BRIEF COMMUNICATION

Effects of Dietary Fatty Acids on Delayed-Type Hypersensitivity in Mice[1]

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ABSTRACT Effects of an essential fatty acid deficient (EFAD) [0% corn oil (CO)] diet and a diet high in polyunsaturated fatty acids [PUFA (50% CO)] on one aspect of in vivo T cell function [delayed-type hypersensitivity (DTH)] were assessed. After a 70-day feeding trial, DTH was reduced by 30% in mice fed the EFAD diet, but the response of mice fed the high PUFA diet equaled that of control mice fed a diet containing 13% CO. The time required for the EFAD diet to reduce DTH was 42 days. Although consumption of the EFAD diet reduced DTH, this reduction was rapidly reversed, within 7 days, by switching the EFAD mice to the control diet. These results indicate that: 1) consumption of the EFAD diet reduces one aspect of in vivo T cell function (DTH), but the effect can be reversed by refeeding the control diet; and 2) a high PUFA diet does not adversely affect DTH. J. Nutr. *111:* 2039–2043, 1981.

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Several recent reports have proposed that polyunsaturated fatty acids (PUFA) regulate, or modulate, the immune response by specifically impairing T cell responses (1–7). For example, mice receiving daily injections of PUFA demonstrated impaired allograft rejection (5, 6) and cytoxicity (5). Addition of PUFA, however, dissolved in ethanol to lymphocyte cultures reduced in vitro T cell mitogenesis (8). In only a few cases has the relationship between dietary PUFA and in vivo T cell responses been evaluated. But these results are difficult to interpret due to improper diet formulation (9) and lack of quantitative presentation of immune response data (10). Effects of dietary saturated fatty acids and PUFA on in vitro T cell transformation have also been reported, but the results are contradictory (7, 11).

We showed earlier that in vivo antibody-mediated immunity was not impaired in mice fed a high PUFA diet [50% corn oil (CO)]. Reduced antibody responses were observed only in mice fed an essential fatty acid deficient (EFAD) diet which had no corn oil (12). The assay (Jerne plaque) used in these experiments measured the number of B cell progeny secreting antibody in response to immunization with sheep red blood cells (SRBC). Since it is well known that this B cell response requires the active participation of T helper (T_H) cells (13), our data indicated that at least one aspect of in vivo T cell function (T_H cell function) was not impaired in mice fed a high PUFA diet.

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Before using in vitro assays to assess mechanisms by which dietary PUFA may affect T cell function, we wished to further determine if the dietary manipulation affected T cell responses in vivo. Taking this approach, we assessed the T cell dependent delayed-type hypersensitivity (DTH) response as described by Vadas and associates (14).

The DTH response is an inflammatory reaction resulting from a complex interaction between sensitized T cells (T_D), which bear the Ly 1 surface alloantigen, macrophages (14, 15), and in some delayed cutaneous reactions, B cells (16). Although B cells and macrophages appear to be involved in this response, their participation depends upon the specific activation of sensitized T_D cells (14, 15). In the experiments reported here, we tested the DTH response of mice fed the EFAD (0% CO), control (13% CO), or high PUFA (50% CO) diets.

MATERIALS AND METHODS

Animals and Diets. Male A/J mice, 21 days old, were housed in plastic solid bottom cages (five mice in each) in a temperature- (24 ± 1°), light- (12 hours of light per day), and humidity-controlled room. Diets and water were provided ad libitum. Three diets were formulated on an equal energy basis, providing 0, 13, or 50% of dietary energy from corn oil, and 24% of dietary energy from casein. Complete composition of the diets has been reported (12).

Experiment 1. To determine effects of the EFAD and high PUFA diets on DTH, mice were fed the diets for 70 days. To measure DTH, a modification of the method of Vadas et al. (14) was used. Body weights were also assessed.

Experiment 2. Two aspects of the relationship between EFAD and T_D cell function were examined: 1) the length of time required to observe losses in the DTH response after introduction of the EFAD diet, and 2) the length of time required to reverse these losses by refeeding the control diet. Mice were fed the EFAD diet (0% CO) or the control diet (13% CO) for 77 days. A third group of mice was fed the EFAD diet for 63 days and then switched to the control diet for 7 or 14 days. In vivo DTH was assessed at 7 day intervals from 14 to 77 days.

