INTERNATIONAL JOURNAL OF LEPROSY
Volume 48, Number 2
Printed in the U.S.A.

Biochemical Alteration in Cells Following Phagocytosis of *M. leprae*—The Consequence—A Basic Concept[1]

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In microbial pathogenesis the basic biological event involves the entry of a microbe into a host cell, leading to either tolerance or rejection of the microbe. This is generally the situation in phage-bacteria, phage-human, or bacteria-human cell systems. Normally, any microbe that enters the cell is not easily tolerated. The defense mechanism connected with the immune resistance of the cell gets into action and eliminates the bacterium. In the case of bacteriophages, they can lyse the host bacteria and continue to multiply if further live host cells are available. The phage can also lysogenize DNA and keep replicating along with bacterial DNA. A stimulus can then lead to the separation of the phage DNA and allow its multiplication, leading to an eventual lysis. On the other hand, where a host cell tolerates a foreign microbe to reside in its fold in whatever fashion, one could expect an altered biochemical character in the host cell. The alteration in biochemical functions in turn leads to morphofunctional changes. Whatever such changes may be, they are the end result of the expression of the genome of the microbe and that of the host cell with the regulatory mechanisms being active. Extending these basic concepts of gene function, one would say that there is a differential activation and repression of genes in both the host cell and the resident microbe.

One would like to look at *Mycobacterium leprae*, an obligate parasite, in several cells of human tissue in light of the above concept. Two cells in human tissues that are very frequently identified as host cells for *M. leprae* are macrophages and Schwann cells. Thus it would be profitable to know more about the biochemical changes that occur due to the presence of *M. leprae* in these host cells so that one could correlate such changes with functional alterations, if any.

MATERIALS AND METHODS

The methods, in general, involve culturing macrophages and studying their protein synthesis in the presence and absence of *M. leprae* and culturing Schwann cells to study their DNA synthesis in the presence and absence of *M. leprae*. The details are as follows:

Macrophage culture technique. Heparinized venous blood (20 ml) was collected in 6% dextran and allowed to settle at 37°C for 45 min. The plasma along with the buffy coat was removed, and the leukocyte pellet after centrifugation at 2000 rpm was washed with Minimal Essential Medium (MEM) and resuspended in MEM containing 40% human AB serum. The leukocytes were distributed into Leighton tubes and incubated in a 5% CO_2 atmosphere at 37°C. After 24 hr the cells were washed, and fresh medium was added. Subsequently, the medium was changed every 48 hr. Using the property of adhesiveness to glass, macrophages were selectively retained in the tubes, and other cells like lymphocytes were removed. Neutrophils, which also adhere to glass, have an *in vitro* survival time of 2 to 3 days and are therefore removed by culturing the cells for 5 days. Thus 5 day old cultures consist predominantly of macrophages.

On the 5th day, 2 culture tubes each were exposed to the various antigens, and 2 tubes were maintained as controls. The antigen dose given (*M. leprae*) *in vitro* was

[1] Received for publication on 15 May 1979; accepted for publication in revised form on 4 January 1980.
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5×10^6 bacilli/tube. Twenty-four hr after *M. leprae* exposure the macrophage monolayer was washed, and fresh medium consisting of leucine-free Eagle's Minimal Medium containing 10% AB serum and 1 μCi/ml of ^{3}H-leucine was added to each tube. After a pulse label of 3 hr, the cells were rinsed in saline and gently scraped off the Leighton tube. A cell count was taken, and 0.05 ml of each suspension was spotted on to 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 of ^{3}H-leucine was considered as an index of protein synthesis.

Schwann cell cultures. Sensory ganglia of 1 to 3 day old Swiss white mice were trypsinized with 0.5% trypsin and explanted on collagen coated glass coverslips. Such coverslips were transferred to plastic petri dishes and fed on alternate days with a mixture of 70% Eagle's MEM containing 600 mg% glucose, 20% fetal calf serum, 10% chick embryo extract, and penicillin (100 IU/ml) and incubated in 100% humidity at 36°C. The explant and emerging cells were observed under the microsope and at appropriate times used for experiments with *M. leprae* or for isotope labelling with metabolites.

