INTERNATIONAL JOURNAL OF LEPROSY

Volume 53, Number 2
Printed in the U.S.A.

Phagocytic and Bactericidal Activities of Macrophages from *Mycobacterium leprae*-infected Normal and Immunosuppressed Mice[1,2]

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Although the precise reason for the difference in behavior of macrophages in different types of leprosy is not known, factors such as heredity ([3]), functional variations ([19]), altered surface membrane ([6]), and inadequate specific sensitization of T cells may be responsible. The reduced synthesis of proteins by human and mouse macrophages in the presence of *Mycobacterium leprae* infection has been documented ([5, 19, 23]).

The phagocytic functions of macrophages from leprosy patients have been found to be decreased ([1–3, 6–8]) although there are contrary reports ([9, 10, 12, 20, 22, 24]). The studies so far available are not longitudinal and do not give any indication of the kinetics of the macrophage functions in leprosy patients or in intact and immune-compromised mice. Although the mouse foot pad model is not an absolute counterpart of human leprosy, it provides a good opportunity for studying macrophage functions from the establishment of the infection to the end of the experiment.

MATERIALS AND METHODS

Weanling Swiss albino mice raised in the animal house of the Postgraduate Institute of Medical Education and Research, Chandigarh, India, were used in the study. Four groups were set up consisting of 50 mice each: 1) Normal controls (NC), 2) normal infected (NI), 3) thymectomized and irradiated controls (TRC), and 4) thymectomized and irradiated infected (TRI). Thymectomy was performed on three-week-old mice just after weaning. Irradiation was carried out at the interval of one week in two sublethal doses of 450 rads each. The thymectomized-irradiated animals were protected with bone marrow cells from normal animals by intravenous route and were thus able to survive for at least nine months. After thymectomy and irradiation, the mice were inoculated with *M. leprae* 24 hours later. *M. leprae* were isolated from human biopsy samples taken from untreated lepromatous patients; 10^4 *M. leprae* were inoculated per foot pad.

The methods of foot pad inoculation, harvesting, and counting of acid-fast bacilli (AFB) were those of Shepard ([25]), Desikan and Venkataramaniah ([8]), and Hanks ([13]), respectively. Five animals were sacrificed from each of the four groups every three months from the third month onward up to nine months. The results are expressed in the histograms (Figs. 2 and 3) as means ± standard errors of five individual experiments (one experiment per mouse).

Macrophage functions. Phagocytic functions were studied in the peritoneal macrophages obtained from mice ([27]) by the method of Lloyd and Levick ([18]), as described previously ([10]), following incubation with phylogenetically different particles such as sensitized sheep red blood cells (SRBC), latex particles, and *Staphylococcus aureus*. Two hundred macrophages engulfing two or more particles were counted for each type of particle, and the percentage of phagocytizing cells in each experiment was determined by the following formula:

[1] Received for publication on 16 April 1984; accepted for publication in revised form on 30 November 1984.

[2] This study forms part of a thesis to be submitted to the Postgraduate Institute of Medical Education and Research, Chandigarh, India, for the award of Doctor of Philosophy.

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$$\frac{\text{Number of macrophages with ingested particles}}{\text{Total number of macrophages counted}} \times 100$$

Bactericidal testing was done according to the method of Suen and Allen ([26]), as described previously ([10]), with modifications. Briefly, 0.5 ml of adjusted macrophages (1×10^5) were incubated with 0.5 ml of adjusted *S. aureus* suspension (1×10^7 organisms) in culture tubes for ½ hr in a shaking water bath of 37°C, allowing ingestion of the bacteria. Colony-forming units/milliliter (CFU/ml) were assessed before and after incubation by pouring a known volume of the serial dilutions made in nutrient broth over a nutrient agar plate ([27]). The numbers of *S. aureus* that were phagocytosed (Np) were determined by subtracting the CFU/ml after ingestion from the CFU/ml before ingestion.

Gentamycin (0.1 ml of 10 μg/ml) was then added to each culture tube and incubated for 1 hr to kill the unengulfed bacteria. After incubation, CFU/ml were again determined in order to measure the number of bacteria that had survived intracellular killing (Ns). The bactericidal index (BcI) of the macrophages was determined by the following formula:

$$\text{BcI} = \frac{\text{Np} - \text{Ns}}{\text{Np}} \times 100$$

RESULTS

Bacterial counts in foot pads (Fig. 1). In the normal infected mice (NI), only a few bacilli were found in the foot pads three months post-inoculation, and counting was not possible. A steep rise in bacillary counts (mean 3.40×10^5 AFB/foot pad) occurred by six months after inoculation which did not change significantly at nine months at the end of the experiment (mean 3.04×10^5 AFB/foot pad).

In thymectomized-irradiated and infected mice (TRI), a mean bacillary count of 1.3×10^6 AFB/foot pad was found at three months. An apparent fall (mean 2.6×10^5 AFB/foot pad) occurred at six months, whereas a slight rise was observed nine months post-infection (mean 1.4×10^6 AFB/foot pad).

