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Phagocytosis in Leprosy. 5. The Effect of the Infection with *Mycobacterium lepraemurium* on the Level of Diverse Hydrolytic Lysosomal Enzymes of Murine Peritoneal Macrophages[1]

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The study of phagocytic function in leprosy is not an easy task because, among other reasons, this function is closely linked to the cell (T lymphocyte) mediated immune (CMI) response. CMI is a complex immunological-regulatory mechanism that commonly becomes affected in patients with lepromatous leprosy (LL). Several research groups have studied the CMI responsiveness in patients with LL. The general consensus is that this form of immune response is indeed diminished ([5, 10, 13, 17, 26]) although it is not yet clear if the defect exists prior to or as a consequence of the leprosy infection, nor if it is solely due to the lack of *Mycobacterium leprae*-reactive T lymphocytes ([16]) or to the triggering of antigen-sensitive suppressor cells ([4, 42]). Aside from this, a defect in the function of phagocytes from LL patients has been claimed by some researchers ([3, 31]); others, however, do not share this opinion ([11, 39]).

Being aware of the primordial role of phagocytes in any infectious disease and therefore in leprosy, some years ago we started to look for an explanation to the apparent paradox of a microorganism that not only is not destroyed by the phagocytic cells but that uses them to secure its existence. Our theory considered that a) infection with *M. leprae* is not a rare event in leprosy endemic areas; b) that the bacillus gets established if the overall conditions within the prospective host are propitious for it (at this point, "the level of cell-mediated immunity" would have a decisive role in the evolution of the disease); and c) that the bacillus proliferates and perpetuates itself if, in some way, it interferes with host-cell metabolism, blocking the cell's bactericidal mechanisms, or at least one that is critical to arrest mycobacterial growth. With these considerations in mind, the study of the phagocytes in LL patients was started.

As some others had done before ([2, 3, 11, 18, 25, 31, 39]), we studied peripheral blood phagocytes which are mainly polymorphonuclear (PMN) leukocytes and found that these cells are not defective in their levels of activity of the hydrolytic lysosomal enzymes: acid and alkaline phosphatases, β-glucuronidase, β-galactosidase, a nonspecific lipase ([15]), ribonuclease, deoxyribonuclease and myeloperoxidase ([37]). Neither are they defective in their ability to reduce nitroblue tetrazolium (NBT) dye or in their capacity to produce normal levels of the anion, superoxide ([36]). In most cases, these physiological parameters were slightly increased in LL patients as compared to healthy controls, suggesting that the peripheral phagocytic cells from the patient group were not intrinsically defective despite the fact that these patients had active LL. Our failure and the failure of some others to detect a defect in these parameters can be explained if we consider that no more than 0.3% of the total circulating leukocytes in the patients are actually parasitized by *M. leprae*, even in patients who are highly bacilliferous ([12]). This "degree" of parasitation is rather low to allow the detection of defects (if there are any) in the cell that has been parasitized by *M. leprae*.

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If such a defect exists, it would be undetectable in the presence of normal non-parasitized cells due to a dilution effect. This pointed out the necessity of studying the function of the cells actually parasitized, especially the macrophage population, since these cells (and not PMN) are the important ones for the control of intracellular microorganisms.

