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Mechanism of Altered B-Cell Response Induced by Changes in Dietary Protein Type in Mice[1]

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ABSTRACT The effect of 20 g/100 g dietary lactalbumin (L) or casein (C) diets or a nonpurified (NP) diet on the immune responsiveness of C57Bl/6J, C3H/HeJ and BALB/cJ mice has been investigated by measuring the response to the T cell–independent antigen, TNP-Ficoll. To investigate the possible influence of dietary protein type on the supply of B lymphocytes, bone marrow lymphocyte production has been examined by a radioautographic assay of small lymphocyte renewal and an immunofluorescent stathmokinetic assay of pre-B cells and their proliferation. The humoral response of all mice fed the L diet was found to be higher than that of mice fed the C diet or nonpurified diet. A similar pattern of dietary protein effect in (CBA/N × DBA/2J) F_1 mice carrying the *xid* defect was observed following challenge with sheep red blood cells (SRBC). An even greater enhancing effect of dietary L was noted in normal (DBA/2J × CBA/N) F_1 mice after immunization with SRBC, but in contrast, the normal large-scale production of B lymphocytes in mouse bone marrow was independent of the type of dietary protein. Dietary protein type did not affect blood level of minerals and trace metals. The free plama amino acid profile essentially conformed to the amino acid composition of the ingested protein, suggesting that the changes in plasma amino acid profile might be a crucial factor in diet-dependent enhancement or depression of the B-cell response. The findings indicate that the observed effects of altered dietary protein type on humoral immune responsiveness are not exerted centrally on the rate of primary B-lymphocyte production in the bone marrow, but may reflect changes either in the functional responsiveness of the B lymphocytes themselves or in the processes leading to their activation and differentiation in the peripheral lymphoid tissues. J. Nutr. *115*: 1409–1417, 1985.

INDEXING KEY WORDS diet • protein • immunity • B-cell response • mice

Previous studies have shown that the type of protein in the diet has a significant effect on the development of humoral immunity to T-cell dependent (TD)[2] and to T-cell independent (TI) antigens. Thus, the humoral immune response to the TD sheep red blood cells (SRBC) and horse red blood cells (HRBC) was found to be significantly greater

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[2]Abbreviations: C, casein; cμ, cytoplasmic μ heavy chains; FITC, fluorescein isothiocyanate; HRBC, horse red blood cells; L, lactalbumin; NP, nonpurified; PFC, plaque-forming cells; sμ, cell surface μ heavy chains; SRBC, sheep red blood cells; TD, T-cell dependent; TI, T-cell independent; TNP-Ficoll, trinitrophenylated-Ficoll; TRITC, rhodamine isothiocyanate; *xid*, X-linked immune deficiency.

in mice fed a lactalbumin (L) diet than that of mice fed with corresponding casein (C) diet (1). Similarly the plaque-forming cell (PFC) response of mice fed L diet to the presumed TI trinitrophenylated (TNP)-Ficoll antigen was substantially higher in mice fed L diet than in their counterparts fed C diet (2). Conversely dietary protein type appeared to have no effect on several aspects of cell-mediated immunity such as the graft-versus-host reaction, delayed-type hypersensitivity reactions, spleen cell mitogen responses, host resistance to *Salmonella typhimurim* (2) or phagocytosis of SRBC by peritoneal macrophages (3). It was thence postulated that the type of protein in the diet influences directly the intrinsic capacity of the B lymphocytes to respond to an immunogenic stimulus. The above-described immune effect of the two tested proteins was obtained at 20 g/100 g concentration at which level both formula diets exhibited similar and normal nutritional efficiency (2).

