INTERNATIONAL JOURNAL OF LEPROSY Volume 37, Number 3
Printed in the U.S.A.

Intracellular Modulation in Cellular Immunity

2. Macrophage Enzymes in Immunized, Protein-Depleted and Control Mice During *M. lepraemurium* Infection[1, 2, 3]

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In chronic granulomatous infectious diseases the defensive mechanism of the body is largely dependent on macrophage function ([1, 10, 16]), rather than on humoral antibodies ([13]). This cellular mechanism is particularly important in instances of intracellular parasitism such as in leprosy and murine leprosy.

A previous report indicated that alteration of the immune status of the host was reflected in morphologic changes within host macrophages ([19]). Enhanced resistance to *M. lepraemurium* infection in immunized animals was associated with rapid mobilization of reactive cells and with active intracellular development of lysosomes of the macrophage. The protein-depleted animals, on the other hand, were unable to achieve either the total cell response or the intracellular responses needed to suppress the dissemination of the infection.

This paper will present the results of radioautographic and biochemical studies of the cellular enzymes of peritoneal macrophages in conditions of enhanced and suppressed immunity.

MATERIALS AND METHODS

Experimental immunization, protein-depletion, preparation and counting of bacillary suspensions and peritoneal cell collections were accomplished as previously reported ([19]). Immunized, protein-depleted and control C_3H inbred female mice were infected intraperitoneally with 10^7 *M. lepraemurium*. Animals were sacrificed at intervals indicated in Table I. Peritoneal cells were counted for each animal and differential counts were made of the cell smears stained with Giemsa stain.

Radioautography. Three hours before sacrifice, each animal was injected intraperitoneally with tritium labelled L-leucine.[5] This had a specific activity of 1.53 millicurie per milligram (200 millicurie/mM). After dilution in physiologic saline to 50 microcuries per ml., this substance was administered at a dose of two microcuries per gram of body weight. Peritoneal cell smears were fixed in acid-alcohol for 30 minutes and dried before coating with radioautographic emulsion (Kodak NTB-3) essentially according to the method described by Kopriwa and Leblond ([8]). After incubation for seven days at 4°C, in light-tight plastic boxes containing Drierite (a desiccant), the slides were developed in Kodak D-19 developer and fixed in acid fixative.

Triff stain ([18]) was used for all smears since it made possible the identification of cell types while also demonstrating acid-fast organisms, and permitting tritium grain counts all on the same slide and same cells. Large macrophages only were counted randomly. A minimum of 20 cells from each animal were double-blind counted by two observers. The average grains per cell and their standard deviation were determined.

Enzyme assay and protein determination. Peritoneal cell suspensions were subjected six times to rapid freeze-thawing, diluted to 1:10 in saline and used for acid phosphatase, esterase and protein determinations. The acid phosphatase released from disrupted peritoneal cells was measured by Gomori's method ([6]) involving incubating the suspension at 37°C at pH 5.00 for 60

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5 From Chicago Nuclear Corporation.

TABLE 1. *Schedule of experiment.*

Duration of infection	Number of animals					
	Immunized		Protein-depleted		Control	
	(1)	(2)	(1)	(2)	(1)	(2)
0 hour	2	4	2	4	2	4
4 hours	2	4	2	4	2	4
1 day	2		2		2	
3 days	2	4	2	4	2	4
5 days	2		2		2	
7 days	2	4	2	4	2	4
9 days	2		2		2	
11 days	2	4	2	4	2	4
13 days	2		2		2	
15 days	2	4	2	4	2	4
17 days	2		2		2	
28 days	2	4		2	2	4
42 days	2	4		2	2	4

(1) = Radioautography.
(2) = Enzyme assays.

minutes with disodium phenyl phosphate in acetate buffer (0.05 M). The reaction was terminated by adding saturated borax in 15 per cent ethyl alcohol solution. The released phenol was coupled with diazo dye (red B salt) and read at 490 mμ on the Gilform microphotometer. Phenol solutions in known concentrations were used to make the standard curve. Each test sample was read with a blank control of water. The optical density readings of the samples were converted to the amount of phenol released by hydrolysis.