Due to the fact that biochemical study of such cells in humans cannot be easily performed (for technical and ethical reasons) and considering the several similarities between human and murine leprosy, we undertook the study of phagocytes in the murine leprosy model, hoping that the findings in this model may help increase our understanding of the role of phagocytic cells in human leprosy. We are aware that human and murine leprosy are not exact counterparts. They are not the same disease, however, they share several characteristics. Their etiological agents (*M. leprae* and *M. lepraemurium*) belong to the same taxonomical group. They are indistinguishable from each other in regard to their tinctorial and morphologic characteristics. Both have intracellular habits and parasitize phagocytic cells. They share several antigenic determinants ([14, 38]). They are not prone to grow on conventional bacteriological media (although some relatively recent reports indicate that *M. lepraemurium*, or a closely related microorganism, has been grown in artificial media, outside its natural environment) ([29]). In both human and murine leprosy, the disease progresses very slowly, and the formation of granulomas (leprous granulomas) is the characteristic tissue alteration that affects skin and internal organs. Subcutaneous and organ-associated lepromas are also common in human and murine leprosy. These similarities justify the study of some aspects of human leprosy in the murine leprosy model. This may be particularly appropriate when the aspect under study is that of the role of phagocytic cells, since phagocytosis is a biological phenomenon which developed very early in evolution and has been retained as a selective advantage.

In this paper, we describe the effect of infection with *M. lepraemurium* on some components of the enzymatic arsenal of cells present in the mouse peritoneal exudate. Most of the cells in the peritoneal exudate are macrophages (adherent, phagocytic and esterase-positive cells) that, in infected animals, become massively parasitized by murine leprosy bacilli, whether the inoculation is intravenous or intraperitoneal. We also give some results on the activity of several hydrolytic enzymes present in purified suspensions of *M. lepraemurium*.

MATERIALS AND METHODS

Animals. Albino CFW mice of either sex and two months old at the time of the infection were used. Animals were maintained under standard conditions until completion of the experiments.

Infection. One hundred animals were intraperitoneally (i.p.) inoculated with 15.5×10^7 *M. lepraemurium*, and a similar number of animals from the same lot and sex-matched were maintained free of infection to be used as controls. *M. lepraemurium* was the Hawaiian strain obtained ten years ago from Dr. Y. T. Chang (Laboratory of Biochemical Pharmacology, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, U.S.A.) and maintained since then in our laboratory by serial infections of mice. The microorganism is periodically inoculated on mycobacterial culture media (Löwenstein-Jensen, PBY and Dorset's media) and has not shown evidence of growth on such media while fully retaining its infectiousness for mice. Suspensions of *M. lepraemurium* were prepared from infected tissue according to Prabhakaran, *et al.* ([33]).

Cell preparations. Four months and six months after infection, 35 to 40 mice (infected and controls) were sacrificed by exsanguination from the retroorbital venous sinus. Some animals died during the course of the infection from undetermined causes. The animals were prepared for abdominal manipulation according to Chang, *et al.* ([6]). Under aseptic conditions, each animal was injected i.p. with 5 ml of ice-cold saline (0.85% w/v NaCl)-citrate (0.4% w/v sodium citrate) solution (SCS) for the collection of peritoneal exudate cells (PEC). The abdominal washing was done with two 5 ml washes per mouse. The individual washes from 35 to 40 infected or control animals were pooled; centrifuged ($250 \times g \times 3$ min) in the cold to pellet the cells;

the cells washed once with SCS before lysing contaminant erythrocytes with hypotonic saline solution; washed two more times with cold SCS; and finally suspended in about 8.0 ml of SCS. Hypotonic lysis of erythrocytes was achieved by resuspending the cell sediment in 3.0 ml of 0.2% w/v NaCl for 30 to 60 sec and immediately restoring isotonicity by adding 3.0 ml of 1.6% w/v NaCl. The final cell suspensions were counted in a Neubauer-type hemocytometer, and the cell concentrations adjusted to 20 to 22 $\times$ 10^6 PEC per ml before dividing them into 2 ml aliquots. These were kept frozen at −70°C until used (within three months after collection).

Enzymatic determinations. Frozen peritoneal cell (PEC) suspensions were thawed, briefly sonicated at a low intensity just to disperse clumps, and used as the source of the following enzymes: acid and alkaline phosphatases, acid (cathepsin D type) proteinase, β-glucuronidase, lipase and deoxyribonuclease. For lysozyme, the sonicates were centrifuged (1900 $\times$ g $\times$ 15 min, 4°C) and the enzymatic activity studied in the cell-free supernatant. The amount of protein in the cell sonicates was determined according to Lowry, *et al.* ([19]), using bovine serum albumin as a standard.