The B lymphocytes, which respond in primary immune responses in the spleen and other peripheral lymphoid tissues of mice, are predominantly newly formed cells continuously supplied to these tissues by the bone marrow (see review in ref. 4). Within the bone marrow, early progenitor cells give rise to large, rapidly proliferating pre-B cells, identified by the presence of free μ heavy chains in their cytoplasm (5–7). These cells, after a terminal mitosis, form small nondividing pre-B cells, which soon mature into B lymphocytes bearing cell surface IgM molecules (8). Together, the small pre-B cells and B lymphocytes comprise a large majority of the small lymphocytes in the bone marrow (9), a substantial population of cells rapidly renewed by precursor cell proliferation (10). Newly formed B lymphocytes leave the bone marrow and circulate rapidly to become immunologically responsive cells in the peripheral lymphoid tissues (4). Thus, the possibility is raised that the changes induced in humoral immune responsiveness by variation in dietary protein type may reflect an effect on the regulation of the production of virgin B lymphocytes in the bone marrow and the supply of these cells to the peripheral lymphoid tissues.

The present study was designed to define the effect of dietary protein type on the production of B lymphocytes in the bone marrow and to explore the nature and mechanism of the observed effect of dietary protein on humoral immunity.

MATERIAL AND METHODS

Mice. Male C3H/HeJ, C57Bl/6J, BALB/cJ were purchased from Jackson Laboratories (Bar Harbor, ME). (DBA/2J Dub × CBA/N Dub) F_1 male normal, and (CBA/N × DBA/2J Dub) F_1 male mice carrying the *xid* defect, were obtained from Dominion Laboratories, Dublin, VA.

Dietary treatment. A detailed composition of the defined formula diets (4.3 kcal/g) is given in table 1. The diets contained 20 g/100 g of L (L diet) or C (C diet) vitamin-free purified protein. Other animals were fed nonpurified (NP) diet (Purina Rodent Chow, Ralston Purina Co., St. Louis, MO; estimated 23 g/100 g protein from various sources).

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 in wire-bottomed cages to reduce coprophagy, were placed on the various diet regimens at 6–8 wk of age, and immunologic studies or blood analysis commenced 2 wk later. Bone marrow studies were performed 3 wk after dietary treatment. In one experiment, immunologic studies were performed in 6-mo-old mice. Dietary treatment was continued throughout the experiment. Each dietary group comprised 10 mice unless otherwise stated.

PFC assay. The method used for assaying IgM PFC was essentially the one described by Cunningham and Szenberg (11) with certain minor modifications (12). Briefly, spleen cell suspensions of 15-ml volume were prepared in a balanced salt solution supplemented with 10% heat-inactivated calf serum. The test consisted of mixing 0.05 ml of spleen cells, 0.15 ml suspension of SRBC (20% concentration) and 0.75 ml guinea pig serum (diluted 1:15). The number of PFC was estimated by multiplying the number of PFC in each sample by 300. The mice were injected i.v. with either 10^8 or 5×10^8 SRBC and assayed for PFC on d 4 when the

TABLE 1

Amino acid composition of test diets[1,2]

Amino acid	Lactalbumin (20 g/100 g)	Casein (20 g/100 g)
Amino acid in g/100 g diet		
Isoleucine*	1.01	0.85
Leucine	1.65	1.50
Valine*	0.88	1.07
Methionine*	0.35	0.42
Cystine*	0.26	0.05
Phenylalanine*	0.54	0.82
Tyrosine*	0.50	0.85
Threonine*	1.12	0.61
Tryptophan	0.22	0.19
Lysine	1.42	1.27
Histidine*	0.27	0.59
Arginine*	0.40	0.56
Glycine	0.32	0.32
Serine	0.85	0.90
Alanine*	0.74	0.56
Proline*	0.93	1.65
Aspartic acid*	1.58	1.38
Glutamic acid	3.23	3.50

