TABLE 2. *Recovery of* M. leprae *isolated from skin biopsies taken from patients with different types of disease.*

Disease type[a]	Biopsy pool: no. of specimens	Tissue homogenates ($\times 10^5$ AFB ml^{-1}) (a)	Two-phase separation ($\times 10^5$ AFB ml^{-1}): Lower layer (b)	Two-phase separation ($\times 10^5$ AFB ml^{-1}): Upper layer (c)	% Recovery $\left(\frac{c}{a} \times 100\%\right)$
LL old cases	5	8.9	0.0	0.0	—
LL new cases	8	281.0	0.0	131.1	47
LL with reactivation	4	131.4	3.1	87.6	67
LL with ENL	4	8.1	0.0	7.0	86
BL	7	71.0	0.0	31.4	44
TT	4	0.0	0.0	0.0	—
BT	3	0.0	0.0	0.0	—
Mouse foot pad	6	3.1	0.0	0.8	26

[a] LL = lepromatous leprosy; ENL = erythema nodosum leprosum; BL = borderline lepromatous; TT = tuberculoid leprosy; BT = borderline tuberculoid.

from armadillo liver tissues (supplied by IMMLEP) in that the former did not react with any of the tuberculosis patients' sera tested, while the latter reacted with 5%–9% of these sera ([2]).

—Sen-Chiew Gan, M.Sc., Dip. Ed., Grad.D.B.A., C.Biol., M.I.Biol., A.I.B.A., A.M.S.A.

Research Officer,
Tropical Diseases
Institute for Medical Research
50588 Kuala Lumpur
Malaysia

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Enzyme Activation in Peritoneal Cells from Mice Infected with *Mycobacterium lepraemurium*

TO THE EDITOR:

In a previous study ([5]) we found that peritoneal cells (PC) from NIH mice inoculated i.p. with 10^8 *Mycobacterium lepraemurium* (*Mlm*) showed increased levels of several lysosomal hydrolases 4 months after inoculation. Two months later, most of the enzyme activities decreased to values equal to or lower than those found in the control group. This suggested a transient state of biochemical activation resulting, very likely ([2]), from the generation of an affective cell-mediated immune response (via lymphokines) toward the mycobacterial antigens, and led us to study the kinetics of such biochemical activation in the PC population (mostly macrophages) during the entire period of infection. We inoculated 150 NIH female mice (8 weeks old, 20–24 g) i.p. with 10^8 *Mlm* bacilli freshly separated ([4]) from lepromas from previously infected animals. Similar, non-inoculated animals served as controls. Groups of 15 animals were sacrificed at 2-week intervals following inoculation to collect PC as described elsewhere ([5]). Four days before PC collection, the animals were injected i.p. with 2.0 ml of light mineral oil (Sigma). Cell suspensions were pooled, separated from the oil in a separation funnel, washed, adjusted to 20 to 22 ×

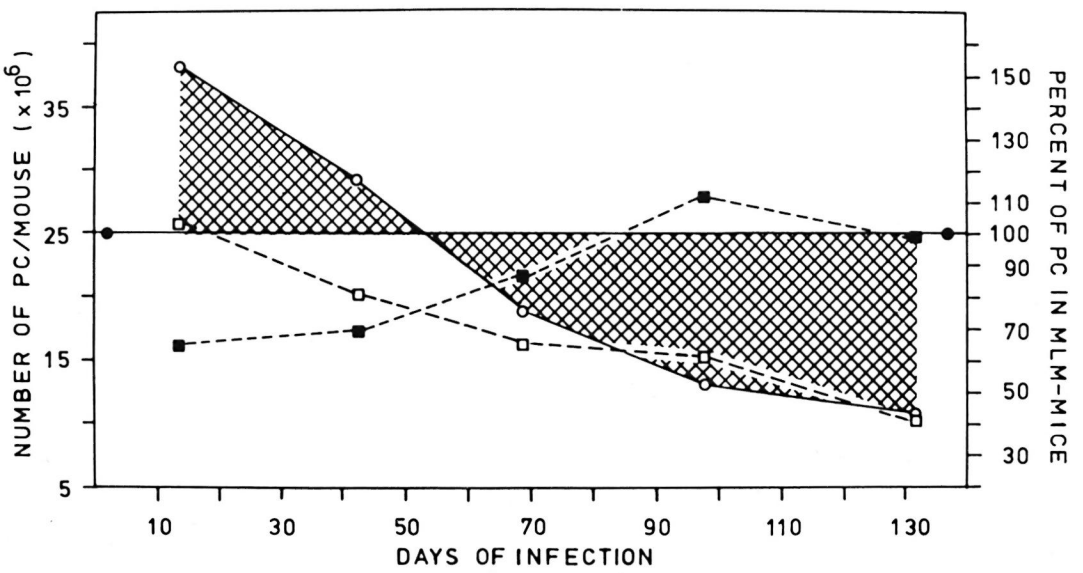

FIG. 1. Number of PC collected from normal (■) and *Mlm*-infected (□) mice at several post-inoculation times. The percent values for infected animals (○) are also given in relation to the values found (here normalized as 100%) in the control animals (●). Each point is the average from 3 determinations.

