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Defective Blood Mononuclear Phagocyte Function in Patients with Leprosy[1]

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The mononuclear phagocyte system normally possesses microbiostatic and microbiocidal functions ([6]). In leprosy, these activities are of great importance since *Mycobacterium leprae* are localized mainly within mature macrophages. It is important to elucidate in leprosy patients the nature of the intrinsic and/or extrinsic deficiency that allows the mycobacterium to survive, grow, and multiply ([10]). Data reported by different investigators concerning the ability of macrophages from leprosy patients to lyse *Mycobacterium leprae* and other microorganisms *in vitro* are controversial ([1,3,7,11]). At present, it is known that the mononuclear phagocyte system not only participates in nonspecific reactions but also plays an important role in the specific immune response ([19]). Specificity is obtained through the interaction between macrophage and lymphocyte, which can be divided into 2 important stages: one is the induction of response to antigens ([24]), and the other is the expression of cell mediated immune reactions ([22]). Hence, a defect in either cell population can account for the failure of the final expression of an immune response. Therefore, a macrophage defect could be involved in the expression of the disease and also in the impairment of cell mediated immune reactions demonstrated in patients with leprosy, mainly those with the polar lepromatous form ([5,14,18,20]).

The purpose of this study was to determine whether blood monocytes from patients with either tuberculoid or lepromatous leprosy were defective in their ability to deal with intracellular organisms. The results indicate that there is a defect in macrophage function for the killing of *C. pseudotropicalis* in leprosy patients, more evident in the polar lepromatous form (LLp) than in the polar tuberculoid form (TT).

MATERIALS AND METHODS

Patients and controls. Monocytes were obtained from 11 normal subjects and 17 patients with leprosy, including 9 with the tuberculoid and 8 with the polar lepromatous form. Their ages ranged from 25 to 70 years with an equal sex distribution. Patients were classified according to the Ridley-Jopling classification ([21]). They were under treatment with dapsone.

Candida pseudotropicalis. The strain used was No. 566 from the Laboratory of Parasitology, Medical College of Paris, France. Candida were cultured for 8–24 hours at 37°C on Sabouraud's glucose agar slants. The yeast cells were collected, washed, and resuspended in Hank's Balanced Salt Solution (HBSS) and adjusted to an appropriate concentration. The reasons for the selection of this particular yeast were the following: a) it can be lysed by myeloperoxidase-independent mechanisms ([17]); b) it provides a simple method for the evaluation of the macrophage killing activity due to the inability of dead candida to stain with Giemsa ([12]); and c) the impossibility of working with *Mycobacterium leprae* due to the difficulties in culturing it *in vitro* and the lack of easy methods to evaluate the viability of the bacilli.

Isolation of peripheral blood monocytes. Mononuclear cells were isolated by Ficoll-Hypaque density gradients ([4]). Cells at the interphase were washed twice with HBSS and finally resuspended in RPMI 1640 medium (Grand Island Biological Co., Grand Island, New York, U.S.A.) and adjusted to a concentration of 5–10 × 10^6/ml.

Monolayer of adherent cells. The procedures used to study blood monocytes have

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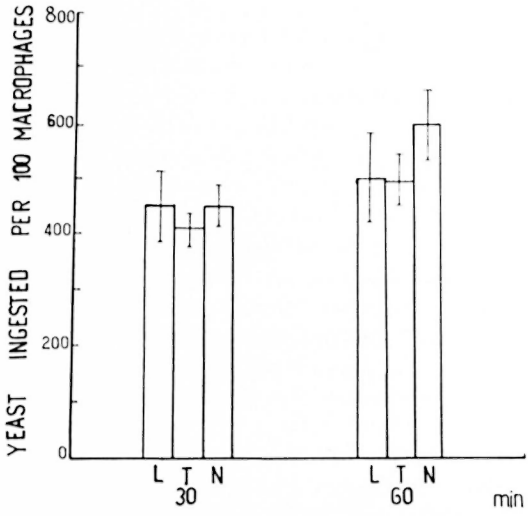

FIG. 1. Phagocytosis of *C. pseudotropicalis* by blood monocytes from patients with leprosy and normal subjects. L: Polar lepromatous form (8); T: Polar tuberculoid form (9); and N: Normal subjects (11). Bars represent mean values ± standard errors.

