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Macrophage Function in Leprosy [1,2]

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Mycobacterium leprae, an intracellular parasite, survives and multiplies in the milieu provided by the macrophages of lepromatous leprosy patients, while it disintegrates and dies in the macrophages from those who have tuberculoid leprosy. Although the precise reason for this difference in behavior is not clear, it has been suggested to be the result of functional variation in the macrophages in the two forms of the disease ([5]).

We investigated the role of macrophages in leprosy; first by assessing the lysosomal activity of tissue macrophages in cutaneous lesions of leprosy, and secondly by estimating the phagocytic and bacteriolytic potential against *M. leprae* of macrophages grown from the peripheral blood monocytes of patients with the two polar disease forms. Protein deficiency has been reported to bring about marked depression in phagocytic and lytic power of macrophages in a variety of laboratory animals ([8, 11, 13]). In order to assess the role of functionally depressed macrophages in the pathogenesis of leprosy, tissue reaction to injection of live *M. leprae* in the foot pad of protein deficient and normal rats was also studied.

MATERIALS AND METHODS

Lysosomal slides. For these studies, cryostat sections of biopsies from cutaneous leprosy lesions from 38 (5 of TT, 12 of BT, 2 of BB, and 7 each of BL and LL, and 5 of histoid) patients, classified according to the criteria of Ridley and Jopling ([14]) were processed by the method of Barka and Anderson ([4]), stained with the Ziehl-Neelsen technic and counterstained with hematoxylin.

Acid phosphatase was quantitated at +, ++, and +++ as assessed visually by intensity of the brown-black color. Paraffin embedded sections were separately processed to determine Bacillary (BI) and Morphologic (MI) Indices.

Macrophage culture. For the second part of the study, blood monocytes from 18 patients (9 each of TT/BT and BL/LL) were cultured *in vitro* in TC 199 medium containing 50% autologous serum using the technic employed by Beiguelman ([5]). On the sixth day of culture, monocytes in Leighton tubes were challenged with 3×10^6 *M. leprae* (obtained from untreated patients having lepromatous leprosy), suspended in one milliliter of TC medium 199 containing 20% of AB serum. Twenty-four hours later, the medium containing *M. leprae* was replaced by fresh, sterile medium.

Monolayers, in duplicate, of macrophages on coverslips were processed on days 1, 7, 14 and 21 (occasionally also on days 2, 17 and 24) after challenge with bacilli to assess the changes in the bacilli and the cells. The coverslips were fixed in buffered formalin and stained by Fite's modification of the Ziehl-Neelsen staining technic. The percentage ratio of the macrophages containing the bacilli on day one was determined and designated as the "Phagocytic Index." The number of bacilli per macrophage, designated as the "Bacteriolytic Index," was determined on days 1, 7, 14 and 21. Both these indices were estimated by counting 200 cells on each coverslip by two independent observers. Monolayers of blood monocytes from two healthy individuals were processed without challenge with *M. leprae* almost daily for a period of 26 days to study the morphologic changes seen in the macrophages.

Animal studies. Five to six week old, inbred albino rats (Wistar) obtained from our stock colony were divided into two dietary groups. Group I (LP) contained six rats and was fed *ad libitum* a diet containing 3% casein. The control group (HP) containing an equal number of rats was fed a protein rich diet containing 18% casein. Each animal in the control group (HP) was pair-fed with the

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TABLE 1. *Relationship between acid phosphatase activity and different types of leprosy.*

Quantitation, acid phosphatase	Number of patients in different types of leprosy					
	TT	BT	BB	BL	LL	Histoid
+	3	3		1	1	
++	2	9	2	6	5	5
+++					1	

TABLE 2. *Relationship between acid phosphatase activity and the Bacterial Index.*

Bacterial Index / Quantitation, acid phosphatase	Number of patients with their Bacterial Index						
	0	1	2	3	4	5	6
+	6				1	1	
++	10	1	1		8	5	4
+++						1	

corresponding animal in the protein deficient group. Except for the protein content the diets were identical and provided adequate vitamins and minerals. Their composition has been described previously ([11]). Four weeks after being placed on the respective diets, each rat was inoculated in the hind foot pad with 5×10^3 live *M. leprae* ([15]), obtained from skin lesions of untreated lepromatous patients. The rats were sacrificed on the tenth day after the injection of bacilli and their foot pads excised and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were stained with hematoxylin and eosin and Ziehl-Neelsen stains.

