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# Influence of Dietary Proteins on the Immune System of Mice[1]

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ABSTRACT The effect of graded amounts of dietary lactalbumin (L) and casein (C) hydrolyzates on the immune responsiveness of C3H/HeN and DBA/2 strain mice has been investigated by measuring both the specific humoral immune response to sheep red blood cells (SRBC) and the nonspecific splenic cell responsiveness to phytohemagglutinin, concanavalin A and *Escherichia coli* lipopolysaccharide after stimulation with *Mycobacterium bovis*, strain BCG. The nutritional efficiency of these diets was similar at both 12 and 28% amino acid levels. The immune responses of mice fed the L diets were found to be significantly greater than those of mice fed the corresponding C diets, especially at the 28% level. Furthermore in the mice fed L diet, increasing the concentration of amino acid in the diet from 12 to 28% greatly enhanced immune responsiveness by both parameters measured. In the C-fed mice, a comparable enhancement of mitogen responsiveness with increasing amino acid level of diet was seen, but there was no change in the humoral immune response. The enhancement of immune responsiveness observed in mice fed the 28% L diet was moderately reduced by the addition of phenylalanine to the diet, indicating that the lower level of this amino acid in the L protein may be of some significance. These dietary effects on immune responsiveness were remarkably similar in both mouse strains tested. J. Nutr. *112:* 1747–1755, 1982.

INDEXING KEY WORDS diet · protein · immunity · mice

The commonplace knowledge that severe protein deficiency predisposes or exacerbates bacterial infections has been verified in several experimental model systems (1–3). In these studies, the test diet containing either 3% (1) or 5% (2) casein, or devoid of protein (3), all brought about substantial weight loss when compared to control diets containing 24% (1) or 20% (2, 3) casein/100 g diet. Observations of the interaction of protein-calorie malnutrition and various immune parameters have led to some understanding of the etiology of the increased susceptibility to infections associated with protein-depleting diets. At the bottom of the scale, a marked depression of both cell-mediated and humoral immunity was observed in rodents fed a protein-free (4, 5) or 3% casein diet (6). Spleen cells of mice fed a 4% casein diet, exhibited either unchanged (7) or decreased (8) responses to phytohemagglutinin (PHA), concanavalin A (Con A) and lipopolysaccharide (LPS) mitogens. On the other hand, a lesser degree of protein deprivation was found to produce a differential immunological effect on the two fundamental components of the immune system. Thus, spleen cells of mice fed a 6% (9) or an 8% (10, 11)

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casein diet exhibited an enhanced response to PHA (9–11) and Con A (11), whereas humoral immune responses were found to be depressed following these restricted diets (6, 7, 10, 12). These growth-inhibiting test diets were compared to a "control" diet containing either 22% (9), 24% (7) or 27% (6, 10–12) casein.

Most studies on protein-calorie nutrition have utilized casein or egg albumin for their high biological value. Our interest in protein-calories and immunity has focused on the established minimum requirement of some essential amino acids. It has been estimated that the provision of about 12% protein of optimal digestibility and biological value, such as casein, is adequate for mice (13–15). However, a recent study has brought new information concerning the minimum requirement of some essential amino acids for mice. Thus, whereas a 12% casein diet contains approximately 0.6 g/100 of phenylalanine (Phe) and 0.3 g/100 of tyrosine (Tyr) (13, 14), the actual requirement of Phe in the presence of about 0.2% Tyr ranges between 0.25 and 0.4% for Swiss mice (16). Accordingly, our previous study has shown that a 12% casein diet with a restriction of Phe to 0.4% and of Tyr to 0.2% provides normal growth of CBA mice (14). Mice fed this diet exhibit a 100% increase in the plaque-forming cell (PFC) response to sheep red blood cells (SRBC) but no change in their delayed-type hypersensitivity (DTH) reaction to this antigen, in comparison to control mice fed 12% casein. A similar increment in humoral immunity, this time accompanied by a concomitant enhancement of the DTH response, was noted when Phe and Tyr were restricted to 0.2 and 0.1%, respectively (14). It is not known whether the availability of a given essential amino acid or its ratio to each of the other amino acids in the protein moiety is the principal factor responsible for the observed immune effect. In the present study we have investigated the effect of graded amounts of dietary lactalbumin hydrolyzate (LAD, Nestlé's S.A., Vevey, Switzerland) on the immune responsiveness of mice. This lactalbumin has a lower concentration of some essential amino acids such as Phe and methionine (Met) (table 1) than does casein. Control groups were fed an equivalent amount of casein hydrolyzate.