Esterase determinations were made by a method adapted from the simplified azo-dye method for the quantititive determination of naphthols developed by Gomori ([7]). Peritoneal cell esterase, which hydrolyzed α-naphthol acetate, was incubated with the substrate in 0.08 M phosphate buffer at pH 7.0 for 30 minutes and the reaction was stopped by Duponal (sodium lauryl sulfate). The released α-naphthol was coupled with red ITRN (diazoatized 4-diethyl-sulfon-aido-A-aminoanisole) and the optical density of the resulting color was read on the Gilform microphotometer at 555 mμ five minutes after mixing. A standard curve was constructed from α-naphthol preparations. Water blanks were used for each sample determination. The final result of the sample was converted to the amount of α-naphthol released from the substrate by enzymatic hydrolysis.

The protein content of peritoneal cell suspensions was determined by Lowry's method ([9]). The suspension was treated with alkaline copper and Folin phenol reagent. The intensity of the blue color developed by the reaction was measured on a Gilform microphotometer at 700 mμ. The control standard was prepared from crystalline bovine serum albumin.

RESULTS

Radioautographic evaluation of enzyme synthesis. These determinations were based on the premise that the cell activity in enzyme synthesis would be reflected by the rate of incorporation of the essential amino acid, L-leucine, into cell protein ([3]). Counting of the black grains on cell smears was done with a light microscope at 970X magnification. Only intact cells which also contained intracytoplasmic acid-fast bacilli were selected for counting. The average counts of the developed radioautographic grains per cell as well as the standard

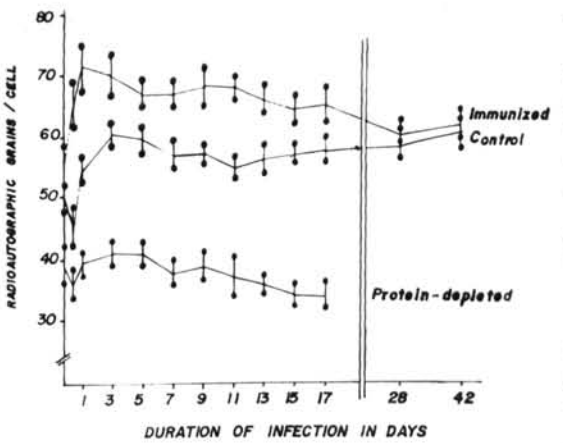

FIG. 1. Radioautography. Average grain counts in peritoneal macrophages of immunized, protein-depleted and control animals.

deviation from the average were calculated (Tables 2, 3). Polymorphonuclear leucocytes usually had less than 10 grains per cell as compared with reactive macrophages which had about 60 grains each. Small mononuclear cells, probably lymphocytes, which comprised about 20-40 per cent of the cell populations in all tested groups, had about 30 grains per cell. The grain counts of these cells and of the polymorphonuclear leucocytes are not included in this presentation.

Since all cell smears were treated under the same conditions, the background counts (less than five grains per area of 400 μ^2) were not deducted from the recorded macrophage grain counts. The operative premise was that it was not the absolute number of grains per cell that was of importance, but rather that the comparative grain counts gave evidence of differences in the uptake of isotope-labeled amino acid between the experimental groups.

The results (Fig. 1) indicate that the macrophages of the immunized animals responded with an immediate increase in uptake of isotope labeled amino acid after infection. This reached the highest level 24 hours after infection and decreased slightly afterwards but still remained at a level of increased activity throughout the entire experimental period of 42 days. Control animals, which were not immunized but were similarly infected, showed an initial decrease in uptake of the amino acid at four hours after infection. After this decrease they responded, as did the immunized group, with an increased uptake of the labelled amino acid and maintained this utilization at a moderately high level. At 28 days of infection the control level reached the same level as that of the immunized animals. The macrophages of the protein-depleted animals showed a markedly suppressed, low level uptake of labelled amino acid throughout the experimental period.

