Differential Effect of Dietary Protein Type on the B-Cell and T-Cell Immune Responses in Mice[1]

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ABSTRACT The effect of 20 g/100 g diet of lactalbumin (L), casein (C), soy (S) and wheat (W) protein on the immune responsiveness of C3H/HeN mice has been investigated by measuring the humoral immune response to the T cell–independent antigen, TNP-Ficoll. The humoral immune response of mice fed the L diet was found to be higher than that of mice fed the C, S and W diets. On the other hand, delayed-type hypersensitivity, and splenic cell mitogen responses to phytohemagglutinin and concanavalin A did not differ among mice fed the various diets. Similarly, the type of diet did not appear to influence host resistance to *Salmonella typhimurium*. It is postulated that the type of protein in the diet influences directly the intrinsic capacity of the B lymphocytes to respond to an immunogenic stimulus. J. Nutr. *115*: 1403–1408, 1985.

INDEXING KEY WORDS diet • protein • immunity • mice

We have recently observed that the type of protein in the diet has a significant effect on the development of humoral immunity to T cell–dependent antigens, namely sheep red blood cells (SRBC) and horse red blood cells (HRBC). Thus, the humoral immune response of mice fed a lactalbumin (L) diet was found to be nearly five times greater than that of mice fed corresponding casein (C), soy (S) or wheat (W) protein diets. The humoral immune response of mice fed C, S and W diets was substantially lower than that of mice fed nonpurified (NP) diets, whereas that of mice fed L diet was higher. However, no difference was seen in spleen cell mitogen responses among the various dietary groups (1).

The purpose of the present study was to analyze the cell type(s) (T cell, B cell) influenced by dietary protein. We have investigated, in mice, the effect of defined formula diets containing either L, C, S or W as the protein source, on several parameters of cell-mediated immunity. To ascertain the possible role of the T-helper cell in the observed effects of dietary protein type on humoral immunity, the plaque-forming cell (PFC) response of diet-fed mice to a T cell–independent antigen was investigated. Trinitrophenyl (TNP)-Ficoll (TNP^{85}-AECM-Ficoll, Biosearch, San Rafael, CA), a type 2 T cell–independent antigen (2), was selected for this study.

MATERIALS AND METHODS

Mice. Male C3H/HeN and C57Bl/6 mice were purchased from Canadian Breeders, Montreal, Que., Canada, at 6–8 wk of age.

Dietary treatment. A detailed composition of the defined formula diets (4.3 kcal/g) is given in a companion article in this journal

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(3). The diets contained 20 g of L, C, S or W vitamin-free purified protein per 100 g diet. These diets are designated L diet, C diet, S diet or W diet, respectively. Other animals were fed NP diet (Purina Rodent Chow, Ralston Purina Co., St. Louis, MO) with an estimated 23 g protein from various sources per 100 g diet.

Diets, refrigerated between feeding, were given thrice weekly. They were continuously available in powder form in stainless-steel feeders designed to avoid spillage. Drinking water was provided ad libitum. The mice, housed two per cage in wire-bottomed cages, to reduce coprophagy, were fed the various diets from 6 to 8 wk of age, and immunological studies commenced 2 wk later. Dietary treatment was continued throughout the experiment. Each dietary group comprised 10 mice.

Graft-versus-host (GVH) reactivity of spleen cells. In one set of experiments 75 $\times$ 10^6 spleen cells from C3H/HeN (H-2^k) mice fed one of the four diets for 2 wk were injected i.v. into lethally irradiated (850 rad) C57Bl/6 (H-2^b) mice fed NP diet. The recipients were killed 5 d later. The spleen index, i.e., the spleen weight to body weight ratio of each experimental group divided by that of the noninjected control group, was calculated (4). In another set of experiments, donor C3H/HeN mice were fed NP diet before their spleen cells (75 $\times$ 10^6) were injected into lethally irradiated (850 rad) C57Bl/6 mice that had been fed one of the four diets for 2 wk. The spleen index was measured 5 d later.

Delayed-type hypersensitivity (DTH) reaction. DTH to SRBC was assessed by the foot pad assay. Mice were immunized by a subcutaneous injection of SRBC emulsified in Freunds Complete Adjuvant (Difco Lab, Detroit, MI) (2 $\times$ 10^8 SRBC/mouse). Four days later, the DTH reaction to SRBC was elicited by injecting 25 μl of 10% SRBC in saline (5 $\times$ 10^7) into the right foot pad of each animal and 25 μl saline into the left foot pad as a control (5). Foot pad swelling was measured immediately before and 24 h after antigenic challenge by using a micrometer (Brown and Sharpe Mfg. Co., N. Kingston, RI). The mean of three foot pad readings was determined for each mouse. A foot pad assay was also performed on a control group of nonimmunized mice from each dietary group. The mean increase of the control group was subtracted from the increase of each animal in the experimental group, and the results were expressed in millimeters (6).