## MATERIAL AND METHODS

*Mice.* Male DBA/2J mice were obtained from Jackson Laboratories (Bar Harbor, ME), and C3H/HeN male mice were purchased from Canadian Breeders, Montreal, Canada, at 6 weeks of age.

*Dietary treatment.* A detailed composition of the purified diets (4.4 kcal/g) is given in table 1. Approximately 50% of essential amino acids in lactalbumin hydrolyzate (L) and 65% in casein hydrolyzate (C) are in the form of free amino acids.

In some experiments 2 g of Phe per 100 g amino acid was added to lactalbumin hydrolyzate to bring the total concentration of Phe in L, to the level present in C. This diet will be designated diet L + Phe. In a few selected experiments, 2 g of Phe plus 0.8 g of Met were added to lactalbumin hydrolyzate to bring the total concentration of both amino acids to the level present in C. This diet is designated L + Phe + Met. Other animals were fed a commercial laboratory diet (Purina rodent chow, Ralston Purina, St. Louis, MO, estimated 20% protein).

Diets, refrigerated between feeding, were given thrice weekly. They were continuously available in powder form in stainless-steel feeders specially designed to avoid spillage and spoilage. Drinking water was allowed ad libitum. The mice, housed in wire-bottomed cages to prevent coprophagy, were placed on the various diets at 6–8 weeks of age, and immunological studies commenced 1, 2 or 3 weeks later. Dietary treatment was continued throughout the experiment. Each dietary group comprised 10 mice.

*PFC assay.* The method used for assaying IgM PFC was essentially the one described by Cunningham and Szenberg (17) with certain minor modifications (14). The mice were injected intravenously (i.v.) with $5 \times 10^6$ SRBC and assayed for PFC on day 5 when the response was shown to peak.

*Mitogen responses.* The tests of mitogenic response to PHA, Con A, and *Escherichia coli* LPS were performed by using the method described by Lapp et al. (18). Several concentrations of the mitogens were used, and the results obtained with the optimum concentrations have been reported here.

*BCG treatment.* Mice were inoculated intraperitoneally (i.p.) with $6 \times 10^6$ CFU (col-

TABLE 1

*Amino acid composition of test diets*[1]

| Amino acid | Casein hydrolyzate (12% C) 12 g/100 g | | Lactalbumin hydrolyzate (12% L) 12 g/100 g | |
|---|---|---|---|---|
| | *g/100 g diet* | *g/100 g amino acids* | *g/100 g diet* | *g/100 g amino acids* |
| Isoleucine | 0.67 | 5.6 | 0.83 | 6.9 |
| Leucine | 1.09 | 9.1 | 1.08 | 9 |
| Valine | 0.83 | 6.9 | 0.71 | 5.9 |
| Methionine | 0.31 | 2.6 | 0.22 | 1.8 |
| Cystine | 0.02 | 0.2 | 0.16 | 1.3 |
| Phenylalanine | 0.55 | 4.6 | 0.31 | 2.6 |
| Tyrosine | 0.22 | 1.8 | 0.30 | 2.5 |
| Threonine | 0.50 | 4.2 | 0.92 | 7.7 |
| Tryptophan | 0.12 | 1.0 | 0.16 | 1.3 |
| Lysine | 0.97 | 8.1 | 1.20 | 10 |
| Histidine | 0.32 | 2.7 | 0.23 | 1.9 |
| Arginine | 0.40 | 3.3 | 0.32 | 2.7 |
| *Total* | | 50.1 | | 53.6 |
| Glycine | 0.26 | 2.2 | 0.23 | 1.9 |
| Serine | 0.61 | 5.1 | 0.64 | 5.3 |
| Alanine | 0.38 | 3.2 | 0.58 | 4.8 |
| Proline | 1.2 | 10.0 | 0.62 | 5.2 |
| Aspartic acid | 0.9 | 7.5 | 1.25 | 10.4 |
| Glutamic acid | 2.6 | 21.8 | 2.34 | 19.5 |

