Systemic Macrophage Mobilization and Granulomatous Response to BCG in the Protein-Deficient Rabbit

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In view of increased susceptibility to tuberculosis in protein malnutrition, the phenomenon of accelerated granulomatous response by the macrophage was studied in rabbits immunized and subsequently challenged with BCG. Dietary protein depletion resulted in marked retardation of macrophage mobilization and granuloma formation in various organs. The granulomatous index was low in most organs. The granulomas were few, small, abortive and ill-formed by loosely knit epithelioid cells and giant cells. This deficiency of macrophage function might limit the effectiveness of BCG vaccination in the malnourished host (Am J Pathol 76:313–322, 1974).

INCREASED SUSCEPTIBILITY to tuberculosis and diminished sensitivity to tuberculin have been observed in protein and protein-calorie malnutrition. This has been reviewed recently.[1]

Macrophages play an important role in tuberculosis.[2–7] In normally fed, BCG-immunized rabbits and mice they are mobilized very rapidly upon intravenous challenge with the organism. The accelerated granulomatous response in the lung closely resembles the tuberculous lesion in humans and is associated with increased resistance to tuberculosis.[3,8–10]

A deficiency of the macrophage function has been seen in protein-deficient mice.[11] This is also evident in the skin and lymph node lesions of protein-deficient guinea pigs immunized with BCG.[1] However, no information is available on the rapidity and intensity of the systemic macrophage response following reinfection. This appears important for the effectiveness of BCG vaccination and has been investigated here in protein-deficient rabbits.

Materials and Methods

Thirty male rabbits weighing 1000 to 1100 g were divided into high-protein (HP) and low-protein (LP) groups and pair-fed diets containing 20% casein and

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no casein, respectively. The diets were adequate in fat, carbohydrate, minerals and vitamins.[1] Each animal, in addition, received 20 g of carrot daily.

At the end of 8 weeks each rabbit was immunized with BCG and tuberculin tested 4 weeks after immunization as described earlier.[1] Three pairs of animals were then sacrificed by etherization and exsanguination to serve as controls for the rest of HP and LP animals, which were challenged with intravenous BCG, 5 mg/kg body weight. They were killed 3 pairs at a time, 4, 7, 14 and 28 days later.

The lungs, liver and spleen were weighed and examined grossly for alteration in consistency and presence of tubercles. The intensity of granulomatous reaction in the lung was assessed using the following formula.[10]

$$\text{Granulomatous index} = \frac{\text{Lung weight/body weight (experimental)}}{\text{Lung weight/body weight (control)}}$$

Pieces of lung, liver, spleen, sternal marrow, BCG nodules and draining lymph nodes were fixed in neutral buffered formalin and processed for 4-μ paraffin sections, and stained with hematoxylin and eosin and Ziehl-Neelsen stain. Epithelioid cell granulomas were enumerated in ten consecutive fields, each with an area of 3.05 sq mm, and expressed as the number per sq cm. The diameters of twenty randomly chosen granulomas were measured by using a calibrated eye piece micrometer (Leitz); the mean diameter in microns was obtained from these observations.

Results

BCG Immunization

The findings were essentially similar to those reported in the guinea pig.[1] In HP rabbits, the primary complex was well developed and consisted of compact epithelioid cell granulomas. The tuberculin response was strongly positive in four-fifths of animals and moderately positive in the others. In the LP rabbits, on the other hand, the primary complex was poorly developed and showed only focal, ill-formed granulomas. The tuberculin sensitivity was weakly positive in one-fourth of the animals and negative in the remainder.

BCG Challenge

The intensity of the granulomatous response in different organs as measured from their mean number per square centimeter and mean diameter in microns is presented in Table 1; the weights of the organs are presented in Text-figure 1. The appearance of the granulomas is shown in Figures 1 through 8. A considerable difference was evident between the two groups of animals.