TABLE 2. *Radioautography. Average grain counts in immunized, protein-depleted and control animals.*

Duration of infection	Immunized	Protein-depleted	Control
0 hour	56.0 (±3.5)	39.0 (±3.0)	50.5 (±2.0)
4 hours	66.0 (±3.5)	36.0 (±2.5)	45.0 (±3.0)
1 day	72.0 (±4.0)	39.5 (±2.0)	55.0 (±2.0)
3 days	70.5 (±4.0)	41.5 (±2.0)	61.0 (±1.5)
5 days	67.0 (±2.0)	41.0 (±2.0)	60.0 (±2.5)
7 days	67.5 (±2.5)	38.0 (±1.5)	57.5 (±2.5)
9 days	68.5 (±3.5)	39.0 (±2.5)	58.0 (±2.0)
11 days	68.5 (±1.5)	37.5 (±3.0)	55.0 (±1.5)
13 days	66.6 (±2.5)	36.0 (±1.5)	57.0 (±2.5)
15 days	65.0 (±2.0)	42.0 (±1.5)	57.5 (±1.0)
17 days	66.0 (±3.5)	34.0 (±2.5)	58.5 (±2.0)
28 days	60.5 (±3.0)		59.0 (±2.5)
42 days	62.5 (±2.5)		61.5 (±3.0)

Each figure is the average counts of two animals. The standard deviations of these figures are given in Table 3.

TABLE 3. *Radioautography. Standard deviations of the grain counts in immunized, protein-depleted and control animals.*

Duration of infection	Immunized		Protein-depleted		Control	
	SD1 (±)	SD2 (±)	SD1 (±)	SD2 (±)	SD1 (±)	SD2 (±)
0 hour (non-inf.)	8.41	6.65	5.32	4.86	7.36	8.18
4 hours	9.19	7.43	4.92	5.33	6.17	7.15
1 day	10.10	9.43	4.51	5.95	8.04	6.14
3 days	9.69	10.13	4.63	5.11	5.76	8.69
5 days	9.98	9.36	6.34	6.62	5.47	7.58
7 days	8.76	9.02	8.40	6.12	5.67	9.19
9 days	10.91	9.86	5.54	5.61	9.06	4.94
11 days	10.46	11.35	5.43	5.73	5.89	6.74
13 days	11.78	12.10	4.34	6.72	7.61	8.79
15 days	9.67	10.11	6.11	5.87	5.77	7.63
17 days	8.43	9.61	5.42	5.90	7.92	6.58
28 days	9.85	7.83			9.41	8.52
42 days	9.85	8.37			8.33	8.51

SD1 = Standard deviation of first animal.
SD2 = Standard deviation of second animal.

Initially the macrophage uptake count of the protein-depleted animals was only 80 per cent that of the control animals. Three days after infection, the differences between the protein-depleted group and the other groups became more apparent because of the marked lack of response of the protein-depleted macrophages. Thereafter the average level of amino acid incorporation of the protein-depleted animals was only two-thirds that of the average level of control group and was only one-half that of the average level of the immunized animals. Because many of the protein-depleted mice died after four weeks of infection (i.e., after about 10 weeks on the protein-free diet), the radioautographic study of peritoneal cells in this group had to be terminated with the 17-day observations.

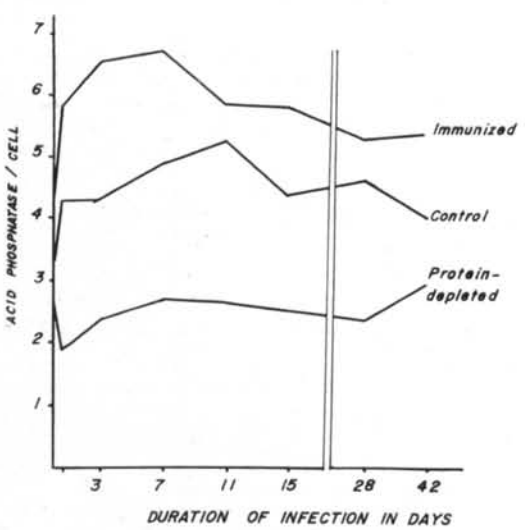

FIG. 2. Acid phosphatase activity of the peritoneal macrophages of immunized, protein-depleted and control animals. Acid phosphatase activity measured as 10^{-6} micrograms of phenol released per cell in 60 min. incubation at 37°C.