Preliminary trials with PEC from normal mice led us to establish the following assay conditions:

Acid phosphatase [Seligman and Chauncey ([40])]. To 0.1 ml of PEC suspension (equivalent to 92.6 μg protein or 2.2 $\times$ 10^6 normal PEC), 0.1 ml of 0.16 M $MnCl_2$ and 2.8 ml of 0.1 M acetate buffer, pH 5.1, were added. The reaction was started by adding 0.1 ml of substrate (β-naphthyl phosphate, 1.5 mg per ml in water) and the mixture incubated at 37°C for 30 min in a water bath. At the end of the incubation period, 1.0 ml was removed to check pH and 2.5 ml of chilled 0.05 M Veronal buffer, pH 8.5, was added to the remaining 2.1 ml. To this, 0.5 ml of tetrazotized o-dianisidine (4 mg per ml in water) was added and allowed to react for 3 min. The diazocoupling reaction was stopped by adding 0.5 ml of 40% trichloroacetic acid (TCA); the color extracted with 6.0 ml of ethyl acetate; and the absorbance of the upper colored phase read at 540 nm against a blank without enzyme.

Alkaline phosphatase [Manning, *et al.* ([22])]. To 0.3 ml of PEC suspension (equivalent to 247 μg protein or 6.6 $\times$ 10^6 normal PEC) contained in 13 $\times$ 100 mm test tubes and diluted to 1.3 ml with physiological saline solution (PSS), 1.0 ml of the substrate mixture was added. The substrate mixture consisted of 1.968 g Tris, 0.030 g phenolphtalein diphosphate, 0.1 g $MgSO_4$, 0.7 g gelatin, 1 N HCl to bring the pH to 9.6, and water to 100 ml. The enzyme-substrate mixture was incubated for 60 min at 37°C in a water bath, and then 4.0 ml of glycine buffer was added to stop the reaction and to develop color. Glycine buffer consisted of 9.19 g glycine, 7.19 g NaCl, 40.0 g sodium pyrophosphate, 30% NaOH to adjust the pH to 11.2, and water to 1000 ml. The test tubes were then spun at 250 $\times$ g for 10 min, and the absorbance of the supernatant was read at 550 nm against a blank without enzyme.

Deoxyribonuclease [Matsokis and Georgatsos ([23])]. To conical 15 ml test tubes containing 0.15 ml of PEC suspension (equivalent to 124 μg protein or 3.3 $\times$ 10^6 normal PEC), 0.1 ml of 0.01 M $MgCl_2$ in water, 1.25 ml of 0.0066 M ammonium acetate buffer, pH 5.0, and 0.1 ml of heat-denatured DNA substrate (5 mg DNA per ml acetate buffer) were added. The reaction was allowed to proceed at 37°C for 60 min and then it was stopped by adding 1.5 ml of 10% TCA. The mixtures were centrifuged at 1900 $\times$ g for 15 min and the absorbance of the supernatants was measured at 260 nm against a blank without enzyme.

Acid (cathepsin D type) proteinase [Dannenberg, *et al.* ([9])]. To 0.3 ml of PEC suspension (equivalent to 247 μg protein or 6.6 $\times$ 10^6 normal PEC) diluted to 1.3 ml with 0.1 M citrate buffer, pH 3.8, 1.3 ml of urea-denatured hemoglobin was added. Immediately after mixing, 1.0 ml of the reaction mixture was removed and admixed with 1.0 ml of 5% TCA to stop the reaction. The remaining 1.6 ml of the reaction mixture was incubated at 37°C for 60 min and then 1.0 ml was removed and transferred to a fresh tube containing 1.0 ml of 5% TCA. The tubes containing the TCA precipitates were reincubated at 37°C for 10 min; centrifuged at 1900 $\times$ g for 15 min; and 1.0 ml of each supernatant was diluted 2-fold with water