Lungs of the HP rabbits were voluminous, heavy, firm and subcrepitant. This was particularly marked at 7 days, when the granulomatous index was between 2.1 and 2.8 At 14 and 28 days numerous tiny tubercles, diffusely distributed on pleural and cut surfaces, were observed. Microscopically, at 4 days, alveolar walls and lumina appeared cellular;

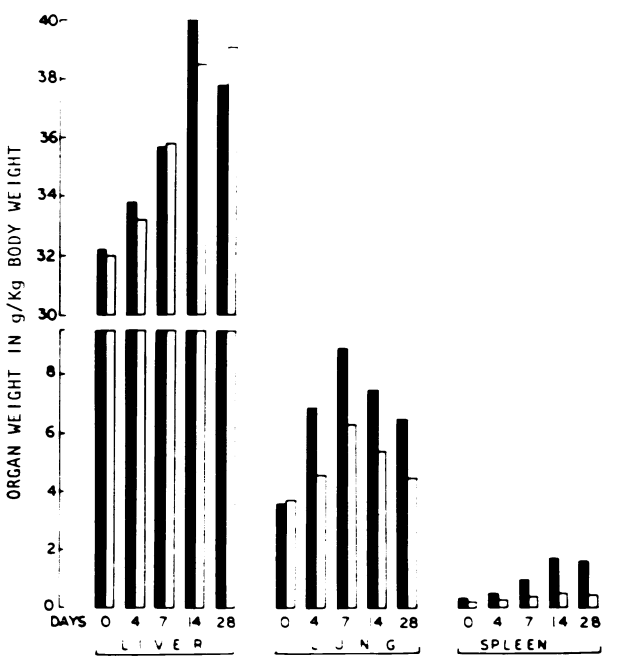
Table 1—Granulomatous Index, Number of Granulomas and Their Diameters in HP and LP Rabbits

Duration of challenge (days)	Exp groups	Lung			Liver		Spleen		Bone marrow
		Index	No.	Diameter (μ)	No.	Diameter (μ)	No.	Diameter (μ)	
4	HP	1.883	G	G	M	M	—	—	—
	LP	1.426	g	g	m	m	—	—	—
7	HP	2.427	528	119	352	43	520	56	G
	LP	1.684	66	44	168	30	m	m	—
14	HP	2.071	660	98	464	58	630	67	G
	LP	1.449	173	58	300	37	122	37	g
28	HP	1.768	429	66	502	66	681	66	G
	LP	1.202	98	43	320	49	132	47	g

G = frequent large ill-defined granulomas; g = occasional small ill-defined granulomas; M = diffuse large aggregates of macrophages; m = focal small collections of macrophages — =absence of granulomas and or macrophage collections. These findings were difficult to quantitate.

Mean of three values in each case.

numerous macrophages and neutrophils and early focal formation of epithelioid cell granulomas were seen. At 7 days numerous large compact and often confluent epithelioid cell granulomas dominated the field (Figure 1). They numbered 430 to 660 per sq cm and measured



TEXT-FIG 1—Graphic presentation of organ weights of liver, lung and spleen of HP (*solid columns*) and LP (*open columns*) rabbits.

90 to 140 μ in diameter. From 2 to 4 weeks the granulomas appeared reduced in size and were associated with moderate lymphoid cell infiltration and fibroplasia. In LP rabbits, on the other hand, the lungs showed only a moderate increase in weight and a granulomatous index of 1.4 to 2.0 at 7 days. In patchy areas the pulmonary tissue felt firm and subcrepitant; this represented areas of increased cellularity of alveolar walls and lumens due to infiltration with numerous neutrophils and moderate number of macrophages. Occasional, ill-defined granulomas consisting of a few loosely knit epithelioid cells began to appear at 7 days (Figure 2). An increase in their number and compactness was seen at 14 days, but they were still small and ill formed. At this time they numbered 130 to 210 per sq cm and measured 40 to 75 μ in diameter. There was mild lymphocytic infiltration and fibroplasia. A reduction in the number and size of the granulomas was observed at 28 days.