Schwann cells were cultured *in vitro* as a component of the organized nerve cultures. Two week old cultures were exposed to 5–8 million *M. leprae* for 96 hr after which the cultures were washed and pulsed with ^{3}H-thymidine (2 μCi/ml) for 24 hr. The coverslips containing the cultures were then washed and fixed in formal-saline for 24 hr, rinsed with xylene, and processed for autoradiography. Ilford K5 emulsion diluted in distilled water (1:1) was used for coating under total darkness, and the preparations were then stored for 12 days. Later, the coverslips were fixed and stained with Ziehl-Neelsen for acid-fast bacilli and with methylene blue for visualizing cellular morphology. Thus, from 1 coverslip, data on thymidine incorporation, presence of acid-fast bacilli, and type of cellular morphology could be recorded.

Blood was obtained from patients attending local clinics. Lepromatous patients with bacterial intlexes (BI) of 1+ and 2+ were grouped in the category LL+. Patients with BI 3+ and above were grouped in the category LL++. Lepromatous patients whose skin smears were negative for acid-fast bacilli at the time of blood collection were included in the group designated LL-ve. The patients were in the age range of 22 to 50 years, 75% were males, and they were clinically free from other infections.

Mycobacterium leprae were obtained from human biopsy material from treated or untreated lepromatous (LL) patients.

Macrophages from patients were not specifically studied to determine their bacillary content before exposure to antigen *in vitro*. Since they were used as controls to which extra *M. leprae* were added (5×10^6), the baseline value is the control value, and difference alone is considered relevant for the purpose of discussion.

RESULTS

M. leprae reduces protein synthesis in macrophages of lepromatous leprosy (LL) patients (Table 1). Such a change is specific with macrophages from LL patients and is not seen with macrophages from tuberculoid patients or normal individuals. That this reduction in protein synthesis is a specific response of LL macrophages to *M. leprae* only and not to other types of live cultivable acid-fast bacilli or autoclaved *M. leprae* is shown by the lack of reduced protein synthesis when the latter were used with LL macrophages (Table 2).

M. leprae on entry into macrophages alter the Fc receptors on these macrophages as seen by the low percent of rosette formation with antibody coated sheep erythrocytes (SRBC). This defect in Fc receptor is not seen when the lepromatous macrophages have not phagocytized *M. leprae* (Table 3).

It should be noted that the defect in Fc receptor is not to that extent in LL macrophages which have not phagocytised *M. leprae* yet they show lower values (28%) than the normals (50%). The lower level of Fc receptors in LL macrophages, i.e., 45% as compared to 66% of normal macro-

TABLE 1. *^{3}H-leucine incorporation in macrophage cultures as an indicator of protein synthesis. Values are expressed as means ± standard deviations of counts per minute.*

Donors	No.[a]	Controls[b]	*M. leprae*	% Difference
Normal	10	1658 ± 159	2319 ± 338	40.4 ± 20.9
Tuberculoid (TT)	7	1343 ± 272	1838 ± 434	37.6 ± 17.5
Lepromatous (LL++)[c]	5	565 ± 61	547 ± 47	−3 ± 2
Lepromatous (LL+)[d]	12	893 ± 333	710 ± 241	−19 ± 8
Lepromatous (LL−ve)[e]	5	1332 ± 168	856 ± 96	−36 ± 3

[a] Number of experiments (with different samples). [b] Controls are macrophage cultures from the respective category of subject not exposed to *M. leprae* and radioactive incorporation determined. [c] LL++ = lepromatous patients with BI 3+ and above. [d] LL+ = lepromatous patients with BI 1+ and 2+. [e] LL−ve = lepromatous patients negative for acid-fast bacilli.

phages, is also noteworthy and may indicate an effect of some factor/factors acting on LL macrophages even in the absence of *M. leprae* inside the cells.