[1] The amino acid content of the 12% diets provides approximately 12% amino acid in the diet. Both diets contained in addition 16% corn oil, 2.8% salt mixture, 0.33% vitamin mixture and 2% fiber. The 12% amino acid diets were then made to 100 g by addition of 66% carbohydrate in the form of partially hydrolyzed cornstarch. The diets designated 28% amino acids contained the same proportion of amino acids listed above with the total amino acid content increased to 28 g per 100 g diet. Carbohydrate was accordingly reduced from 66% to 50% per 100 g diet. The vitamin mixture provided in milligrams per 100 g diet: ascorbic acid, 31.5; niacin, 5.04; riboflavin, 0.38; thiamin, 0.32; folic acid, 0.063; vitamin B-6, 0.25; biotin, 0.032; pantothenic acid, 1.9; choline, 53.2; and per 100 g diet: vitamin A, 1007 IU; vitamin D, 253 IU; vitamin E, 6.3 IU; vitamin B-12, 1.26 μg; and phylloquinone, 63 μg. The mineral content of ions or cations (expressed in milligrams per 100 g diet) and the actual chemical compounds fed, were: 378 Ca ($CaHPO_4 \cdot 2H_2O$ and $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$); 208 P ($K_2HPO_4 \cdot 2H_2O$); 7.7 Fe ($FeSO_4 \cdot 2H_2O$); 44 Mg (MgO); 0.38 Cu ($CuSO_4 \cdot 5H_2O$); 2.5 Zn ($ZnSO_4 \cdot 7H_2O$); 0.63 Mn ($MnSO_4$); 840 Cl ($C_5H_{14}ClNO$); 1050 K ($K_2HPO_4 \cdot 2H_2O$); 245 Na (NaI).

ony-forming units) of living *Mycobacterium bovis*, strain BCG (TMC # 1029, Phipps, strain, Trudeau Institute, Saranac Lake, NY). The BCG was given at varying intervals after commencement of dietary treatment and 1 week before killing the animals.

*Statistical analysis.* Statistical evaluation of differences between groups was done by Student's *t*-test or by analysis of variance (*F* test).

## RESULTS

*Nutritional data.* In table 2 data are presented on the nutritional efficiency of the different diets. Mice fed Purina chow and the 12 or 28% C and L diets increased in body weight by approximately the same amount with similar food consumption ranging from 3.5 g to 3.8 g/24 hours. No significant differences were observed between dietary groups in serum protein values and white cell counts (data not shown). The spleen weights of all mice fed the L diets were higher than those of mice fed the corresponding C diets. With the exception of mice fed the 28% L diet, the relative spleen weights of mice on purified diets tended to be lower than those of mice on laboratory diet.

*Humoral immune response.* After inoculation with $5 \times 10^6$ SRBC, mice fed the L diets produced more PFC to SRBC in the spleen than mice fed the C diets (fig. 1). The protein quality effect is particularly striking at the 28% amino acid level where the L diet–fed mice exhibited nearly five times more PFC in their spleens than their C-fed counterparts. This impressive enhancement

TABLE 2

*Effect of 4 weeks dietary regimen on body growth and spleen weight of 6-week-old male mice*

| Mouse strains and measurements | Dietary treatment | | | | |
|---|---|---|---|---|---|
| | Stock diet[1] | 12% C[2] | 12% L[3] | 28% C | 28% L |
| DBA/2 | | | | | |
| Initial weight, *g* | 20.2 ± 0.7[4] | 22.9 ± 0.6 | 19.5 ± 0.6 | 22.1 ± 0.4 | 21.5 ± 0.15 |
| Final weight, %[5] | 123.0 ± 1.8 | 122.8 ± 4.4 | 117.7 ± 2.3 | 124.7 ± 3.0 | 122.2 ± 1.5 |
| Spleen weight:body weight ratio | | | | | |
| Immunized[6] | 56.3 ± 1.2 | 37.1 ± 1.2 | 50.7 ± 1.0[a] | 38.4 ± 0.9 | 48.1 ± 0.6[a] |
| Unimmunized | 44.4 ± 0.9 | 32.5 ± 2.1 | 41.8 ± 0.5[a] | 31.6 ± 0.5 | 46.9 ± 0.4[a] |
| C3H/HeN | | | | | |
| Initial weight, *g* | 19.7 ± 0.6 | 21.5 ± 0.2 | 20.8 ± 0.22 | 21.6 ± 0.13 | 20.1 ± 0.31 |
| Final weight, %[5] | 133.3 ± 1.6 | 124.7 ± 2.2 | 127.5 ± 1.9 | 131.0 ± 2.9 | 126.3 ± 0.9 |
| Spleen weight:body weight ratio | | | | | |
| Immunized[6] | 55.8 ± 2.2 | 38.9 ± 1.3 | 48.0 ± 1.6[a] | 39.1 ± 1.3 | 54.4 ± 2.6[a] |
| Unimmunized | 43.9 ± 1.9 | 36.8 ± 0.4 | 41.1 ± 2.4[a] | 37.3 ± 0.5 | 50.6 ± 0.6[a] |