Livers in the HP group contained small to large clusters of macrophages and neutrophils throughout but concentrated mostly in portal and periportal regions. At 1 week these were transformed into granulomas consisting mainly of foreign body and Langhans' giant cells and a few loosely knit epithelioid cells. At 2 and 4 weeks they were more compact and contained epithelioid cells, numbering 370 to 530 per sq cm and measuring up to 70 μ in diameter (Figure 3). In the LP group the liver sections showed fatty change and very small clusters of macrophages and neutrophils were distributed as in the HP group. Very small, ill-formed granulomas consisting of a few epithelioid and giant cells were seen at 2 and 4 weeks (Figure 4). These numbered 220 to 340 per sq cm and measured 30 to 55 μ in diameter.

In the HP animals, the spleen showed fivefold enlargement, from 300 to 1600 mg/kg body weight. At the end of 1 week, both the white and red pulp were diffusely infiltrated by macrophages. Numerous well-defined granulomas, similar to those in the liver, were observed at 2 to 4 weeks (Figure 5). In contrast, the spleens in the LP animal were small and atrophic and showed only moderate increase in weight, from 200 to 450 mg/kg body weight. Ill-defined, small, abortive granulomas, mostly in the white pulp and rarely in red pulp, were seen in about two-thirds of animals at 2 and 4 weeks (Figure 6).

The sternal marrow in the HP rabbit was cellular and showed focal formation of loosely knit granulomas (Figure 7). It was hypocellular and atrophic in the LP rabbit and in one-third of animals contained rare focal collections of macrophages showing attempted granuloma formation (Figure 8).

In both HP and LP groups, acid-fast bacilli were frequently demonstrable in macrophages and giant cells at 4 days of BCG challenge. They were seen in much diminished number at 7 days and were difficult to detect during later periods. No consistent difference regarding bacillary persistence or disappearance was observable between the two groups of animals.

Discussion

In an earlier study, BCG vaccination of protein-deficient guinea pigs resulted in poorly formed primary complexes and weakly positive or negative tuberculin responses.[1] This was associated with delayed and deficient mobilization and activation of macrophages. These features were evident in protein-deficient rabbits also.

Further evidences of macrophage dysfunction in protein deficiency were observed in immunized rabbits after intravenous challenge with BCG. A poor, patchy granulomatous response in the lung contrasted sharply with an intense reaction in the lungs of HP rabbits. The granulomatous reaction in the liver was, however, quite appreciable, though much less pronounced in comparison with the HP group. This might have resulted from the richness of locally resident macrophages.[6,7] The predominant giant cell response, moreover, appeared effective in entrapment and destruction of the limited number of circulating mycobacteria.[3] This would also explain the absence of consistent differences between the two groups of animals regarding the presence or absence of acid-fast bacilli in stained sections. Culture studies would be essential for a definite conclusion on this point. In contrast to that in the liver, the macrophage response in the spleen and bone marrow of malnourished rabbits was very poor. The above findings strongly suggest a relative ineffectiveness, in continued protein malnutrition, of active immunization in evoking the accelerated granulomas response.

As effector cells, macrophages play a crucial role in mycobacterial infections.[2–7] In normal states of nutrition, immunization with BCG results in mobilization and activation of macrophages which might undergo local and systemic proliferation and development of cell-mediated immunity (CMI) as judged from the positivity of tuberculin response.[3,6–8] A larger pool of activated macrophages are mobilized faster on reinfection and transformed earlier under the influence of CMI.[9,12] The process of transformation into epithelioid cells is associated with increased metabolic activity and synthesis of lysozomal enzymes.[8,13,14]

These cellular functions appeared adversely affected by dietary protein depletion; this was evident during BCG immunization, and, more

noticeably, in the internal organs during reinfection. The dual deficiency of macrophage mobilization and epithelioid cell transformation could have resulted from a reduced pool of macrophages undergoing inadequate stimulation and proliferation during active immunization and a depression in the development of CMI. Limited availability of "building blocks" for cellular enzyme synthesis could have further affected the process of epithelioid cell transformation.[3,15] This continued deficiency of macrophage function might limit the effectiveness of BCG vaccination against tuberculosis in the malnourished host.