before reading their absorbance at 280 nm against a blank of water. The absorbance readings were corrected by subtracting 0 hr absorbance readings from the 1.0 hr readings, and the results compared to that obtained with blanks without enzyme. The urea-denatured hemoglobin substrate was prepared by thoroughly dissolving 0.44 g of bovine hemoglobin (Type III, Sigma Chemical Company, St. Louis, Missouri, U.S.A.) in 14.4 ml water and adding 7.2 g urea, 1.6 ml 1 M NaOH, and 2.0 ml water, in that order. After mixing for 60 min (magnetic stirrer), 0.8 g of additional urea was added and, finally, the pH in the mixture was adjusted to 3.8 by adding 2.56 ml 1 M citric acid and 0.04 ml 1 M sodium citrate.

Lipase [Nachlas and Seligman ([28])]. To 15 × 125 mm test tubes containing 0.3 ml PEC suspension (equivalent to 247 μg protein or 6.6×10^6 normal PEC) and 0.2 ml of PSS, 3.0 ml of freshly prepared substrate was added. The substrate was prepared by dissolving 10 mg of β-naphthyl laurate in 10 ml acetone, adding this solution (submerging the tip of the pipette) to 50 ml of 0.06 M Veronal buffer, pH 7.4, and finally bringing the volume to 100 ml with water. The reaction mixture was incubated at 37°C for 60 min and then 0.5 ml of the coupling NDBB (tetrazotized orthodianisidine) reagent was added to develop color. After shaking, the tubes were left undisturbed for 3 min and the coupling reaction was stopped by adding 0.5 ml of 40% TCA. The coupling NDBB solution was prepared by dissolving 4 mg of NDBB per ml of water. To the mixtures treated with TCA, 5.0 ml of ethyl acetate were added with energetic shaking to extract the color, the absorbance of which was determined at 540 nm after a brief centrifugation ($1900 \times g \times 5$ min) to clear the supernatant, against a blank without enzyme.

Lipase in the presence of sodium taurocholate [Cohn and Wiener ([7])]. The assay was as described above except that 0.1 ml of 2.8 M sodium taurocholate in 0.06 M Veronal buffer, pH 7.4, was added before the substrate. According to Cohn and Wiener, this amount of sodium taurocholate (0.08 M final concentration) increases the lipase activity from 10% to 15% in whole homogenates and, at the same time it inhibits the esterase activity up to 85%.

Lysozyme [Shugar ([41])]. This assay was performed in ½ inch test tubes (Bausch and Lomb Spectronic 20) containing 2.5 ml of substrate prepared by suspending 5.0 mg of *Micrococcus lysodeikticus* in 20 ml of 0.066 M phosphate buffer, pH 6.24. This suspension gives an absorbance of 0.7 at 540 nm in the Bausch and Lomb spectrophotometer (zero-time reading). The suspension is preincubated at 25°C for 5 min and then 45 μg of protein (equivalent to 2.2×10^6 normal PEC) is added to start the reaction. This is allowed to proceed for 30 min at 25°C and then the absorbance at 540 nm of the suspension is measured (30 min reading). Blanks without enzyme are always included to correct results.

β-Glucuronidase [Yarborough, *et al.* ([43])]. To 15 × 100 nm test tubes containing 0.3 ml of PEC suspension (equivalent to 247 μg protein or 6.6×10^6 normal PEC), 0.9 ml of PSS and 1.5 ml of 0.1 M sodium acetate buffer, pH 4.5; 0.3 ml of substrate (0.01 M phenolphthalein glucuronic acid, pH 7.0) was added. The reaction mixture was incubated for 120 min at 38°C to allow the reaction to proceed and, at the end, 2.0 ml was removed and mixed with 5% TCA to stop the reaction. The remaining 1.0 ml was used to check pH. The TCA-treated mixtures were centrifuged ($1900 \times g \times 15$ min) and 1.0 ml of the supernatant was mixed with 2.0 ml of alkaline reagent (see acid phosphatase assay), and the color developed was read at 540 nm against a blank without enzyme.