[1] Ralston Purina Co., St. Louis, MO. [2] Casein hydrolyzate. [3] Lactalbumin hydrolyzate. [4] Mean of 10 mice ± SEM. [5] Percentage of initial weight. [6] Five days after immunization of mice with $5 \times 10^6$ sheep red blood cells. [a] Spleen-weight-to-body-weight ratio of L-fed group vs. corresponding C-fed groups: $P < 0.025$ or less by Student's *t*-test.

of the PFC response cannot be ascribed to presensitization of the L diet–fed group with cross-reacting antigens present in the lactalbumin hydrolyzate because only low numbers of PFC per spleen ($0.4–0.6 \times 10^{-3}$) were found in nonimmunized mice and, moreover, these did not differ between the two dietary groups.

*Mitogen responses.* In diet-fed mice not subjected to an immunogenic stimulus, the splenic mitogen responses to PHA, Con A and LPS do not differ in any marked degree between mice fed the L diets and those fed the C diets although, in general, the responses tend to be slightly higher in the L diet–fed groups (table 3). This is true for both strains of mice tested.

When the mice are stimulated with BCG 1 week prior to death, it is found that increasing the dietary level of amino acid from 12 to 28% enhances the responsiveness of the spleen cells to all of the mitogens tested (fig. 2). In the groups stimulated with PHA and Con A, mice fed the L diets exhibit higher mitogen responses than mice fed the corresponding C diets, and this difference is particularly evident in the groups fed 28% diet and stimulated with PHA. No significant difference is seen between the L and C diet groups in their response to LPS. Strikingly similar results were obtained in both strains (DBA and C3H) of mice tested but, because of space limitations, only the data for DBA mice is shown.

*Effect of Phe and Met.* Our data (fig. 3) show in both strains of mice a 30% drop in splenic PFC response to SRBC when the Phe level in the L diet is raised to that present in the C diet. Similarly a 20% (DBA mice) and a 23% (C3H mice) decrease in the mitogen response to PHA was produced by the addition of Phe (fig. 3). The addition of Met to the L + Phe diet, on the other hand, failed to influence the immune response.

*Time requirement of dietary treatment.* In the above described experiments, immunological studies were begun after 3 weeks of dietary treatment. To establish the minimum period of dietary exposure necessary to obtain a significant effect, immune assays were commenced, in selected experiments, after 1, 2 or 3 weeks of dietary treatment. The results of these experiments (table 4) clearly indicate that the enhancing effect of L on immune responsiveness increases with time, and the values after 2 weeks on a diet are intermediate between those observed after 1 and 3 weeks, respectively.

