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Depression of T-cell function and normality of B-cell response in protein calorie malnutrition

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Summary. Antibody response to a T-cell dependent antigen, sheep erythrocytes and to a B-cell mitogen, purified lipopolysaccharide (LPS), has been studied in mice kept on protein deficient (2 and 4 per cent casein) diets. The number of plaque-forming cells (PFC) to SRBC were $20{\cdot}5 \pm 7{\cdot}7$ per million spleen cells in protein-deficient animals compared to $216{\cdot}0 \pm 31{\cdot}1$ in parallel controls maintained on a protein rich diet (18 per cent casein). No difference was observed in number of PFC formed in controls and deficient animals to LPS, values were $161{\cdot}4 \pm 19{\cdot}7$, $158{\cdot}5 \pm 14{\cdot}2$, & $162{\cdot}3 \pm 31{\cdot}9$ in control (18 per cent casein) and deficient groups (4 per cent and 2 per cent casein) respectively. The delayed hypersensitivity skin reaction to SRBC measured in foot pads was significantly lower in mice on 4 per cent casein diet compared to controls. These studies suggest that the effect of protein deficiency is primarily on T-cell function and not on the B-cell response.

INTRODUCTION

Protein calorie malnutrition (PCM) is widely prevalent in developing countries. Scarcity of protein during growth interferes with the development of many tissues (Smythe, Schonland & Breveton-Stiles, Coovadia, Grace, Loening, Mayfoyane, Parent & Vos, 1971). The immune system appears also to be adversely affected in PCM (Faulk, Demaeyer & Davies, 1974). Cell-mediated immunity (CMI) has been shown to be depressed in both experimental animals, and in human subjects (Bhuyan & Ramalingaswami, 1973; Harland, 1965). Some investigators have however observed that CMI may be enhanced in mice rendered moderately protein deficient (Cooper, Good & Mariani, 1974). The status of humoral immunity appears variable and related to the nature of the antigen used. In man, immunization with diptheria toxoid (Scrimshaw & Behar, 1971) and tetanus toxoid (Cooper, Good & Mariani, 1974) elicited normal antibody responses whereas response to typhoid–paratyphoid vaccination was impaired in PCM (Chandra, 1972). Response to viral antigens has also been observed to be variable. Immunization with polio and smallpox vaccine evoked a normal antibody response, vaccination with yellow fever on the other hand elicited low antibody titres (Brown & Katz, 1965).

In recent years, evidence has been presented to show that certain antigens and mitogens directly stimulate B cells which then undergo differentiation. Other antigens require T-cell help to stimulate the B cells for the production of antibodies. It is not clear from earlier reports on PCM whether the

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Table 1. Composition of diet

	Control	Protein deficient	
	(18 per cent casein)	(4 per cent casein) g/100 g	(2 per cent casein)
Casein	18	4	2
Starch	63	77	79
Sucrose	10	10	10
*Groundnut oil	5	5	5
†Salt mixture	4	4	4

* Vitamin mixture: 1. To each kg of diet, 12000 u of vitamin A, 200 u of vitamin D and 60 mg of vitamin E were added. Thiamine 50 mg, riboflavine 25 mg, pyridoxine 10 mg, niacinamide 100 mg, calcium pentothenate 25 mg, folic acid 1 mg, ascorbic acid 1 g, inositol 200 mg, para-amino benzoic acid (PABA) 1 g, choline chloride 1 g, biotin 1 mg, vitamin B_{12} 5 μg, menadione (vitamin K) 100 mg.

† Salt Mixture: $CaCO_3$ 300 mg, K_2HPO_4 320 g, $CaH_2PO_4 \cdot 2H_2O$ 80 g, $MgSO_4 \cdot 7H_2O$ 100 g, NaCl 160 g, $Fe(C_6H_5O7)6H_2O$ 36 g, $MnSO_4 \cdot 4H_2O$ 3·4 g, $ZnCl_2$ 200 mg, $CuSO_4 \cdot 5H_2O$, 350 mg, KI 50 mg.

difference in the nature of antigen is responsible for the differential response of the deficient host to immunizations with various agents.

Data presented in this communication show that the lesion in PCM is primarily with respect to T-cell dependent antigens and that the response to B-cell mitogens is essentially unimpaired.