The changes that *M. leprae* bring about in the Schwann cells derived from dorsal root ganglia of Swiss white mice grown in culture are as follows: a) phagocytosis of *M. leprae* by Schwann cells leads to the lack of interaction of Schwann cells with axons as seen in the *in vitro* cultures; b) reduction in DNA synthesis (Table 4) which appears to be completely blocked; and c) no clear indication of any effect of *M. leprae* on the protein synthesis of Schwann cells (data not included).

Furthermore, it has also been shown that no other mycobacteria, including cultivable bacilli isolated from leprosy patients and heat killed *M. leprae*, exhibit these effects in these Schwann cells. Only Schwann cells show a reduction or a block in DNA synthesis in the presence of *M. leprae* and not the other type of phagocytizing cell, i.e., flat cells (Table 4).

DISCUSSION

Our data clearly indicate that the presence of *M. leprae* in lepromatous macrophages reduces protein synthesis in the cell while the presence of *M. leprae* within macrophages from tuberculoid patients or from normal individuals increases protein synthesis ([2]).

The data also clearly indicate that lepromatous macrophages do not show such reduced protein synthesis in the presence of any other mycobacteria except *M. leprae* isolated from untreated lepromatous patients. The probable reason for this may be that the cultivable nature of these bacteria has led to the loss of this feature of *M. leprae*.

Similarly, Schwann cells have been shown to have an inability to divide follow-

TABLE 2. *Comparison of ^{3}H-leucine incorporation in lepromatous macrophages using different types of bacilli. Values expressed as means ± standard deviations of 3 separate samples of percent differences between controls and cells exposed to bacilli.*

Types of mycobacteria		% Difference
Autoclaved *M. leprae*		+17 ± 2
ICRC-C44[a]		+68 ± 16
MO2[b]	Strains received from Prof. L. Kato.	+140 ± 9.3
D[c]		+177 ± 77
R 56[d]		+175 ± 19
HI-75[e]		+228 ± 46
AG[f]		+114 ± 39
A5[g]		+87 ± 16

[a] ICRC: (Strain received from Dr. C. V. Bapat) Cultivable acid-fast bacilli isolated from lepromatous nodules.

[b] MO2: Mycobacteria isolated from a lepromatous patient. [c] D: Mycobacteria isolated from a lepromatous patient from Dakar, Senegal. [d] R 56: Mycobacteria isolated from a subcutaneous murine leproma. [e] HI-75: Mycobacteria isolated by Dr. O. K. Skinsnes from a lepromatous patient. [f] AG: Mycobacteria isolated from an armadillo infected with *M. leprae* isolated in turn from a lepromatous patient. [g] A5: Mycobacteria isolated from an armadillo infected with *M. leprae*.

TABLE 3. *Mean percent of macrophages with Fc receptors as measured by rosette formation with antibody coated sheep erythrocytes.*

Source of macrophage	No. of experiments	Macrophages cultured without *M. leprae*	Macrophages cultured with *M. leprae*	
			With intracellular *M. leprae*	Without intracellular *M. leprae*
Normals	3	66	47	50
Tuberculoid	3	50	40	50
Lepromatous	7	45	14	28

ing phagocytosis of *M. leprae*. Schwann cells containing *M. leprae* do not incorporate ^{3}H-thymidine into acid insoluble material due to a lack of DNA synthesis (Table 4). This is not the case with protein synthesis in this cell, however.

The following hypothesis is proposed based on the above findings:

1. Macrophage with *M. leprae*	→ lose capacity to fully process the antigen.	→ cell-mediated immunity (CMI) is depressed.
2. Schwann cells (after *M. leprae* engulfment)	→ lose capacity to synthesize DNA, divide, and associate with axons.	→ non-myelination of axons. C-fibers' function affected.