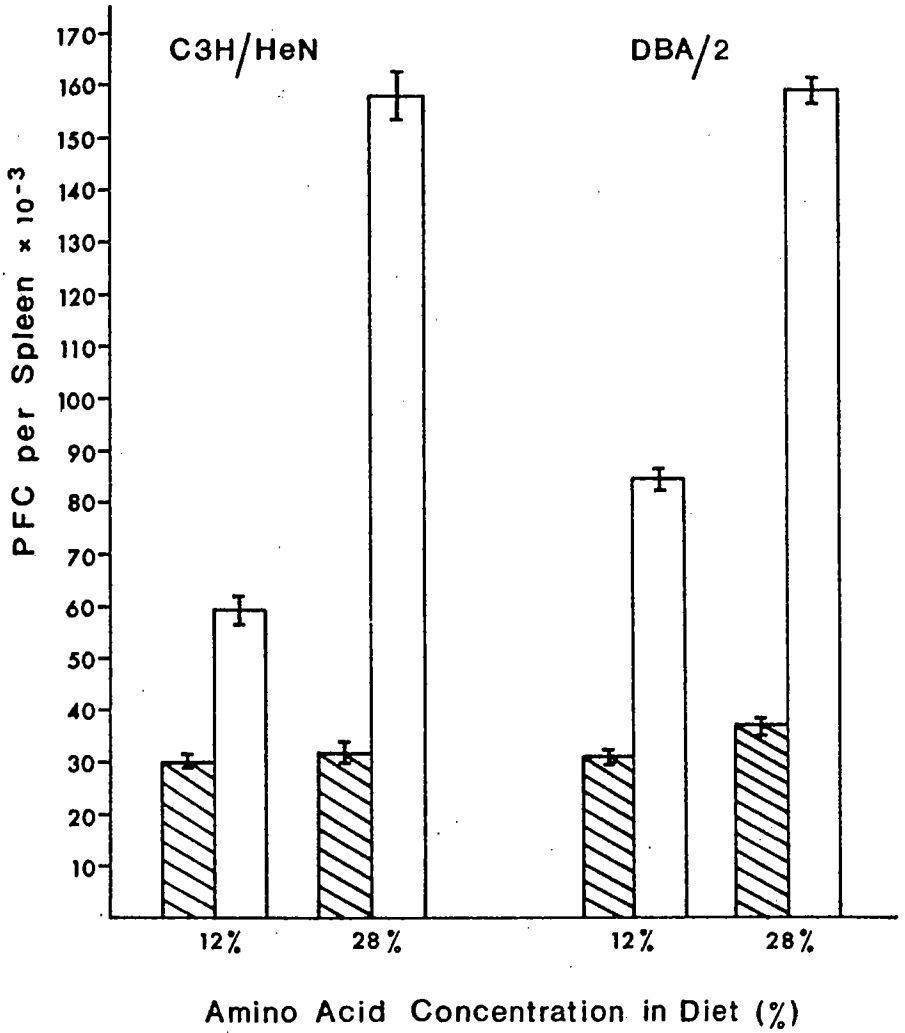


Fig. 1 Effect of 3 weeks of dietary treatment with lactalbumin hydrolyzate (open bars) or casein hydrolyzate (hatched bars) on the number of plaque-forming cells (PFC) per spleen 5 days after immunization with $5 \times 10^6$ sheep red blood cells. Each value represents the mean of 10 mice ± SEM. 28% L diet vs. 12% L diet: $P < 0.01$ by Student's $t$-test. By the two-way analysis of variance ($F$ test) for both strain of mice, the effect of the quality of protein (L vs. C) is highly significant, $P < 0.001$.

## DISCUSSION

Our nutritional studies show that 12% L and C diets and stock diet all sustain normal growth of mice and that 28% diets, with higher amino acid content, do not enhance body growth beyond that of the 12% diets (table 2). In the 12% C and L diets, the concentrations of arginine (Arg), lysine (Lys), tryptophan (Try) and Phe are either similar to, or in excess of, the established minimal concentration for optimal growth of Swiss mice (16). The concentration of Met in the 12% L diet is approximately half of the 0.5% value suggested for Swiss mice (19). In the latter study (19), however, dietary cystine (Cys) was considered negligible, whereas in the 12% L diet, the concentration of Met-Cys reaches 0.37%. Moreover, a recent study showed that a diet that contained 0.317% Met supported growth of weanling DBA/2J mice with no evidence of fatty liver even in the absence of Cys (20). The National Academy of Sciences' recent report (21) on maximum possible weight gain in 26 inbred male mouse strains indicates that, at a starting weight of 21 g, the average weight gain from age 6 to 8 weeks is 0.22 g/day and from 6 to 16 weeks

TABLE 3

*Effect of 3 weeks of dietary treatment with lactalbumin (L) or casein hydrolyzate (C) on the mitogen response of mouse spleen cells*

| Mouse strain and mitogen | Dietary treatment | | | |
|---|---|---|---|---|
| | 12% C | 12% L | 28% C | 28% L |
| DBA/2 | | | | |
| PHA (5 μg) | 42.0 ± 1.7[1] | 40.5 ± 1.1 | 36.5 ± 0.7 | 43.2 ± 1.1 |
| Background value | 5.1 ± 0.1 | 3.6 ± 0.1 | 4.8 ± 0.7 | 4.0 ± 0.1 |
| Con A (5 μg) | 47.2 ± 0.9 | 54.5 ± 1.3 | 50.3 ± 1.1 | 55.1 ± 0.7 |
| Background vlaue | 5.1 ± 0.1 | 3.6 ± 0.1 | 4.8 ± 0.1 | 4.0 ± 0.1 |
| LPS (10 μg) | 23.2 ± 1.4 | 28.0 ± 0.3 | 29.8 ± 0.8 | 33.7 ± 0.7 |
| Background value | 5.1 ± 0.1 | 3.6 ± 0.1 | 4.8 ± 0.1 | 4.0 ± 0.1 |
| C3H/HeN | | | | |
| PHA (5 μg) | 44.3 ± 0.7 | 41.6 ± 0.9 | 39.8 ± 0.9 | 39.6 ± 0.4 |
| Background value | 4.5 ± 0.1 | 3.9 ± 0.2 | 4.0 ± 0.1 | 4.1 ± 0.3 |
| Con A (5 μg) | 46.8 ± 0.7 | 49.8 ± 0.9 | 51.5 ± 0.9 | 52.1 ± 0.7 |
| Background value | 4.5 ± 0.1 | 3.9 ± 0.2 | 4.0 ± 0.1 | 4.1 ± 0.3 |
| LPS (10 μg) | 26.2 ± 0.9 | 25.9 ± 0.9 | 27.3 ± 0.6 | 31.6 ± 1.5 |
| Background value | 4.5 ± 0.1 | 3.9 ± 0.2 | 4.0 ± 0.1 | 4.1 ± 0.3 |