[1]The amino acid content of the 20 g/100 g protein diets provides approximately 17% amino acid in the diet. All diets contained in addition 18% corn oil, 2.8% salt mixture, 0.33% vitamin mixture and 2% fiber. The 20 g/100 g protein diets were then made to 100 g by addition of 57% carbohydrate in the form of partially hydrolyzed cornstarch. The presence of lactose, ash and moisture in lactalbumin (20 g/100 g) and ash and moisture in casein, (15 g/100 g) was taken into consideration. [2]The vitamin mixture plus the vitamins contained in the basal diet provided *in milligrams per 100 g diet:* ascorbic acid, 45.0; niacin, 7.2; riboflavin, 0.54; thiamin, 0.45; folic acid 0.09; vitamin B-6, 0.36; biotin, 0.045; pantothenic acid, 2.7; choline, 76 *and per 100 g diet:* retinyl palmitate, 1439 IU; ergocalciferol, 360 IU; vitamin E (*dl*-tocopheryl acetate), 9.0 IU; vitamin B-12, 1.8 μg; and vitamin K (phylloquinone), 90 μg. The mineral content of ions or cations (expressed in milligrams per 100 g diet) and the actual chemical compounds fed were: Ca, 378 ($CaHPO_4 \cdot 2H_2O$ and $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$); P, 208 ($K_2HPO_4 \cdot 2H_2O$); Fe, 7.7 ($FeSO_4 \cdot 2H_2O$); Mg, 44 (MgO); Cu, 0.38 ($CuSO_4 \cdot 5H_2O$); Zn, 2.5 ($ZnSO_4 \cdot 7H_2O$); Mn, 0.63 ($MnSO_4$); Cl, 840 (C_5H_{14} ClNO); K, 1050 ($K_2HPO_4 \cdot 2H_2O$); Na, 245 (NaCl). *Difference in amino acid concentration between diets ≥ 10%.

response was shown to peak (12). Data on plaque-forming responses is presented as plaques per spleen rather than as plaques/10^6 cells. This is a valid measure of the B-cell response of the host because we are measuring the total response of the whole organ, regardless of any variation in its size. In earlier studies, we have calculated our results both ways (i.e., per spleen and per 10^6 cells) and both sets of results have provided similar data.

PFC response to TNP-Ficoll. Hapten-coupled SRBC were prepared by the method of Rittenberg and Pratt (13). Immunization consisted of a single i.v. injection of 20 μg TNP-Ficoll dissolved in saline. The PFC assay was performed on d 4 after TNP-Ficoll injection, when the response has been shown to peak.

Radioautographic assay of bone marrow lymphocyte production. C3H/HeJ mice were fed either C diet, L diet or NP diet for 3 wk from 6 wk to 9 wk of age. Mice were then given [^{3}H]thymidine (sp act, 6.7 Ci/mmol; New England Nuclear Corp., Boston, MA) by an initial i.p. injection (25 μCi) followed by continuous infusion through a subcutaneous polyethylene cannula (2 μCi/g body weight per day) as detailed elsewhere (10). At intervals of 12, 24, 48 and 72 h after starting [^{3}H]thymidine administration pairs of mice were killed by cervical dislocation. Bone marrow cells from each femur were suspended, counted, smeared, processed for radioautography, exposed for 28 d and stained with MacNeal's tetrachrome as described (8, 10).

In radioautographic bone marrow smears small lymphocytes were identified as described previously (8, 10). The incidence of small lymphocytes was determined in differential counts of 2500–3000 nucleated marrow cells, scanning longitudinally along the entire length of each smear to ensure representative cell sampling. The proportion of small lymphocytes labeled by [^{3}H]thymidine (labeling index) was found by examining 1000 consecutive small lymphocytes, recording the number of photographic grains overlying each nucleus. Cells with more than 3 grains were scored as positively labeled, well above the level of background grains.

Immunofluorescence assay of bone marrow pre-B cells. After 3 wk of dietary treatment, 9-wk-old C3H/HeJ mice were given an i.p. injection of vincristine sulfate (1 mg/kg body weight; Eli Lilly, Toronto, Ont.) at 0800–0900 to arrest dividing cells in metaphase, as described (6). Femoral bone

