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The effect of protein malnutrition on the IgA immune response in mice

D. W. McGEE & D. N. McMURRAY *Department of Medical Microbiology and Immunology, Texas A & M University, College Station, Texas, U.S.A.*

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SUMMARY

The influence of protein malnutrition on the IgA immune response was determined in BALB/c mice fed isocaloric diets containing 20% (control), 4%, or 2% protein. We describe here a severely protein-deficient state (6 weeks on a 2% protein diet) and a moderately malnourished state (6–8 weeks on a 4% protein diet). The total IgA concentration in intestinal washes, as determined by radial immunodiffusion, was reduced at 6 and 8 weeks in the 4% diet group and at 6 weeks in the 2% diet group, compared to the controls. Serum IgA levels were significantly elevated in both the 2% and 4% diet-fed groups at all time intervals. The IgA anti-sheep red blood cell (SRBC) plaque-forming cell (PFC) response generated after oral immunization with SRBC did not differ between the control and the 4% diet group at any time interval, yet the IgA PFC/spleen response was significantly reduced in the 2% diet group at all time intervals studied. However, the IgA PFC/10^6 spleen cells was reduced only with the 2% protein diet group at 6 weeks. Severely protein-deficient mice replenished with the control diet for 3 weeks showed a recovery to values similar to the 8-week control group of both the IgA PFC response and the total IgA concentration in intestinal washes. These results suggest that protein deprivation leads to a reversible reduction in the IgA response to antigens encountered at the intestinal mucosa.

INTRODUCTION

Protein and protein-energy malnutrition in children are commonly associated with an increased incidence of mucosal infections and diarrhea, suggesting a malnutrition-induced defect in the mechanisms for protection of mucosal surfaces (Scrimshaw, Taylor & Gordon, 1968; Watson & McMurray, 1979). Various studies have documented a reduction in the concentration of IgA, the major immunoglobulin found in external secretions (Chodirker & Tomasi, 1963), in tears, salvia, and nasal secretions, of malnourished children (Reddy, Raghuramulu & Bhaskaram, 1976; McMurray *et al.*, 1977; Sirisinha *et al.*, 1975; Chandra, 1975). However, the IgA levels in intestinal fluids have been found to be both increased (Bell *et al.*, 1976b) and decreased (Reddy *et al.*, 1976).

The response to specific antigens is also depressed with malnutrition. Normally, antigens encountered by the oral route will induce sensitization in the Peyer's patches of specific IgA-producing cells, which then migrate via the thoracic duct and circulatory system to various mucosal tissues (Strober & Jacobs, 1985). Chandra (1975) has found reduced levels of virus-specific IgA antibodies in nasopharyngeal washes of malnourished children previously immunized with oral vaccines. Barry & Pierce (1979) have reported a decrease in the number of cholera toxin-specific immune cells in the thoracic duct and lamina propia of orally immunized protein-malnourished rats.

In this report, diets containing various amounts of protein were used to determine the effect of protein deprivation on the IgA immune system. Since the spleen is also a target organ for the systemic IgA response to an orally introduced antigen (Bazin, Levi & Doria, 1970; Andre, Bazin & Heremans, 1973), the response of mice on diets to orally introduced SRBC was determined as the number of anti-SRBC PFC produced in the spleen. The levels of IgA present in the serum and intestinal washes of mice on the diets were also compared.

Corespondence: Dr D. W. McGee, Dept. of Medical Microbiology and Immunology, Texas A & M University, College Station, TX 77843, U.S.A.

MATERIALS AND METHODS

Experimental animals and diets

Female BALB/c mice (Harlan Sprague-Dawley; Houston, TX), 3–4 weeks of age, were placed on isocaloric diets containing 20% (control), 4%, or 2% egg-white protein (Dyets Inc., Bethlehem, PA). The composition of this diet has been published in detail elsewhere (Carlomagno & McMurray, 1983). Mice were given food and tap water *ad libitum*.

Assessment of nutritional status

At weekly intervals mice were weighed to monitor weight changes. Prior to killing, the mice were anaesthetized with sodium pentobarbital and blood was collected via the retro-orbital plexus. Total serum protein concentration was deter-

mined by the method of Lowry *et al.* (1951) and cellulose-acetate electrophoresis was used to determine the concentration of serum albumin.

