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Protein-Calorie Malnutrition Impairs the Anti-Viral Function of Macrophages[1] (40289)

LLOYD C. OLSON[2], DOUGLAS R. SISK, AND EUGENE IZSAK

Department of Microbiology, Indiana University School of Medicine, Indianapolis, Indiana 46202

Human malnutrition is accompanied by decreased resistance to at least certain infectious diseases (1). This enhanced susceptibility may be mediated by such factors as altered complement metabolism (2), deficient cellular immune responses (3, 4) and decreased production of secretory immunoglobulins (5). Little information is available as to whether macrophage antimicrobial mechanisms are also affected by malnutrition. As an important member of primary host defenses and as an effector cell for many of the cellular immune processes, the macrophage plays a critical role in infectious diseases (6). Consequently any degree of impairment of the efficiency with which this cell performs these roles might be expected to result in considerable reduction in host resistance.

Douglas and Schopfer (7) reported that monocyte phagocytic indices are not altered by severe protein-calorie malnutrition. Passwell *et al.* (8), however, noted that phagocytic capacities of macrophages do seem to be impaired in mice which were protein-deprived. Nevertheless, neither of these reports described the total microbicidal capability of the macrophage. Keusch *et al.* have recently reported (9) that macrophages from mice with kwashiorkor kill *Staphylococcus aureus, E. coli* and salmonella normally *in vitro*. These authors infer that *in vivo* however, it appears likely that macrophage contributions to host defenses are impaired.

We have recently described a model in mice which demonstrates that age-specific resistance to ip infection with Wesselsbron virus (WBV) is macrophage-mediated (10). Resistance by mice is essentially complete by age 2–3 weeks, and represents the acquired ability of peritoneal macrophages to phagocytose and to destroy infectious virus. The present report represents studies on the resistance of protein-calorie malnourished mice to WBV infection and whether macrophage antiviral function is concomitantly affected.

Materials and methods. Mice. Randombred 3-week old white mice were individually caged and allowed free access to water and food. Experimental mice were placed on protein-depletion diet USP XV composed of 84% white dextrin, 9% corn oil, 4% salt mixture, 2% agar, 1% cod liver oil and vitamin supplement (ICN Nutritional Biochemicals). Control mice were fed a normal protein diet containing 27% casein. Under these conditions normal mice showed a mean increase in body weight of 5.3% after 5 days while protein-depleted mice lost a mean of 12.9% body wt during the same period. Unless otherwise noted resistance to infection was determined in mice that had been fed protein-depletion diet for 5 days and continued on this diet during the observation period.

Virus. The source and preparation of WBV was as previously published (10). Infectivity was assayed by inoculating 1-day old mice intracerebrally (i.c.); serial tenfold dilutions were made and each dilution was inoculated into one litter (9–14 mice). End-points were determined by summarizing mortality 14 days later and calculated by the method of Reed and Muench (11).

Results. Of 21 normal mice inoculated ip with WBV (10^8 LD_{50} as assayed in suckling mice), none developed signs of illness nor died. In contrast, each of 11 protein-depleted mice developed symptoms of encephalitis; three of these mice were sacrificed on day 5 and brain tissue was assayed for WBV while the other 8 mice succumbed within 7 days of inoculation. WBV, $10^{5.3}$ LD_{50} per 0.1 g tissue was recovered from each of the brains tested. One of 12 protein-depleted mice observed as uninoculated controls died after 5 days but no virus could be recovered from its brain.

To study how rapidly susceptibility to WBV developed, a series of mice were inoc-

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[2] Present address: The Children's Mercy Hospital, Kansas City, MO 64108.

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ulated ip with WBV at various times before or after switching them to the protein-depletion diet. The details and results of this experiment are shown in Table I. Mice that were inoculated with WBV 3 days or longer before initiating protein depletion showed normal resistance to virus. However, susceptibility to ip infection with WBV rapidly developed in relation to protein depletion such that even animals inoculated one day previously showed decreased resistance to infection. Signs of encephalitis appeared 5–7 days after inoculation in all mice succumbing to infection.

These data implied that some event occurred relatively early in the initial stage of infection that was sensitive to protein depletion. To support this idea it was necessary to determine how soon after ip inoculation WBV could be detected in the central nervous system. Groups of mice on normal diet or after 5 days on depletion diet were inoculated ip with 10^8 LD_{50} of WBV as before, and brain tissue from two animals of each group was assayed daily thereafter (Table II). In normal mice very small amounts of WBV were recovered 1–3 days postinoculation, while samples collected on days 4–7 contained no detectable virus. Simultaneously collected samples of blood obtained by section of the axillary vessels contained similar concentrations of virus and presumably represented the source of virus present in the brain samples. In contrast, much greater concentrations of WBV could be detected in blood from protein-depleted mice. Brain samples also had concentrations of virus of similar magnitude on days 3 and 4, although the presence of viremia made the origin of this virus uncertain. By day 5, however, the titer of virus in the brain significantly exceeded that in the blood and by day 6 the animals had developed encephalitis.

Unstimulated peritoneal macrophages were collected from normal and from PCM mice and the susceptibility of WBV to inactivation by these cells was studied by the methods previously described (10). Briefly, cells were collected by washing the peritoneal cavity with phosphate–buffered saline (PBS, pH 7.2). The cell suspension was inoculated into glass bottles and allowed to attach for 2 hr at 37°. Nonadherent cells were removed by repeated vigorous washing with PBS. The adherent cells were resuspended by scraping and cultured in medium 199 at a concentration of 10^6 cells per ml. WBV was added at a multiplicity of infection of one and allowed to adsorb 1 hr at 37°. The cultures were then washed 3 times and fresh medium 199 was

TABLE I. DEVELOPMENT OF SUSCEPTIBILITY TO WBV IN MICE PLACED ON PROTEIN-DEPLETION DIET BEFORE OR AFTER VIRUS INOCULATION.