marrow cells were sampled 2.5 h later, suspended, counted and exposed to fluorescein isothiocyanate (FITC)-conjugated affinity-purified goat antibody to mouse μ heavy chains (Kirkegaard & Perry Laboratories, Gaithersburg, MD) for 30 min on ice to label cell surface μ chains (sμ) (6). The cells were then washed, cytocentrifuged, fixed in cold 5% acetic acid in ethanol, and exposed to rhodamine isothiocyanate (TRITC)-conjugated anti-μ antibody (Kirkegaard & Perry Laboratories) for 30 min at room temperature to label cytoplasmic μ heavy chains (cμ) in addition to sμ (6). After overnight washing in phosphate-buffered saline, pH 7.2, individual cells were examined by epifluorescence microscopy, scoring pre-B cells, which exhibited rhodamine fluorescence alone (cμ only), and B cells, double labeled with fluorescein plus rhodamine (sμ ± cμ). Sufficient nucleated bone marrow cells were examined to score at least 100 cells of each phenotype (7). Each pre-B cell was examined by phase contrast microscopy to measure its diameter, by means of an ocular micrometer scale, and to detect the percentage of pre-B cells blocked in metaphase (metaphase index).

Amino acid analysis of purified dietary protein. Purified proteins were analyzed, following enzymatic hydrolysis, in a Beckman amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA) with an expected coefficient of variation of ±5%.

Free plasma amino acids. After 2 wk of dietary treatment, mice were anesthetized with diethylether, and blood was collected from the retroorbital sinus into heparinized syringes. Plasma was separated from whole blood by centrifuging at 15,000 × g at 4°C for 15 min. Blood from three mice was pooled together. To prepare for amino acid analysis, proteins were precipitated by sulfosalicylic acid. After centrifuging to remove precipitated proteins, aliquots of supernatant containing free amino acids were analyzed in a Beckman amino acid analyzer, Model 119 CL.

Plasma minerals and trace metals. The plasma zinc and copper contents were determined by atomic absorption in a Perkin-Elmer Model 306 (Norwalk, CT) spectrophotometer. Plasma phosphorus, magnesium and calcium were determined in the University hospital laboratory with standard reproducible procedures (coefficient of variation less than 5%).

Statistical analysis. Statistical evaluation of differences between groups was done by Student's *t*-test.

RESULTS

Nutritional data and blood analysis. The average body growth over a 3-wk period expressed as percentage of initial weight in C3H/HeJ, C57Bl/6J, BALB/cJ, (DBA/2J Dub × CBA/N Dub) F_1 normal and (CBA/N × DBA/2J Dub) F_1 defective mice was 113.3 ± 2.8, 116.9 ± 3.0, 112.1 ± 2.0, 117 ± 3.6 and 118 ± 1.5, respectively, when fed the 20 g/100 g L diet. No significant differences were noted in the rate of growth between mice of the same strain fed the L, C or NP diet. Food consumption was similar varying from 3.5 ± 0.3 g/24 h in the C3H/HeJ group to 3.9 ± 0.4 g/24 h in the BALB/cJ group. Moreover, no significant differences were observed in serum protein values.

The plasma free amino acid pattern in the mice fed L diet or C diet conformed to a great extent to the amino acid profile of the corresponding ingested protein. For example, when the difference between L and C in the concentration of a specific amino acid was 10% or more (indicated by asterisks in table 1), the plasma concentration of the corresponding amino acid in diet-fed mice changed accordingly. This involved seven essential and three nonessential amino acids. However, in the case of cystine and isoleucine the protein composition differences (>10%) were not reflected in the plasma value where no difference was seen between mice fed L or C diet in the plasma concentration of these two amino acids.

The plasma values of all measured minerals and trace metals were similar or identical in mice fed L or C diet (data not shown).

Humoral immune response. The mean number of PFC per spleen 4 d after i.v. injection of 20 μg TNP-Ficoll in the L diet–fed C57Bl/6J mice was 166% of that noted in C diet–fed and 133% of that noted in the NP diet–fed counterparts. The corresponding values observed in C3H/HeJ mice were 185