Enzyme activities. For the enzymes acid phosphatase, alkaline phosphatase, deoxyribonuclease, acid proteinase, lipase and β-glucuronidase, one unit of enzyme is defined as the amount of enzyme required to produce a change of 1.0 in the absorbance, under the particular assay conditions. For lysozyme, one unit is the amount of enzyme needed to observe an absorbance change of 0.1 under the assay conditions. For all of the above enzymes, the specific activity is the number of units per mg protein.

Chemicals. Most chemicals were from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Some were from Calbio-

chem, LaJolla, California, U.S.A.; Worthington Biochemicals Corp., Freehold, New Jersey, U.S.A. and E. Merck AG, Darmstadt, Germany.

Statistics. Since the above enzyme determinations were done in pooled PEC suspensions from normal or *M. lepraemurium*-infected mice, no statistical treatment of data was possible. For each enzyme, however, quintuplicate or at least triplicate determinations were performed, with negligible variations among them, and the results are expressed as the mean values.

RESULTS

Enzyme activities. Enzyme activities in PEC suspensions from animals after four months of infection for acid phosphatase (AcPh), acid proteinase (AcPr), β-Glucuronidase (β-Glu), deoxyribonuclease (DNase), lipase (Lip), alkaline phosphatase (AlPh) and lysozyme (Lys) are shown in Table 1, both in the form of units per 1×10^6 PEC and in units per mg protein. In either case, it can be observed that, compared to the normal controls, most of the enzyme activities are increased in the group of mice infected with *M. lepraemurium* four months earlier. The differences between groups are more evident when the results are expressed in the form of units per 1×10^6 PEC. This is probably because the PEC from the infected animals had a higher amount of protein per cell, part of which corresponds to the increased levels of the various enzymes themselves. The average amount of protein in the normal and infected animals four months after infection was 926 μg and 1204 μg per 22×10^6 PEC, respectively.

TABLE 2. *Changes in the activities of hydrolytic enzymes in peritoneal cells from mice infected with* Mycobacterium lepraemurium *for four and six months.*[a]

Enzyme	L/N ratio[b] at	
	Four months	Six months
Acid phosphatase	3.16	0.80
Acid proteinase	2.00	1.80
Deoxyribonuclease	2.42	1.53
Lipase	2.93	0.74
β-Glucuronidase	3.74	1.97
Alkaline phosphatase	2.72	1.22
Lysozyme	2.31	0.85

[a] This table is derived from the data in Table 1.
[b] L/N ratio is defined as the ratio between the enzyme activity per 10^6 cells from leprosy-infected mice and that from 10^6 cells from normal mice.

The enzyme activities in mice with a six-month-old infection are also shown in Table 1 and compared to those of the control group. Different patterns are evident at the two stages of the infection. After six months of infection some of the enzyme activities are still increased but only slightly (AcPr, β-Glu, DNase); others are within normal values or are slightly decreased (AcPh, Lip, AlPh and Lys).

In Table 2, the changes in the ratios between leprosy and normal (L/N) enzyme activities at four months and at six months of infection are presented. The L/N ratios range from 2.0 (acid proteinase) to 3.74 (β-glucuronidase) at four months of infection. At six months of infection, these L/N

TABLE 1. *Hydrolytic enzyme activities in peritoneal exudate cells from mice infected with* Mycobacterium lepraemurium *for four and six months.*

