

Dietary *Trans* Fatty Acids Suppress the Development of Spontaneous Atopic-Like Dermatitis in NC/Nga Mice

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(Received March 19, 2009)

Summary Although several studies have shown that the type and degree of unsaturation of fatty acids alter the production of proinflammatory mediators, there have been few studies in which the effects of *trans* isomers on allergic responses were evaluated. NC/Nga (NC) mice raised in conventional conditions develop spontaneous atopic dermatitis-like lesions with IgE hyperproduction. We used this model to evaluate the effect of dietary *trans* fatty acids (TFAs) on the development of spontaneous dermatitis. NC mice were fed a 4 g TFAs/kg diet (4 g TFA), a 8 g TFAs/kg diet (8 g TFA) or a control diet with no TFA content for 10 wk. The dermatitis condition improved with the increasing intake of TFAs, and there was a significant difference from week 5 of the experimental period between the dermatitis conditions in 8 g TFA-fed mice and control mice. Production of total IgE was also suppressed in mice fed TFAs compared with that in control mice, correlating with the skin lesions. IFN- γ , but not IL-4, production was significantly greater in TFA-fed mice than in control mice. Our results suggest that intake of TFAs suppresses the development of atopic dermatitis-like lesions in NC mice. Even though we observed a decreased production of IgE levels, mechanisms involved in the development of dermatitis-like lesions seem to be IL-4-independent.

Key Words *trans* fatty acids, NC/Nga mice, dermatitis, IgE, allergy

Several studies have shown that the type and degree of unsaturation as well as the *trans* configuration of dietary fatty acids affect lipid profiles, eicosanoid production and inflammatory responses, though the results are controversial (1–5). There is, however, limited information concerning *trans* isomers in allergic responses.

Trans fatty acids (TFAs) occur naturally in the fat of ruminants and in dairy products and some vegetables, although the main source of TFAs is foods containing industrially hardened vegetable and marine oils. The level of dietary TFAs in the Western diet has increased remarkably in the past few decades due to increased consumption of industrial products such as cakes and other baked products, fried foods, snacks, instant foods and prepared foods containing margarine, vegetable shortening and salad dressing.

NC/Nga (NC) mice raised under conventional conditions develop spontaneous skin lesions characteristic of inflammatory responses and a high concentration of total IgE in the plasma (6). The aim of this study was to determine whether intake of TFAs has an effect on immune responses. For this purpose, we evaluated spontaneous dermatitis in NC mice fed an experimental diet with TFAs and maintained under conventional con-

ditions.

MATERIALS AND METHODS

Experimental design. Seven-week-old female NC mice (Japan SLC, Inc., Shizuoka, Japan) were randomly divided into three groups ($n=7$). Mice were kept under conventional conditions in a room with a 12-h light/dark cycle and a controlled temperature (23°C), with free access to food and water. After 1 wk of ambientation, mice were fed an isoenergetic (3,964 kcal/kg) diet containing 100 g/kg of lipids with different ratios of shortening, olive oil and soybean oil to supply 4 g TFAs/kg diet (4 g TFA), 8 g TFAs/kg diet (8 g TFA), or no TFA content (Table 1). Analysis of the content of the fatty acids was carried out by the Research Institute for Q.P. Corporation. The main fatty acid composition of each experimental diet is summarized in Table 2. Changes in dermatitis-related skin condition and scratching behavior were evaluated weekly. Serum samples were collected at baseline and in weeks 6 and 8. At the end of week 10, mice were killed by cervical dislocation and blood and spleen samples were collected for analyses. The study protocol was approved by the Committee on Animal Welfare of The University of Tokushima.

Dermatitis score. NC mice were allowed to develop spontaneous atopic dermatitis, and the severity of symptoms was evaluated weekly. Dermatitis scores were

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Table 1. Composition of the experimental diets (g/kg).