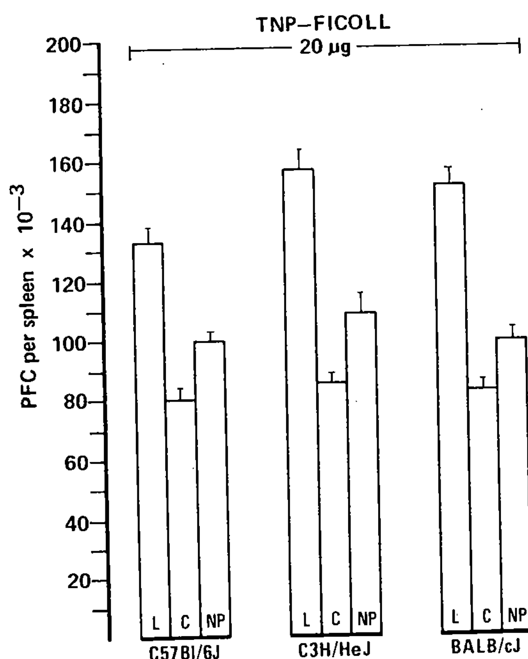

Fig. 1 Number of plaque-forming cells (PFC) per spleen after immunization of C57Bl/6J, C3H/HeJ and BALB/cJ mice with trinitrophenylated (TNP)-Ficoll. Effect of 2 wk of dietary treatment with 20 g lactalbumin/100 g diet (L), casein (C) (20 g/100 g) or nonpurified diet (NP). Each value represents the mean ± SEM; $n = 10$ mice. By Student's t-test, the effect of the type of protein after 20 µg of TNP-Ficoll is: L vs. C, L vs. NP and NP vs. C: $P < 0.025$.

and 145% and in BABL/cJ mice were 184 and 153% respectively (fig. 1). The mean number of PFC per spleen 4 d after i.v. injection of 10^8 SRBC in L diet-fed CBA/N defective mice was 167% of that of C diet-fed and NP diet-fed mice. Following 5×10^8 SRBC, the mean number of PFC in L diet-fed CBA/N defective mice was 229% of that noted in C diet-fed and 175% of that noted in NP diet-fed counterparts (fig. 2*A*). As expected, the normal F_1 mice produced a greater number of PFC per spleen after injection of the same amount of SRBC. Moreover, the number of PFC per spleen in L diet-fed was 437% of that of C diet-fed and 168% of that of NP diet-fed mice. The pattern of response was essentially similar after 5×10^8 SRBC (fig. 2*B*).

Bone marrow lymphocyte production. The number and renewal of small lymphocytes in the bone marrow are summarized in table 2. The total cellularity of femoral marrow was unaffected by 3 wk of treatment with either C diet or L diet, while small lymphocytes formed the usual substantial subpopulation of bone marrow cells in each animal group. Moreover, the turnover the newly formed small lymphocytes proceeded at a similar rate in all cases, as revealed by [^{3}H]thymidine labeling. Bone marrow small lymphocytes are themselves postmitotic nondividing cells, which consequently do not directly incorporate [^{3}H]thymidine in DNA synthesis; they are rapidly renewed from the division of proliferating precursor cells, however, and thus soon become replaced by labeled progeny of such progenitors during continuous administration of [^{3}H]thymidine (4, 5). Mice fed the NP diet showed the customary rapid increase in the proportion of small lymphocytes labeled by [^{3}H]thymidine during infusion of the isotope for 12 h (data not shown), 24 h and 48 h (table 2), which represented the production of a substantial total number of newly formed small lymphocytes per femur (table 2). Groups of mice fed C or L diet showed incidences and absolute numbers of [^{3}H]thymidine-labeled small lymphocytes, which were closely comparable with those in the mice fed nonpurified diet (table 2). Thus, the total production of small lymphocytes per femur had not been altered by either of the two dietary treatments.

An incidental observation of note was that the intensity of labeling of small lymphocytes with time during [^{3}H]thymidine administration appeared to differ to some extent in both groups fed purified diets, compared with the group fed the NP diet. In mice fed NP diet there was a distinct increase in the labeling intensity of individual small lymphocytes during [^{3}H]thymidine infusion; the mean proportion of the total labeled small lymphocytes that had high grain counts (≥20 grains) was 79% at 48–72 h. In C- and L-fed mice, however, despite an increasing percentage of labeled cells with time, the labeling intensity of small lymphocytes tended not to increase to the usual extent (labeled small lymphocytes having ≥20 grains: 43% at 48–72 h).