MATERIALS AND METHODS

Male mice of Swiss strain weighing 20–35 g were used in this study. The mice were divided into three groups. Group I formed the control group and were fed *ad libitum* 18 per cent casein diet. Groups II and III were fed *ad libitum* 4 per cent and 2 per cent casein diet respectively and constituted the protein deficient groups. The composition of diets fed to three groups is given in Table 1. The animals were kept on the prescribed diet for 5–6 weeks. Body weights were recorded weekly. Protein deficiency was monitored by fall in body weight and loss of hair.

Serum proteins were estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). Serum albumin and globulins were measured by electrophoretograms of serum on paper strips which were stained with 0·1 per cent bromophenol blue and subjected to subsequent densitometric scanning.

Antigens

SRBC were collected in Alsever's solution. Before use, these were washed three times in 10 mM sodium phosphate-buffered saline (PBS) pH 7·4.

Purified Lipopolysaccharide (LPS) was a kind gift of Dr Fritz Melchers, Basel Institute for Immunology, Basel, Switzerland.

Plaque-forming cells (PFC)

(i) *PFC to SRBC.* 10^8 SRBC were injected i.p. into mice of 20–25 g body weight and the number of PFC determined per million spleen cells essentially by the method of Jerne & Nordin (1963).

(ii) *PFC to LPS.* LPS was dissolved in 10 mM PBS pH 7·4 and heated in a boiling water bath for 60 min before use.

100 μg of LPS was injected into mice of 20–25 g body weight i.p. Specific PFC to LPS were determined by the method of Watson & Riblet (1974).

In brief, 1 ml of LPS (1 mg/ml) was added to 0·2 ml of packed SRBC and incubated for 90 min at 37°. The coated SRBC were washed five times with 10 mM PBS pH 7·4. Coated cells at 1/15 dilution in PBS were used for the demonstration of plaques and at 1/100 dilution for the estimation of haemagglutinating antibody titre. 1/15 dilution of coated cells were layered on agar plates. Parallel controls were run with uncoated SRBC. The specific PFC to LPS were determined by subtracting PFC to uncoated cells from PFC to LPS coated cells.

Lyophilized guinea-pig complement (Miles Laboratories, Kankakee, code no. 64-283) was used for both assays at 1/10 dilution.

Delayed hypersensitivity reaction (DHR)

DHR *in vivo* was studied essentially by the method of Larange, Mackaness & Miller (1974). 10^7 SRBC were injected into the foot-pad of mice weighing 15–20 g. 4 days after immunization, an eliciting dose of 10^8 cells in 50 μl was injected into the other foot-pad and thickness measured after 24 h using Schnelltaster Kroplin Calipers.

Antibody titres

Blood was drawn from mice by retro-orbital bleeding prior to killing for estimation of PFC (i.e. on day 4 from mice immunized with SRBC and on day 7 from mice immunized with LPS) and sera were separated. The antibodies against SRBC and LPS coated SRBC were determined by haemagglutination methods described elsewhere (Herbert, 1973; Watson & Riblet 1974).

RESULTS

Adult mice weighing from 25–33 g were pooled and distributed randomly in two groups. The mice kept on 4 per cent and 2 per cent casein diet lost weight progressively as shown in Fig. 1. These were taken for immunological studies at 6 and 5 weeks of maintenance on 4 per cent and 2 per cent casein diets. The serum proteins in three groups of animals at the time of sacrifice were estimated and given in Table 2. The decrease in serum proteins in group II was 36 per cent and in Group III 40 per cent. (1) Group I and II *P* value for total proteins, < 0·001; albumin, < 0·001; globulin < 0·001; (2) Group I and III *P* value for total proteins, < 0·001; albumin < 0·001; globulin < 0·001. (3) Groups II and III *P* value for total proteins, n.s.; albumin = 0·001; globulin < 0·05. The total serum proteins were lower by 36–40 per cent in animals on protein deficient diet. The fall is more prominent in the albumin content.