	Control	4 g TFAs	8 g TFAs
Cornstarch	423	423	423
Sucrose	212	212	212
Casein	200	200	200
Shortening	0	34	68
Olive oil	75	37.5	0
Soybean oil	25	28.5	32.0
Cellulose	20	20	20
Vitamin mix ¹	10	10	10
Mineral mix ²	35	35	35

¹ Vitamin mixture was composed of (mg or IU/kg diet): retinal acetate, 5,000 IU; ergocalciferol, 1,000 IU; tocopherol acetate, 50 mg; manadion, 52 mg; thiamine chloride, 12 mg; riboflavin, 40 mg; pyridoxine-HCl, 8 mg; vitamin B12, 0.005 mg; ascorbic acid, 300 mg; D-biotin, 0.2 mg; folic acid, 2 mg; calcium pantothenate, 50 mg; *p*-amino benzoic acid, 50 mg; niacin, 60 mg; inositol, 60 mg; choline chloride, 2,000 mg.

² Mineral mixture was composed of (mg/kg diet): CaHPO₄/2H₂O, 7,280; KH₂PO₄, 12,860; NaH₂PO₄, 4,680; NaCl, 2,330; Ca-lactate, 17,750; Fe-citrate, 1,590; MgSO₄, 3,590; ZnCO₃, 55; MnSO₄/4–5 H₂O, 60; CuSO₄/5H₂O, 15; KI, 5.

based on the Scoring Atopic Dermatitis (SCORAD) System (7). Four areas of the mouse body (right ear, left ear, head, and back) were graded from 0 to 3 (0, no symptoms; 1, mild; 2, moderate; 3, severe) according three criteria: skin dryness, eruption and wounds. The degree of dermatitis was determined from the sum of all individual scores.

Lymphocyte proliferation. Spleen cell suspensions (2×10^6 cells/well) were cultured with anti-mouse CD3 mAb (2 μ g/mL) in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 50 μ M 2-mercaptoethanol, 100 μ g/mL streptomycin and 100 U/mL penicillin. Plates were incubated at 37°C in humidified, 5% CO₂ conditions for 72 h. Cells were pulsed with 0.5 μ Ci tritiated methylthymidine ([³H]-TdR) for the final 16 h of incubation. Culture supernatants were collected and stored at –20°C until analysis. Cells were harvested onto glass fiber filters and dried overnight. [³H]-TdR incorporation into DNA was measured by a Matrix beta counter (Packard Instrument B.V. Chemical Operations, Netherlands).

IL-4 and IFN- γ production. Spleen cell suspensions (1×10^7 cells/mL) were cultured in 24-well plates with either a vehicle or anti-mouse CD3 mAb (2 μ g/mL). Plates were incubated at 37°C in humidified, 5% CO₂ conditions for 72 h. After culture, supernatants were collected and frozen at –20°C until analysis. Quantitative determination of cytokine production was assessed by an enzyme-linked immunosorbent assay (ELISA) using commercial kits following the manufacturer's experimental protocol (e-Bioscience, San Diego, CA, USA).

Total IgE in serum. Total IgE was determined by an

Table 2. Fatty acid composition of the experimental diets (g/kg).

