

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 bio-

chemical 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 $22 \times$

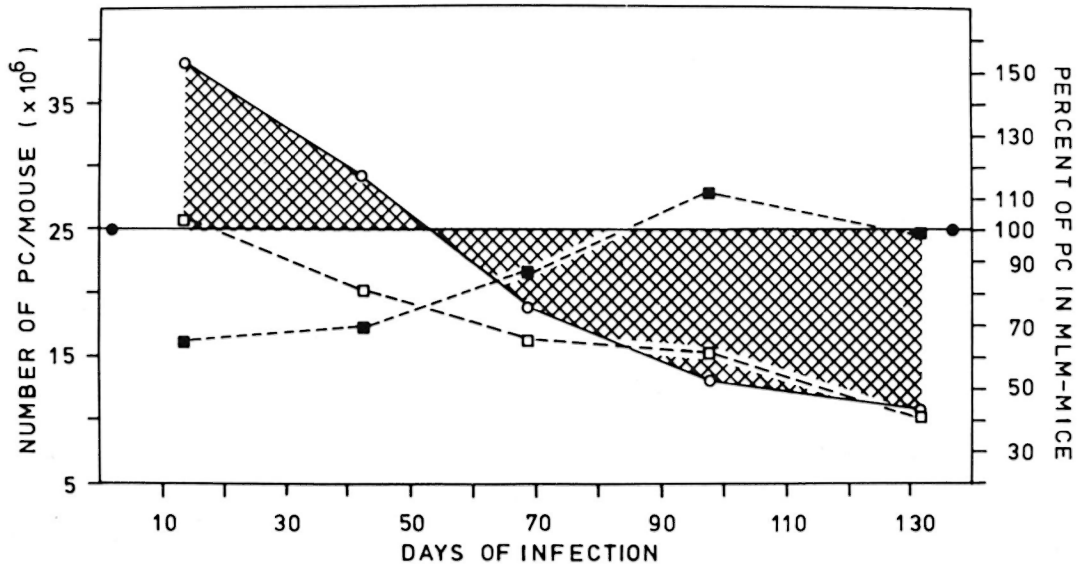


FIG. 1. Number of PC collected from normal (■) and *Mlm*-infected (□) mice at several post-inoculation times. The percent values for infected animals (○) are also given in relation to the values found (here normalized as 100%) in the control animals (●). Each point is the average from 3 determinations.

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 ($3000 \times g \times 10$ 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 non-specifically 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-

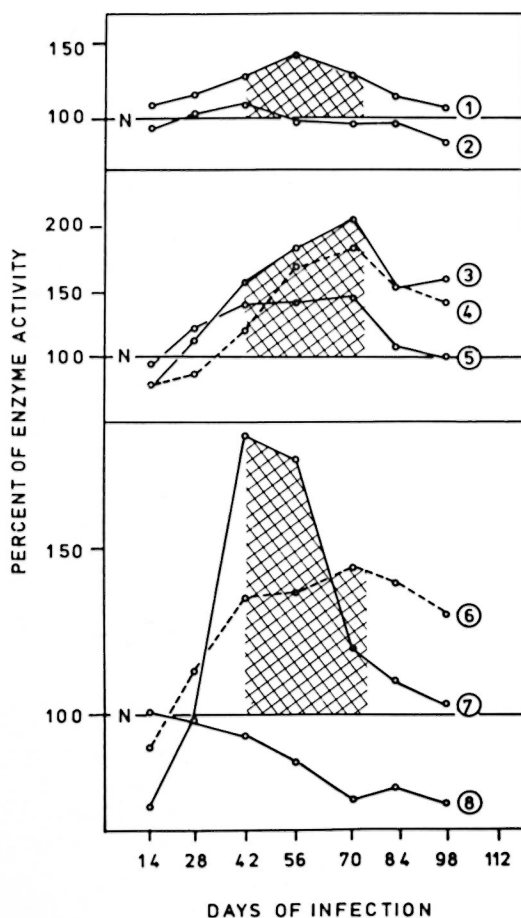


FIG. 2. Enzyme activities in PC from normal (N) and *Mlm*-infected mice at several post-inoculation times. The enzyme activities (and amounts of protein) tested were: 1 = DNase (240 μ g); 2 = RNase (.10 μ g); 3 = β -Glu (240 μ g); 4 = AcPh (240 μ g); 5 = Lip (240 μ g); 6 = Lys (112 μ g); 7 = Pro (329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

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

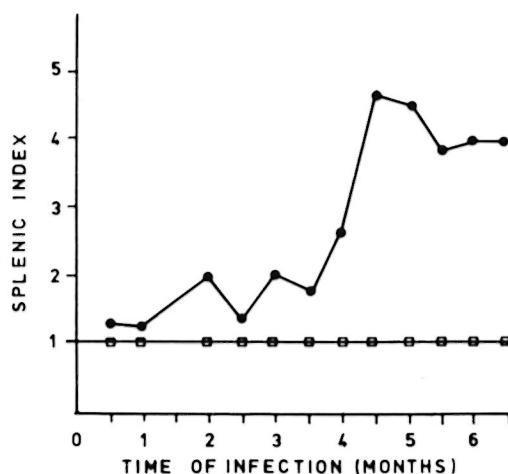


FIG. 3. Progress of the *Mlm* infection in terms of spleen enlargement. At each post-infection time shown, 3 *Mlm*-inoculated (\bullet) and 3 normal controls (\square) were randomly selected to calculate the splenic indexes according to the formula:

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

Points are averages of 3 determinations.

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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