Phagocytic activity (Fig. 2). No significant difference was observed in the phagocytic activity toward *S. aureus* at 3, 6, or 9 months in NI and NC mice. However, a slight decrease ($p < 0.05$, Student's *t* test) was seen in TRI mice as compared to the TRC and NI groups at three months post-inoculation. No signficant difference was seen in either the uninfected NC or TRC group at any stage of infection.

No significant difference was found between any of the groups at any period of infection in the phagocytosis of latex particles.

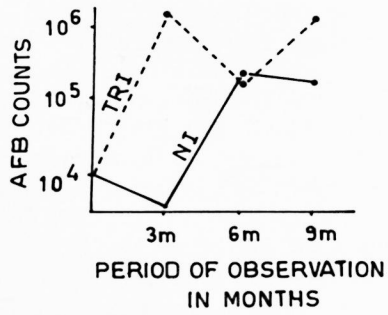

FIG. 1. Acid-fast bacilli per foot pad of normal (NI) and thymectomized-irradiated (TRI) mice after inoculation with *M. leprae*.

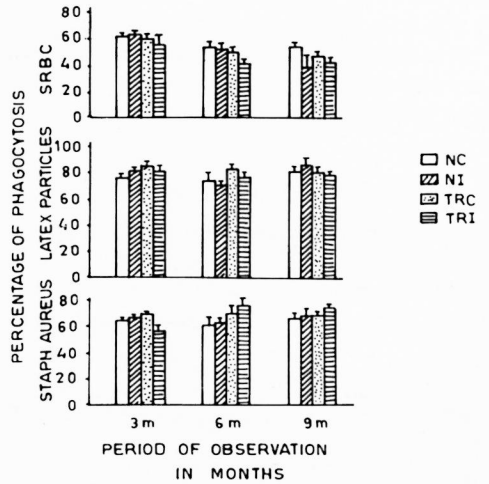

FIG. 2. The percentage of peritoneal macrophages from normal control (NC), normal *M. leprae*-infected (NI), thymectomized-irradiated control (TRC), and thymectomized-irradiated infected (TRI) mice phagocytized sheep erythrocytes (SRBC), latex particles, and *Staphylococcus aureus* (Staph aureus). Each bar is the mean ± S.D. of the sample from each of five mice at the times indicated after inoculation with *M. leprae*.

No significant difference was found in the control groups (NC and TRC) in the phagocytosis of antibody sensitized (Fc coated) SRBC. However, a transient decrease was seen at six months in the TRI group as compared to the NI group ($p < 0.05$).

Bactericidal activity (Fig. 3). The bactericidal activity was significantly decreased in the NI group as compared to the NC group at six months ($p < 0.01$) and at nine months ($p < 0.05$) after inoculation. The activity was decreased at all test periods in the TRI as compared to the TRC group ($p < 0.01$ to <0.001). A significant depression of bactericidal functions was found in the TRI group compared with the NI mice at three ($p < 0.001$) and nine months ($p < 0.05$).

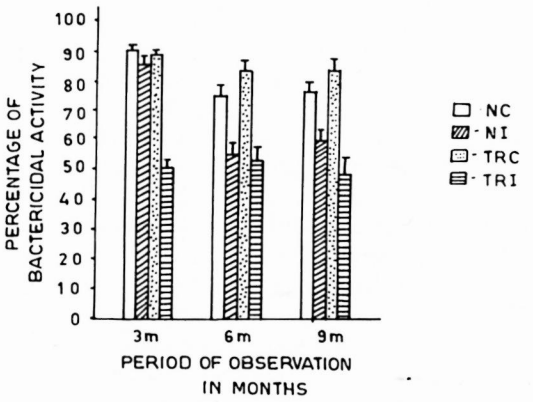

FIG. 3. The percentage of phagocytized *Staphylococcus aureus* killed in 1 hr at 37°C by peritoneal macrophages from normal control (NC), normal *M. leprae*-infected (NI), thymectomized-irradiated control (TRC), and thymectomized-irradiated infected (TRI) mice. Each bar is the mean ± S.D. of the sample from each of five mice at the times indicated after inoculation with *M. leprae*.

DISCUSSION

Macrophage functions in leprosy are closely linked with T cell-mediated immune responses ([17, 22]). Various mechanisms such as surface membrane changes in lepromatous macrophages affecting the adherence of *M. leprae* ([16]), reduced protein synthesis by macrophages in lepromatous leprosy, and a relative increase in tuberculoid patients ([19]), etc., have been used to explain the deficiencies. The lack of response in lepromatous disease is considered to be due to the failure of the macrophages in presenting *M. leprae* antigens in an immunogenic form ([15]). All of these changes, individually or collectively, might affect the phagocytic, bactericidal, and other activities of macrophages.