Determination of total IgA in body fluids

Intestinal fluids were obtained by removing the entire intestine and washing the contents out with 1 ml of cold Hanks' balanced salt solution (KC Biologicals, Lenexa, KS). The resulting fluid was centrifuged to sediment solid material, and the supernatant collected stored frozen. The concentration of IgA in serum or intestinal washes was determined by a radial immunodiffusion assay (RID) (Mancini, Carbonera & Heremans, 1965) with rabbit anti-mouse IgA antiserum (Miles Scientific, Naperville, IL). Purified mouse myeloma IgA (TEPC 15, Sigma Chemical Co., St Louis, MO) was used as a standard. All samples were tested in duplicate. The values for the intestinal wash IgA were adjusted for the volume of fluid recovered and reported as the total IgA recovered.

Oral immunization and spleen-cell preparation

The procedure used for oral immunization was similar to that of Andre *et al.* (1973). Mice deprived of food overnight were immunized by gastric intubation using a 22-guage intubation needle (Popper and Sons Inc., New Hyde Park, NJ). Each mouse was given 0·28 ml of a 50% SRBC suspension in 0·9% saline on 4 consecutive days.

Eight days after the initial intubation, mice were killed by cervical dislocation. The spleens were removed and placed in RPMI-1640 containing 10% fetal bovine serum (Irvine Scientific, Santa Anna, CA), 2 mM L-glutamine, 0·1 mg/ml streptomycin, and 100 U/ml penicillin (KC Biologicals). Spleen-cell suspensions were prepared using a Ten Broek glass homogenizer (Thomas Scientific, Swedesboro, NJ) followed by passing the suspension through a 50-guage metal mesh. Viable cell numbers were determined by trypan blue exclusion and counting on a haemocytometer.

Plaque-forming cell assay

Anti-SRBC PFC were enumerated using a modification of the plaque assay described by Jerne *et al.* (1974). Plaques were obtained by mixing a 0·1-ml spleen suspension with 0·6% agarose (0·7 ml), 50% SRBC (0·05 ml), guinea-pig serum (0·05 ml) and tissue-culture medium (0·05 ml), A 0·2-ml drop of this mixture was then added to a petri-dish and a 24 × 30 mm cover slip placed over the drop. The plates were incubated at 37° in a himidified chamber for 2 hr and plaques counted in the specific volume under the cover slip were used to calculate the PFC per spleen. The IgA or IgG PFC were determined indirectly by adding rabbit anti-mouse IgA (Miles Scientific) or goat anti-mouse IgG1 (heavy-chain specific; Sigma Chemical Co.) antisera in the place of the tissue culture medium and, after incubation and counting, subtracting the number of direct IgM PFC. Controls containing no guinea-pig serum were subtracted from all results.

Statistical analysis

The data were expressed as the mean ± SE. The Duncan's new multiple range test, with the probability level set at 95%, was used to test for significant differences between the mean values (Ott, 1984).

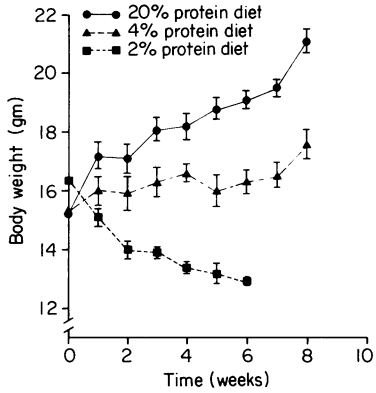

Figure 1. The effect of dietary protein content on the cumulative body weight of mice. Vertical bars indicate the SE for six to seven animals.

Table 1. Effect of dietary protein content on total serum protein and serum albumin concentrations

Weeks on diet		Dietary protein treatment		
		20%	4%	2%
2	Serum protein*	4·52±0·16	4·71±0·14	4·17±0·29
	Serum albumin	3·10±0·12	3·13±0·17	2·18±0·22
4	Serum protein	4·32±0·17	4·72±0·27	3·84±0·13
	Serum albumin	2·88±0·16	3·22±0·31	2·53±0·12
6	Serum protein	4·55±0·36	4·90±0·34	3·39±0·17†
	Serum albumin	3·28±0·34	3·23±0·27	2·25±0·09†
8	Serum protein	4·94±0·20	5·36±0·34	ND
	Serum albumin	3·25±0·09	3·60±0·19	ND

* Values are mean ± SE in g/100 ml for five to nine animals. ND, not determined.

† Significant difference from values of mice on control diets ($P < 0·05$).