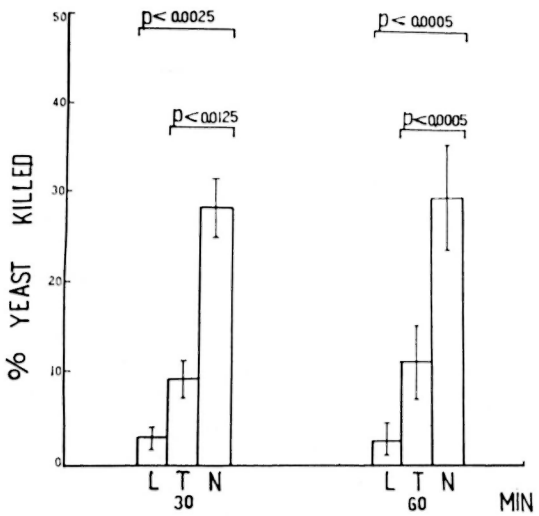

FIG. 2. Killing of *C. pseudotropicalis* by blood monocytes from patients with leprosy and normal subjects. L: Polar lepromatous form (8); T: Polar tuberculoid form (9); and N: Normal subjects (11). Bars represent mean values ± standard errors. Significance was determined by the Student's t test.

been described previously (9). In brief, adherent cell monolayers were prepared in Leighton tubes with coverslips by adding first 0.2 ml of heat-inactivated human AB serum and subsequently 3×10^6 blood mononuclear cells in 0.8 ml RPMI 1640 with gentamycin. The contents of the tubes were mixed gently and cultured 18–24 hours at 37°C. After incubation, the remaining nonadherent cells were washed off with warm Medium 199, and *C. pseudotropicalis* were added in a proportion of 3×10^6 candida/ml in Medium 199 with 10% of fresh AB serum in each Leighton tube and incubated for 30 and 60 min at 37°C. It was calculated that 3×10^6 candida per tube were enough to cover all the area of the coverslip.

Evaluation of phagocytosis and killing. Phagocytosis and killing of *C. pseudotropicalis* were ascertained by a modification of a leukocyte killing assay based on Giemsa staining described by Lehrer (16). To determine the peak or maximum intracellular activity by blood monocytes, phagocytosis and killing were allowed to proceed for 30 and 60 min. These time intervals were optimum for the study since after 90 min we found that some adhered monocytes showed signs of deterioration. At the end of the incubation period, the yeast cells that remained extracellular or in suspension were removed by washing the coverslip 3 times with warm Medium 199. The coverslip monolayers were examined morphologically with Giemsa staining. Duplicate monolayers were observed to evaluate phagocytosis and killing of candida by the adherent cells. Phagocytosis was expressed as the mean number of candida ingested by 100 macrophages or monocytes. Killing was expressed as the percentage of the candida which were unstained (dead) within the mononuclear phagocytes.

RESULTS

Phagocytosis by blood monocytes. The study of phagocytosis of *C. pseudotropicalis* by peripheral blood monocytes from patients with leprosy and normal controls is illustrated in Fig. 1.

In LLp patients, after 30 min of incubation with the yeast cells, the number of ingested candida per 100 macrophages was 452 ± 63 (mean ± SEM). At 60 min, phagocytosis was 505 ± 81. In TT patients at 30 min, the number of ingested candida was 409 ± 32 and at 60 min, 500 ± 44. These values, after 30 and 60 min of incubation with candida, were similar to those found

in normal individuals, 454 ± 35 and 643 ± 76, respectively.

When the phagocytic activity was compared in LLp and TT patients, there was no statistically significant difference among them or between them and normal subjects. Thus, the phagocytic activity for *C. pseudotropicalis* in patients with leprosy was not altered.