In the present study with the mouse foot pad model, no significant and persistent deficiency in phagocytic functions was seen at any period post-infection. A significant decrease in phagocytosis of *S. aureus* which was noticed at three months in the TRI compared to the TRC group was transient and did not persist subsequently. Among infected groups a slight but significant increase in the phagocytosis of *S. aureus* was observed in the TRI as compared to the NI group at six months post-infection. A transient decrease in phagocytosis of SRBC was seen at six months in the TRI group compared to the NI mice. On detailed analysis, phagocytosis of latex particles remained unaltered in all of the experimental groups at all stages of infection.

Fluctuations in phagocytic activity toward some particles could be mediated through separate receptor systems. For example, it is possible that only the Fc receptor mediating phagocytosis of SRBC was affected.

In human leprosy a wide spectrum of phagocytic activity has been reported. Some workers have shown an increase in phagocytosis ([4, 14]), while others have reported normal ([9, 11, 12]) or depressed phagocytosis ([1–3]). The TRI group may more closely resemble human lepromatous leprosy ([21]) than any model other than the nude mouse and the armadillo.

The bactericidal activity was significantly low in both infected groups as studied in the mouse peritoneal macrophages. It was observed that when the foot pad counts were highest in the TRI group at 3 and 9 months (with a fluctuation at 6 months), the bactericidal activity for *S. aureus* was the lowest. It could be presumed that heavy *M. leprae* infection in the mice led to immunosuppression and, thus, the lowering of bactericidal activity (Figs. 1 and 3). The onset of the diminished bactericidal activity occurred earlier in the TRI as compared to the NI group.

SUMMARY

Phagocytic and bactericidal activities were studied in *Mycobacterium leprae*-infected normal (NI) and thymectomized/irradiated

(TRI) mice at different time periods. No significant differences were seen in the phagocytic activity for *Staphylococcus aureus* at 3, 6, and 9 months in the normal infected (NI) and normal control (NC) mice. A slight but significant decrease in the phagocytosis of *S. aureus* was seen in the TRI as compared to the NI group at 3 months which recovered at 6 months. Phagocytosis of sheep erythrocytes was depressed in the TRI as compared to the NI group at 6 months only. No significant differences in the phagocytosis of latex particles were seen in any of the groups at any time of infection.

Bactericidal activity was significantly reduced in the NI group as compared to the NC group and, similarly, in the TRI group as compared to the TRC group at all periods of infection. The *M. leprae*-infected T/R 900 group (TRI) showed more decrease in bactericidal activity when compared to the normal infected (NI) group.

RESUMEN

Se estudiaron las actividades fagocítica y bactericida en ratones intáctos (NI) y en ratones timectomizados e irradiados (TRI) infectados con *Mycobacterium leprae* a diferentes intervalos de tiempo. No se encontraron diferencias significativas en la capacidad fagocítica con *Staphylococcus aureus* entre los grupos intácto-infectados (NI) y control normal (NC) a los 3, 6 y 9 meses. En comparación con el grupo NI, el grupo TRI mostró una ligera pero significante disminución en la fagocitosis de *S. aureus* a los 3 meses la cuál se recuperó a los 6 meses. En comparación con el grupo NI, la fagocitosis de eritrocitos de carnero en el grupo TRI estuvo deprimída sólo a los 6 meses. No se observaron diferencias significativas en la fagocitosis de partículas con látex a ningún tiempo y en ninguno de los grupos.

La actividad bactericida en los grupos NI (en comparación con NC) y TRI (en comparación con TRC) estuvo significativamente reducida a todos los tiempos de infección. El grupo T/R 900 (TRI) infectado con el *M. leprae* mostró mayor disminución en su actividad bactericida que el grupo normal infectado (NI).

RÉSUMÉ

Chez des souris normales (NI) et des souris thymectomisées et irradiées (TRI) inoculées avec *Mycobacterium leprae,* on a étudié les activités phagocytaires et bactéricides à différentes périodes Aucun différence significative n'a été observée dans l'activité phagocytaire à l'égard de *Staphylococcus aureus,* après 3, 6, et 9 mois, chez les souris normales infectées (NI) et chez les souris témoins (NC). Une diminution significative, encore que légère, dans la phagocytose de *S. aureus* a été relevée chez des souris thymectomisées et irradiées lorsqu'on les comparait au groupe de souris normales, 3 mois après l'inoculation; cette différence n'était plus notée après 6 mois. La phagocytose des érythrocytes de mouton était diminuée chez les souris TRI, par comparaison au groupe NI; ceci cependant n'était noté qu'après 6 mois. Aucune différence significative dans la phagocytose de particules de latex n'a été constatée dans les différents groupes, à n'importe quel moment après l'infection.

L'activité bactéricide était significativement réduite dans le groupe NI, comparé au groupe NC. De même, elle était diminuée dans le groupe TRI comparé au groupe TRC, et ceci à toutes les périodes après inoculation. Le groupe T/R 900 (TRI) infecté par *M. leprae* a présenté une diminution plus prononcée de l'activité bactéricide, par rapport au groupe de souris normales infectées (NI).

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