	WBV inoculated on day[a]					
	−5	−3	−1	+1	+3	+5
No. mice inoculated	10	10	10	10	10	10
No. mice surviving	10	10	2	0	0	0

[a] Mice were switched from normal to protein-depletion diet on day 0. WBV, $10^8 LD_{50}$ (suckling mouse assay) was inoculated intraperitoneally on days indicated before (minus days) or after (plus days) switching diets. Mortality for each group of mice was summarized 14 days after inoculation with WBV.

TABLE II. WBV TITER IN BLOOD AND BRAIN TISSUE AFTER INTRAPERITONEAL INOCULATION OF NORMAL AND PCM MICE.

	Days postinoculation						
	1	2	3	4	5	6	7
Mice							
Normal: blood[a]	1.2	1.0	1.3	0.6	0	0	0
brain[b]	0.9	1.2	0.3	0	0	0	0
PCM: blood[a]	2.2	3.3	3.3	3.3	4.6		
brain[b]	2.6	2.9	4.1	4.8	8.3		

[a] Log_{10} LD_{50} per 0.05 ml serum.
[b] Log_{10} LD_{50} per 0.1 g tissue.

added at time 0. At intervals some cultures were rapidly frozen and thawed 3 times, debris was removed by centrifugation and the supernatant virus content was assayed. The experiments were run in triplicate and assay results were pooled by summing mortality of the individual titrations. In no instance did the end–points of individual titrations disagree by as much as one $\log_{10}$ dilution. The results of these experiments are illustrated in Fig. 1. Whereas infectious WBV had completely disappeared by 24 hr in macrophages obtained from normal animals there was an obvious difference in the ability of macrophages from PCM mice to inactivate WBV. This suggests that the extraperitoneal dissemination that occurred to a much greater extent in PCM mice (Table II) resulted from the decreased capacity of local defense mechanisms to inactivate and contain the infectious inoculum. Presumably, macrophages were a major contributor in the population of cells studied.

Discussion. The effects of malnutrition on host resistance to virus infections may be variable. Measles infections are a classic example of the increased susceptibility to severe and often fatal effects of disease occurring in the malnourished host (12).

Experimentally, malnourished mice have been shown to have decreased resistance to coxsackie B3 virus (13) but apparently increased resistance to pseudorabies virus (14). Host resistance to different viruses represents a complex interaction of many mechanisms, each affected to varying degrees by malnutrition. Thus, contrasting results with individual viruses with distinctive pathogenetic schemes is not surprising. The model employed here attempts to isolate the effect of the macrophage.

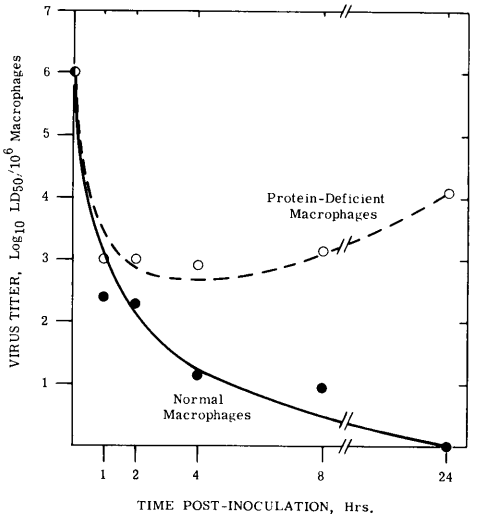

FIG. 1. Infection of macrophages with WBV. Titer of infectious virus present at intervals after inoculating cultures of macrophages obtained from normal and protein-deficient mice.

Although mice normally develop age-specific resistance to WBV the target organ (central nervous system) remains susceptible to infection and disease (10). Thus, the data presented here on the loss of resistance to peripheral (ip) infection in protein-calorie malnourished mice implies that local resistance is impaired, and that moreover the events mediating resistance are rapidly sensitive to the deleterious effects of this malnutrition. In normally nourished mice only limited amounts of virus gain access to the circulation after ip inoculation, and this is apparently below the threshold required to initiate infection in the central nervous system. Local restriction of the virus inoculum was not effective with protein-calorie malnutrition; large amounts of virus appeared in the circulation and encephalitis ensued.

The direct interaction of WBV and macrophages cultured *in vitro* suggested that a primary effect of protein-calorie malnutrition was on the ability of macrophages to restrict WBV infection. Significant levels of infectious virus presisted throughout the time period studied and in fact WBV may have replicated in the macrophages from malnourished mice. This must at least in part account for the susceptibility of these mice to WBV infection. In this regard they would be similar to newborn mice which are susceptible to infection for similar reasons (10). These results suggest that impaired macrophage function is an additional feature of protein-calorie malnutrition that contributes to the susceptibility of such individuals to certain virus diseases. Since the mechanism by which macrophages exert antiviral effects is not understood the cellular basis of the defect is obscure.

Summary. Mice which are normally resistant to infection with Wesselsbron (WBV)

virus became rapidly susceptible to disease due to this agent after being placed on protein-depletion diet. After ip inoculation large amounts of virus appeared in the circulation followed by fatal encephalitis. In normally fed mice only small amounts of virus could be detected in blood and no disease developed. This suggested that local defense mechanisms which normally restrict the extent of infection was sensitive to the early effects of protein-calorie malnutrition. That this was due at least in part to impaired antiviral function of macrophage under these conditions was confirmed by *in vitro* macrophage studies. Over the course of 24 hr infectious WBV disappeared after inoculation into cultures of normal macrophages whereas infectivity persisted at high titers in macrophages from protein-depleted mice.

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