Intracellular killing of *C. pseudotropicalis*. To evaluate killing, the number of "ghost" candida organisms (unstained yeast cells) per 100 ingested candida was determined in patients with leprosy and in normal controls after 30 and 60 min incubation (Fig. 2).

In LLp patients, the percentage of killed candida at 30 min was 4.0 ± 1.3 and at 60 min, 2.5 ± 1.0 (mean ± SEM). The values in TT patients were 9.4 ± 3.2 and 11.2 ± 3.7 at 30 and 60 min, respectively. In normal subjects, the values obtained at 30 and 60 min were 25.3 ± 4.6 and 29.8 ± 7.8, respectively.

The killing activity at 60 min was different between the 2 polar forms of leprosy; it was decreased in LLp as compared to TT patients ($p < 0.05$). When compared with normal controls, the killing function in normal monocytes was significantly greater than that of monocytes from leprosy patients of either the LLp or the TT types (Fig. 2). There was a statistically significant difference between LLp and normal subjects at 30 and 60 min of incubation. When compared with cells from normal controls, those from TT patients also showed a decrease in their killing capacity.

DISCUSSION

The results show significant differences between patients with leprosy and normal individuals in the fungicidal activity of peripheral blood monocytes, with no important differences in their phagocytic function.

Although blood macrophages from the 2 polar forms of leprosy have lower candidacidal activity than monocytes from normal controls, the failure to kill *C. pseudotropicalis* in the polar lepromatous form is more severe than in the polar tuberculoid form.

In patients with LLp, abnormalities in T-lymphocyte mediated immunity have been repeatedly encountered, one related to a specific anergy for *M. leprae* [14,20] and the other to a less severe nonspecific depression of T-cell mediated immunity [5,13,18].

It has been postulated that the inability of macrophages to digest *M. leprae in vitro* may explain the impairment of cell mediated immunity in patients with leprosy since the resistance to intracellular pathogens is dependent upon interactions between lymphocytes and macrophages [8].

Although Beiguelman [3] and Barbieri and Correa [1] reported that macrophages from patients with LLp were unable to digest *M. leprae*, other investigators have failed to confirm this observation [8,11]. Studies concerning the phagocytic and killing capacity of monocytes from patients with leprosy with regard to other microorganisms are meager. Drutz, *et al.* [7,8] have shown that monocytes from patients with leprosy possess a normal microbiocidal function against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris*; they also studied the candidacidal capacity against *C. albicans* of polymorphonuclear leukocytes from normal subjects and patients with various forms of leprosy and could not find any difference among them.

There is evidence that multiple microbiocidal mechanisms operate in monocytes and neutrophils [17]; hence in order to evaluate the monocyte lytic system, it is important to consider not only the type of cells studied but also the bacteria or fungus employed. *C. pseudotropicalis* can be lysed by mature monocytes that lack peroxidase activity [12]. Moreover, monocytes from patients with myeloperoxidase deficiency are impaired in their ability to kill *C. albicans* while *C. pseudotropicalis* are killed more effectively by myeloperoxidase-deficient monocytes [17], indicating the presence of different lytic mechanisms in monocytes, depending on the strain and species of organism used in the test system. In this paper, *C. pseudotropicalis* was selected because it can be lysed by mechanisms different from *C. albicans*.

All patients studied were under sulfone treatment. Although there is no direct evidence that dapsone affects candida-digestion abilities of macrophages, there are reports that sulfonamides affect candida-

cidal activity of neutrophils ([15]), and there is indirect evidence that dapsone inhibits lysosomal enzymes ([2]). Dapsone has been recently reported to inhibit polymorphonuclear leukocyte cytotoxicity ([23]). The effects of sulfonamides and dapsone were on myeloperoxidase-dependent mechanisms ([2,15,23]), and they did not occur when cell suspensions were washed free of drug. Since in our study prior to culture the mononuclear cells were extensively washed and were incubated with normal human AB serum, we suggest that the finding of a defect in blood monocytes in patients with leprosy is not due to the effect of dapsone.