Enzyme assay. The results of the quantitative determinations of the representative lysosomal enzymes, acid phosphatase and esterase, of the immunized, protein-depleted and control animals are shown in Figures 2, 3, 4, 5 and Tables 4, 5. The cellular enzyme responses of these three groups of animals bore a remarkable resemblance to the response patterns of protein synthesis revealed by radioautography. This suggests that the protein synthesis in these macrophages represents, at least to some degree, the synthesis of lysosomal

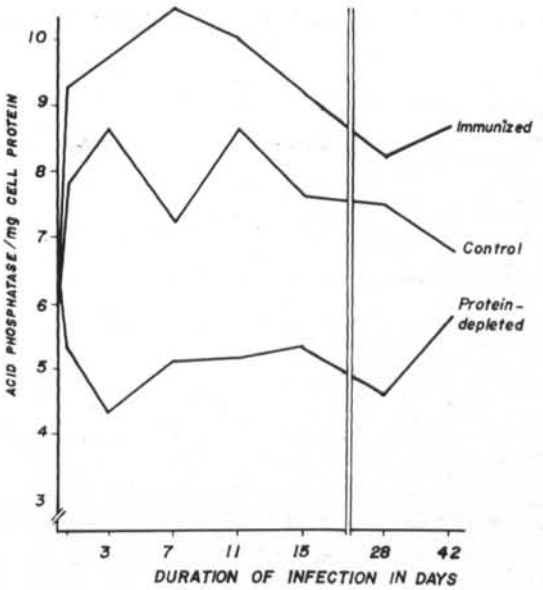

FIG. 3. Acid phosphatase activity of the peritoneal macrophages of immunized, protein-depleted and control animals. Acid phosphatase activity measured as micrograms of phenol released in 60 min. incubation at 37°C per mgm. of cell protein.

enzymes. High resolution electron microscope radioautography provided further evidence that these newly synthesized proteins, in fact, were incorporated into phagocytic vacuoles ([20]).

Acid phosphatase. The immunized animals showed an immediate sharp increase in cellular acid phosphatase titer at four hours, three days and seven days after infection, achieving the highest level at the seventh day with an increase of about 70 per cent of activity (Figs. 2, 3). Cellular acid phosphatase activity of the control infected animals increased at a slower rate than did that of the immunized animals and reached the highest level at the 11th day after infection with an increase of 50 per cent. Thereafter the enzyme activity gradually decreased. Within the experimental period of 42 days, the enzyme activities of both groups were maintained at levels higher than in the noninfected mice (zero hour groups). The rate of increase in the immunized group was more rapid than that of the controls and the highest level of enzyme activity achieved by the immunized group occurred several days earlier than that of the control group. From the second to the third weeks of infection the enzyme activity levels of these two groups tended to converge, perhaps because of a gradual drop in the enzyme concentration of the immunized animals. In the protein-depleted animals, it is evident that there was significantly less acid phosphatase content as compared with the control and immunized animals. At first the enzyme activity decreased a little and then gradually returned to the original level. This low level of response was in agreement with the result of the radioautographic indication of suppressed cellular protein synthesis (Fig. 1). It also correlated with the assay of the macrophage esterase activity (Figs. 4, 5).