Enzyme	Four months		Six months	
	Normal	Infected[a]	Normal	Infected[a]
Acid phosphatase	0.186[b] (4.70)[c]	0.587[b] (10.05)[c]	0.200[b] (3.31)[c]	0.160[b] (2.86)[c]
Alkaline phosphatase	0.002 (0.07)	0.007 (0.09)	0.004 (0.06)	0.004 (0.06)
Acid proteinase	0.030 (0.77)	0.060 (0.96)	0.020 (0.28)	0.036 (0.59)
Deoxyribonuclease	0.144 (3.56)	0.348 (5.64)	0.067 (0.90)	0.103 (1.59)
Lipase	0.098 (2.34)	0.287 (3.83)	0.132 (1.84)	0.098 (1.63)
β-Glucuronidase	0.031 (0.77)	0.116 (1.88)	0.037 (0.51)	0.074 (1.18)
Lysozyme	1.260 (62.39)	2.920 (61.95)	2.500 (56.75)	2.140 (60.37)

[a] Each animal received 15.5×10^7 bacilli, intraperitoneally.
[b] Units per one million cells (mean value from three to five determinations).
[c] Units per mg protein (mean value from three to five determinations).

ratios decreased considerably for most enzymes. Acid phosphatase, lipase, and lysozyme were the enzymes showing the most dramatic changes; others were moderately affected (deoxyribonuclease, β-glucuronidase, alkaline phosphatase). The L/N ratios for acid proteinase at four months and at six months of infection were essentially the same.

In the determinations of lipase activity in the presence of sodium taurocholate, the taurocholate interfered with the total extraction of color by ethyl acetate. The results presented here are those in the absence of such a reagent.

Cell composition of peritoneal exudates. Duplicate cell counts were made (1100 cells each) of Giemsa-stained smears prepared from pooled peritoneal cell suspensions from the animals after four months of infection. These cell counts showed that the peritoneal cells of normal and *M. lepraeinfected* mice were similar, mononuclears being the predominant cell type. In both groups, roughly 80% of the cells had the appearance of monocytes (50% to 60%) or mature macrophages (20% to 30%); less than 5% were probably lymphocytes and 6% to 15% were PMN leukocytes. We were unable to classify approximately 2% of the PEC. No other attempts (biochemical or functional) were made to precisely define cellular types, but this morphologic approach suggests that the differences in enzymatic activities between normal and infected animals are not due to differences in the types of cells in the peritoneal cavity. No differential counts were performed in the six-month infection experiment, but work in progress has revealed gradual changes in the peritoneal cell population that become clearly evident in the far advanced stages of the disease. These changes include a diminution in the total number of peritoneal cells and an almost complete disappearance of cells other than macrophages.

As for the degree of parasitation, one month after infection with *M. lepraemurium* about 20% of the mouse PEC population contained acid-fast bacilli (2% of the total cell population contained more than ten bacilli per cell). The number of infected peritoneal cells increased to about 50% at 3.5 months after inoculation (about 20% of the cells contained more than ten bacilli per cell). In spite of the progressive infection, the infected animals did not experience any higher mortalities than the uninfected mice for the first seven months. After this time, the mortality rate in the infected animals rose considerably.

TABLE 3. *Hydrolytic enzyme activities in* Mycobacterium lepraemurium *suspensions.*[a]

Enzyme	Per 10^8 bacilli[b]	Per mg protein[c]
Acid phosphatase	0.000[d] (378)[e]	0.000[d] (0.103)[f]
Alkaline phosphatase	0.010 (378)	0.000 (0.308)
β-Glucuronidase	0.000 (378)	0.000 (0.308)
Lipase	0.018 (252)	0.000 (0.308)
Deoxyribonuclease	0.000 (378)	0.468 (0.103)
Acid proteinase	0.000 (378)	0.000 (0.205)
Lysozyme	0.001 (378)	0.000 (0.103)

[a] Isolated from infected mouse mesenteric lepromas according to Prabhakaran, *et al.* ([33]).
[b] Bacillary suspension sonicated for 30 sec (see text).
[c] Bacillary suspension sonicated for 12 min and then centrifuged to eliminate cell debris.
[d] Average values from two determinations.
[e] Number of bacilli, in millions, used in the assay.
[f] Milligrams of protein used in the assay.