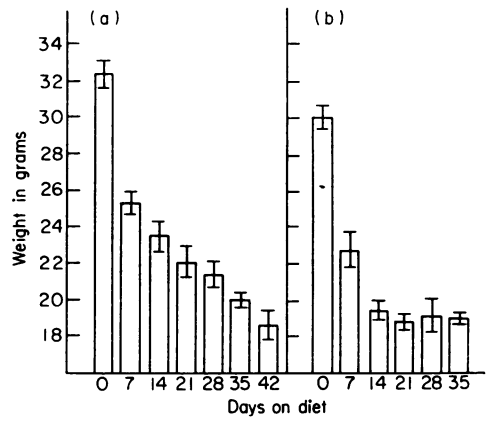

Figure 1. Body weights of mice fed protein deficient diet. Each point on the histogram represents mean weight of twenty five to thirty animals. Values are mean ± s.e. (a) Four per cent casein diet; (b) 2 per cent casein diet.

Antibody response to SRBC

The kinetics of PFC to SRBC in normal mice is shown in Fig. 2. The optimum response was 4 days after immunization and subsequent studies were done on day 4. There was a ten-fold decrease in

Table 2. Serum proteins in control and protein-deficient mice

Group	Total proteins	Albumin g/100 ml	Globulins
I 18 per cent casein diet	8·0 ± 0·22 $n = 20$	4·4 ± 0·10 (3·1–5·1) $n = 20$	3·61 ± 0·01 (2·9–4·6) $n = 20$
II 4 per cent casein diet	5·1 ± 0·14 $n = 25$	2·4 ± 0·12 (1·6–3·5) $n = 30$	2·73 ± 0·08 (1·6–3·5) $n = 30$
III 2 per cent casein diet	4·8 ± 0·22 $n = 14$	1·77 ± 0·11 (0·74–2·6) $n = 18$	3·0 ± 0·11 (2·2–4·0) $n = 18$

Values are mean ± s.e; figures in parentheses give the range and n denotes the number of animals studied in each group.

PFC to SRBC in mice maintained on 4 per cent casein diet (Table 3). The haemagglutinating antibody titres were also significantly lower in these animals (Table 3).

Antibody response to LPS

The kinetics of PFC to LPS in normal mice is shown in Fig. 2. The optimum response was 7 days after immunization and subsequent studies were done on day 7. Table 4 summarizes the results of PFC to a T-cell independent antigen LPS. It is clear that the response to LPS is similar in all the groups of animals. It is interesting that even in the group of animals fed 2 per cent casein for 5 weeks and in whom serum albumin levels had decreased to 1·77 g per 100 ml, there was no reduction in PFC to LPS. Similarly, the antibody titres in the two protein deficient groups were not reduced to any significant extent.

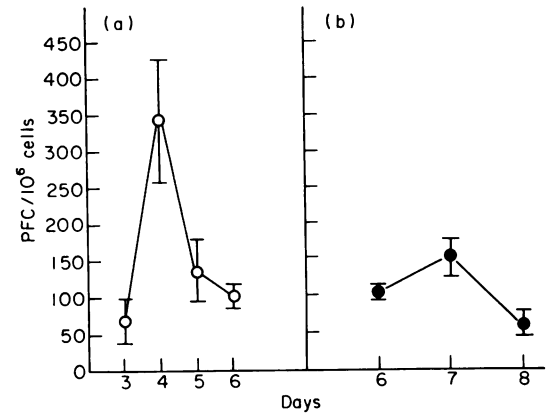

Figure 2. Kinetics of PFC to SRBC and LPS. Each point on the figure represents the mean PFC of five to eight animals. Values are mean±s.e. (a) PFC to SRBC; (b) PFC to LPS.

Table 3. Antibody response to sheep erythrocytes in mice fed on protein-rich and deficient diet

	18 per cent casein	4 per cent casein	*P* value
I Plaque-forming cells per 10^6 spleen cells	216·0±31·1 *n* = 9	20·5±7·7 *n* = 10	<0·001
II Haemagglutinating antibody titre			
10–40	0	9	
80–320	8	2	
640–1280	6	—	
	n = 14	*n* = 11	

n Denotes number of animals studied in each group, values are mean±s.e. Antibody titres are expressed as the reciprocal of the highest dilution of the sera causing haemagglutination.