Esterase. The esterase activity of the peritoneal exudate cells is shown in Figures 4 and 5. The esterase values of these three groups of animals were quite similar to those of the acid phosphatase responses noted above. The effect of immunization was reflected consistently in the promptness and in the greater degree of enzyme activity increase in the macrophage of the immunized group as compared with the control group. The effect of protein starva-

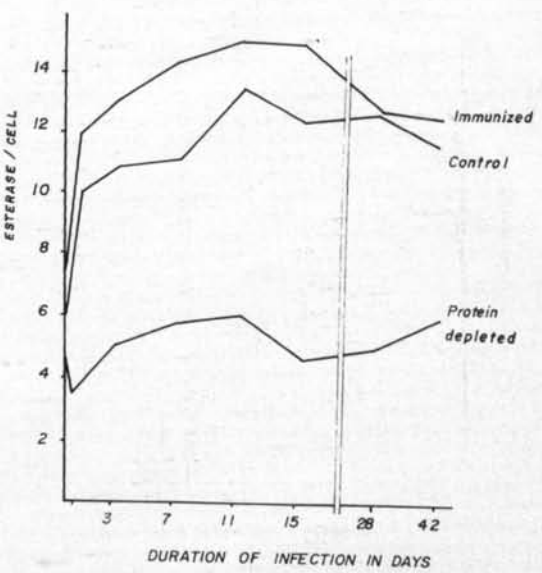

FIG. 4. Esterase activity of the peritoneal macrophages of immunized, protein-depleted and control animals. Esterase activity measured as 10^{-5} microgram α-napthol released per cell in 30 min. incubation at 37°C.

TABLE 4. *Peritoneal cell counts, cell protein, cell enzymes in immunized, protein-depleted and control animals.*

Duration of infection	Cell No. 3 (cell/mm.)	Protein (mgm./ml.)	Acid-ptase[a]	Esterase[b]
		Immunized animals		
0 hour	2400–2800	1.30–1.78	10.0–11.0	1.80–1.98
4 hours	3950–4250	2.22–2.70	22.5–25.5	4.62–4.93
3 days	3650–4300	2.55–2.73	24.6–27.1	5.00–5.42
7 days	3500–3900	2.19–2.58	24.4–26.2	5.24–5.60
11 days	3950–4300	2.39–2.57	22.8–26.1	5.93–6.35
15 days	3700–3950	2.33–2.52	21.0–24.2	5.63–5.97
28 days	3450–3750	2.14–2.56	18.2–20.6	4.41–4.71
42 days	3550–4050	2.10–2.51	19.4–21.4	4.60–4.82
		Protein-depleted animals		
0 hour	500–800	0.20–0.29	1.58–1.90	0.28–0.34
4 hours	900–1150	0.27–0.43	1.61–1.99	0.30–0.39
3 days	600–850	0.34–0.50	1.58–2.04	0.34–0.42
7 days	550–850	0.34–0.42	1.82–2.08	0.33–0.47
11 days	600–800	0.31–0.43	1.77–2.01	0.35–0.49
15 days	700–850	0.29–0.39	1.77–2.11	0.32–0.38
28 days	650–900	0.34–0.50	1.82–2.08	0.32–0.44
42 days	450–700	0.26–0.36	1.62–1.92	0.32–0.39
		Control animals		
0 hour	2050–2475	1.03–1.45	6.9– 8.6	1.26–1.42
4 hours	2560–4050	1.96–2.26	15.1–17.7	3.66–3.99
3 days	3450–3950	1.72–1.91	15.4–17.9	3.93–4.17
7 days	3050–3200	1.87–2.32	14.8–16.0	3.38–3.67
11 days	3300–3700	2.01–2.37	17.1–19.9	4.55–4.86
15 days	3400–3850	1.95–2.21	9.2–11.0	4.42–4.61
28 days	3600–4100	2.17–2.62	16.6–19.2	4.78–5.11
42 days	3300–3650	1.80–2.24	13.2–15.6	3.92–4.22

[a] Acid phosphatase: micro-gram phenol released/60 min./ml. cell suspension.
[b] Esterase: 10^{-4} gram α-Naphthol released/30 min./ml. cell suspension.

tion was reflected in the depression of the macrophage esterase response. During most of that experimental period, the enzyme titers of protein-depleted animals were at the base line level.