RESULTS

Assessment of nutritional status

Figure 1 shows the cumulative body weights of mice fed each diet over 6–8 weeks. As expected, mice fed the control (20% protein) diet gained weight over the entire period studied. Those fed the 4% protein diet increased only slightly in body weight. Mice fed the 2% protein diet lost weight consistantly over the 6-week period, indicating a severely malnourished state.

Table 1 shows the effect of the various diets on the total serum protein and serum albumin levels. Only the mice fed the 2% protein diet for 6 weeks showed a significant reduction in both values, compared to the control diet-fed mice.

Measurement of the weekly food consumption per mouse showed that mice fed the control diet consumed 16·8±0·7 gm/mouse/week, whereas mice fed the 4% or 2% protein diets consumed 18·6±0·5 and 22·6±1·8 gm/mouse/week, respectively. This confirms that the effects seen with the protein deficient-mice were due to a reduction in protein consumption and not a reduction of calories or other dietary factors.

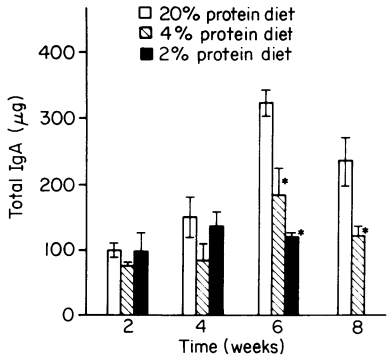

Figure 2. Total IgA content in intestinal washes of mice fed diets varying in the quantity of protein. Values are the means ± SE of four to eight samples. Asterisk indicates a significant difference from the control (20% protein) group ($P < 0.05$).

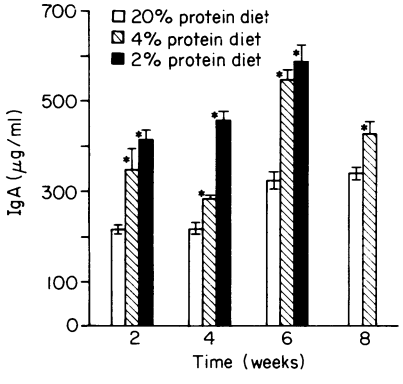

Figure 3. Serum IgA content of mice fed diets varying in the quantity of protein. Values are means ± SE of five to seven samples. Asterisk indicates a significant difference from the control (20% protein) group ($P < 0.05$).

Effect of diet on the IgA content in body fluids

A radial immunodiffusion assay was used to compare the amounts of IgA in intestinal washes and serum of mice fed the control and 4% or 2% protein diets. Intestinal washes from the 4% protein diet-fed mice yielded significantly reduced total IgA levels compared to controls, at both 6 and 8 weeks (Fig. 2). Intestinal washes from the 2% protein diet-fed mice also gave values significantly lower than the controls at 6 weeks (Fig. 2).

As illustrated in Fig. 3, the RID assay showed significant increases in the serum IgA levels of mice fed either the 2% or 4% protein diet, compared to controls. These increases were apparent at all intervals of diet feeding.

Effect of diet on the response to antigen

The response of mice to orally introduced SRBC is presented as the IgA anti-SRBC PFC response in the spleen (Fig. 4). Mice fed the 4% protein diet showed no significant difference in the spleen IgA PFC/spleen response as compared to controls at any diet-feeding interval. However, mice fed the 2% protein diet showed

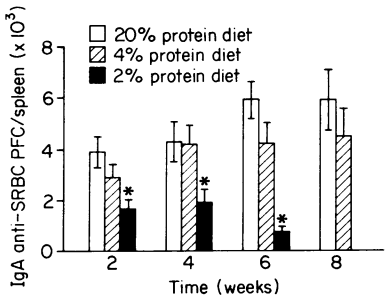

Figure 4. Spleen IgA anti-SRBC PFC response of mice fed diets varying in protein content and immunized orally with SRBC. Values are means ± SE of five to nine animals per treatment. Asterisk indicates a significant difference from the control (20% protein) group ($P < 0.05$).