	Control	4 g TFAs	8 g TFAs
14:0	0.0	0.1	0.2
15:0	0.0	0.0	0.0
16:0	10.8	11.8	12.8
16:1	0.6	0.4	0.1
16:1 <i>t</i> 9	0.1	0.0	0.0
17:0	0.0	0.1	0.1
18:0	4.0	6.8	9.6
18:1 <i>c</i> 9	61.8	42.4	23.0
18:1 <i>t</i> 9	0.0	3.4	6.8
18:1 <i>c</i> 11	2.1	1.9	1.7
18:2 <i>c</i> 9 <i>c</i> 12	17.2	25.3	33.5
18:2 <i>c</i> 9 <i>t</i> 12	0.1	0.3	0.6
18:2 <i>t</i> 9 <i>c</i> 12	0.1	0.2	0.3
18:2 <i>t</i> 9 <i>t</i> 12	0.0	0.0	0.1
18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	2.1	2.8	3.6
18:3 <i>c</i> 9 <i>c</i> 12 <i>t</i> 15	0.1	0.1	0.2
18:3 <i>c</i> 9 <i>t</i> 12 <i>c</i> 15	0.0	0.0	0.0
18:3 <i>t</i> 9 <i>c</i> 12 <i>c</i> 15	0.1	0.1	0.2
18:3 <i>t</i> 9 <i>c</i> 12 <i>t</i> 15	0.0	0.0	0.0
20:0	0.4	0.4	0.4
20:1	0.2	0.2	0.1
21:0	0.0	0.0	0.1
22:0	0.2	0.3	0.3
Other	0.0	3.1	6.2

ELISA method using commercial kits following the experimental protocol of the manufacturer (Bethyl Laboratories Inc., Montgomery, TX, USA).

Statistical methods. Data are expressed as means $\pm$ standard error (SE). Analysis of variance (ANOVA) was used to evaluate differences between groups. Games-Howell's test was used for post hoc comparisons between groups. Data processing and statistical analyses were conducted using the SPSS version 10.0 software package (SPSS Inc., IL, USA).

RESULTS

There were no differences between the groups in mean weight or weight gain. However, spleen weight was significantly greater in mice fed a 8 g TFA diet than in the control group expressed as absolute weight (178 ± 6 mg vs 159 ± 18 mg, $p=0.035$) or as weight of 100 g/kg body weight (652 ± 19 mg vs 591 ± 56 mg, $p=0.030$). Flow cytometric analysis showed that percentage of cells expressing CD4, CD8, DX5 and B220 Ag is not different between the three groups.

Dermatitis score

The dermatitis score was evaluated in NC mice fed the experimental diet (Fig. 1). Dermatitis lesions appeared in week 4 of the experimental period in 4 g TFA-fed mice and control mice, being more severe in the control mice. No signs of dermatitis lesions were observed in 8 g TFA-fed mice during the study period. Differences between 8 g TFA-fed mice and control mice were statistically significant in week 5 and from week 7 to week 10 ($p<0.05$) and between 4 g TFA-fed mice and

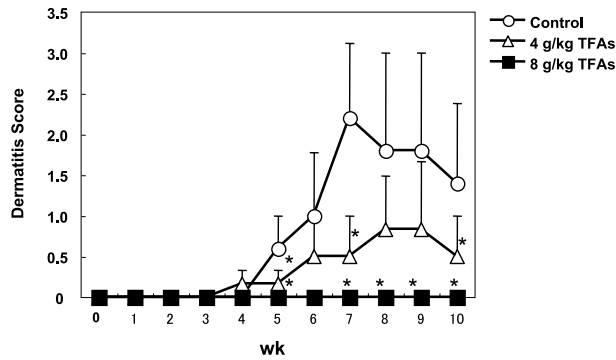


Fig. 1. Skin conditions in conventional NC mice fed TFAs. Conventional NC mice were fed 0, 4 or 8 g TFAs/kg diet for 10 wk. Total severity score for skin lesions and were quantitated according to the methods described in "Materials and Methods." Each value represents the means $\pm$ SE ($n=7$). * $p<0.05$, when compared with age-matched controls.