RESULTS

Acid phosphatase in cutaneous lesions. Acid phosphatase was seen as brownish-black granular or confluent deposits present in association with or independent of bacilli. Tables 1 and 2 give results of the quantity of acid phosphatase and its relationship to the Bacterial Index in different types of leprosy.

In vitro culture of blood-derived macrophages. Monocytes and polymorphonuclear leucocytes tended to stick to the coverslips in 30 to 45 minutes and the former assumed a rounded form in the first 24 hours and, in some cases, had formed a syncytium (Fig. 1). By 72 hours, the polymorphs had come off and some of the monocytes had assumed a spindle shape with abundant cytoplasm and elongated nuclei (Fig. 2). From the fourth day onwards, a tendency to form giant cells was seen. Nuclei were large, pale staining

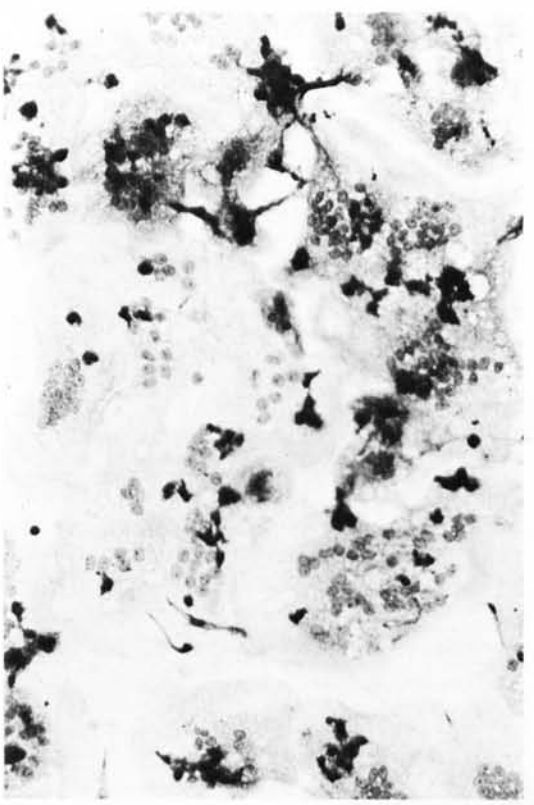

FIG. 1. Twenty-four hour old culture showing syncytium formation.

and vesicular with prominent nucleoli. By the 14th day, the vacuolation in the cytoplasm of the cells was quite conspicuous. Nuclei in the giant cells were arranged around the periphery, as in the Langhan's giant cell, or irregularly as in foreign body type giant cells (Fig. 3).

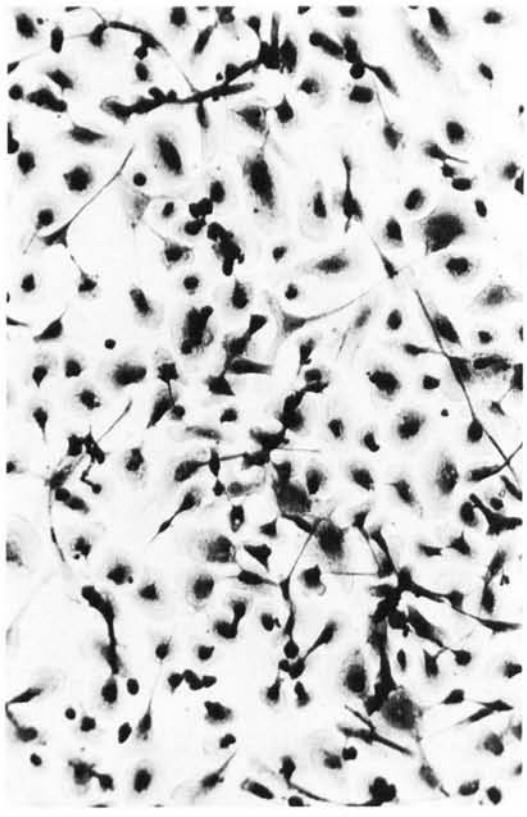

FIG. 2. Seventy-two hour old culture with spindly and rounded macrophages.