Table 2. Effect of dietary protein content on the spleen IgA anti-SRBC PFC/10^6 cells

Dietary protein	IgA PFC/10^6 spleen cells*		
	2 weeks	4 weeks	6 weeks
20%	49·5 ± 10·6	54·5 ± 10·8	62·7 ± 4·3
4%	47·6 ± 6·7	65·9 ± 8·2	58·3 ± 7·4
2%	52·0 ± 11·2	55·8 ± 12·2	24·9 ± 7·4†

* Mean ± SE of five to nine animals.
† Significantly different from 20% protein diet value ($P < 0.05$).

a drastic reduction in the spleen IgA anti-SRBC PFC/spleen response at all intervals, compared to controls (Fig. 4). This reduction of IgA PFC/spleen obtained from mice fed the 2% protein diet for 6 weeks was also found when protein-deficient mice were killed at 6, 7, 9, and 10 days after the onset of immunization (data not shown). The IgM and IgG anti-SRBC PFC/spleen responses were consistantly low in all cases (approximately 400 IgM PFC/spleen and 100 IgG PFC/spleen for controls, with values for mice fed the 2% protein diet being slightly lower) and therefore were not included.

When the above values were converted to IgA anti-SRBC PFC/10^6 spleen cells, only the mice fed the 2% protein diet for 6 weeks had significantly reduced values when compared to the controls (Table 2). Values for the mice fed the 4% protein diet did not vary from the controls at any time interval. As with the PFC/spleen data, the IgM and IgG PFC/10^6 spleen cells were consistantly low and therefore were not included.

Effect of dietry replenishment

Mice fed the 2% protein diet for 6 weeks were replenished with the control diet for 3 weeks. Figure 5 shows that a switch to the control diet was accompanied by a rapid increase in body weight. When killed after 9 weeks, these replenished mice had serum protein levels of 4·44 g/100 ml (SE ± 0·04), and serum albumin levels of 3·07 g/100 ml (SE ± 0·07), both of which were not significantly different from the values of control mice at 8 weeks.

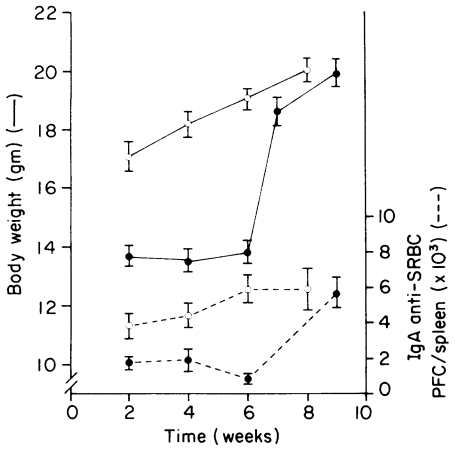

Figure 5. The effect of replenishment on the cumulative body weight and spleen IgA anti-SRBC PFC response. Mice fed the 2% protein diet for 6 weeks followed by the control (20% protein) diet for 3 weeks (●) are compared to mice fed the control diet (○). Vertical bars represent the SE for five to nine animals.

Also shown in Fig. 5 is the increase in the IgA anti-SRBC PFC/spleen response seen with renourished mice compared to mice on the control diet. The spleen IgA PFC response after 3 weeks of replenishment was not significantly different from the control values at 8 weeks (5658 ± 905). The IgA content in the serum and intestinal washes from the replenished mice (300 ± 18 μg IgA/ml and 276 ± 22 total μg IgA, respectively) was not significantly different from the control values at 8 weeks.

DISCUSSION

Severe protein malnutrition in humans is characterized by a reduction in both body weight and visceral protein stores (Suskind *et al.*, 1977). Moderate protein malnutrition, however, constitutes a transitional area somewhere between the severe form and the norm. Using the mouse as a model, we have devised a set of experimental conditions which produces a severely protein-deficient state (6 weeks on a 2% protein diet) characterized by weight loss and significant reduction in total serum protein and albumin (Table 1). We have also induced a moderately protein malnourished state (6–8 weeks on a 4% protein diet) in which reduced growth velocity is the only evidence of nutritional deprivation. With this model, we have studied the effect of protein deprivation on the IgA immune system.

Both severe and moderate protein deprivation resulted in a reduction in the amount of IgA found in intestinal fluids (Fig. 2), suggesting that the IgA immune system of the intestine is sensitive to both forms of malnutrition. Reddy *et al.* (1976), studying malnutrition in children, and Lim, Messiha & Watson (1981) using mice, have also found a reduction in intestinal fluid IgA levels with malnutrition. However, Bell *et al.* (1976b) reported increased levels of IgA in duodenal fluids of malnourished children, but suggested that this may be due to an increased incidence of gastrointestinal infections in these children.