Further examination of these data revealed that the total protein of the peritoneal cell suspension in the protein-depleted animals approximated only about 25 per cent that of the control animals. That of the immunized animals was slightly higher than that of the controls. When these values were converted to amount-of-protein-per-cell on the basis of total peritoneal cell counts, the value was still significantly lower in the protein-depleted animals than in the other groups. This reflected the differences in cell sizes and intracellular lysosome production between the experimental groups as seen by light microscopic and electron microscopic studies ([19]). There was an increase of the calculated protein-per-cell in the protein-depleted group after infection was initiated. Knowing that there was no significant increase of uptake of the labelled amino acid and no increase of acid phosphatase and esterase titers, it is probable that this calculated increase represented an increased lysis of the debilitated cells. Cell smears taken from infected protein-depleted animals showed increased numbers of broken, smudged cells. Peritoneal macrophages from debilitated animals were found by electron microscopy to form abnormal intracellular inclusion bodies and

TABLE 5. *Cell enzymes and cell protein in immunized, protein-depleted and control animals.*

Duration of infection	AP/C	AP/P	E/C	E/P	P/C
		Immunized			
0 hour	4.01	6.61	7.39	1.14	6.08
4 hours	5.87	9.33	11.7	1.89	6.32
3 days	6.56	9.80	13.0	1.96	6.62
7 days	6.77	10.50	14.3	2.22	6.42
11 days	5.93	10.00	15.0	2.52	5.93
15 days	5.90	9.20	15.1	2.36	6.37
28 days	5.37	8.23	12.7	1.96	6.50
42 days	5.42	8.70	12.6	2.03	6.20
		Protein-depleted			
0 hour	2.65	6.90	4.73	1.24	3.34
4 hours	1.86	5.34	3.40	0.98	3.48
3 days	2.39	4.30	5.01	0.91	5.50
7 days	2.75	5.09	4.72	1.05	5.42
11 days	2.70	5.12	6.00	1.13	5.42
15 days	2.57	5.32	4.67	0.97	4.81
28 days	2.40	4.59	5.00	0.94	5.20
42 days	3.00	5.79	6.00	1.16	5.17
		Control			
0 hours	3.46	5.78	6.02	1.04	6.02
4 hours	4.31	7.82	10.2	1.86	5.51
3 days	4.36	8.66	10.9	2.18	5.04
7 days	4.94	7.22	11.5	1.68	6.85
11 days	5.28	8.70	13.6	2.20	6.12
15 days	4.40	7.65	12.4	2.15	5.80
28 days	4.68	7.50	12.7	2.04	6.22
42 days	4.05	6.80	11.7	2.01	5.85

AP/C = Acid phosphatase activity per cell.
AP/P = Acid phosphatase activity per mgm. cell protein.
E/C = Esterase activity per cell.
E/P = Esterase activity per mgm. cell protein.
P/C = Amount of protein (10^{-4} microgram) per cell.

myelin figures such as are generally accepted by electron microscopists as representing focal cytoplasmic degeneration (17)

This evidence tends to suggest that protein-starvation caused cellular degeneration and made the cells more liable to lysis when the cells were infected by *M. lepraemurium.*

DISCUSSION

The peritoneal cell infection model provides an opportunity for *in vivo* study of cell-pathogen interactions and also provides an opportunity for contrasting the early cellular and intracellular reactions in previously immunized and in protein-depleted animals with similar factors in control conditions of unaltered defense mechanisms. The model presents an advantage over cell culture technics in that the infection takes place *in vivo* and no artificial environmental factors are introduced that might affect the usual function of the cells (14). It has, however, the disadvantage inherent in attempting evaluation of specific cell functions in the presence of a mixed cell population such as is inevitable in intraperitoneal infection. Nevertheless, the differential cell counts showed a remarkable similarity in the relatively small percentage of polymorphonuclear leucocytes at various stages of the infection of the control, protein-starved and