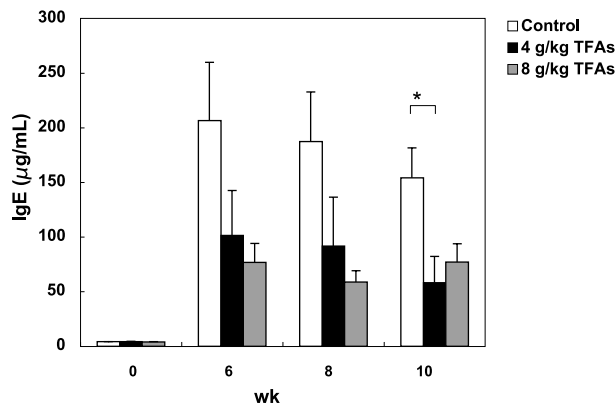


Fig. 2. Serum IgE levels in conventional NC mice fed TFAs. Conventional NC mice were fed 0, 4 or 8 g TFAs/kg diet for 10 wk. Total serum IgE concentrations were determined according to the methods described in "Materials and Methods." Each value represents the means $\pm$ SE ($n=7$). * $p<0.05$, when compared with age-matched controls.

control mice in weeks 5, 7 and 10 ($p<0.05$).

Serum IgE levels

We measured the serum concentrations of total IgE at baseline and weeks 6, 8 and 10 of the experimental period (Fig. 2). Total IgE levels increased remarkably in weeks 6 to 10 in all groups compared with the baseline levels. However, IgE production was clearly suppressed in mice fed the TFA diet, with differences bordering on statistical significance in weeks 6 and 8. Differences were statistically significant in week 10 of the experimental period between 4 g TFA-fed mice and control mice ($p=0.039$).

Cytokine production

The effect of TFAs on cytokine production was examined in NC mice. Since IFN- γ and IL-4 have been shown to play a role in the pathogenesis of allergies, we determined the production of these cytokines by culturing splenocytes with anti-CD3 mAb. IL-4 production level against anti-CD3 mAb stimulation was not significantly

Table 3. Effects of TFAs on the production of IFN- γ and IL-4 in NC mice.

	Control	4 g/kg TFAs	8 g/kg TFAs
IFN- γ (pg/mL)			
Medium	16 $\pm$ 6	21 $\pm$ 6	20 $\pm$ 8
Anti-CD3 mAb	168 $\pm$ 63 ^{ab}	1,563 $\pm$ 450 ^{a*}	1,828 $\pm$ 114 ^{b**}
IL-4 (pg/mL)			
Medium	<0.07	<0.07	<0.07
Anti-CD3 mAb	6.6 $\pm$ 1.8	11.8 $\pm$ 2.2	11.9 $\pm$ 1.9

Values are means $\pm$ SE ($n=7$). Means sharing superscript letters differ (* $p<0.05$, ** $p<0.01$) as determined by the ANOVA test with post hoc Bonferroni multiple comparison.

different between the groups. In contrast to IL-4, IFN- γ production level was remarkably higher in mice fed TFAs than in control mice (Table 3). Although not statistically significant, the lymphocyte proliferation response of splenocytes upon stimulation with anti-CD3 mAb was slightly suppressed in TFA-fed groups compared with that in the control group (data not shown).

DISCUSSION

In humans, skin lesions of atopic dermatitis are characterized in the initiating phase by infiltrating Th2 cells, producing IL-4 and other Th2-related cytokines. IL-4 regulates IgE production, and consequently IgE levels are elevated in most patients with atopic dermatitis. The NC mouse is a murine model of atopic dermatitis, developed from a Japanese fancy mouse by K. Kondo in 1957, and NC mice have been widely used as an animal model of human atopic dermatitis (7–9). NC mice develop lesions comparable to those of human atopic dermatitis when kept under conventional conditions. IgE levels in NC mice increase remarkably from the age of 8 wk and the lesions show marked lymphocyte infiltration with a high CD4/CD8 ratio. There is also an increase in cytokine IL-4 production by mast cells in the lesions, whereas CD4⁺ cells produce IL-4 first and also IFN- γ in later stages (7). On this basis, we evaluated the evolution of skin lesions in NC mice raised in a conventional environment and two diets with different amounts of TFAs. Control mice developed skin lesions from week 4, gradually increasing in severity until the end of the experimental period. However, skin lesions in the both 4 g and 8 g TFA groups were remarkably suppressed (Fig. 1). Accordingly, total serum IgE levels in the control group increased remarkably, while IgE production was significantly suppressed in mice fed TFAs (Fig. 2). The secretion of IL-4 was slightly increased in TFA-fed mice compared with that in the control group, but Th1-related cytokine IFN- γ production increased remarkably in mice fed TFAs (Table 3), and this might have accounted for inhibition of the production of IgE in these mice. IgE responses are enhanced by IL-4 and other Th2-related cytokines, while inhibitors of these cytokines would consequently reduce the production of IgE (10). Even though IgE levels were clearly suppressed