References

1. Bhuyan UN, Ramalingaswami V: Immune responses of the protein-deficient guinea pig to BCG vaccination. Am J Pathol 72:489–500, 1973
2. Rich AR: The Pathogenesis of Tuberculosis, Second edition. Springfield, Ill, Charles C Thomas Publishers, 1952
3. Lurie MB: Resistance to Tuberculosis: Experimental Studies in Native and Acquired Defensive Mechanisms. Cambridge, Mass, Harvard University Press, 1965
4. Mackaness GB: Editorial, The immunology of antituberculous immunity. Am Rev Resp Dis 97:337–344, 1968
5. Youmans GP, Youmans AS: Recent studies on acquired immunity in tuberculosis. Curr Top Microbiol Immunol 48:129–178, 1969
6. Nelson DS: Macrophages and Immunity. Frontiers of Biology, Vol 11. Amsterdam, North-Holland Publishing Company, 1969.
7. Pearsall NN, Weiser RS: The Macrophage. Philadelphia, Lea and Febiger, 1970
8. Oshima S, Myrvik QN, Leake ES: The demonstration of lysozyme as a dominant tuberculostatic factor in extracts of granulomatous lungs. Br J Exp Pathol 42:138–144, 1961
9. Myrvik QN, Leake ES, Oshima S: A study of macrophages and epithelioid like cells from granulomatous (BCG-induced) lungs of rabbits. J Immunol 89:745–751, 1962
10. Kawata H, Myrvik QN, Leake ES: Dissociation of tuberculin hypersensitivity as mediator for an accelerated pulmonary granulomatous response in rabbits. J Immunol 93:433–438, 1964
11. Yang H-Y, Skinsnes OK: Intracellular modulation in cellular immunity. II. Macrophage enzymes in immunized protein depleted and control mice during M. lepraemurium infection. Int J Leprosy 37:263–269, 1969
12. David JR, David RR: Cellular hypersensitivity and immunity: inhibition of macrophage migration and the lymphocyte mediators. Prog Allergy 16:300–449, 1972
13. Sutton JS, Weiss JS: Transformation of monocytes in tissue culture into macrophages, epithelioid cells, and multinucleated giant cells: an electron microscope study. J Cell Biol 28:303–332, 1966
14. Epstein WL, Krasnobrod H: The origin of epithelioid cells in experimental granulomas of man. Lab Invest 18:190–195, 1968