Enzyme activities in suspensions of isolated *M. lepraemurium*. Since the possibility existed that the bacilli infecting the PEC could affect the results by contributing to the activity levels of the enzymes analyzed, fresh suspensions of bacilli were prepared from mesenteric lepromas from heavily infected mice according to Prabhakaran, *et al.* ([33]), and tested for enzyme activity. Table 3 shows that some enzyme activity was detected in assays for DNase, Lip, and AlPh. For all the other hydrolytic enzymes, no activity was found. Although it is possible that these detectable enzyme activities are of bacillary origin, a more likely explanation is that they result from contaminant host tissue.

DISCUSSION

The present results indicate that mice with a four-month infection with *M. lepraemurium* have macrophages which are activated biochemically. Such cell activation could be non-immunological (due to the mere presence of digestible materials derived from the bacilli) or immunological (due to lymphokines from *M. lepraemurium*-reac-

tive T lymphocytes) in origin. Although the first mechanism of activation probably exists, the second mechanism is perhaps predominant. This is deduced from the tendency of most of the enzyme activities to decrease during the interval between four months and six months after infection. These diminutions could be explained by a depression in the level of cell-mediated immunity during this interval, since it has been demonstrated that this type of immune response becomes impaired in animals with murine leprosy ([1,32,34]). Because macrophage activation is one of the expressions of cell-mediated immune stimulation ([21]), both phenomena should exhibit parallel changes. If this is the case here, the levels of enzyme activity at four months after infection, although still elevated, could well correspond to levels in the process of diminution, diminution that becomes more apparent two months later. In support of this view, Lefford's group ([32]) has established that infection of mice with *M. lepraemurium* (10^8 bacilli per mouse in the hind foot pad) leads to the development of delayed type hypersensitivity (DTH) over the first month of infection; that DTH remains high up to month 2; and that it then drops to a low level and remains at a low level for several more months. The actual correlations among cell-mediated immunity, macrophage activation, and biochemical changes of macrophages remain to be demonstrated in the murine leprosy model although this has been clearly done in other animal models since the pioneering work of Lurie and his group in tuberculosis ([20]). From our results, it is clear that mice infected with *M. lepraemurium* possess macrophages with a high degree of biochemical activation, as judged by increased lysosomal enzyme activities. Despite this state of activation, the murine leprosy bacillus proliferates unrestrictedly within the cell. Whether or not this infected, activated cell is able to destroy other microorganisms is a matter currently under investigation in our laboratory.

How can a highly activated cell allow an apparently unlimited proliferation of a microorganism such as *M. lepraemurium*? What is the meaning, in terms of protection, of the elevated levels of lysosomal enzymes in macrophages of murine leprosy infected animals? Recently, Rea and Taylor ([35]) determined serum and tissue lysozyme levels in leprosy patients. Mean serum lysozyme values were elevated in untreated leprosy patients, especially in patients with severe reversal reaction or Lucio's phenomenon. They also found that, histologically, the enzyme was present in epithelioid and giant cells and in lepromatous histiocytes. Previously, Myrvik's group had evidence of a substance isolated from serum and granulomatous lung extracts with the characteristics of lysozyme and with bacteriostatic activity towards BCG, *M. tuberculosis* H37Ra and H37Rv and *M. phlei* ([30]). Large amounts of lysozyme in alveolar macrophages provided a possible explanation for the origin of serum lysozyme in rabbits undergoing a granulomatous response induced by BCG ([27]). To our knowledge, no other lysosomal enzyme activity has been particularly invested with mycobacteriostatic or mycobactericidal properties, although some evidence that suggests a protective role of peroxidase has been given by McKeever, *et al.* ([24]). By electron microscopy, they studied lepromatous tissue from armadillos bearing a 36-month infection with *M. leprae*. Cytochemically stained lepromas consisted of two subpopulations of macrophages in regard to their peroxidase content. There were relatively few cells containing peroxisomes and many cells containing bacilli, but no cells containing both peroxidase and bacilli. They also found macrophages containing both acid phosphatase activity and bacilli, and concluded that this enzyme is unlikely to be involved in the protection of host cells from leprosy bacilli. We are conducting *in vivo* and *in vitro* studies on several enzymes during the course of infection with *M. lepraemurium* to define the relationships of cell-mediated immunity, macrophage activation, and bactericidal function of macrophages in leprosy.