Table 4. Antibody response to lipopolysaccharide in mice fed on protein-rich and deficient diets

	18 per cent casein	4 per cent casein	2 per cent casein	*P* value
I LPs-specific plaque forming cells per 10^6 spleen cells	161·4±19·7 *n* = 12	158·5±14·2 *n* = 15	162·3±31·9 *n* = 11	n.s.
II LPs-specific haemagglutinating antibody titres				
10–80	0	0	0	
160–640	9	8	8	
1280–5120	12	13	6	
	n = 21	*n* = 21	*n* = 14	

n denotes number of animals studied in each group; values are mean±s.e. Antibody titres are expressed as the reciprocal of the highest dilution of the sera causing haemagglutination of LPS-coated sheep erythrocytes.

Table 5. Delayed hypersensitivity reaction to sheep erythrocytes *in vivo*

	18 per cent casein	4 per cent casein	*P*
Foot-pad swelling 24 h (mm)	0·46±0·022 (0·4–0·5) *n* = 12	0·25±0·026 (0·2–0·4) *n* = 8	<0·001

n denotes number of animals studied in each group; values are mean±s.e.

Delayed hypersensitivity to SRBC (DHR)

Pilot experiments showed that the optimum DHR was obtained in the normal animals on 4th day after immunization (Fig. 3). Thus on day 4 after foot-pad immunization, an eliciting dose of 10^8 cells in 50 μl was administered in the contralateral foot-pad. Delayed hypersensitivity reaction was measured by thickness of foot-pad after 24 h. The results are summarized in Table 5. The foot-pad thickness was reduced significantly in protein-deficient group as compared to controls on 18 per cent casein diet ($P < 0{\cdot}001$).

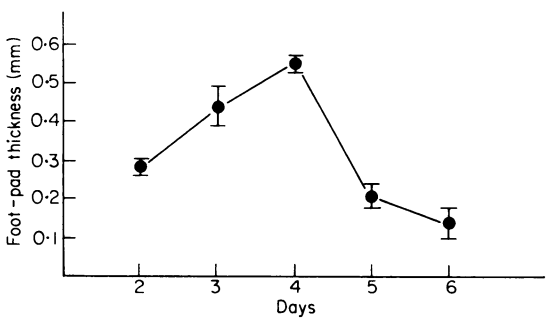

Figure 3. Kinetics of delayed hypersensitivity reaction to SRBC. Foot-pad thickness was measured 24 h after injection of eliciting dose of SRBC. Each point is a mean of five animals. Values are mean±s.e.

DISCUSSION

This study provides evidence for a differential humoral response of protein-deficient mice to thymus-dependent and thymus-independent antigens. Mice were maintained on 4 per cent and 2 per cent casein diets for 6 weeks and immunized with a T-cell dependent antigen, sheep erythrocytes (Katz & Benacerraf, 1972) and a B-cell antigen, purified lipopolysaccharide (Andersson, Sjoberg & Moller, 1972). It was noted that protein deficient mice showed a ten-fold decrease in plaque-forming cells to SRBC but gave normal response to lipopolysaccharide (Tables 3 and 4).

Thymus-dependent T cells interact or help antibody-forming cells in the immune response to SRBC (Katz & Benacerraf, 1972). Early in this response depending on the dose and route of antigenic administration, a transient delayed hypersensitivity also develops (Larange, Mackaness & Miller, 1974). It is evident from our data that both these functions of T cells e.g. the helper function and the delayed hypersensitivity reaction are significantly reduced in animals subjected to selective protein insufficiency. These results are analogous to the human situation, where populations suffering from protein-calorie malnutrition show impaired tuberculin response to BCG (Harland, 1965; Harland & Brown, 1965).

The unimpaired humoral response to the thymus-independent antigen, lipopolysaccharide indicates that if T-cell helper function is bypassed by an antigen, then protein insufficiency has no effect on the B-cell response. Normal production of plaque-forming cells to LPS were noted not only in the mice subjected to 4 per cent casein diet but also in those receiving 2 per cent casein diet. Thus even severe protein insufficiency does not impair the humoral response to thymus-independent antigen. These results may further explain relatively unaltered immunoglobulin levels noted in PCM. The lymphoid cells of the mesenteric and cervical lymph nodes are constantly stimulated by endotoxin-like material from the gastrointestinal tract and a cumulative effect might result in maintenance of a normal or even raised immunoglobulin levels.

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