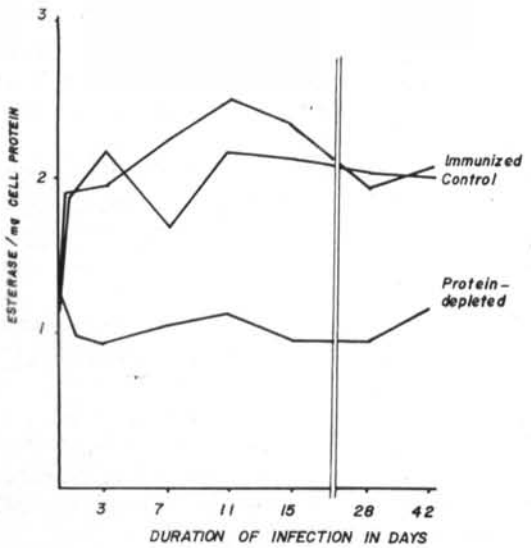

FIG. 5. Esterase activity of the peritoneal macrophages of immunized, protein-depleted and immunized animals. Esterase activity measured as 10^{-4} microgram α-napthol released per mgm. of cell protein in 30 min. incubation at 37°C.

immunized animals (Fig. 6). This uniformity makes it possible to retain some confidence in comparisons of macrophage total cell enzyme content as well as in extrapolating such total content to calculations regarding the average individual cell enzyme content and the enzyme concentration per milligram of protein. The radioautographic study, in this respect, is particularly useful

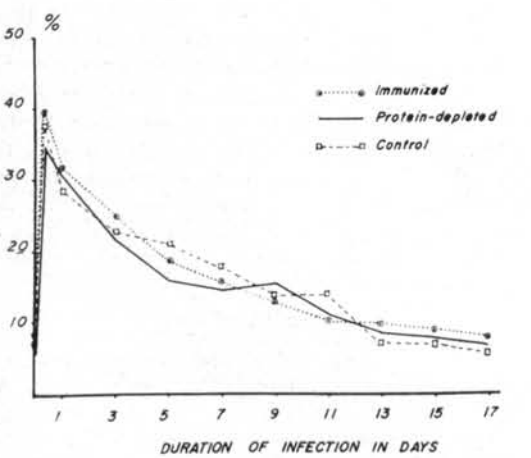

FIG. 6. Percentage of polymorphonuclear leucocytes in peritoneal cells of immunized, protein-depleted and control animals.

in serving as a general reference guide to the study of specific macrophage responses. Its grain counts on cells harboring acid-fast bacilli allowed the evaluation of actively phagocytosing macrophage population. By this sensitive specific method, interestingly enough, the results turned out to be remarkably similar to those noted by enzyme assay, thus adding confidence to the interpretation of the macrophage intracellular responses in enzyme comparisons.

The macrophages of the protein-depleted animals were so debilitated that their cellular content of acid phosphatase and esterase were reduced to about one-half that of the control level. Both the acid phosphatase and the esterase were similarly suppressed. The suppressive effect of protein depletion on enzyme synthesis was further demonstrated by the inability of these cells to respond normally when they were challenged by infection. Radioautography revealed also a significantly lower degree of utilization of labeled L-leucine and this was reflected at least in part in the lower production of enzymes.

The macrophages of both the immunized animals and the control animals responded with a notable increase in the cellular enzymes after infection. A considerable portion of these enzyme increases (presumably that portion of increase common to both groups), may simply reflect the nonspecific, normal functional adaptability of the macrophages. Investigation of macrophage differentiation *in vitro* [4] has led to the conclusion that cell maturation is associated with an increase of their enzyme content and new enzyme synthesis. The ingestion of yeast cell walls or heat-killed BCG by the rabbit alveolar macrophages [5, 12] and BCG infection of peritoneal macrophages [15] also has been shown to be accompanied by an increase of cell acid phosphatase content. This nonspecific macrophage enzyme increase is apparently associated in large measure with observed cross resistance or cross protection [11] of host in infections caused by facultative intracellular parasites. In the present experiments, the results indicate that there was a small difference in enzyme titers between control and immunized groups.

