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Surface membrane changes in lepromatous macrophages affecting the adherence of *Mycobacterium leprae*

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Abstract. Macrophages from lepromatous leprosy patients showed poor adherence to *Mycobacterium leprae.* The phagocytic activity of the macrophages was not correlated to the influence on the adherence ability. Based on the phagocytic behaviour of macrophages from normal individuals and from lepromatous leprosy, patients as well as the action of neuraminidase in reversing the extent of adherence, it is suggested that macrophages from lepromatous leprosy patients differ from those from normal individuals in regard to their surface properties. There was no relationship between the degree of adherence and the concentration of Fc receptors of the macrophages. It was also shown that an extract of lysed macrophages from lepromatous leprosy patient was able to reduce the adherence of *Mycobacterium leprae* to normal macrophages. This study shows that adherence is a good indicator of the surface property of macrophages which in turn could play an important role in the cell mediated immunity of the patient. The observations suggest altered macrophage membrane structure in the long term-treated, otherwise normal, lepromatous leprosy patients.

Keywords. *Mycobacterium leprae;* adherence; macrophage membrane; lepromatous leprosy.

Introduction

In an earlier report (Lad and Mahadevan, 1982), we had shown that *Mycobacterium leprae* adhered to human macrophages and that this adherence was based on chemical interaction. The ability of adherence of *M. leprae* to macrophages from lepromatous leprosy patient was significantly reduced and this defect was specific to *M. leprae.* One of the changes induced in the macrophages of lepromatous leprosy patients, was the alteration in the membrane structure indicated by changes in the levels of various receptors (to be published elsewhere). Furthermore, an extract from lysed macrophages of lepromatous leprosy patients, induced changes in the membranes of macrophages from normal individuals (Salgame *et al.,* unpublished observations). Studies on factors such as the phagocytic ability, the effect of soluble factors affecting adherence, role of Fc receptors and the innate membrane alterations that could be identified using

Abbreviations used: Fc, Receptor for Fc region of immunoglobulins; BI, bacteriological index; T, tuberculoid type of leprosy patient; MEM, minimal essential medium; N- and L-lysate, lysate from macrophages from bacteriologically positive lepramatous patients and from macrophages from normal subjects to *M. leprae in vitro* respectively; SRBC, sensitized sheep red blood cells.

'adherence ability' as an indicator which influence the property of adherence of *M. leprae* to the macrophages from lepromatous patients and the relationship of this property to the membrane structure are reported in this communication.

Materials and methods

Normal subjects

These were healthy volunteers residing in a leprosy endemic area having varying degrees of exposure to leprosy bacilli.

Patients

Leprosy patients used in this study were classified according to the Ridley and Jopling (1966) classification.

Lepromatous leprosy bacteriologically positive patients

These were lepromatous leprosy patients who were bacillary positive but receiving treatment, duration of which varied from a few months to several years. The degree of positivity was indicated by bacteriological index (BI) from skin smears when necessary (Ridley, 1964). The long-term treated, bacteriologically negative lepromatous leprosy patients are referred to as lepromatous negative. Tuberculoid type of leprosy patients are referred to as T.

Culturing of macrophages

Macrophages were cultured from peripheral blood as described by Lad and Mahadevan (1982). Briefly, packed leukocytes were obtained from peripheral blood. It was washed with minimal essential medium (MEM), (Centron Laboratories, Bombay) and dispensed in Leighton tubes after suspending in the culture medium (40% human AB serum in MEM with 100 units/ml of penicillin). On the 5th day, almost pure, glass-adherent cells, typical of macrophages, were obtained. The macrophages obtained from the peripheral blood of the leprosy patients are referred to as lepromatous macrophages or tuberculoid macrophages and those from normal individual as normal macrophages. *M. leprae* from human sources were obtained from freshly collected nodules from untreated lepromatous patients (Acworth Leprosy Hospital, Bombay) while *M. leprae* from Armadillo spleen (preserved continuously at −90°C) were obtained from tissues supplied by Dr E. Storrs (Melbourne, Florida,USA). The bacilli were harvested as described earlier (Lad and Mahadevan, 1982).