15. Alleyne GAO, Young VH: Adrenal function in malnutrition. Lancet 1:911–912, 1966

Acknowledgments

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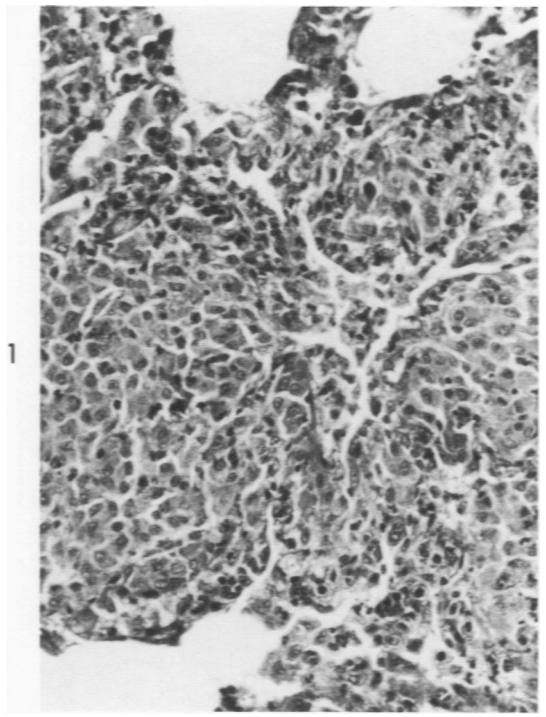
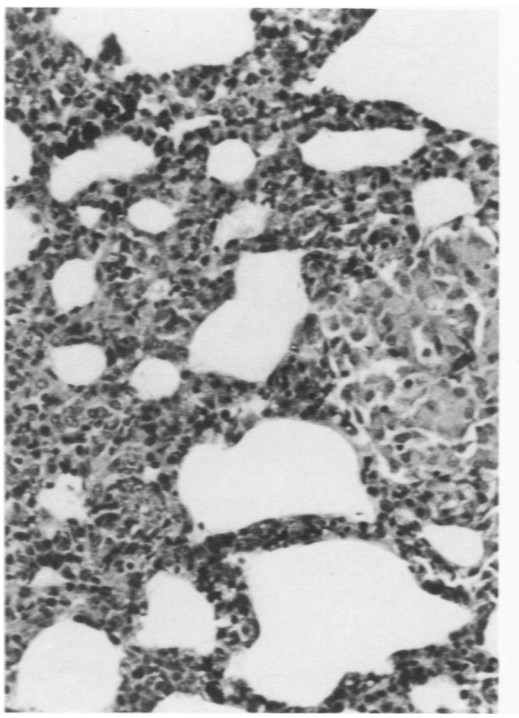
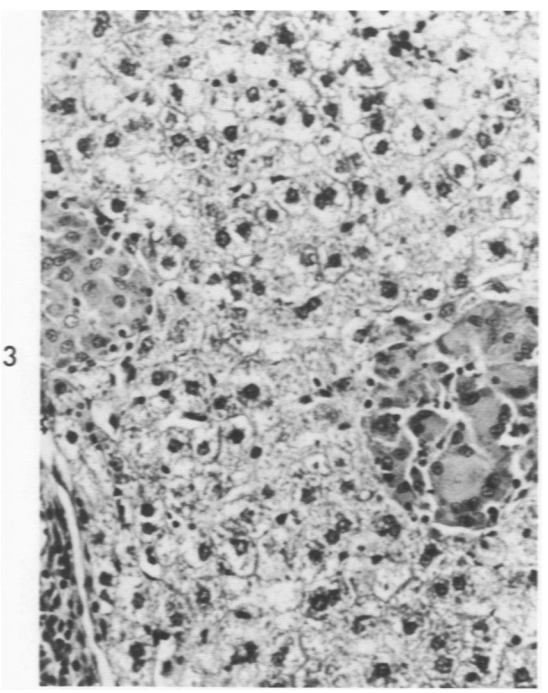
[Illustrations follow]







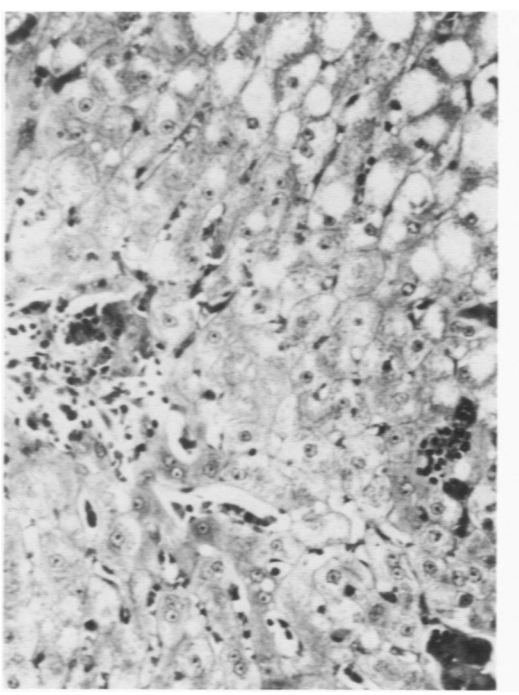
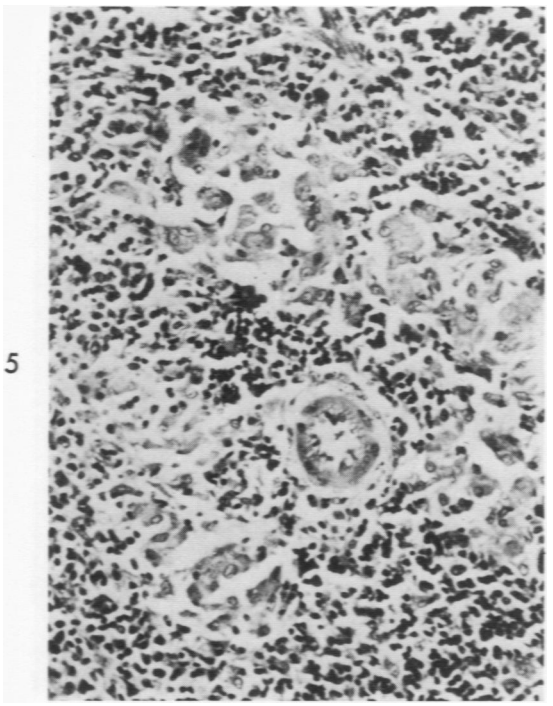
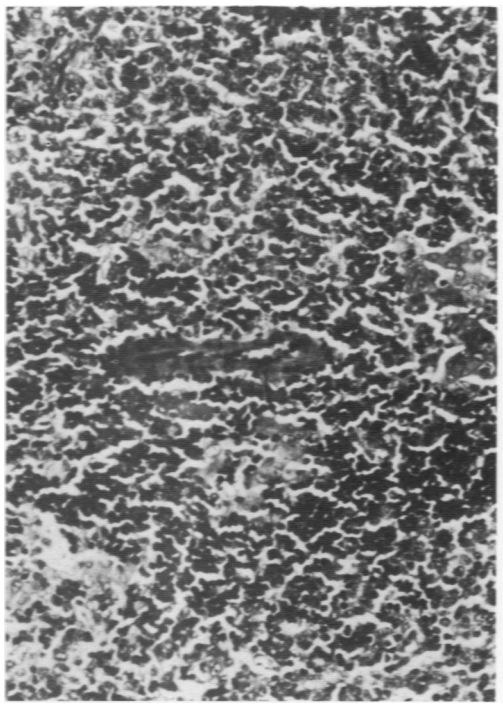