It can be concluded that peripheral blood monocytes from patients with leprosy possess an impaired enzymatic candidacidal activity, more pronounced in the polar lepromatous form than in the polar tuberculoid form. Whether the deficiency is due to the presence of leprosy bacilli, to an immune deficiency existing prior to infection, and/or to a genetic predisposition remain as hypotheses for future investigation.

SUMMARY

Patients with lepromatous leprosy possess a defective lymphocyte function *in vivo* and *in vitro* that is less evident in the tuberculoid form. Data concerning their macrophage ability to digest *Mycobacterium leprae* are controversial.

The purpose of this study was to determine whether monocytes from patients with either tuberculoid or lepromatous leprosy were altered in their enzyme systems, that is myeloperoxidase-dependent and myeloperoxidase-independent systems. The ability of adherent blood monocytes to ingest and kill *Candida pseudotropicalis* after 30 and 60 min of incubation with yeast cells was tested. Mononuclear phagocytic cells from patients with either principal form of leprosy functioned similarly to normal monocytes in phagocytosis while their fungicidal activity for *C. pseudotropicalis* was statistically significantly altered and was more evident in the lepromatous than in the tuberculoid type. The results indicate that peripheral blood monocytes from patients with leprosy possess an impaired enzymatic candidacidal activity.

RESUMEN

Los pacientes con lepra lepromatosa presentan una defectuosa función linfocítica tanto *in vivo* como *in vitro*. Este defecto es menos aparente en los pacientes tuberculoides. Los datos relativos a la capacidad de sus macrófagos para digerir al *M. leprae* son controversiales.

El propósito de este estudio fue el de determinar si los monocitos de los pacientes con lepra tuberculoide o lepromatosa estaban alterados en sus sistemas enzimáticos dependientes e independientes de mieloperoxidasa. Se estudió la habilidad de los monocitos adherentes de la sangre para ingerir y matar a *Candida pseudotropicalis* después de 30 y 60 min de incubación con la levadura. Los fagocitos de los pacientes con cualquiera de las formas principales de la lepra, endocitaron de manera similar a los monocitos normales. Sin embargo, la actividad fungicida estuvo alterada de manera significativa, más en el tipo lepromatoso que en el tuberculoide. Los resultados indican que los monocitos de sangre periférica de los pacientes con lepra presentan una alteración en su actividad enzimática candidacida.

RÉSUMÉ

Les malades souffrant de lèpre lépromateuse possèdent un défaut de la fonction lymphocytaire *in vivo* et *in vitro* qui est moins marquée que dans la forme tuberculoïde. Les données concernant la capacité des macrophages à digérer *Mycobacterium leprae* sont controversées.

Le but de cette étude a été de déterminer si les monocytes provenant de malades atteints de la forme lépromateuse de la lèpre ou de la forme tuberculoïde, étaient altérés en ce qui concerne leur système enzymatique, selon que celui-ci dépende ou ne dépende pas de la myélooxidase. On a étudié la capacité des monocytes adhérents du sang à ingérer et à tuer *Candida pseudotropicalis* après 30 et 60 minutes d'incubation avec des levures. Les cellules phagocytaires provenant de malades atteintes de l'une ou l'autre des formes principales de la lèpre, ont montré des activités similaires aux monocytes normaux. Par contre, l'activité fongicide de C. *pseudotropicalis* était altérée et cette altération était statistiquement significative. Cette différence était plus évidente en ce qui concerne les malades lépromateux que les tuberculoïdes. Les résultats indiquent que les monocytes du sang périphérique provenant des malades atteints de lèpre, présentent une altération des activités enzymatiques candidacides.

