Influence of Protein Deficiency on 19S Antibody-forming Cells in Rats and Mice

MEERA MATHUR, V. RAMALINGASWAMI AND M. G. DEO
Department of Pathology, All-India Institute of Medical Sciences, New Delhi-16, India

ABSTRACT Influence of protein deficiency on immune response to sheep red blood cells was investigated in rats and mice with Jerne's plaque-forming cell technique. Protein deficiency resulted in a reduction in the number of antibody-producing cells consequent upon a prolongation of cell generation time in rats. Injection of syngeneic thymocytes brings about better improvement in the immune response of the X-radiated protein-deficient mice as compared to the controls. It is suggested that depressed immunity in malnutrition may be partly due to a disturbance in thymic function. J. Nutr. *102:* 841–846, 1972.

INDEXING KEY WORDS protein deficiency · antibodies

Protein malnutrition which is widespread throughout the developing countries of the world, increases susceptibility of the host to infection both in man and experimental animals (1, 2). Although data on production of antibodies in malnutrition in man are controversial (3, 4), in animals synthesis of antibodies is said to be depressed (5–7).

Earlier studies from this laboratory have indicated that one of the fundamental lesions of protein deficiency is a marked depression of the rates of cellular proliferation in the organs of high cell turnover such as the intestine, bone marrow and growing ends of bones (8, 9). Proliferation of the antibody-producing cells may likewise be affected.

Recent work of Mitchell and Miller (10) indicates that in mice thymocytes, which are essential for the formation of antibodies against sheep red blood cells (SRBC), are the antigen reactive cells. Protein deficiency is usually associated with atrophy of thymus which may depress immune response to certain antigens (11).

Using Jerne's plaque-forming cell (PFC) technique (12) we have investigated the influence of protein deficiency on the primary immune response and the kinetics of proliferation of PFC in rats. A study has also been made of the improvement brought about in PFC in X-radiated deficient mice given syngeneic thymocytes.

MATERIALS AND METHODS

Two sets of experiments were performed. In the first, male albino rats weighing 40 to 50 g obtained from the stock colony were used. They were divided into two dietary groups, a protein-deficient and a control group. The animals in the deficient group were fed ad libitum a diet deficient in protein (3% casein) but otherwise adequate in other nutrients. The animals in the control group were fed a similar diet except that it contained 16% casein. Each animal in the control group was pair-fed with the corresponding animal in the deficient group. The compositions of the diets fed to the rats are shown in table 1. The compositions of vitamin and mineral mixtures were the same as used in this laboratory and described earlier by Bhuyan et al. (13).

After consuming the diets for 3 to 4 weeks, animals in both the groups were given intraperitoneally a single dose of 10^9 SRBC, and direct PFC which denotes 19S antibody producing cells was measured on days 3, 4 and 6. The rate of division of PFC was assessed with the use of colchicine after the method of Rowley et al. (14) in five deficient and five control animals. The animals received 1 mg/kilogram body weight of colchicine intraperitoneally on day 3, and PFC was measured 4 hours after administration of the drug.

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TABLE 1

Composition of diets — rats [1]

Constituents	High protein	Low protein
	%	
Casein	16	3
Sago	10	10
Sucrose	63	76
Ground nut oil	5	5
Vitamin mixture [2]	2	2
Salt mixture [3]	4	4

[1] To every 100 g of the diet, 1 g of choline and 1.5 g of a mixture of vitamin A, D and E (cod liver oil, 500 g + vitamin E (α-tocopheryl acetate), 1 g) were added.
[2] Vitamin mixture (g): thiamin 0.15, riboflavin 0.25, pyridoxine 0.15, vitamin B_{12} 0.005, folic acid 0.05, biotin 0.005, inositol 5.00, nicotinic acid 0.50, calcium pantothenate 0.5, ascorbic acid 0.1, PABA 1.0, vitamin K_3 (menadione) 0.05, sucrose to make 1000.
[3] Salt mixture (g): $CaCO_3$ 390.0, $CaHPO_4 \cdot 2H_2O$ 682.0, $Na_2HPO_4 \cdot 2H_2O$ 375.0, KCl 392.0, $MgSO_4 \cdot 7H_2O$ 121.0, ferric ammonium citrate 34.0, $MnSO_4 \cdot 4H_2O$ 1.8, $CuSO_4 \cdot 5H_2O$ 2.8, $ZnSO_4$ 0.1, KI 0.1, NaF 1.0, $KAl(SO_4)_2 \cdot 12H_2O$ 0.2.