Determination of DTH. DTH was measured by a modification of the method of Vadas et al. (14). Mice were sensitized to dinitrofluorobenzene (DNFB) by spreading 50 μl of a 2% solution of DNFB onto shaved areas (2 cm diameter) of their backs and abdomens. Five days later 10 μl of a 1% DNFB solution was applied to the right ear and a nonspecific agent (turpentine) was applied to the left (control) ear. Ten hours after the second administration of DNFB, mice were injected with 2 μCi of ^{125}I-iodo-deoxyuridine (5 Ci/mmole, Amersham Searle Corp., Arlington Heights, IL). Twenty-six hours after the second administration of DNFB, mice were killed and both ears were removed and counted in a gamma counter. Results were calculated as follows:

$$\text{Stimulation index} = \frac{\text{cpm (test ear)-cpm (background)}}{\text{cpm (control ear)-cpm (background)}}$$

Data analysis. All data were treated statistically by the student's *t* test or one way analysis of variance with treatment differences assessed by Tukey's test (17).

RESULTS

Experiment 1. As we had observed previously (12), body weights of mice fed the EFAD diet for 70 days were reduced compared to weights of mice in the control and high PUFA groups (table 1). Food intake was not measured, but in our earlier study (12) mice fed the high PUFA and control diets consumed slightly, but not significantly, more than mice fed the EFAD diet. DTH was impaired in mice fed the EFAD diet (table 1), but the response of mice fed the high PUFA diet did not differ from the controls. These findings agree with our previous report on the humoral response (12) and demonstrate that when relatively high levels of PUFA are fed, no inhibitory effects on in vivo immune functions are seen. On the

TABLE 1

Body weight and delayed-type hypersensitivity response of mice fed 0, 13, and 50% of energy from corn oil for 10 weeks (experiment 1)[1]

	Dietary energy from corn oil		
	0%	*13%*	*50%*
Body wt,[2] g	23.0 ± 0.7[a]	27.5 ± 1.2[b]	29.8 ± 1.4[b]
SI[3]	1.73 ± 0.13[a]	2.51 ± 0.20[b]	2.33 ± 0.13[b]

[1] Means for 10 mice. Numbers with different superscripts are significantly different ($P < 0.05$). [2] Mean initial body weights were 9.6 grams.

[3] $SI = \frac{\text{cpm } ^{125}\text{I-iododeoxyuridine incorporated into stimulated ear-cpm (background)}}{\text{cpm } ^{125}\text{I-iododeoxyuridine incorporated into control ear-cpm (background)}}$.

See materials and methods section for a description of the method.

other hand, when mice consume an EFAD diet, in vivo immunity is impaired.

Experiment 2. To examine the temporal relationship between consumption of an EFAD diet and in vivo T cell function we monitored the DTH response of mice over a 77-day period (figure 1). After 42 days, a significant reduction in DTH was observed in mice fed the EFAD diet. This reduction remained fairly constant at about 75% of the control response for the remainder of the experiment (77 days).

To determine if the inhibitory effects of the EFAD diet on DTH were reversible, two groups of mice fed the EFAD diet for 63 days were switched to the control diet (13% CO). After only 7 days of refeeding, the DTH response was completely restored (figure 1), and even exceeded the control response by about 15%. The DTH response after two weeks of refeeding was approximately equal to the control response. At both time points the DTH response of mice remaining on the EFAD diet was significantly reduced compared to the response of the control or the refed groups. Thus, the deleterious effects of the EFAD diet on DTH are reversible, with recovery occurring rapidly (within 7 days) after refeeding a diet containing EFAs.

DISCUSSION

This report extends previous observations from our laboratory (12) related to the influence of EFAD and high PUFA diets on in vivo immune responses. In both studies, consumption of an EFAD diet (0% CO) depressed in vivo responses, whereas consumption of a high PUFA diet (50% CO) for an extended period of time (70 days) had no adverse effects on in vivo responses. These findings, particularly those related to PUFA and in vivo T cell responses, do not agree with the conclusions of others (4, 7). Several reasons for these differences are possible.