Adherence of bacilli to macrophages

The adherence of bacilli was carried out as described earlier (Lad and Mahadevan, 1982). Briefly, five day old macrophage cultures in Leighton tubes were exposed to 20×10^6 bacilli at 8°C for 1 h. The cells were washed, fixed with 2.5% glutaraldehyde and stained by the Zeihl-Neelson technique to identify the acid-fast bacteria adherent to macrophage. The enumeration of macrophages with adherent bacilli was carried out as described earlier (Lad and Mahadevan, 1982)

Phagocytosis

One set of macrophage cultures were exposed to *M. leprae* (5×10^6 bacilli per tube) directly at 37°C for 1 h. The second set of cultures were exposed to the bacilli at 8°C for 1 h and then incubated at 37°C for an additional 1 h. At the end of the incubation period, the cells, were washed, fixed and stained for acid-fast bacilli. One hundred macrophages were counted and those having phagocytosed bacilli were expressed as a percentage of the total.

Colchicine treatment of macrophages

Five-day old macrophages cultures from positive lepromatous leprosy patients were exposed to 10^{-5}M colchicine solution for 1 h at 37°C. At the end of the incubation period the cells were washed twice with saline and were then exposed to *M. leprae* to assess their ability to adhere.

Preparation of lysate

Lysate was prepared from macrophages of bacteriologically positive lepromatous patients (L-lysate) as described by Salgame *et al.* (1980). Lysate was also prepared from normal macrophages exposed to *M. leprae in vitro* (N-lysate). This is to simulate as closely as possible conditions prevailing in the lepromatous macrophage.

Treatment of macrophage with lysate

Five day old macrophage cultures were exposed to the above lysate (derived from 10^5 macrophages) at 8°C for 1 h. At the end of the incubation period, the cells were washed once with saline and used to determine the level of adherence to *M. leprae* (Lad and Mahadevan, 1982).

Evaluation of Fc receptors in relation to adherence

Adherence of *M. leprae was* carried out with macrophages pretreated with uninactivated human AB serum at 37°C for 1 h. Untreated macrophages served as control.

The blocking effect of Fc receptors by the antibodies in the serum was confirmed by using sensitised sheep red blood cells (SRBC). SRBC were sensitised by treating them with 1:3000 dilution of amboceptor (obtained from Haffkine Institute, Bombay), at 37°C for 1 h. At the end of the incubation period SRBC were washed thrice with saline and suspended to make a final concentration of 2% (v/v). The cells were assayed for the presence of Fc receptor as described by Birdi *et al.* (1980). Macrophages were exposed to sensitised SRBC at 8°C for 1 h to simulate the condition existing at the time of carrying out the adherence test.

Neuraminidase treatment of macrophages

Five day old macrophage cultures were exposed to neuraminidase (5 units/ml). (Sigma Chemical Co., St. Louis,Missouri, USA) at 37°C for 12 min according to the method described by Wier and Ogmudsdottir (1977). At the end of the incubation period, the cells were washed with saline-and the adherence was determined as described above.

Results

Relationship of phagocytosis to adherence

Adherence does not appear to be related to the level of phagocytosis by the macrophages. This conclusion was based on the following data.

1) The level of adherence was very low in macrophages from bacillary-positive lepromatous patients and high in macrophages from bacillary-negative patients. Nevertheless the level of phagocytosis in the two types of macrophages was almost the same under both the experimental conditions. During direct incubation at 37°C for 1 h, the mean level of phagocytosis was close to 3% in macrophages of lepromatous leprosy-positive patients and near to 8% in macrophages of lepromatous leprosy-negative patients. During the tempeature shift from 8°C to 37°C it was 20 and 23% respectively (table 1).

Table 1. Level of phagocytosis of *M. leprae* by macrophages.

Source of macrophages	Per cent phagocytosis (Mean ± S.D.)	Incubation at 37°C/1 h
	Pre incubation at 8°C	Not preincubated
Normal	8 ± 1.7	33 ± 9.2
LL (+)	20.30 ± 4.0	2.67 ± 2.1
LL (−)	23.00 ± 12.0	7.7 ± 8.3
TT (III)	5.30 ± 2.5	11.30 ± 1.5

2) The level of phagocytosis in normal individuals is higher than in treated tuberculoid patients when incubated at 37°C for 1 h (normal–33%, tuberculoid–11%).

Normally, macrophages do not phagocytose ingestible materials, when incubated at low temperatures (24°C) (Pesanti and Nugent, 1981). This was clearly seen by the failure of normal macrophage to phagocytose armadillo-grown *M. leprae* when they were incubated in the cold (8°C) and then shifted to 37°C (8% phagocytosis).

