

Role of Macrophages in Defective Cell Mediated Immunity in Lepromatous Leprosy. II. Macrophage and Lymphocyte Interaction¹

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Our earlier studies (²) have shown that there is a reduced incorporation of leucine in macrophages derived from susceptible Swiss white mice in the presence of intracellular *M. leprae*, but this was not observed in the more resistant C57BL mice. It was also indicated at that time that macrophages from lepromatous leprosy patients in culture also showed a lower quantity of leucine incorporation in the presence of *M. leprae* when compared to macrophages from healthy volunteers or tuberculoid leprosy patients. The data in the earlier paper showed clearly that macrophages from lepromatous leprosy patients, in the presence of *M. leprae*, become biochemically altered and produce soluble factors that affect protein synthesis and lymphocyte transformation in a normal leukocyte population (¹¹).

We have further extended these observations to determine the ability of macrophages to form antigen specific rosettes with lymphocytes. Since antigen-specific binding of lymphocytes to macrophages occurs before lymphocyte activation, the enumeration of such rosettes would indicate the capacity of macrophages to process the antigen. The effect of intracellular *M. leprae* on the surface membrane of macrophages has also been studied using the Fc receptor as a marker. Further, in this paper

we present a detailed discussion of our results covering both the previous (¹¹) and present papers.

MATERIALS AND METHODS

Source of viable *M. leprae*. Freshly collected biopsies from untreated lepromatous patients were trypsinized, and after differential centrifugation the *M. leprae* were obtained and stored at 4°C to be used within 1 week.

Subjects. The leprosy patients were classified clinically according to the Ridley and Jopling classification (⁹).

Macrophage culture technique and macrophage exposure to *M. leprae*. These were as described in the accompanying paper (⁸).

Macrophage-lymphocyte (ML) interaction. Mononuclear cells were separated from heparinized peripheral blood by Ficoll-Hypaque gradient centrifugation and washed twice with Eagle's Minimal Essential Medium (MEM). These preparations contained 5%–10% monocytes. The rest were mainly lymphocytes. The mononuclear cell pellet was then resuspended in MEM containing 20% human AB serum to a final cell concentration of 5×10^6 cells/ml and distributed in Leighton tubes containing coverslips. Bacilli (5×10^6 /ml) were added and the tubes incubated in 5% CO₂ at 37°C for 20 hr. The culture was then fixed with glutaraldehyde and the coverslip removed and stained. Two hundred consecutive monocytes were counted, and the percentage of rosettes was determined as a measure of interaction. A rosette was defined as a monocyte with 2 or more lymphocytes adherent to it.

Fc receptor (EA rosette). Sheep erythrocytes (SRBC) in Alsever's solution were

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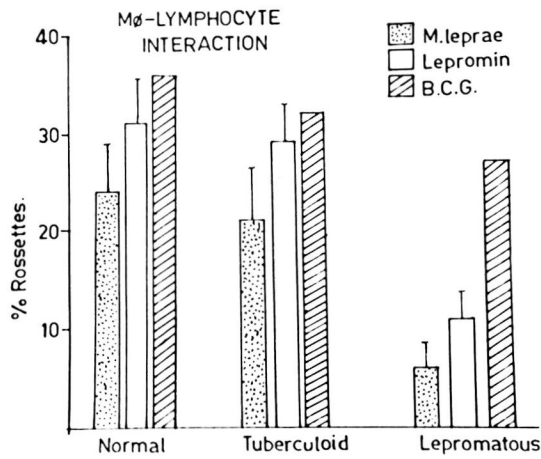


FIG. 1. The results of the macrophage-lymphocyte interaction have been expressed as mean \pm S.D. Five experiments with each antigen (except only 1 with BCG) were carried out in normal individuals and lepromatous and tuberculoid patients.

washed and resuspended to 5% (v/v) and incubated with an equal volume of MEM containing amboceptor (a commercially available mixture of IgG and IgM) for 30 min at 37°C. The SRBC were again washed and resuspended to 0.5% in MEM. To enumerate the rosettes, coverslips from mononuclear cell cultures were removed, covered with Ig-coated SRBC, and incubated at 37°C for 30 min. They were then fixed with gluteraldehyde and the rosettes counted. Attachment of 3 or more SRBC to a monocyte signified a rosette.

RESULTS

Macrophage-lymphocyte (ML) interaction. The interaction was studied in the presence of viable *M. leprae*, lepromin, and BCG. When no antigen was added, a baseline of 7% (\pm 3%) ML rosettes was obtained. As seen in Fig. 1, both tuberculoid patients and healthy volunteers showed an increased percentage of interaction to all 3 antigens. However, lepromatous patients did not respond to viable *M. leprae* or lepromin, and only the baseline percentage of rosettes was obtained. ML rosettes were formed in response to BCG with lepromatous patients.