[1] Mean of 10 mice ± SEM.

of age is 0.13 g/day. This rate of growth is consistent with the overall average growth of 0.19 g a day in 4 weeks observed in our 6-week-old mice on purified diets (table 2).

The immune responses in mice fed stock diet were found to vary with different batches of commercial laboratory diet, although they were always significantly lower than those of mice fed the 28% L diet (data not shown). In contrast, the pattern of immune responses in mice fed defined formula diets were always consistent. The amino acid distribution of the protein moiety is a noncontrollable variable in standard laboratory mouse feeds, since these are made of varying proportions of animal and vegetable proteins. To evaluate correctly the effect of lactalbumin hydrolyzate on immune responsiveness, we have, therefore, chosen to compare this protein moiety with casein hydrolyzate. The latter amino acid mixture is derived from the type of protein most commonly used both in laboratory and clinical settings.

In mice not challenged with an immunogenic stimulus, diet alone was found to have little or no effect on a variety of parameters examined. Thus, serum protein, circulating leukocyte number and the spleen cell mitogen responses to Con A, PHA and LPS (table 3) were all within normal limits. The only difference noted was a higher spleen weight:body weight ratio in the L diet–fed mice.

After challenging mice with an immune stimulus and measuring either the specific humoral immune response to SRBC (fig. 1) or the nonspecific splenic cell responsiveness to mitogens after stimulation with BCG (fig. 2), it was observed first of all that the responses of the mice fed the L diets were consistently greater than those of the mice fed the corresponding C diets. These differences were particularly evident with the 28% amino acid diets. Secondly, increasing the concentration of amino acids in the diet from 12 to 28% greatly enhanced both parameters measured, namely, specific humoral immunity and splenic mitogen responses. In the C-fed mice, a comparable enhancement was seen in mitogen responsiveness. However, increasing the dietary concentration of C from 12 to 28% failed to produce any change in the splenic PFC response to SRBC, in either strain of mice tested. This latter finding is consistent with a report by José and Good showing that C3H mice on 15 and 28% casein diets did not differ significantly in their capacity to develop hemagglutinating antibody (22).