Mitogen responses. The mitogen responses of spleen cells (3.3 $\times$ 10^6 cell/ml) to phytohemagglutinin (PHA) and concanavalin A (Con A) were measured by using the method described by Lapp et al. (7). Several concentrations of the mitogens were used, and the results obtained with the optimum concentrations have been reported here.

Salmonella typhimurium *experiments.* Mice were injected by i.g. inoculation with 3.2 $\times$ 10^5 CFU (colony-forming units) of *S. typhimurium*, "Keller" strain (8), after 2 wk of dietary treatment, which was continued through the experiment. The total number of viable *S. typhimurium* found in the spleen and liver was measured 10 d postinfection, by plating out serial dilutions of organ homogenates on tryptose agar and counting the colonies formed after incubation overnight at 37 °C.

PFC response to TNP-Ficoll. Hapten-coupled SRBC were prepared by the method of Rittenberg et al. (9). Briefly, packed SRBC were added dropwise to 2,4,6-trinitrobenzene sulfonic acid dissolved in cacodylate buffer, and the mixture was stirred for 10 min. Reacted cells were washed three times with modified barbital buffer (containing glycyl-glycine for the second wash) and stored briefly in the refrigerator if not used immediately. The haptenation reaction was carried out in foil-wrapped containers. Immunization consisted of a single i.v. injection of either 10, 20, 30 or 100 μg TNP-Ficoll dissolved in saline. The PFC assay was performed on d 4 after TNP-Ficoll injection, when the response was shown to peak. The method used for assaying IgM PFC was essentially the one described by Cunningham and Szenberg (10) with certain minor modifications (5), as outlined in a companion paper (3).

Statistical analysis. A two-way analysis of variance (ANOVA) was used to compare the four types of proteins across the three antigen doses. Because the protein $\times$ dose interaction was significant, it was necessary to perform three separate one-way ANOVA, one for each dose to compare the effect of

dietary proteins. Multiple comparisons among the four protein means were performed by using a Sidak *t*-test at a significance level of 0.01 for each pair of means.

RESULTS

Growth. Our previous studies (1) have shown that mice fed the above described 20 g protein/100 g diets and nonpurified diet increased in body weight by approximately the same amount, with similar food consumption. In the current studies, body growth was similar for all dietary groups. The initial body weights, in grams, of mice fed L diet or C, S or W diet were 18.8 ± 0.4 (mean ± SEM), 19.4 ± 0.4, 19.1 ± 0.4 and 19.2 ± 0.5, respectively. The corresponding values after 3 wk of the dietary regimen were 22.9 ± 0.5, 23.7 ± 0.3, 23.5 ± 0.3 and 23.4 ± 0.4, respectively. Moreover, no significant differences in serum protein values were observed among these dietary groups. The values of total serum protein (grams/100 ml) were 5.8 ± 0.49, 5.7 ± 0.26, 6.1 ± 0.30, 6.0 ± 0.40 and 5.9 ± 0.33 in mice fed NP, L, C, S and W diets, respectively.

Cell-mediated immune responses. Among C3H/HeN mice, spleen cells from those fed L or C diets elicited a slightly lower GVH reactivity than those from mice fed S or W diets, when injected into lethally irradiated C57Bl/6 mice that had been fed NP diet ($P < 0.05$) (table 1). On the other hand, no diet had a significant effect on the GVH reaction induced in C57Bl/6 mice by spleen cells from C3H/HeN mice that had been fed NP diet (table 1). The DTH reaction and the splenic mitogen responses to PHA and Con A did not differ among mice fed the various diets. Similarly, the type of dietary protein did not influence the resistance of the host to infection with *Salmonella.*

Humoral immune response. The mean number of PFC per spleen 4 d after i.v. injection with 10 μg TNP-Ficoll in the L diet–fed mice was 183% of that noted in their C diet–fed counterparts. The value for the latter group was approximately twice the corresponding values observed in the S and W diet groups (fig. 1). The mean values for the S and W diet groups were similar. The pattern of immune responses in relation to protein type was strikingly similar after immunization with 20 μg TNP-Ficoll. However, when the dose of TNP-Ficoll was raised to 30 μg, the effect of dietary protein type on the PFC response was found to differ somewhat from that observed with lower doses of the antigen. Whereas the number of PFC in the L diet–fed group was still 164% of that noted in the C diet–fed group, no significant difference was seen among mice fed the C, S and W diets (fig. 1). The type of diet did not influence, to any marked degree, the PFC response after immunization with 100 μg TNP-Ficoll (data not shown).