There is ample evidence ([6]) to show that Schwann cells have to divide and reorient their relationship with axons so as to reach a 1 to 1 relationship before myelination. Thus blockage of division of Schwann cells due to the presence of *M. leprae* would interfere with the normal process of myelination even in the early stages of *M. leprae* infection. It is well established that demyelination of peripheral nerves can alter their conduction velocity. Nonmyelinated "C" fibers could also get similarly affected due to lack of normal association with their Schwann cells ([1,5]).

The above results confirm the concept that entry of *M. leprae* into certain cells like macrophages and Schwann cells leads to some basic biochemical changes in these cells which result in depression of cell-mediated immunity through defective macrophages ([3,4]) and nonmyelination of nerves through inactivation of the Schwann cells.

In both these cases, the results indicate some basic alteration in the host cells at the molecular level. It is hoped that more details of these basic molecular events will be identified following the future experiments being carried out in our laboratory.

TABLE 4. *Relationship of thymidine incorporation to presence of bacilli.*

Sr. No.[a]	Bipolar cells (Schwann cells)				Flat cells			
	^{3}H-labelled cells		Unlabelled cells		^{3}H-labelled cells		Unlabelled cells	
	Without bacilli	With bacilli	Without bacilli	With bacilli	Without bacilli	With bacilli	Without bacilli	With bacilli
1	74	8	110	68	8	6	3	2
2	114	0	165	88	0	8	8	0
3	60	2	112	199	9	4	11	17
4	68	2	157	153	17	9	44	62
5	91	2	135	56	22	14	30	20
6	66	3	177	144	4	2	18	1
Total	473	17	856	708	60	43	114	102

[a] Each serial number represents collective data from 3 cultures. Cells were counted microscopically from several fields of each culture under oil immersion.

SUMMARY

When macrophages from lepromatous leprosy patients are exposed to *M. leprae*, the macrophages show reduced protein synthesis. Such a phenomenon is not seen with macrophages from tuberculoid patients or normal individuals. *M. leprae* phagocytized by Schwann cells affect the incorporation of DNA precursor in the cells, leading to failure of Schwann cell association with axons in *in vitro* cultures. These 2 observations form a basis of proposing that basic biochemical events take place when *M. leprae* are associated with host cells, which in turn can be amplified to physiologically functional defects.

RESÚMEN

Cuando los macrófagos de los pacientes con lepra se exponen al *M. leprae*, las células muestran una reducida capacidad de síntesis protéica. Este fenómeno no se observa en los macrófagos de los pacientes tuberculoides o de los individuos sanos. Los *M. leprae* fagocitados por las células de Schwann afectan la incorporación celular de precursores del DNA y ocasionan fallas en la asociación de las células de Schwann con axones en cultivos *in vitro*. Estas dos observaciones constituyen la base para proponer que la interacción del *M. leprae* con las células del huésped induce en ellas cambios bioquímicos que pueden ser amplificados hasta convertirse en defectos fisiológicos o funcionales.

RÉSUMÉ

Lorsque des macrophages provenant de malades atteints de lèpre lépromateuse sont exposés à *M. leprae*, ils montrent une synthèse protéinique réduite. Un tel phénomène n'est pas observé avec les macrophages provenant de malades tuberculoïdes ou d'individus normaux. Les bacilles de la lèpre phagocytés par les cellules de Schwann ont un effet sur l'incorporation du précurseur du DNA sur les cellules, ce qui interfère avec l'association entre cellules de Schwann et axones en culture *in vitro*. Ces deux observations forment la base pour suggérer que des évènements biochimiques élémentaires se déroulent lorsque que *M. leprae* est associé avec les cellules hôtes, ceci pouvant alors être amplifié et entraîner des défauts de la fonction physiologique.

Acknowlegement. The authors are very grateful to their colleagues, Dr. (Mrs.) Rama Mukherjee, Miss Tannaz J. Birdi, and Miss Padmini R. Salgame, for their continued interest in carrying out the experiments related here, constant discussion, and cooperation.

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