3) Lepromatous macrophages, both bacillary-positive and bacillary-negative, do not behave like normal macrophages. Incubation at 8°C enhanced their phagocytic capacity unlike that of the normal macrophages (table 1) indicating differential phagocytic ability of the macrophages.

The level of adherence of *M. leprae* to normal macrophages and treated tuberculoid patients macrophages have been determined earlier as 14.33%±5.85 and 14.17% ± 5.42 respectively (Lad and Mahadevan, 1982).

Effect of lysate on adherence

The lysate derived from lepromatous leprosy bacillary-positive macrophages was able to reduce the level of adherence of the normal macrophages from 14% to 3%. On the other hand, lysate derived from normal macrophages failed to inhibit adherence ability of macrophages from normal individuals (figure 1).

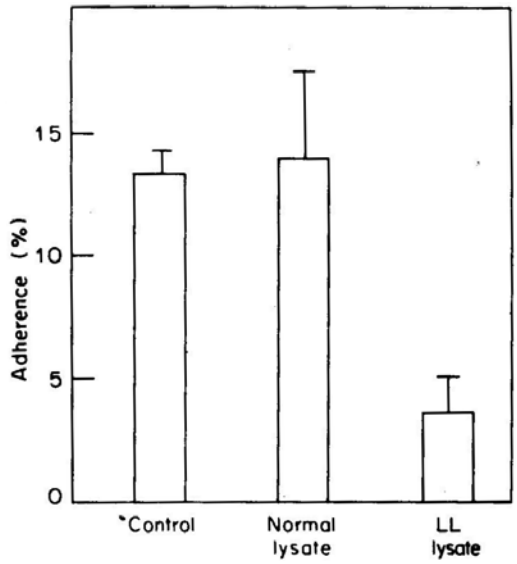

Figure 1. Effect of lysate on adherence of *M. Leprae* to normal macrophages.

The bar chart represents the mean of three experiments and standard error of mean.

Fc receptors are different from the receptors for M. leprae adherence

Pretreatment of macrophages with antibody inhibited the Fc receptor expression of macrophages to antibody coated SRBC, but failed to inhibit the ability of *M. leprae* to adhere to macrophages (table 2) indicating that Fc receptors may not be involved in adherence of the bacteria.

Effect of colchicine on adherence

The receptors present on the macrophages from normal individuals and treated bacillary-negative lepromatous patients were sensitive to colchicine treatment (figure 2), since it reduced the adherence ability.

In bacillary-positive lepromatous patients, colchicine treatment restored the level of adherence to near normal values (figure 2). Similar treatment failed to bring back the level of adherence in the macrophages of lepromatous patients who were only slightly positive (BI 1+) .

Effect of neuraminidase on adherence

Neuraminidase treatment increased the level of adherence of *M. leprae* to macrophages from normal individuals (table 3). In the case of the macrophages from bacillary-positive lepromatous patients, there was also an increase in the level of adherence. It is interesting to note that neuraminidase failed to increase.

Table 2. Effect of neuraminidase on adherence ability of *M. leprae* to macrophages.

Type of macrophages	Expt. no	Control cells		Neuraminidase treated cells	
		Per cent adherence*	Number of bacilli bound to 100 cells	Per cent adherence	Number of bacilli bound to 100 cells
Normal	1	14	n.d.	31	n.d.
	2	35	143	52	250
	3	15	55	26	155
LL (−ve)	1	35	114	26	55
	2	18	82	14	46
	3	14	44	13	38
LL (+ve)	1	2	14	22	76
	2	2	n.d.	15	n.d.
	3	2	9	25	94

n.d.- Not done.

« Number of macrophages adhering *M. leprae.*

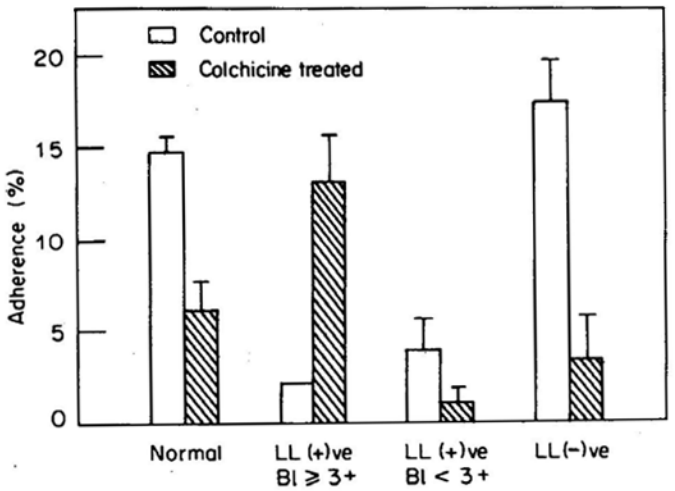

Figure 2. Effect of colchicihe on adherence of *M. leprae* to macrophages.