DISCUSSION

Our previous studies have shown that feeding mice different types of dietary protein influences the humoral immune response of the animals to heterologous erythrocytes (1). Since this type of response involves both the B lymphocyte and the T-helper cell, the dietary protein type may be exerting its effect on either of the major classes of lymphocytes, namely T or B cells, or on both. To investigate this further, in the present studies the influence of dietary protein type on T cell–mediated immune responses was examined. Also, the effect on the T-helper cell was explored indirectly, by determining the effect of dietary protein type on the humoral immune response to a T cell–independent antigen.

For the measurement of cell-mediated immunity, a variety of different tests was selected in which the T cell is known to mediate the response. Numerous experiments have demonstrated convincingly that T lymphocytes are the cells that initiate the GVH reaction (11). Similarly, in mice, PHA and Con A are T cell–specific mitogens in vitro, while DTH reactivity is a measure of the T-cell response in vivo (11). Although total resistance to *S. typhimurium* may be a composite of both humoral and cellular events, previous studies strongly indicate that effective anti-*Salmonella* resistance is primarily cellular in nature (12, 13). In the present experiments it was observed that, except for a slight drop in GVH reactivity of spleen cells when tested in L or C diet–fed donors, dietary protein type appears to have

TABLE 1

Effects of 2-wk dietary regimen on various parameters of cell-mediated immunity in C3H/HeN mice[1]

Response measured	Dietary treatment							
	Lactalbumin		Casein		Soy protein		Wheat protein	
GVH reactivity (spleen index)								
Donor[2]	4.1 ± 0.3*		4.2 ± 0.2		5.2 ± 0.1		5.5 ± 0.2	
Recipient[3]	4.4 ± 0.2		5.0 ± 0.2		4.1 ± 0.3		4.1 ± 0.3	
DTH reaction,[4] *mm*	3.4 ± 0.15		3.1 ± 0.12		2.8 ± 0.12		3.2 ± 0.13	
	Con A	PHA	Con A	PHA	Con A	PHA	Con A	PHA
Spleen cell mitogen responses[5]								
Mitogen	51.1 ± 0.9	40.6 ± 1	48.8 ± 0.7	42.3 ± 0.9	52.6 ± 1.7	44.9 ± 1.4	50.3 ± 1.0	43.8 ± 3.5
Background value	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.2	4.0 ± 0.1	5.0 ± 0.2	4.4 ± 0.08	5.1 ± 0.3	5.1 ± 0.3
Salmonella infection[6]								
Liver	5.31 ± 0.4		5.68 ± 0.3					
Spleen	5.20 ± 0.36		5.09 ± 0.23					

[1]Values are means ± SEM; $n = 10$. Unless otherwise specified, the experiments were performed in C3H/HeN mice. [2]C3H/HeN mice were fed one of the four diets for 2 wk before their spleen cells were injected into irradiated C57Bl/6 mice that had been fed nonpurified diet. [3]C3H/HeN mice were fed nonpurified diet before their spleen cells were injected into irradiated C57Bl/6 mice that had been fed one of the four diets for 2 wk previously. [4]Foot pad swelling is expressed in millimeters (mean increase of the control unimmunized group was subtracted from the increase of each experimental group). [5]The spleen cell response to 5 μg mitogen is expressed as cpm × 10^{-3}. Mean value of triplicate cultures ± SEM. [6]The number of microorganisms is expressed as $\log_{10}$ *Salmonella* per organ on d 10 after i.g. inoculation. *GVH reactivity of spleen cells from mice fed lactalbumin or casein diets vs. soy or wheat diet-fed groups: $P < 0.05$ by one-way ANOVA. In all other experiments no significant difference was noted between the various dietary groups by using one-way ANOVA.