The reason for the divergent immune ef-

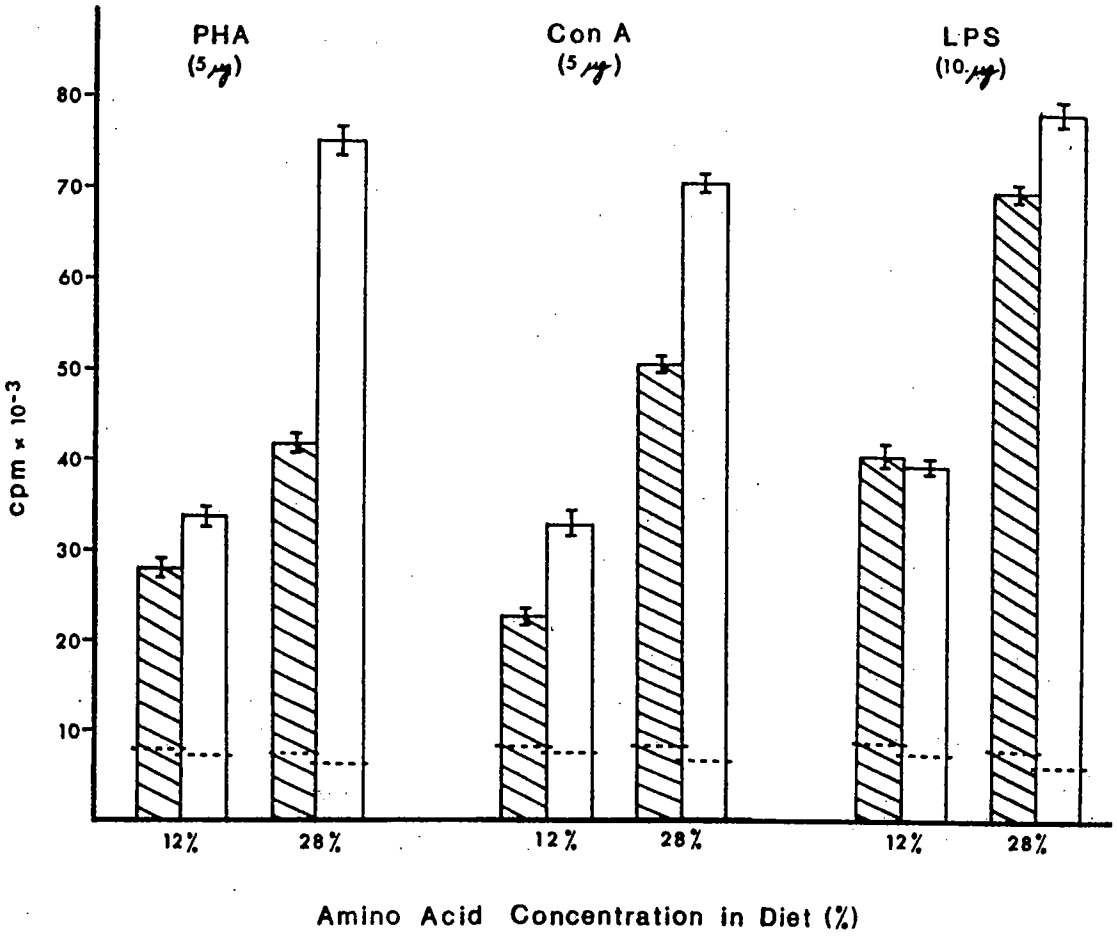


Fig. 2 Effect of 3 weeks of dietary treatment with lactalbumin hydrolyzate (open bars) or casein hydrolyzate (hatched bars) on the spleen cell mitogen responses of BCG-stimulated mice. Dotted line indicates background (no mitogen) values. Each value represents the mean of 10 mice ± SEM. By the two-way analysis of variance ($F$ test), for PHA the effect of the quality of protein (L vs. C) and the effect of the concentration (%) of amino acid were both significant, $P < 0.05$, and $P < 0.01$, respectively; for Con A both effects were highly significant, $P < 0.001$; for LPS, the concentration of amino acid effect was highly significant, $P < 0.001$.

fect of these two dietary proteins is a matter of speculation. The relative concentration of some essential amino acids is higher, similar to, or lower in L than in C (table 1). Although any single difference between these two proteins could theoretically be construed as being crucial to the immune effect, our previous study (14) led us to consider first the restricted amino acids. The major relative deficiency in the L diet involves Phe and Met, which are respectively 44 and 30% lower than in the C diet. Of these, Phe attracted our attention since our previous experiments (14) showed that a 34% restriction of Phe in a 12% casein diet enhanced the PFC response in CBA, C3H and C57BL/6 mice by approximately the same degree as that noted in our 12% L diet group when compared to the 12% C diet mice (fig. 1).

Despite a slight drop in immune reactivity, the abolition of the difference in Phe level between L and C diets, fails to lower the immune response of L + Phe fed mice to the level of mice fed C diets (fig. 3). Indeed, other amino acids must be implicated. For example, lactalbumin contains significantly more threonine than casein.

Our data further indicate that the determining factor in the immune effect of Phe does not appear to be related to its nutritional deficiency, but rather the ratio of this essential amino acid to one or more of the other