10^6 PC per ml, divided into 2 ml aliquots, and kept frozen until used.

Enzyme determinations have been previously detailed ([5]) and include: deoxyribonuclease (DNase), ribonuclease (RNase), β-glucuronidase (β-Glu), acid phosphatase (AcPh), lipase (Lip), acid proteinase (Pro), and lysozyme (Lys). An additional enzyme activity, that of peroxidase (PO), was assayed in conical, 5 ml glass tubes to which the following were added: 0.1 M phosphate buffer, pH 6.0 (0.3 ml); 0.02 M o-dianisidine hydrochloride in water (0.05 ml); the appropriately diluted enzyme preparation (0.3 ml) (After thawing, the cell suspension was sonicated for 10 sec at a low intensity just to disperse clumps.); distilled water to make 2.4 ml; and 0.03 M H_2O_2 (0.1 ml). After shaking and incubation of the tubes at 25°C for 20 min, the reaction was stopped by adding 0.5 ml of 40% trichloroacetic acid (TCA), the tubes centrifuged (3000 × *g* × 10 min), the supernatant discarded, the resulting brownish precipitate dissolved in 3.0 ml dioxan, and the optical density (OD) of the colored supernatant from a final centrifugation was read at 460 nm against a dioxan blank. PO activity is given in reference to authentic PO samples (P8375, Sigma) as units per 1 × 10^6 cells (1 unit is the amount of enzyme needed to produce an OD change of 0.1 under the assay conditions).

The progress of the infection was periodically monitored by measuring the spleen weights in relation to the body weights. (The spleen is the organ most affected following i.p. inoculation of mice with *Mlm*.)

We found a progressive loss of total PC in the infected group with a proportional increase in the macrophage population (Fig. 1). Shortly after the inoculation, the number of PC per infected mouse increased nonspecifically in response to the i.p. deposition of *Mlm* but decreased steadily thereafter. By day 132 post-inoculation, this value was nearly 60% below the one found in the control group with almost 100% of the cells being morphologically and functionally macrophages.

In spite of these facts, most of the assayed enzyme activities in the PC from the *Mlm*-infected animals showed a progressive elevation that peaked between 40 and 75 days post-inoculation, with a tendency to decrease thereafter until the end of the experiment (Fig. 2). The infected animals that were not further manipulated, and which survived the infection for over 6 to 8 months, showed increasing signs of the disease that eventually killed them. RNase and PO ac-

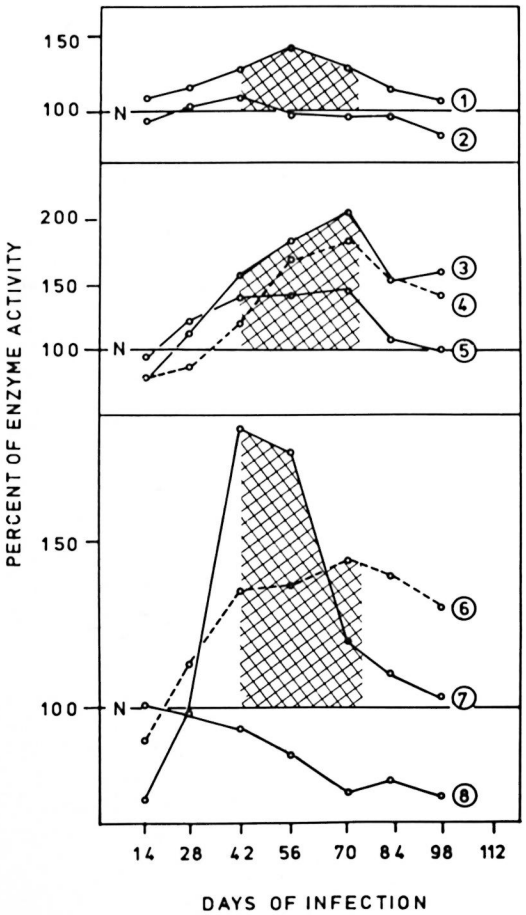

FIG. 2. Enzyme activities in PC from normal (N) and *Mlm*-infected mice at several post-inoculation times. The enzyme activities (and amounts of protein) tested were: 1 = DNase (240 μg); 2 = RNase (10 μg); 3 = β-Glu (240 μg); 4 = AcPh (240 μg); 5 = Lip (240 μg); 6 = Lys (112 μg); 7 = Pro (329 μg); 8 = PO (400 μg). Each point is the average of 3 determinations. Results are shown normalized as percent values. Shadowed areas show the period of peak activation.