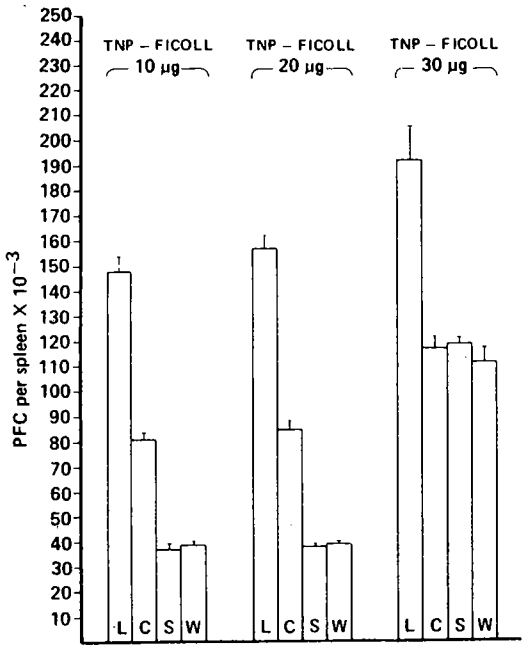

Fig. 1 Number of plaque-forming cells (PFC) per spleen after immunization of C3H/HeN mice with trinitrophenylated (TNP)-Ficoll; effect of 2 wk of dietary treatment with 20 g of lactalbumin (L), casein (C), soy (S) or wheat (W) protein in 100 g diet. Each value represents the mean ± SEM, n = 10 mice. Two-way ANOVA of dietary protein and antigen dose showed a significant ($P < 0.0001$) protein × dose interaction. The individual one-way ANOVAs of protein for each dose produced highly significant differences among the proteins ($P < 0.0001$ at each dose). Multiple comparisons of these significantly different means by Sidak's t-test at significance level 0.01 showed the ordering of the type of protein after 10 or 20 μg TNP-Ficoll is: L ≠ C ≠ W = S (where ≠ means significantly different and = means not significantly different). After immunization with 30 μg TNP-Ficoll, the effect of the type of protein is: L ≠ C = W = S.

no effect on GVH reactions, the DTH reaction, spleen cell mitogen responses or host resistance to *Salmonella* (table 1).

These data indicate that cell-mediated immune responses do not appear to be influenced substantially by dietary protein type, at least not after short-term dietary exposure.

To investigate the effect of dietary protein on the humoral immune response to a T cell–independent antigen, the TNP conjugate of Ficoll was used as the challenge antigen. Until recently, it was thought that all antigens that could elicit responses in vivo in athymic mice, or in vitro in the relative absence of T lymphocytes, could be designated as thymus-independent antigens. More recent studies in mice have, however, shown that even putatively T cell–independent antigens such as TNP-Ficoll are, in fact, unable to stimulate B lymphocytes directly, without some ancillary help (2). Nonetheless, TNP-Ficoll was used in our studies because it can stimulate B lymphocytes in the relative absence of T lymphocytes (14). Our present results indicate clearly that, after challenge with low doses of this antigen, an effect of dietary protein type on the PFC response is seen (fig. 1). This effect is similar to that observed after immunization with the T cell–dependent antigen, SRBC (1); in particular, the L diet has a markedly enhancing effect on the humoral immune response to both types of antigens.

At higher doses of antigen the difference in humoral immunity to TNP-Ficoll among C, S and W diet–fed mice is no longer seen. The impaired response of S and W diet–fed mice as compared to C diet–fed mice is overcome by increasing the dose of the antigen. Under natural conditions, the body is more often exposed to relatively low levels of antigen so that dietary protein type may well be relevant to the immunological response of the host in naturally occurring circumstances.

Since none of the cell-mediated immune responses tested in the present study appear to be greatly affected by source of dietary protein, while the humoral immune response is substantially influenced, even to T cell–independent antigens, the dietary protein effect is apparently exerted at the level of the B lymphocyte and probably has an insignificant effect, if any, on the T lymphocyte. Moreover, our previous studies have shown that there is no difference between L diet–fed and NP diet–fed mice in the phagocytosis of ^{51}Cr-labeled opsonized SRBC by peritoneal macrophages (15), so that, by this criterion, macrophage function appears to be unaffected by dietary protein. If so, it may be inferred that the effect of the type of dietary protein is confined to the B lymphocyte. It is conceivable that dietary protein may influence directly the intrinsic capacity of the B lymphocytes to respond to an immunogenic stimulus. The allegedly shorter

life span of B cells (16) could make them more readily susceptible to changes in the nutritional status of the host.

Whereas the nonpurified diet contains a variable mixture of vegetable and animal proteins, the present study has allowed us to show the specific effect of each individual purified protein on the immune reactivity of the host.

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