Others reported a consistent pattern of immunosuppression when mice fed stock diets were injected daily with 10 μl of PUFA in the form of linoleic or arachidonic acid (4–6). Based on a daily intake of 12 kcal/day (12), and assuming 90% absorption of PUFA (18), mice fed our control diet [13% of energy from CO (60% linoleic acid)] consumed about 94 mg linoleic acid/day and mice fed the high PUFA diet received about 360 mg linoleic acid/day. If the daily intake of stock diet fed to mice injected with PUFA contained approximately 100 mg PUFA, injection of 10 mg PUFA increased intake only 10%. Since mice fed our high PUFA diet increased PUFA intake by nearly 300% without a reduction in in vivo T cell function, it is difficult to attribute the immunosuppression ob-

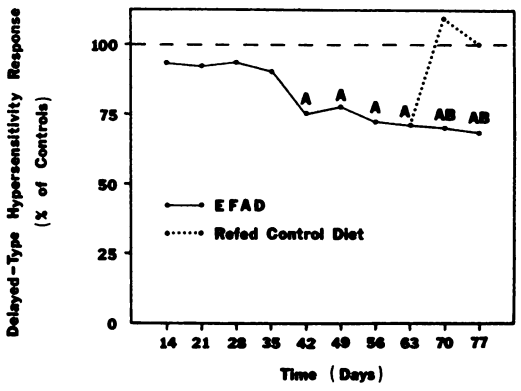

FIG. 1. Delayed-type hypersensitivity response of mice fed an essential fatty acid deficient diet (0% corn oil). Response expressed as a percentage of control (13% corn oil) response. Each point represents the mean of 10 mice. A = significantly different than control ($P < 0.05$); B = significantly different than refed ($P < 0.05$).

served after injection of PUFA to PUFA alone. Although two reports suggest that dietary PUFA suppress in vivo T cell responses, interpretation of these studies is difficult because in one case the experimental diets were grossly different in their nutrient density (9) and, in the other, quantitative assessments of T cell responses were not used (10).

Conflicting reports have appeared on effects of high PUFA diets on T cell mitogenesis in vitro (7). Erickson et al. (7) reported that a high PUFA diet (approximately 44% of energy from safflower oil) impaired mitogenic responses of spleen cell from young mice to Con A. Ossman et al. (11), however, presented data showing that Con A responses of spleen cells from adult mice increase with increasing amounts of corn oil in the diet. Another in vitro approach has been to add PUFA, or PUFA bound to albumin, directly to cell cultures. Addition of PUFA has been shown to decrease (8, 19), increase (20), or have no effect on mitogen responses (21). Thus, these data have not helped clarify effects of high PUFA intake on T cell function.

It has also been proposed that PUFA "deficiency" is immunopotentiating (5) because graft rejection was faster in mice fed an autoclaved (ostensibly to destroy PUFA double bonds) diet. We have never found our EFAD diet (which could be termed "PUFA deficient") to be immunopotentiating in any of our in vivo studies. On the contrary, in vivo immunity has been consistently depressed in mice fed the EFAD diet. And, because autoclaving would be expected to destroy several water soluble vitamins in addition to PUFA double bonds, it is difficult to see how this diet could enhance the immune response.

We found that DTH and antibody-mediated responses were reduced 21–28 days after feeding the EFAD diet. This reduced capacity to respond in vivo plateaued at approximately 60–75% of the control response, where it remained for the duration of both experiments (70–77 days). Even though we have not studied the effect of EFAD beyond 77 days, it appears that despite an extended period of EFA deficiency, the immune system maintains some capacity to respond in vivo. Also, after 63 days of consuming the EFAD diet, the DTH response of mice switched to the control diet was rapidly (7 days) restored to control levels. This is consistent with our previous findings (12) and indicates that EFAD-induced alterations in immune functions are rapidly reversed by feeding a diet containing EFA.

Results presented here, and in our previous report (12), indicate that feeding mice high levels of PUFA has no adverse effects on in vivo immune functions. In both reports, however, in vivo immune functions were reduced in mice fed the EFAD diet. Both in vivo antibody-mediated immunity and DTH responses result from complex interactions among several cell types, including T and B lymphocytes and macrophages (13, 14). Because of the complexity of these responses, it is not possible from available data to identify the precise cell population affected by the EFAD diet. Further studies are needed to determine the cell type and mechanisms responsible for the effects of EFAD on immune functions.

Our results do not agree with previous reports proposing that PUFA decrease immunity (2, 4–7) and "PUFA deficiency" increase immunity (5), but this disparity is probably due to differences in experimental approach. PUFA may impair lymphocyte responses under certain experimental conditions such as subcutaneous injection (4, 6) or direct addition to cell cultures (8). But, when PUFA are consumed in the diet, no adverse effects on immune functions are seen.

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