interaction avec *M. leprae*, un ou des facteurs qui diminuent la synthèse des protéines dans les macrophages et qui bloquent la transformation lymphocytaire chez les leucocytes normaux

en culture en présence de *M. leprae* comme antigène. Ces observations suggèrent que les macrophages sont déficients chez les malades souffrant de lèpre lépromateuse.

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