If the lack of interaction in lepromatous patients was due to the active inhibition by *M. leprae*, then only viable *M. leprae* would have caused a negative interaction.

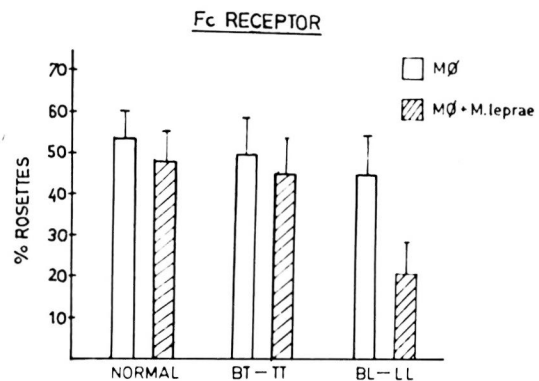


FIG. 2. The results of the percentage of macrophages forming "EA" rosettes are expressed as mean \pm S.D. Three experiments were carried out in normals and tuberculoid patients and 7 with lepromatous patients.

Since even in the presence of autoclaved *M. leprae* there is no interaction, it appears that the surface antigens of *M. leprae* are not recognized by lepromatous macrophages.

Fc receptor. Fig. 2 compares the erythrocyte-antibody or "EA" rosetting capacity of macrophages, in the absence and presence of *M. leprae*, from lepromatous and tuberculoid patients and healthy volunteers. From each individual, macrophage cultures were set up in 4 Leighton tubes. To 2 cultures, *M. leprae* were added while the other 2 were maintained as controls. Note that in all 3 groups of individuals the "EA" rosetting capacity of macrophages from cultures to which no *M. leprae* were added was similar. There was no significant difference in the rosetting property between cultures infected with *M. leprae* and their control counterparts from tuberculoid patients and healthy volunteers. However, the rosetting capacity of lepromatous macrophages in cultures infected with *M. leprae* was greatly reduced. These observations are similar to those recorded by Ridley, et al. (10).

DISCUSSION

The defect in cell mediated immunity of lepromatous leprosy patients has attracted attention for a long time, and a generally accepted reason for such a deficiency is attributed to T cells (5). However, our earlier observations (2) had indicated that macro-

POSSIBLE SEQUENCE OF EVENTS FOR A DEFECTIVE CMI IN LEPROMATOUS LEPROSY.

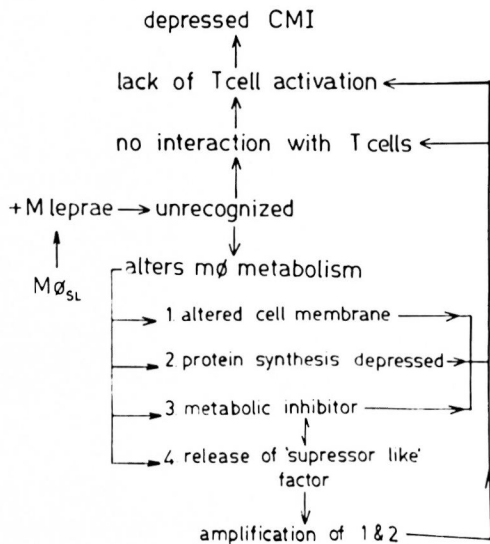


FIG. 3. The point at which leprosy infection becomes effective in an individual has been denoted by "+*M. leprae*" in the diagram.

$M\phi_{SL}$ —macrophage from individuals susceptible to lepromatous leprosy.

- 1.—as seen from the Fc receptor experiments.
- 2.—as observed in the ^3H -leucine uptake studies.
- 3.—as concluded from the lysate experiments.
- 4.—unpublished data.

The factor(s) mentioned in 3 and 4 may be the same or different.

phages should also be studied to explain the defective CMI in lepromatous leprosy patients. A similar opinion had been expressed by other workers (4).

The preceding (11) and present papers show experimental findings that lead us to conclude as follows. From the study of macrophages of lepromatous leprosy patients it appears that there are 2 types of events taking place:

a) There is production of soluble factor(s) by lepromatous leprosy macrophages in the presence of *M. leprae* that reduce their protein synthesis. Such factor(s) are also active on normal macrophages. Besides, these factor(s) also block lymphocyte transformation that would normally occur in leukocyte culture in response to an antigen (11).

b) There are structural changes in lep-

romatous macrophages due to entry of *M. leprae*. Normal levels of "EA" rosette forming cells were observed in lepromatous macrophages without *M. leprae*. However, the percentage was reduced in cultures with *M. leprae* added *in vitro*. Furthermore, macrophage-lymphocyte interaction is absent in lepromatous patients in response to *M. leprae*.