in mice fed TFAs, this response did not correlate with IL-4 cytokine levels. Moreover, IL-4 production increased slightly in both the 4 g and 8 g TFA groups. This apparent contradiction might be related to the increased level of Th1-related IFN- γ (11). We observed in our study a remarkable and significant increase in the production of IFN- γ in mice fed TFAs, which might have caused the inhibition of IgE responses in these groups. At present, the exact mechanism for inhibition of IgE responses by TFAs is not known. Although *cis*-9, *trans*-11-conjugated linoleic is not contained in the experimental diet used in this study, it has been reported that *cis*-9, *trans*-11-conjugated linoleic exerts anti-inflammatory effects in vivo. A recent study by Jaudszus et al. shows that *cis*-9, *trans*-11-conjugated linoleic acid reduces IgE production and allergen-induced airway hyperresponsiveness (12). Furthermore, bovine milk fat enriched in *cis*-9, *trans*-11-conjugated linoleic also reduces circulating allergen-specific IgE and IgG1 levels in OVA-immunized C57BL/6 mice (13).

Spleen weight was significantly greater in mice fed the 8 g TFA diet. Han et al. investigated the effects of diets containing hydrogenated fat on cellular immune response and production of inflammatory cytokines in human subjects. In this study, it was shown that consumption of a diet high in hydrogenated fat increases production of inflammatory cytokines that have been associated with the pathophysiology of atherosclerosis (14). It is possible that TFAs increase inflammatory cytokines and then increase spleen weight. Since we did not determine inflammatory cytokines and the contribution of inflammatory cytokines to the development of allergy is not fully understood, we cannot discuss this point at present.

There have been few studies in which the effect of TFA in allergic responses was evaluated. However, the effects of these *trans* isomers on lipid profiles, metabolism of fatty acids and cardiovascular diseases have been intensively investigated in the past few decades. It was previously reported that TFAs suppress the production of proinflammatory prostaglandins via competitive inhibition of the activity of Δ -6 desaturase, a key enzyme in the metabolic conversion of linoleic acid to arachidonic acid (2, 5, 11). That is, TFAs compete with linoleic acid as a substrate for Δ -6 desaturase, reducing the availability of arachidonic acid for the production of inflammatory prostaglandins. Results reported by Larqué et al. support this theory (2). In a murine model, they observed elevated levels of linoleic acid and low levels of its polyunsaturated fatty acid metabolites in pregnant rats fed TFAs. They also observed that *trans* isomers inhibited the activity of Δ -6 desaturase. Similar results were reported by Kummerow et al. in pregnant pigs (5). In consideration of these studies, one plausible mechanism for the suppressive effect of TFAs on allergic responses is the competitive inhibition of Δ -6 desaturase, which is crucial in the degranulation of mast cells. Another possibility is that TFAs suppress T cell function, resulting in improvement of dermatitis, since proliferation response to stimulation with anti-CD3 mAb of sple-

nocytes from TFA-fed mice is reduced compared to that of splenocytes from control mice.

In summary, our results suggest that *trans* fatty acid intake suppresses the development of dermatitis-like lesions in NC mice via IL-4-independent IgE responses.

Acknowledgments

We gratefully thank M. Ariizumi and K. Migita (Q.P. Corporation) for analysis of TFAs.

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