In the second set of experiments a total of 32 inbred A (strong) strain of male mice, 4 to 5 weeks old were used. It was essential to use inbred animals since a part of the experiment involved transfusion of thymocytes from a syngeneic donor. The animals were divided into two equal groups fed low and high protein diets. Each animal in the high protein group was pair-fed with the corresponding animal in the low protein group. The compositions of the diets used for mice are shown in table 2. Compositions of vitamin and mineral mixtures were the same as used for rats. Primary immune response, as judged by the number of PFC formed on day 3 of intraperitoneal administration of 10^9 SRBC, was measured in six animals in each group. Using a cobalt unit, the rest of the animals in both the groups were subjected to 850 r X-radiation with marrow protection. On the day of X-radiation, five animals from both the groups were given intravenous transfusion of 50×10^6 thymocytes from a normal syngeneic donor on the stock diet along with 10^9 SRBC. As a control, SRBC were given alone to an equal number of animals in both the groups. The immune response (PFC) was measured on day 8.

Protein deficiency is associated with marked atrophy of spleen. As such, PFC expressed per spleen would have been low in the deficient animal merely because of splenic atrophy. In such situations the results expressed after equalization of cells are more informative since they denote the size of the differentiated clone of cells. Therefore, the results both in rats and mice were expressed as PFC per 10^6 spleen cells.

RESULTS

Experiments with rats. The animals on the low protein diet stopped growing within 3 to 4 days of being placed on the diet. On the other hand the animals fed the high protein diet grew well. At the end of 4 weeks of dietary regime the protein-deficient animals weighed less than half of the control animals (table 3).

There was a marked atrophy of the lymphoid tissue in the protein-deficient

TABLE 2

Composition of diets — mice [1]

Constituents	High protein	Low protein
	%	
Casein	16	1
Sago	20	20
Sucrose	53	68
Ground nut oil	5	5
Vitamin mixture [2]	2	2
Salt mixture [3]	4	4

[1] To every 100 g of diet 1 g of choline and 1.5 g of the vitamin A, D and E mixture were added (see footnote 1, table 1).
[2,3] See footnotes 2 and 3, table 1.

TABLE 3

Body weights of rats on low and high protein diets

Diet		Basal	Weeks on the experimental diet 1	2	3	4
Low protein (16) [1]	Mean (g)	42.3	38.7	35.7	33.4	33.1
	SD	±5.329	±4.416	±4.324	±3.661	±4.796
High protein (16)	Mean (g)	43.2	50.0	59.3	69.5	76.5
	SD	±5.771	±3.082	±4.243	±4.919	±5.621

[1] Figures in parentheses denote the number of animals.

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animals. The mean ratio of the weights of thymus/spleen was only 0.18 in the deficient animals as opposed to 0.41 in the high protein controls, indicating that the thymus was preferentially affected in protein deficiency (table 4). Atrophy of thymus was predominantly due to depletion of cortical lymphocytes. There was a marked atrophy of germinal centers of the spleen in the deficient animals. In some, the change was so marked that the lymphoid follicles were represented by collections of a few lymphocytes.

The average numbers of PFC in the deficient animals were markedly depressed on all days, compared with the control animals (table 5).

Figure 1 shows the effect of administration of colchicine on the number of PFC. Four hours after administration of the drug, the average number of PFC in the high protein animals fell from a value of 78.6 to 24. The rate of disappearance of antibody forming cells in the protein-deficient animals was tardy. During the same interval, the deficient group showed a fall to 9.6 from the original value of 14.4.

Experiments with mice. As in the rats, feeding a protein-deficient diet resulted in a reduction in the number of PFC in mice (table 5).

Both in the controls and deficient animals, administration of syngeneic thymocytes resulted in a marked improvement in the response of X-radiated animals to SRBC. The net improvement of 184.3% in the protein-deficient group was about three times that observed in the controls (table 6).

TABLE 4

Influence of protein deficiency on weights of thymus and spleen

Group	No. of animals	Thymus	Spleen	Thymus / spleen
		mg	*mg*	
Low protein (LP)	5			
Mean		11.4	60.8	0.18
Range		8–16	41– 79	
High protein (HP)	5			
Mean		67.2	156.8	0.41
Range		49–90	86–210	
LP vs. HP		$P < 0.01$	$P < 0.01$	

TABLE 5

Plaque-forming cells in protein-deficient rats and mice

	Days following injections of SRBC [1]					
	3		4		6	
	LP [2]	HP	LP	HP	LP	HP
Rats						
Mean	14.4(5) [3]	78.6(5)	42.0(6)	188.6(6)	33.2(5)	108.0(5)
SD	±12.43	±31.38	±14.11	±49.39	±14.36	±13.26
	$P < 0.01$		$P < 0.001$		$P < 0.01$	
Mice						
Mean	48.0(5)	166.0(5)				
SD	±22.09	±47.74				
	$P < 0.001$					

[1] Results expressed as PFC/10^6 spleen cells.
Student t test for rats:
LP day 3 vs. day 4: $P < 0.01$
day 4 vs. day 6: $P < 0.2$ (not significant)
HP day 3 vs. day 4: $P < 0.01$
day 4 vs. day 6: $P < 0.01$

[2] LP: low protein; HP: high protein.

[3] Figures in parentheses denote the number of animals used.