Mahadevan and Antia (8) have suggested that residence of *M. leprae* in macrophages in lepromatous leprosy would lead to regulation of metabolism, and the above changes described in the preceding (11) and present papers are most probably the results of such an event. On the basis of these observations, the possible sequence of events resulting in lepromatous leprosy can be postulated as follows (Fig. 3):

Whatever may be their route of entry, *M. leprae* are engulfed by the lepromatous macrophages, which are, genetically or otherwise, disposed to interact with *M. leprae*, leading to molecular changes inside the macrophages that result in a) structural changes reflected in membrane function (Fc receptor) as well as b) the production of factor(s) that alter the metabolism of the macrophages as studied by protein synthesis. This would lead to failure in processing antigens like *M. leprae*. Further, these factor(s) probably make the macrophages so altered that even normal T cells are unable to interact with macrophages in the presence of *M. leprae* and undergo blastogenesis—an essential step in cell mediated immunity (CMI).

It may be argued that the basic defect is in T cells only and that the macrophages in lepromatous leprosy are immature because of lack of proper interaction with normal T cells, which are deficient in lepromatous patients. However, the macrophage lysate experiments (11) show that even normal macrophages and T cells will not function as expected in the presence of "suppressor" factor(s) obtained from the lepromatous macrophages.

The activation of sensitized T cells and the effective induction of a CMI response to many antigens and mitogens involve the cooperation of lymphocytes and macrophages (7). Recently, evidence has been presented that animals that are poor responders to a particular antigen may be so

because their macrophages lack the ability of processing or presenting antigens to T cells (¹²). A similar situation seems to be present in lepromatous leprosy patients. Some recent observations by others support this view (⁶). Our results thus explain the defect in the macrophages in lepromatous patients in some molecular terms.

A genetic basis for leprosy infection has previously been suggested by Chakravarti and Vogel (³) and Beiguelman (¹) but is still debated. From our experiments a clear inability of macrophages from lepromatous patients to react with *M. leprae* is seen. Can this be under genetic control?

SUMMARY

Macrophages from lepromatous patients after phagocytosis of *M. leprae* showed alteration in their surface property as determined by their ability to express Fc receptors. The same macrophages without intracellular *M. leprae* show normal Fc receptors. The lepromatous macrophages also show very poor interaction with lymphocytes in the presence of *M. leprae* while they are able to interact with lymphocytes when exposed to other antigens. These observations along with earlier ones on macrophage defects have indicated a probable reason for defective cell mediated immunity (CMI) in lepromatous leprosy patients. There appears to be a defective macrophage population in lepromatous patients that is unable to process *M. leprae* antigens and initiate the CMI response.

RESÚMEN

Los macrófagos de los pacientes lepromatosos después de fagocitar al *M. leprae* presentaron alteraciones en la actividad de sus membranas, según se determinó por su capacidad para expresar receptores para el fragmento Fc. Los mismos macrófagos sin el *M. leprae* intracelular mostraron la existencia normal de receptores para el Fc. Los macrófagos lepromatosos también muestran una muy pobre interacción con linfocitos en presencia del *M. leprae* en tanto que son capaces de interactuar adecuadamente con los linfocitos cuando se exponen a otros antígenos. Estas observaciones, junto con otras anteriores sobre defectos en los macrófagos sugieren una probable razón de la defectuosa inmunidad celular (IC) en los pacientes con lepra lepromatosa. Parece ser que hay una población de macrófagos defectuosos en los pacientes lepromatosos que son incapaces de procesar adecuadamente a los antígenos del *M. leprae* e incapaces de iniciar la respuesta inmune celular.

RÉSUMÉ

Après phagocytose de *M. leprae*, les macrophages de malades lépromateux présentent des altérations dans leur propriété de surface, ainsi qu'on peut le mettre en évidence par la capacité à exprimer le récepteur Fc. Les mêmes macrophages, lorsqu'ils ne contiennent pas de *M. leprae* intracellulaire, présentent des récepteurs normaux Fc. Les macrophages lépromateux témoignent également d'une interaction très limitée avec des lymphocytes en présence de *M. leprae*, alors qu'ils sont cependant capables de réagir avec les lymphocytes lorsqu'on les expose à d'autres antigènes. Ces observations, de même que les observations antérieures concernant les déficiences des macrophages, indiquent la raison probable du défaut de l'immunité à médiation cellulaire (CMI) chez les malades atteints de lèpre lépromateuse. Il apparaît qu'il existe une population de macrophages déficients chez les malades lépromateux, ces macrophages étant incapables de réagir avec les antigènes de *M. leprae* et de déclencher la réponse immunitaire à médiation cellulaire.

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