Fig 1—Section of lung showing large compact epithelioid cell granulomas in a HP rabbit 7 days after BCG challenge (H&E, × 200). **Fig 2**—A focal, loosely knit epithelioid cell granuloma and interstitial infiltration with numerous neutrophils and macrophages in the lung of a LP rabbit 7 days after BCG challenge (H&E, × 200). **Fig 3**—Granulomas in the liver with predominant giant cell reaction. HP animal 14 days of BCG challenge (H&E, × 200). **Fig 4**—Ill-formed granulomas consisting predominantly of giant cells in the fatty liver of a LP rabbit, 14 days after BCG challenge (H&E, × 200).





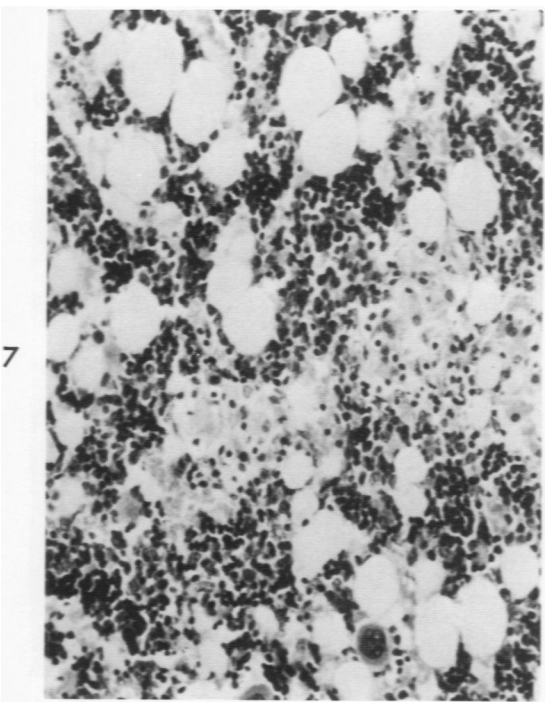


Fig 5—White pulp of the spleen in a HP animal showing well-formed granulomas around a central arteriole, 28 days of BCG challenge (H&E, × 200). **Fig 6**—Small abortive granulomas, two at the right mid-portion in the white pulp of spleen. LP animal 28 days of BCG challenge (H&E, × 200). **Fig 7**—Loosely knit granulomas in the bone marrow. HP rabbit, 28 days of BCG challenge (H&E, × 200). **Fig 8**—Hypocellular marrow of a LP rabbit. An ill defined loose collection of macrophages with attempted granuloma formation is seen at the right mid-portion of the field (H&E, × 200).