Phagocytosis of *M. leprae* began by 30 minutes, but was uniform and consistent at 24 hours (Fig. 4) when observations were made to determine the Phagocytic Index. The Phagocytic Index on day one and the Bacteriolytic Index on different days in the two groups of the patients are given in Tables 3 and 4. We failed to detect any significant differences in these two parameters or in any culture in which the bacilli had been completely lysed during the period of observation, i.e., up to three weeks post-infection.

Animal studies. Rats showed, at the site of injection of live *M. leprae* by the tenth day, formation of granulomas composed of histiocytes, some of them vacuolated, with scattered plasma cells and lymphocytes. The

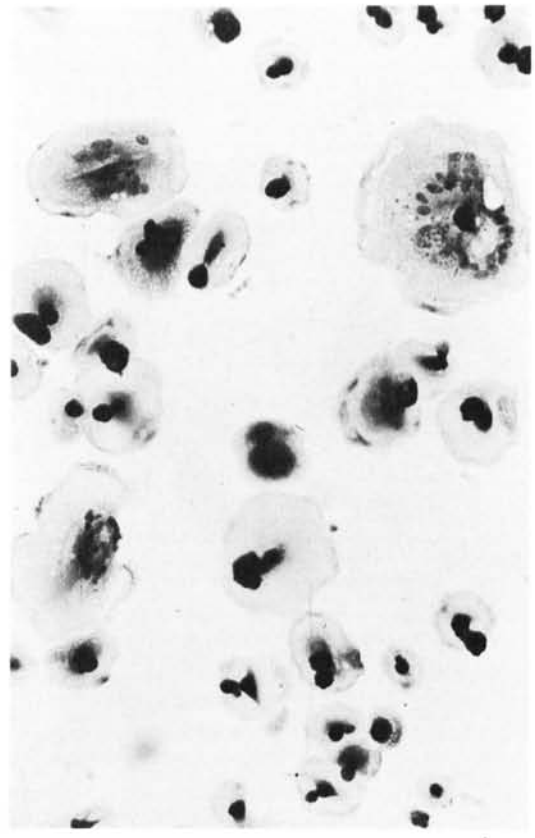

FIG. 3. Three giant cells and many histiocytes (17th day).

granulomas had no particular predilection for nerves or skin appendages. The infiltrate was sparse and present chiefly deep in the subcutis and at places infiltrating in between the muscles. Acute inflammatory component was not present. A fair number of *M. leprae* could be demonstrated in the granulomas.

No differences were observed between the protein deficient (LP) and the control (HP) animals, as far as the tissue reaction or the bacteriology were concerned.

DISCUSSION

The study was an attempt at assessing the role of macrophages in defense against *M. leprae*. Histochemical demonstration of acid phosphatase in the cutaneous lesions was selected as a marker of lysosomal activity. The *in vitro* culture of blood monocytes followed by challenge with *M. leprae* was attempted to study the phagocytic and bacteriolytic capability of macrophages derived from blood monocytes from the two polar forms of leprosy. Tissue responses to injection of

TABLE 3. *Phagocytic Index of macrophages on day one, after challenge with 3 × 10⁶ untreated* M. leprae.

Type of disease	Mean value	SD	t value
TT/BT	27.0	18.43	1.54 NS
BL/LL	48.2	24.5	

NS = not significant.

TABLE 4. *Bacteriolytic Index of macrophages after challenge with 3 × 10⁶* M. leprae.

	Day 1	Day 7	Day 14	Day 21
TT/BT	7.1 ± 3.8	8.9 ± 4.4	9.54 ± 3.85	9.82 ± 4.91
BL/LL	8.5 ± 3.05	11.28 ±3.85	11.10 ±2.16	10.86 ±4.53
t value	0.64 NS	0.90 NS	0.78 NS	0.35 NS

NS = not significant.