SUMMARY

The effect of the infection with *M. lepraemurium* on the activity of several lysosomal enzymes of mouse peritoneal cells was studied. The enzymes studied were acid- and alkaline-phosphatases, acid (cathepsin D-type) proteinase, β-glucuronidase, deoxyribonuclease, a nonspecific lipase, and lysozyme. Enzyme determina-

tions were carried out four months and six months after the infection with 15.5×10^7 bacilli per mouse. Clear differences between *M. lepraemurium*-infected and normal animals were observed at four months of infection, with all of the mentioned enzyme activities well above the normal values. At six months of infection, a tendency to decrease to normal values of the enzyme activities was observed. It is suggested that this biochemical activation of mouse peritoneal cells reflects the effect of the cell-mediated immune response triggered by the infection with the murine leprosy bacillus. *M. lepraemurium*-infected mice possess macrophages in a high state of biochemical activation; yet, they are unable to get rid of the infecting microorganism.

RESUMEN

Se estudió el efecto de la infección con *M. lepraemurium* sobre la actividad de varias enzimas hidrolíticas lisosomales de las células peritoneales del ratón. Las enzimas estudiadas fueron las fosfatasas ácida y alcalina, una proteinasa ácida (tipo catepsina D), β-glucuronidasa, desoxirribonucleasa, una lipasa no específica y lisozima. Las determinaciones enzimáticas se efectuaron a los cuatro y a los seis meses de la infección con 15.5×10^7 bacilos por ratón. A los 4 meses de infección se observaron marcadas diferencias entre los animales normales y los infectados. En estos últimos todas las actividades enzimáticas mencionadas estuvieron bien arriba de los valores normales. A los 6 meses de la infección, se observó una tendencia de las actividades enzimáticas a disminuir hasta valores normales. Se sugiere que esta activación bioquímica de las células peritoneales del ratón refleja el efecto de la respuesta inmune celular inducida por la infección con el *M. lepraemurium*. Los ratones con la infección leprosa poseen macrófagos con un elevado grado de activación bioquímica y sin embargo son incapaces de eliminar al microorganismo infectante.

RÉSUMÉ

Nous avons étudié l'effet de l'infection avec *M. lepraemurium* sur l'activité de plusieurs enzymes lysosomales des cellules péritonéales de souris. Les enzymes étudiés ont été: phosphatases acide et alkaline, protéinase acide (type cathépsine D), β-glucuronidase, deoxyribonuclease, une lipase non spécifique et lysozyme. Les activités des enzymes ont été déterminées au quatrième et sixième mois après l'infection par 15.5×10^7 bacilles par souris. De claires différences entre les animaux normaux ont été observées a quatre mois de l'infection avec toutes les activités des enzymes mentionnées bien au-dessus des valeurs normales. Au sixième mois de l'infection on à observé une tendance des activités des enzymes à retourner aux valeurs normales. On suggère que cette activation biochimique des cellules péritonéales de la souris reflete l'effet de la réponse immunologique cellulaire provoquée par l'infection avec *M. lepraemurium*. Les souris infectées de la lèpre possèdent des macrophages dans un êtat élevé d'actívatíon biochimique qui sont cependant incapables de détruíre les microorganismes infectants.

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