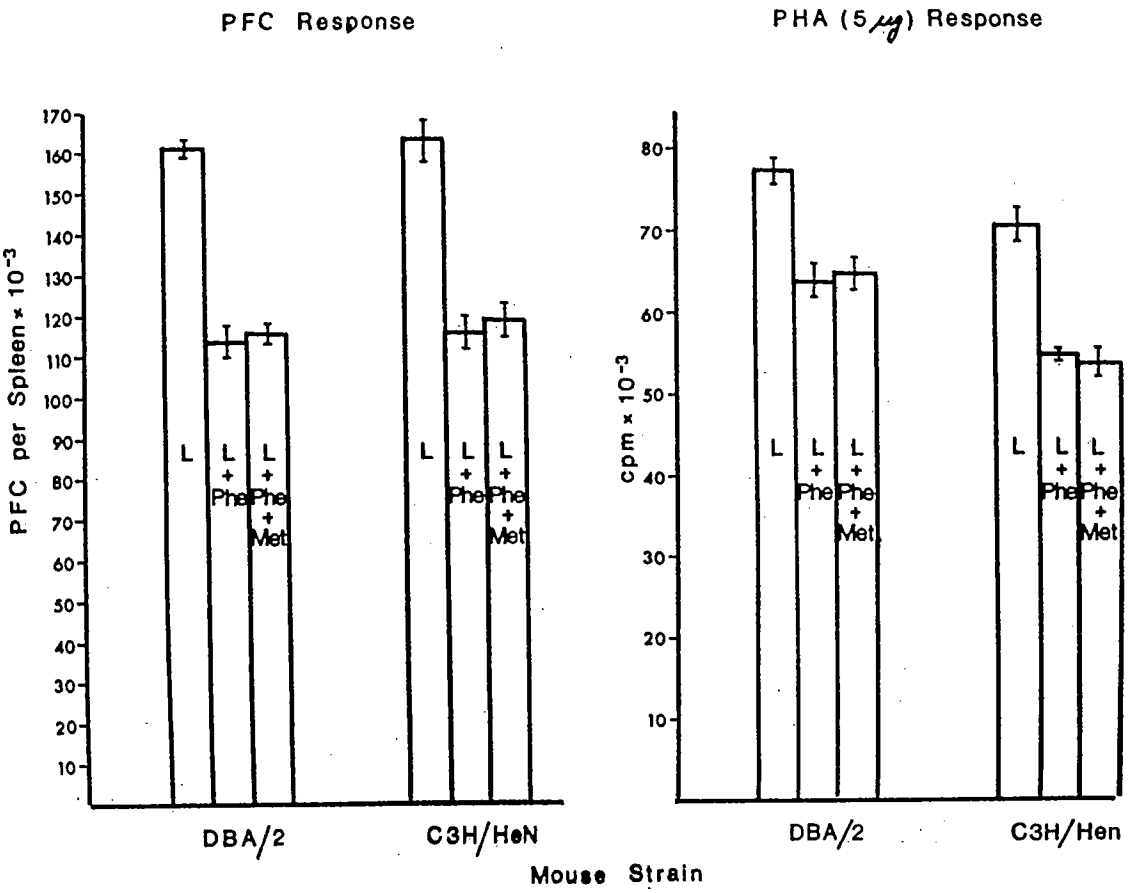


Fig. 3 Effect of 3 weeks of dietary treatment with lactalbumin hydrolyzate (L), L + Phenylalanine (Phe), or L + Phe + methionine (Met) on the PFC response to SRBC (as in fig. 1) or the mitogen response of BCG-stimulated mice to PHA (as in fig. 2). Each value represents the mean of 10 mice ± SEM. L diet vs. L + Phe: $P < 0.005$ by Student's $t$ test.

amino acids in the diet. This assumption is strengthened by the fact that the immune-enhancing effect of L diets is considerably greater with the 28% L diet, which contains Phe well in excess of minimum requirement. It is noteworthy that this immune effect of dietary Phe was previously (14) found in CBA, C3H and C57BL/6 mice; hence the

TABLE 4

*Effect of 1, 2 or 3 weeks of dietary treatment with 28% lactalbumin hydrolyzate*

| Mouse strain | PFC[1] per spleen × $10^{-3}$ | | | Mitogen response[2] (cpm × $10^{-3}$) | | |
|---|---|---|---|---|---|---|
| | 1 week[3a] | 2 weeks[b] | 3 weeks[c] | 1 weeks[a] | 2 weeks[b] | 3 weeks[c] |
| DBA/2 | 93 ± 5.6 | 127 ± 2.9 | 160 ± 0.6 | 26.6 ± 0.8 (6.7) | 62.8 ± 1.4 (6.4) | 75.5 ± 1.3 (6.9) |
| C3H/HeN | 89 ± 4.7 | 139 ± 6.4 | 159 ± 3.5 | 29.4 ± 6.3 (6.2) | 56.3 ± 0.9 (6.5) | 69.0 ± 1.0 (7.5) |

[1] Number of plaque-forming cells (PFC) per spleen 5 days after immunization of mice with 5 × $10^6$ sheep red blood cells. [2] Mitogen response of mouse spleen cells to 5 μg PHA. BCG-stimulated mice were tested 7 days after inoculation with *M. bovis*. Background values obtained without mitogen are shown in parentheses. Values are means cpm ± SEM. [3] Significance comparing by superscripts a vs. b; b vs. c: $P < 0.01$ or less by Student's $t$-test.

same phenomenon appears to apply to four unrelated strains of mice. Although the underlying mechanism remains totally unknown, the practical importance of these findings is self-evident.

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