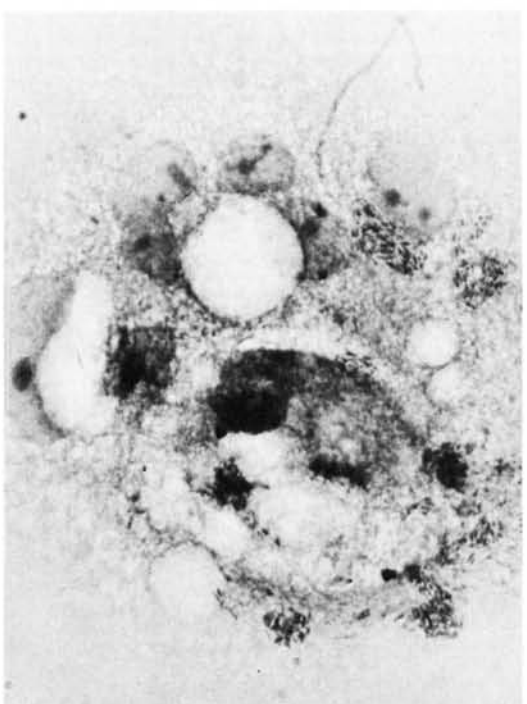

FIG. 4. A giant cell with vacuolated cytoplasm and many *M. leprae*.

M. leprae in protein deficient and control animals were studied to assess the effect of protein deficiency on macrophage functions.

The results of the present study show that the quantum of acid phosphatase was almost equally distributed in different forms of leprosy (Table 1). In fact, the acid phosphatase content was also quite independent of the bacterial load (Table 2) or even the Morphologic Index. The results suggest that the quantity of lysosomal enzyme (and macrophage function) had little to do with determining the form of leprosy the host will suffer from. This is contrary to the expected findings, since patients with the tuberculoid form of leprosy, assumed to have activated macrophages, should be expected to show high levels of acid hydrolases. The possible explanation for the findings in this study could be that either the lysosomes have little role to play in the disposal of *M. leprae* or an enzyme other than acid phosphatase, for instance lipase, may be responsible for defense against these bacilli. Such a hypothesis was also put forth by Job ([10]) when he similarly found no differences in the acid phosphatase activity in various forms of leprosy. Aquino and Skinsnes ([1]) also demonstrated by electron microscopy acid phosphatases in lysosomal organelles of macrophages of seven lepromatous cases.

The results of Phagocytic Index of blood monocyte-derived macrophages cultured *in vitro* from the two polar forms of leprosy revealed wide differences in individual patients. No significant differences between the two groups could, however, be detected.

Again, studied over a period of three weeks, the macrophages from either of the two polar forms of the disease failed to reveal any differences in their capacity to lyse *M. leprae*. This is contrary to earlier reports ([3, 5]) in which a very significant difference in the lytic potential of macrophages between the two polar forms of leprosy was shown. The authors had, in fact, held that the basic fault in the two forms resided in the functional activity of the macrophages, in that the macrophages in lepromatous patients specifically lacked the ability to lyse *M. leprae*. Godal and Rees ([9]), in leprosy patients, and Delville ([7]) in normal individuals, however, failed to demonstrate the dimorphism of macrophage behavior postulated by Beiguelman ([5]), and Barbieri and Correa ([3]).

Animal studies also failed to reveal any significant differences between the protein deficient and control groups. Granulomatous infiltrate in the rats in response to *M. leprae* was, in many ways, similar to that observed in borderline (BB/BL) variety of human leprosy except that no special predilection was observed for skin appendages or the nerves. Moderate protein deficiency did not alter tissue response of rodents to *M. leprae*. This may, at first sight, seem surprising since protein deficiency is known to depress macrophage function ([6, 8]). It has, however, also been shown that the level of the circulating small lymphocyte, the key cell in cell-mediated immunity, is reduced only after 70 days of protein deficiency ([2]). Recent studies from this laboratory also indicate that homograft rejection, a T cell function, is not altered in moderate protein deficiency ([12]). This may also hold true for macrophage function.

From the evidence presented in the present study it would seem that the macrophages per se are incapable of lysing *M. leprae* within three weeks of ingestion, and that in the two polar forms the macrophages are equally efficient (or inefficient) in dealing with *M. leprae*. The differences observed in the human subjects may, therefore, be the result of macrophage-lymphocyte interaction. One wonders if the addition of lymphocytes as such or lymphokines to the monocyte culture after challenge with *M. leprae* would alter the behavior of the macrophages. The animal studies suggest that moderate protein deficiency over a limited period does not alter the behavior, *in vivo* of host responses in the rats.