The bar chart represents, the mean of three experiments and standard error of mean.

adherence of *M. leprae* to macrophages from bacillary-negative patients. These observations clearly indicate that the lepromatous macrophages, (from bacillary negative patient) though they exhibit the same degree of receptor-mediated adherence, possibly the receptors being chemically identical to those of normal macrophages, are perhaps not exactly the same as normal macrophages in all respects.

Table 3. Fc receptor in relation to adherence of *M. leprae* to macrophages.

	Expt. no. 1	2	3
	Per cent adherence		
Control macrophages	14	35	16
Antibody-treated macrophages	27	29	40
	Per cent macrophages with Fc receptors[a]		
Control macrophages	46	25	35
Antibody-treated macrophages	10	3	00

[a] Per cent macrophages having two or more sensitised SRBC attached.

Discussion

Phagocytosis is an important property of macrophages and since the same macrophages also exhibit adherence to pathological organisms like *M. leprae,* it was essential to understand the relationship between these two phenomena. The observation that adherence ability of the macrophage of patients suffering from lepromatous leprosy has greatly reduced is of considerable significance. Our observations indicate that receptors involved in adherence do not seem to regulate the level of phagocytosis by the macrophages. Further more, the phenomenon of phagocytosis of macrophages from lepromatous patients is quite distinct from those of the normal. At 37°C normal macrophages showed good phagocytic ability which was absent when incubated at 8°C and remained so even after shifting the culture to 37°C after the prior cold incubation. At 37°C macrophages from the lepromatous-positive and negative patients showed low phagocytosis. The decreased level in this case could be due to a different membrane disposition that does not help phagocytosis. However when incubated at 8°C and then shifted to 37°C unlike the normal macrophages, those from both types of patients showed higher phagocytosis. This perhaps indicates that cold temperature brings about some favourable disposition in the membrane that enables increased phagocytosis at 37°C. This also indicates a possible innate difference between the two types of macrophages, the normal and that of the patient.

It has also been shown by the present study that the ability of adherence of normal macrophages is greatly reduced when such macrophages are exposed to the lysate of macrophage derived from lepromatous patients. The changes brought about by the lysate on the membrane may be responsible for the reduced adherence of bacteria similar to the effect with other receptors (Salgame *et al.,* 1980; unpublished observations). It could be possible that such products produced inside the macrophages make the cells exhibit poor adherence to *M. leprae* in the patient.

Our earlier results showed that the reduced level of adherence was reversed by treatment of the macrophages with trypsin (Lad and Mahadevan, 1982). In the present work, it has been shown that colchicine can also reverse it. This reversal by colchicine indicates the role of the cytoskeletal elements of the macrophages like the microtubules in the membrane alteration induced by *M. leprae.*

Fc receptors of macrophages do not play a role in adherence, since antibody treatment of macrophages failed to alter the ability of *M. leprae* to adhere to macrophages. Hence the reduced adherence in normal macrophages treated with the lysate is a clear indication that it is due to modification of receptors for adherence and not through alteration of receptors for Fc or phagocytosis.

Our data also demonstrate that neuraminidase treatment increased the level of adherence of *M. leprae* to macrophages from normal individuals. On the other hand, the long term-treated bacillary-negative lepromatous patients, whose macrophages otherwise behave normally, are not altered by neuraminidase treatment as regards their adherence ability.

These observations, like the different phagocytic ability of lepromatous macrophages from that of the normal, and that neuraminidase treatment of these two types of macrophages elicit different responses, indicated to us that the macrophage of patients who are susceptible to, but not suffering from lepromatous disease, could have an altered membrane structure. This alteration may be due to a preexisting state in the individual which could be due to a gene function genetic or otherwise. Our future studies are being directed towards clarification of the above possible concept.

In conclusion, it can be stated that the study of the phenomenon of adherence of *M. leprae* to macrophages, reveals some basic differences between the macrophages of the lepromatous leprosy patients and the normal. This may have relevance to the cell mediated immunity of the host.

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