Although the differences between the control and immunized animals, with respect to intracellular enzyme titers, were not as striking as the differences between these groups and the protein-depleted group, they were significant and evidently represented an immunization-induced acceleration and enchancement of response. The use of the sensitive, indirect, radioautographic technic made it possible to further differentiate the reactions of these two groups. The macrophages of control animals showed a lag period in response to protein synthesis while the immunized macrophages responded with alacrity. This difference in response between immunized and control animals reflected a similarity to the anamnestic response of the antibody-producing mechanism ([2]). It is not unlikely that this accelerated response could represent a "cellular anamnestic" or remembering reaction of the macrophages comparable with the anamnestic antibody responses.

It is emphasized that the enzymes here studied reflect only the probable response of an intricate total enzyme pattern. It is not assumed that acid-phosphatase and esterase are the enzymes required for the digestion of mycobacteria and their capsules. In all probability, there are other unmeasured and undetermined enzymes which carry the major burden of this performance. Nevertheless, the measurements here presented suggest strongly that enzyme response and function are an integral part of the mechanisms of cellular immunity and that absence of such immunity in diseases such as lepromatous leprosy may profitably be sought in terms of possible enzymatic deficiency in responding macrophages.

SUMMARY

Quantative analysis of peritoneal macrophage enzymes (acid phosphatase and esterase) in immunized mice, protein depleted mice and in control mice showed that whereas the enzyme content of the macrophages of protein-depleted animals fell following infection with M. *lepraemurium* both immunized animals and control animals were able to respond rapidly with an increase of enzyme titers. These titers were subsequently maintained at a higher level in immunized animals than in control animals.

Radioautographic studies of the uptake of H^3-L-leucine by the macrophages indicated that the rate of protein synthesis (probably enzymes) in the protein-depleted animals is depressed. The remarkable difference between the immunized animals and the control animals was reflected in the early responses (at 4 hours and 1 day after infection) of leucine uptake. The macrophages from the control animals had an initial lag period of one day while those of the immunized animals showed a rapid response without a lag period.

RESUMEN

Análisis cuantitative de las enzimas de macrófagos peritoneales (fosfatose ácida y esterase) en ratones inmunizados, ratones deprivados de proteina y en ratones de control, reveló que, mientras que el contenido de las enzimas de los macrófagos de animales deprivados de proteina disminuye despues de infección por *M. lepraemurium*, animales inmunizados tanto que animales de control podrían responder rapidamente por aumento de títulos de enzimas. Estos títulos se mantenieron en un nivel mas alto en animales inmunizados que en animales de control.

Estudios radioautográficas de recogido de H^3L-leucina por los macrófagos indicaron que la razon de síntesis de proteinas (probablemente enzimas) en los animales deprivados de proteina se deprime. La notable diferencia entre los animales inmunizados y los animales de control fue reflejado en las respuestas de recogido de leucina (a los cuatro horas y una hora despues de infección). Los macrófagos de los animales de control tenian un período de retraso de un dia, mientras que aquellos de los animales inmunizados demonstraban una respuesta sin un período de retraso.

RÉSUMÉ

L'analyse quantitative des enzymes des macrophages péritonéaux (phosphatase acide et estérase) chez des souris immunisées, chez des souris déficientes en protéine, et chez des souris témoins, a montré que, alors que le contenu enzymatique des macrophages d'animaux déficients en protéine à la suite de l'infection par *M. lepraemurium.* les animaux immunisés comme les animaux témoignaient d'une ca-

pacité à répondre rapidement à un accroissement des titres enzymatiques. Ultérieurement, ces titres se maintenaient à un niveau plus élevé chez les animaux immunisés que chez les animaux témoins.

Les études radio-autographiques de l'incorporation (uptake) de H^3-L-leucine par les macrophages ont rèvélè que la taux de la synthèse des protéine (probablement des enzymes) chez des animaux déficients en protéine était abaissé. La différence remarquable notée entre les animaux immunisés et les animaux témoins se manifestait également par les réponses rapides de l'incorporation de leucine (quatre heures et un jour après l'infection). Les macrophages provenant des animaux témoins présentaient une latence initiale d'un jour, tandis que les macrophages d'animaux immunisés montraient une réponse rapide sans période de latence.

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