Interestingly, the IgA levels in the serum from both moderately and severely protein-malnourished mice were significantly higher than the control values, even at 2 weeks (Fig 3). Many studies of malnutrition in children have shown this same phenomenon (Newmann *et al.*, 1975; McMurray *et al.*, 1977; Watson, Reyes & McMurray, 1978; McMurray, Watson & Reyes, 1981). This excess serum IgA may represent an impairment of a mechanism for the removal of IgA from the serum which is extremely sensitive to protein deprivation.

One mechanism for the removal of serum IgA in mice involves transport of IgA in the bile secretions (Jackson, Lemaitre-Coelho & Vaerman, 1977). A malfunction in this transport mechanism may lead to a reduction in intestinal-wash IgA levels, with a concomitant increase in serum IgA levels. Alternatively, this inverse relationship between high serum and low secretory IgA may reflect an inability of malnourished mucosal epithelial cells to export secretory IgA, resulting in accumulation in serum. Quantification of the monomeric (serum) and multimeric (secreted) forms of IgA in serum and intestinal washes of malnourished animals could contribute a valuable insight regarding this.

Watson *et al.* (1985) have found a reduction in the free secretory component levels in the tears of severely malnourished children. These authors suggested that malnutrition caused a reduction in the availability of secretory component, resulting in lowered secretory IgA levels in tears. Perhaps a mechanism such as this is causing the reduction of IgA levels in other mucosal secretions of malnourished subjects.

A reduction in the number of IgA plasma cells in the intestinal lamina propia, as seen in malnourished children (Green & Heyworth, 1980; Chandra, 1983a) and rats (Barry & Pierce, 1979), may also be responsible for the reduced levels of IgA in intestinal fluids with malnutrition. A variety of mechanisms could explain this decrease in IgA-producing cells at mucosal surfaces. The ability of the cells committed to IgA production to home to mucosal tissues may be impaired (McDermott *et al.*, 1982; Chandra, 1983b). Malnutrition may also result in a reduced capacity to generate the IgA-committed cells. Lopez *et al.* (1985) found that severely protein-deficient rats have fewer IgM- and IgA-bearing B cells in the Peyer's patches. Barry & Pierce (1979) reported decreased numbers of cholera toxin-specific cells in the thoracic duct of protein-deficient rats after oral immunization.

In this report, we have demonstrated that severe protein malnutrition results in a reduction of the specific IgA PFC response in the spleen after oral immunization (Fig. 4). The reduction in the IgA PFC/spleen could be influenced by the general reduction in the spleen weight which we (data not shown) and others (Bell, Hazell & Price, 1976a) have seen with protein malnourished mice. However, since the IgA B cells are produced in the Peyer's patches, a reduction in spleen weight without a reduction in the production of IgA B cells would be seen as an increase in IgA PFC/10^6 spleen cells (perhaps as indicated in Table 2 by the mice fed the 4% protein diet for 4 weeks). The significant reduction in the IgA PFC/10^6 cells after 6 weeks on the 2% protein diet (and perhaps the equal value after 4 weeks on the 2% protein diet) confirm that the specific IgA PFC response was reduced by protein malnutrition. Since the migration of radiolabelled mesenteric lymph node cells to the spleen appears to be unaffected by malnutrition (Chandra, 1983b), our results support the hypothesis that malnutrition somehow impairs the production of IgA-committed cells to antigens encountered at the intestinal mucosa.

Moderate protein deficiency, on the other hand, had little or

no effect on the production of specific IgA-committed cells. This suggests that the decrease in intestinal fluid IgA found in the moderately malnourished animals was not due to a reduction in the ability to generate immune cells but perhaps to alterations in the homing of these cells, their production of IgA, or even the transport of IgA across the mucosa.

Finally, a common occurrence seen with malnourished subjects is the return to normal of many immune responses, including the IgA responses, when the subject is replenished (Reddy *et al.*, 1976; McMurray *et al.*, 1981; Watson *et al.*, 1985). We have tested the reversibility of the depressed IgA responses in the severely protein-deficient mice by refeeding the animals with the control diet. These mice showed a rapid (3-week) and complete recovery of both the nutritional status and the IgA immune response (Fig. 5), suggesting that the loss of the IgA-producing capacity is directly related to the nutritional status of the animal.

In this report, we have outlined a model system using the mouse which mimics in many ways the reported findings for severely and moderately malnourished human subjects. Our goal is to use this system to further elucidate the mechanisms by which protein malnutrition affects the IgA immune response to oral challenge.

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