Bone marrow pre-B cells and B lymphocytes. As detailed in table 3 the number of $c\mu^+$ pre-B cells and of $s\mu^+$ B lymphocytes in

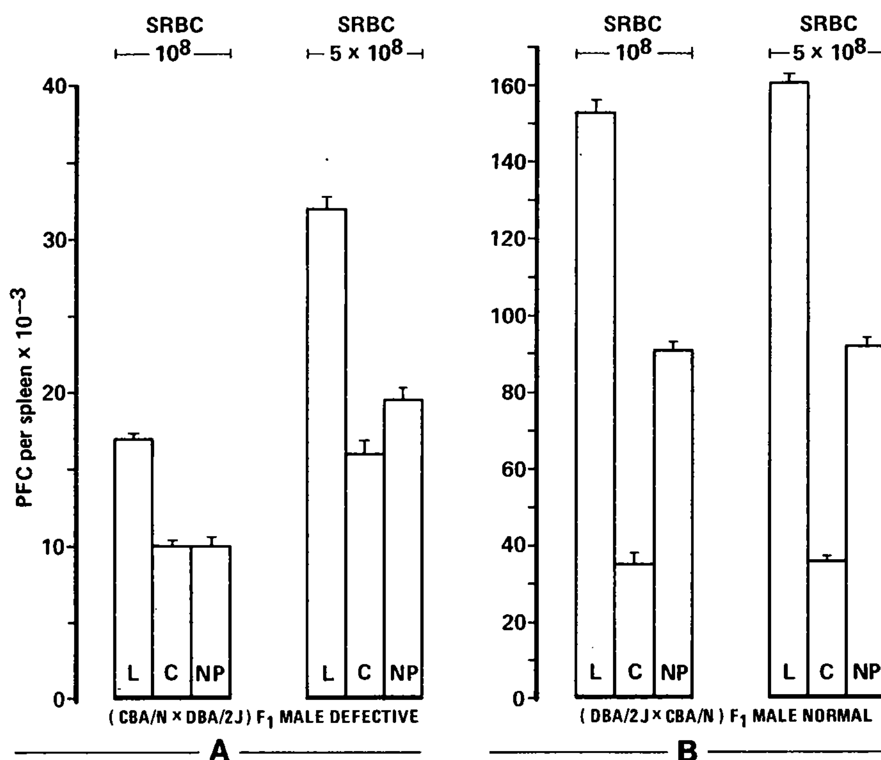

Fig. 2 Number of plaque-forming cells (PFC) per spleen after immunization of (CBA/N × DBA/2J)F_1 defective (2*A*) and (DBA/2J × CBA/N)F_1 normal (2*B*) mice with sheep red blood cells (SRBC). Effect of 2 wk of dietary treatment with 20 g/100 g diet of lactalbumin (L), casein (C) or with nonpurified diet (NP). Each value represents the mean ± SEM; $n = 10$ mice. By Student's *t*-test, the effect of the type of protein on PFC of F_1 defective mice after 10^8 SRBC is: L vs. C: $P < 0.005$, and in F_1 normal mice it is: L vs. C, L vs. NP and NP vs. C: $P < 0.0005$. After 5×10^8 SRBC, the effect of the type of protein on PFC of F_1 defective and F_1 normal mice is: L vs. C, L vs. NP and NP vs. C: $P < 0.025$.

the femoral bone marrow of mice fed C or L diet were closely similar to those of mice fed NP diet. The proportion of large dividing cells (≥10 μm diameter) among the pre-B cell population in the mice fed C or L diet (22–25%) also resembled that in mice fed NP diet (33%). The incidence of cells arrested in metaphase after vincristine administration indicated the proportion of cells entering mitosis per unit time. Under the conditions of the present experiment the mitotic index of pre-B cells has been shown to increase linearly from 2 to 4 h after vincristine injection (7). The metaphase index at 2.5 h, as used in the present work, thus provided a measure of the rate of pre-B cell proliferation. The metaphase index of pre-B cells, in conjunction with their population size, revealed no apparent difference in the total production of pre-B cells between groups of mice receiving the 3 types of diets (table 3).