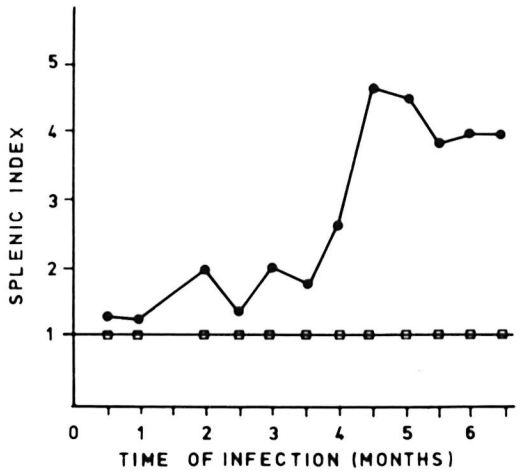

FIG. 3. Progress of the *Mlm* infection in terms of spleen enlargement. At each post-infection time shown, 3 *Mlm*-inoculated (●) and 3 normal controls (□) were randomly selected to calculate the splenic indexes according to the formula:

$$\frac{\text{spleen weight/body weight in } \textit{Mlm}\text{-infected mice}}{\text{spleen weight/body weight in control mice}}.$$

Points are averages of 3 determinations.

tivities did not increase in response to the infection. On the contrary, PO activity gradually fell and completely disappeared by day 110 following inoculation. This behavior of PO was related to the diminution of cells other than macrophages, including PO-rich neutrophils.

The progress of the infection was never arrested (Fig. 3). Spleen enlargement increased until day 135 (4.5 months) post-inoculation, after which it did not change significantly.

From these data, several conclusions can be drawn: a) infection of mice with *Mlm* apparently induces biochemical activation in their PC population; b) such PC activation is not permanent but transitory, and peaks between 40 and 75 days post-infection, depending on the enzyme; c) some enzyme activities increase more than the others, with somewhat different kinetics (In this study, RNase did not show evidence of activation.); d) the total number of PC and the proportion of cells other than macrophages fall in proportion to the duration of infection; e) PO activity does not increase but falls steadily and completely disappears by the end of the fourth month of infection; f) despite these changes, the murine disease progresses in an apparently unlimited manner.

These observations suggest that a) *Mlm* infection of mice induces an early state of cell-mediated immunity (CMI) ([3]) which influences macrophage metabolism ([1]), including their microbicidal capabilities ([2]); b) shortly after (or simultaneously with) the development of CMI, suppressor mechanisms are induced which turn off the "protective effects" of CMI ([6]) including macrophage activation, allowing the "few" surviving bacilli to take over and assuring

the progress of the infection; c) the parallel and progressive decreases in PO activity and neutrophils from the PC of *Mlm*-infected animals suggest an effect of the infection on this cell population. Further work is in progress.

—Oscar Rojas-Espinosa, Sc.D.
—Antonina Oltra, Q.B.P.
—Eira Quevedo, Q.F.I.
—Patrice Arce, I.B.Q.

Departments of Immunology and Biochemical Engineering
Escuela Nacional de Ciencias Biologicas
I.P.N. Carpio y Plan de Ayala
Col. Santo Tomas
11340 Mexico, D.F., Mexico

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Lepromin Skin Test in Normal People in Singapore—A One-year Follow Up

To the Editor:

The lepromin test is an intradermal skin test used to classify a case of Hansen's disease into the tuberculoid or lepromatous variety ([5]). This survey was prompted by the fact that the incidence of lepromin positivity in Singapore is unknown. Singapore is a highly urbanized island in South East Asia which is endemic for Hansen's disease.

We prepared our human lepromin solution in the manner recommended by WHO ([5]). The solution contained 160×10^6 bacilli per ml prepared from a nodule of a lepromatous leprosy patient, and 0.1 ml was injected in the usual manner intradermally in the volar forearms of 120 normal volunteers. The Mitsuda reaction was read at 21 days by two people. Simultaneously, a tuberculin test was done on the other forearm. One year later, 30 of the subjects were recalled and re-tested with armadillo-derived lepromin containing 160×10^6 bacilli per ml.

A positive result was defined as per WHO criteria as being any induration 3 mm or greater in diameter ([1]). Table 1 gives the number of cases according to age. There was an overall positivity rate of 70.3% which corresponds to the rate found in most endemic countries where similar studies have

Table 1. *Lepromin test (human) in 120 normal subjects.*

Age group	Positive	Negative	Total
10–19	6	2	8
20–29	48	11	59
30–39	19	10	29
40–49	14	3	17
50–59	1	5	6
60–69	0	1	1
Total	88	32	120