SUMMARY

The macrophage function in patients with leprosy was assessed by estimating histochemically the acid phosphatase activity in skin biopsies and by assessment of phagocytic and lytic capability of *in vitro* cultured macrophages derived from peripheral blood monocytes, challenged with live *M. leprae*.

Acid phosphatase was demonstrated in skin biopsies of different groups of leprosy patients classified according to the Ridley and Jopling scale. The degree of acid phosphatase positivity was correlated with clinical spectrum, Bacterial and Morphologic Indices and treatment status.

Peripheral blood monocytes from patients with leprosy, either tuberculoid or lepromatous, were cultured in monolayers and challenged with *M. leprae*. The phagocytosis and lysis of mycobacteria by macrophages was observed at different time intervals from the 1st to the 28th day. The morphology of the macrophages in different types of leprosy was also studied.

The results suggest that macrophages from patients with either tuberculoid or lepromatous leprosy are not by themselves capable of lysing live *M. leprae*.

Live *M. leprae* injected into the foot pad of Wistar strain of rats evoked similar responses on the tenth day, in normal and protein deficient animals.

RESUMEN

La función macrofágica en pacientes con lepra fue evaluada estimando histoquimicamente la actividad de la fosfatasa ácida en biopsias cutaneas y la determinación de la capacidad fagocítica y lítica de macrófagos cultivados, derivados de monocitos de sangre periférica y enfrentados con *M. leprae*.

La fosfatasa ácida fue demonstrada en biopsias cutaneas de diferentes grupos de pacientes leprosos clasificados de acuerdo a la escala de Ridley y Jopling. El grado de positividad de la fosfatasa ácida fue correlacionado con el espectro clínico, con los índices bacteriológicos y morfológicos y con el estado terapéutico.

Monocitos de sangre periférica de pacientes leprosos, tanto tuberculoides como lepromatosos, fueron cultivados en mono-capas y enfrentados con *M. leprae*. La fagocitosis y lisis de micobacterias por macrófagos fue observada a diferentes intervalos, desde el primero hasta el vigésimo

octavo dia. La morfología de los macrófagos en diferentes tipos de lepra fue también estudiada.

Los resultados sugieren que los macrófagos de pacientes con lepra tuberculoide o lepromatosa no son capaces por si mismo de producir la lisis de las *M. leprae.*

M. leprae vivos inyectados en el panículo podal de ratas Wistar evocaron respuestas similares al décimo dia en animales normales y en aquellos deficientes en proteina.

RÉSUMÉ

On a procédé à une évaluation de la fonction macrophagique chez des malades atteints de lèpre, en estimant par des méthodes histochimiques l'activité en phosphatase acide dans des biopsies cutanées. On a également évalué la capacité phagocytaire et la capacité lytique observées dans des cultures *in vitro* de macrophages obtenus à partir de monocytes du sang périphérique, stimulés par *M. leprae* vivant.

Une activité en phosphatase acide a été mise en évidence dans les biopsies de peau, dans différents groupes de malades de la lèpre classés selon la classification de Ridley and Jopling. Le degré de positivité en phosphatase acide présentait une corrélation avec le spectre clinique, avec les indices bactériens et morphologiques, de même qu'avec la réponse au traitement.

Les monocytes du sang périphérique obtenus de malades atteints de lèpre, que ceux-ci soient tuberculoïdes ou lépromateux, ont été cultivés en couches monocellulaires et stimulées par *M. leprae.* On a relevé la phagocytose et la lyse des mycobactéries par les macrophages à différents intervalles de temps, depuis le 1e jour jusqu'au 28e. La morphologie des macrophages dans différents types de lèpre a également été étudiée.

Les résultats obtenus suggèrent que les macrophages de malades atteints de lèpre tuberculoïde ou de lèpre lépromateuse ne sont pas par eux-mêmes capables de détruire *M. leprae* vivant.

Des bacilles de la lèpre vivants injectés dans le coussinet plantaire d'une souche suisse de souris ont induit au dizième jour des réponses semblables à celles que l'on peut relever chez des animaux normaux et chez des animaux présentant une déficience en protéine.

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