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DISCUSSION

Conflicting views have been expressed regarding the status of humoral immunity in protein malnutrition. Thus it has been reported that antibody formation against Kahn lipid antigen, and typhoid and paratyphoid endotoxins was normal in undernourished children (15, 16). On the other hand, Olarte et al. (17) found depression of antibody formation to diphtheria toxoid. There is also no unanimity regarding the levels of serum IgG which have been reported to be elevated, normal or decreased (18–20) in protein malnutrition. Very little is known about the status of thymus-dependent immune responses in protein deficiency.

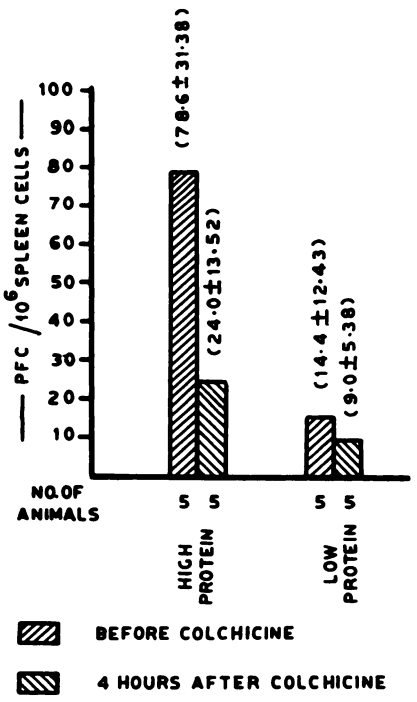

Fig. 1 Rate of disappearance of PFC following administration of colchicine. Figures in parentheses denote mean and standard deviation.

The present observation of a depression of PFC is in agreement with those of Kenney et al. (6) and Munro (7). In kwashiorkor, turberculin reaction is subnormal or absent even in the presence of an active pulmonary focus (21). Both the formation of the PFC and tuberculin reaction are thymic-dependent immune responses (22). These observations suggest that thymic-dependent immune responses are affected in malnutrition.

Depression of immune response could be either due to diminished differentiation of the immunological competent cells to PFC and/or poor proliferation of differentiated clones of PFC. Rowley et al. (14) have recently used colchicine, which blocks PFC in metaphase and makes them ineffective, to study the rate of division of PFC. Slower rate of disappearance of PFC in the protein-deficient animals following administration of colchicine, in the present study, indicates that the cell generation time of the PFC is prolonged in malnutrition. Depression of cellular proliferation which is

TABLE 6

Effect of injection of syngeneic thymocytes on production of PFC in X-radiated protein-deficient and control mice

Groups	PFC per 10^6 spleen cells on day 8 after 850 r		Percentage improvement
	SRBC [1]	SRBC + Thymocytes	
Low protein (LP)			
Mean	19.2(5) [2]	54.6(5)	184.3
Range	0–32	31–90	
		$P < 0.01$	
High protein (HP)			
Mean	68.4(5)	110(5)	60.8
Range	50–90	80–129	
		$P < 0.02$	
P (LP vs. HP)	< 0.01	< 0.01	

[1] SRBC or SRBC + thymocytes were given immediately after X-radiation. Thymocytes were obtained from syngeneic animals on stock diet.
[2] Figures in parentheses denote the number of mice used.

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also observed in other tissues appears to be the fundamental lesion of protein deficiency (8, 23).

As opposed to the generation time, the doubling time as calculated from the number of PFC on days 3 and 4 of administration of SRBC is lower in the deficient as compared with the control animals. This paradox may be explained on the basis of lower rate of "death" of PFC in the deficient animals. Although there is no information on this, it may be mentioned that the effective life span of the jejunal villus cells is prolonged in protein-deficient monkeys and rats (9, 23). This may be true also of the PFC. It is believed that in the log phase, besides proliferation of already differentiated PFC, there is a considerable extent of "recruitment" of immunologically competent cells to PFC (24, 25). The lower doubling time of the PFC in protein deficiency may be also due to a relative higher rate of "recruitment."

The role of macrophages in induction of the primary immune response is now well recognized (26). Recent studies from this laboratory indicate that the phagocytic activity of the reticulo-endothelial cells of the liver and spleen is markedly depressed in malnutrition in monkeys and rats (27). Besides the macrophages, cooperation of antigen-reactive thymocytes and antibody-producing bone marrow-derived lymphocytes is essential for induction of immune response by certain antigens (10, 28). Under certain conditions such as administration of hydrocortisone, bone marrow-derived antibody-producing cells are selectively destroyed (29). Malnutrition results in atrophy of both the thymus and bone marrow (11, 30). Depression of immune response in malnutrition may be due to a reduction in the number of thymocytes and/or bone marrow-derived cells.

Injection of syngeneic thymocytes results in better improvement of immune response in X-radiated protein-deficient animals as compared with the controls. A part of the depression in the formation of PFC in protein deficiency may be, therefore, attributed to the atrophy of the thymus. The role of other factors such as depression of macrophage function and atrophy of bone marrow-derived antibody-producing cells in bringing about depression of antibody formation in malnutrition is being currently investigated.

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