DISCUSSION

We have recently shown that the type of protein in diet influences the humoral immune response in C3H/HeN mice (2). Our current experiments (fig. 1) indicate that the same phenomenon may be observed in at least three unrelated strains of mice.

Alterations in humoral immune responsiveness after feeding various types of dietary protein might be due to either a central effect on the bone marrow, with consequent changes in the supply of newly formed B lymphocytes, or a peripheral effect in the lymphoid tissues. In the current work, however, marked changes in humoral responsiveness and numbers of PFC in the

TABLE 2

Number and renewal of small lymphocytes in the bone marrow of mice fed diets of various protein types[1]

Bone marrow cell	Diet NP	Diet C	Diet L
Total nucleated cells/femur, $\times 10^{-6}$	17.9 ± 3.1	18.0 ± 3.8	17.0 ± 1.5
Small lymphocytes			
Incidence, %	22.1 ± 1.1	21.1 ± 5.3	20.1 ± 2.9
Cells/femur, $\times 10^{-5}$	39.3 ± 7.8	36.8 ± 6.5	34.0 ± 7.3
[^{3}H]Thymidine-labeled small lymphocytes[2]			
Labeling index, %			
24 h	25.1	32.0	28.3
48 h	65.1	71.6	69.0
Labeled cells/femur, $\times 10^{-5}$			
24 h	9.8	9.3	10.5
48 h	31.9	32.0	28.3

[1]Values after ± are SD from groups of six mice. Bone marrow taken from C3H/HeJ mice, 9 wk old, after 3-wk treatment with nonpurified (NP) diet, casein diet (C) (20 g casein/100 g diet), or lactalbumin diet (L) (20 g/100 g). [2]Labeled small lymphocytes (≥3 grains/cell) derived from bone marrow cells pooled from two mice at each time interval during continuous infusion of [^{3}H]thymidine.

spleen of mice fed the various diets were not associated with any comparable changes in the magnitude of bone marrow B-lymphocyte genesis, as assayed either by pre-B cell proliferation or by small lymphocyte production. Functional changes in responsiveness of the individual B lymphocytes generated centrally in the bone marrow are not excluded. Alternatively, the results would be consistent with a peripheral effect of altered dietary protein type, either producing qualitative changes in the B lymphocytes themselves or modifying the complex sequence of events in B-lymphocyte activation, clonal expansion and antibody secretion.

The mechanisms regulating B-lymphocyte production in the bone marrow may normally include external environmental factors. The production of bone marrow lymphocytes is reduced in magnitude in mice either raised under germfree conditions or fed an "elemental" purified diet (14) containing most nutrients in their simple molecular form. Conversely, NP diet–fed mice given a variety of foreign agents show increased bone marrow pre-B cell proliferation and small lymphocyte production (15, 16). Thus a basal level of bone marrow lymphocyte genesis, regulated by as yet ill-defined endogenous mechanisms, appears to be modulated by exogenous factors. While the latter may include gross changes in dietary intake, the regulatory mechanisms are evidently independent of the type of protein in the diet, at least under the conditions of the present experiments.

In our previous article (2) we postulated that dietary protein type may influence directly the intrinsic capacity of the B lymphocyte to respond to an antigenic stimulus. To substantiate this assumption, we have investigated the effect of dietary protein type in mice presenting an accessory cell–B cell interaction defect. The CBA/N mice have an X-linked absence of a subpopulation of mature or late-developing B lymphocytes (17). Our results (fig. 2A) are consistent with observations by Scher (17) showing that the primary in vivo IgM anti-SRBC responses of these immune-defective male mice were from 10 to 50% of normal and their IgG responses were from 1 to 10% of normal after immunization with relatively high numbers of SRBC. The abnormally low TD responses of the B cells are apparently related to a diminution in the