REFERENCES

1. Barbieri, T. A. and Correa, W. M. Human macrophage culture: The leprosy prognostic test (LPT). Int. J. Lepr. **35** (1967) 377–381.
2. Barranco, V. P. and Okla, T. Inhibition of ly-

sosomal enzymes by dapsone. Arch. Dermatol. **110** (1974) 563–566.
3. Beiguelman, B. Leprosy and genetics. A review of past research with remarks concerning future investigations. Bull. WHO **37** (1967) 461–476.
4. Boyum, A. Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. **21** Suppl **97** (1968) 7.
5. Bullock, W. E. and Fasal, P. Studies of immune mechanisms in leprosy. J. Immunol. **106** (1971) 888–899.
6. Cline, M. J., Lehrer, R. I., Territo, M. C. and Golde, D. W. Monocytes and macrophages; Functions and diseases. Ann. Int. Med. **88** (1978) 78–88.
7. Drutz, D. J. and Cline, M. J. Polymorphonuclear leukocyte and macrophage function in leprosy. Int. J. Lepr. **38** (1970) 352–353.
8. Drutz, D. J., Cline, M. J. and Levy, L. Leukocyte antimicrobial function in patients with leprosy. J. Clin. Invest. **53** (1974) 380–386.
9. Estevez, M. E. and Sen, L. Capacidad funcional de los monocitos humanos normales: Una simple técnica para su exploración. Sangre **23** (1978) 870–875.
10. Godal, T. Immunological aspects of leprosy. Present status. Prog. Allergy **25** (1978) 211–242.
11. Godal, T. and Rees, R. J. W. Fate of *Mycobacterium leprae* in macrophages of patients with lepromatous or tuberculoid leprosy. Int. J. Lepr. **38** (1970) 439–441.
12. Golde, D. W., Territo, M., Finley, T. N. and Cline, M. J. Defective lung macrophages in pulmonary alveolar proteinosis. Ann. Int. Med. **85** (1976) 304–309.
13. Han, S. H., Weiser, R. S. and Kan, S. T. Prolonged survival of skin allografts in leprosy patients. Int. J. Lepr. **39** (1971) 1–6.
14. Han, S. H., Weiser, R. S., Wang, J. J., Tsai, L. C. and Lin, P. P. The behavior of leprous lymphocytes and macrophages in the macrophage migration-inhibition test. Int. J. Lepr. **42** (1974) 186–192.
15. Lehrer, R. I. Inhibition by sulfonamides of the candidacidal activity of human neutrophils. J. Clin. Invest. **50** (1971) 2498–2505.
16. Lehrer, R. I. Measurement of candidacidal activity of specific leukocyte types in mixed cell populations. I. Normal, myeloperoxidase-deficient and chronic granulomatous disease neutrophils. Infect. Immun. **2** (1970) 42–47.
17. Lehrer, R. I. The fungicidal mechanisms of human monocytes. I. Evidence for myeloperoxidase-linked and myeloperoxidase-independent candidacidal mechanisms. J. Clin. Invest. **55** (1975) 338–346.
18. Lim, S. D., Kiszkiss, D. F., Jacobson, R., Choi, Y. S. and Good, R. A. Thymus dependent lymphocytes of peripheral blood in leprosy patients. Infect. Immun. **9** (1974) 394–399.
19. Mackaness, G. B. Delayed hypersensitivity and the mechanism of cellular resistance to infection. Prog. Immunol. **1** (1971) 413–424.
20. Myvang, B., Godal, T., Ridley, D. S., Frö-land, S. S. and Song, Y. K. Immune responsiveness to *Mycobacterium leprae* and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy. Clin. Exp. Immunol. **14** (1973) 541–553.
21. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity: a five-group system. Int. J. Lepr. **34** (1966) 255–273.
22. Simon, H. B. and Sheagren, J. N. Enhancement of macrophage bactericidal capacity by antigenically stimulated immune lymphocytes. Cell. Immunol. **4** (1972) 163–174.
23. Stendahl, O., Molin, L. and Dahlgren, C. The inhibition of polymorphonuclear leukocyte cytotoxicity by dapsone. J. Clin. Invest. **62** (1978) 214–220.
24. Unanue, E. R. and Cerottini, J. C. The immunogenicity of antigen bound to the plasma membrane of macrophages. J. Exp. Med. **131** (1970) 711–725.