TABLE 3

Number and proliferation of pre-B cells in the bone marrow of mice fed diets of various protein types[1]

Bone marrow cells	Diet NP	Diet C	Diet L
Pre-B cells			
Incidence, %	11.5	11.7	11.5
Cells/femur, $\times 10^{-5}$	23.0	19.0	20.0
Pre-B cells in metaphase[2]			
Incidence, %	4.0	5.8	4.0
Cells/femur, $\times 10^{-4}$	9.2	11.0	8.0
B lymphocytes			
Incidence, %	4.9	4.1	3.6
Cells/femur, $\times 10^{-5}$	9.0	6.0	6.0

[1]Values from cells pooled from groups of three mice. Bone marrow taken from C3H/HeJ mice, 9 wk old, after 3-wk treatment with nonpurified diet (NP), casein diet (C) (20 g casein/100 g diet), or lactalbumin diet (L) (20 g/100 g). [2]Cells arrested in metaphase 2.5 h after i.p. injection of vincristine sulfate.

number of B lymphocytes (18) as well as to the inability of the CBA/N B cells to be activated by antigen-presenting accessory cells (19) and to accept the help provided by T cell–replacing factor (17). Our data indicate that, in spite of the expected lower response, the effect of dietary protein type on humoral immunity in mice with an accessory cell–B cell interaction defect follows the pattern noted in normal mice (fig. 2*A*). However, the fourfold difference in the number of PFC between L diet–fed mice and C diet–fed mice noted in normal F_1 mice after immunization with SRBC (fig. 2*B*) is remarkably similar to that previously reported in C3H (1) and DBA (20) mice after immunization with SRBC. On the other hand, the effect of dietary protein type in normal mice challenged with TI TNP-Ficoll or in CBA/N defective mice after SRBC is less profound. In both these situations the PFC response of L diet–fed mice is only twice that of the C diet–fed counterparts (figs. 1 and 2*A*). Although the characteristic response to dietary protein type may be an intrinsic property of the antigen specific B cells, it appears to be more dramatically expressed when T-helper cell–derived factors become operational as in the normal mouse challenged with SRBC.

The enhancement of PFC response in L diet–fed mice cannot be ascribed to presensitization of the L diet–fed group with cross-reacting antigens present in L because only very low numbers of PFC per spleen were found in nonimmunized mice and, moreover, these did not differ between the groups fed L or C diet (20). A possible immune-enhancing effect produced by intestinal absorption of intact L molecules is also unlikely for the following reasons: neonatal intestinal protein uptake and transport to the circulation has been shown to be of importance in suckling mammals, but direct observation of protein transport has been more difficult in the adult (21). Our studies show that the differential effect of L and C protein on the immune response is still present in 6-mo-old mice (the number of PFC per spleen following 10^6 SRBC in C3H mice fed L diet wat $156 \pm 3.6 \times 10^{-3}$ and in those fed C diet it was $33 \pm 1.8 \times 10^{-3}$). Moreover, the same marked difference in PFC response was noticed when lactalbumin hydrolyzate or casein hydrolyzate (approx. two-thirds free amino acids and one-third small peptides) were fed to mice (20).

Because minerals and trace metals including zinc (22) and copper (23) have been found to influence the immune response, it was felt important to analyze plasma level of several minerals and trace metals in relation to dietary protein type. The study was undertaken in view of the possibility that the protein source might influence the rate of absorption or the bioavailability (24) of minerals and trace metals in spite of the fact that identical amounts of minerals were added in the two purified diets. Our data clearly show no difference between the groups fed two purified diets in the plasma concentration of all of the five measured minerals and trace metals. Our previous study (1) had shown that the principal factor responsible for the observed differential effect of dietary L and C on humoral immunity was not the availability or concentration of single essential amino acids but rather the composite effect of the specific amino acid distribution in the protein. Our current data, showing that free plasma amino acid levels essentially conform to the amino acid composition of the ingested protein, indicate that the diet-dependent changes in plasma amino acid profile might represent the crucial factor responsible for the observed effect of protein type on the B cell response.

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