Note
Lack of Effect of the Abnormal Fatty Acid Metabolism in NC/Nga Mice on Their Atopic Dermatitis

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Although clinical evidence has suggested that dysregulated fatty acid metabolism is associated with atopic disorders, the molecular basis for such a correlation remains to be demonstrated. In the present study, we analyzed the fatty acid composition in peripheral blood cells of NC/Nga mice, a model for atopic dermatitis (AD). We found that arachidonic acid significantly accumulated in mice with the AD manifestation. In addition, the leucotriene B4-releasing ability upon calcium ionophore A23187 stimulation was potentiated in blood cells. An arachidonic acid accumulation was not apparent in the non-atopic BALB/c strain, but was still observed in healthy NC/Nga mice fed under specific pathogen-free conditions. These results indicate that a disturbed fatty acid metabolism in NC/Nga mice was not a trigger factor for their dermatitis development.

Key words: arachidonic acid; atopic dermatitis; leucotriene B4; NC/Nga mice; polyunsaturated fatty acid

Fatty acid is an essential component of the plasma cell membrane, and also plays an important role in the control of inflammation. The n-6 series of fatty acids are finally metabolized to the polyunsaturated fatty acid (PUFA), arachidonic acid (AA, C20:4, n-6). AA metabolites produced via intracellular AA cascade activation include proinflammatory eicosanoids which are potent chemical mediators involved in the progression of atopic diseases. Thus, excess n-6 fatty acid feeding and/or turbulent fatty acid metabolism might increase the susceptibility to the allergy.

Clinical evidence has suggested an association of the fatty acid metabolism with the allergy. Patients with atopic dermatitis (AD) often exhibit an increased linolenic acid (LA, C18:2, n-6) concentration in their plasma. It has also been pointed out that plasma LA metabolites were substantially down-regulated in atopic individuals. These changes in fatty acid composition have been recognized to result from impaired delta-6 fatty acid desaturation. However, this assumption still seems to be controversial, because contradictory clinical results have been reported which indicate that there was no LA metabolite reduction in atopic patients. Moreover, the actual molecular basis for such a PUFA metabolic defect is largely unknown, since the mechanism underlying the PUFA biosynthesis pathway has yet to be fully elucidated. To clarify the involvement of fatty acid metabolism in allergic disorders, further rigorous analyses with a suitable animal model would be required. In the present study, we examined the PUFA metabolism in AD-prone NC/Nga mice by analyzing their fatty acid composition.

The NC/Nga mouse has recently been introduced as an animal model for human AD. This strain of mouse spontaneously develops AD-like skin lesions accompanied by typical itching behavior and hyper-IgE production. Such an AD-like phenotype has been seen when kept under conventional but not in specific pathogen-free (SPF) conditions. Tsuzuki and his colleagues have proposed that dermatitis in NC/Nga mice was controlled by one gene, whereas their hyper-IgE production was driven by two autosomal recessive genes. This evidence suggests that, together with an atopy-prone genetic background, environmental factors might trigger AD-like symptoms.

NC/Nga mice (five-week old, male; purchased from SLC, Shizuoka, Japan) were maintained under conventional conditions (conv. group, n = 8) or in a SPF facility (SPF group, n = 8). Non atopic BALB/c mice (Charles River Japan, Kanagawa, Japan) were kept under conventional conditions (BALB/c group, n = 6). All groups of mice were fed with a certified diet (CRF-1, purchased from Oriental Yeast, Tokyo, Japan) for 17 weeks. The AD-like clinical skin severity score was determined by the method of Matsuda.
Briefly, five AD-like symptoms (itching, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness) were scored into four graded severities from 0 (none) to 3 (severe). The sum of these points is defined as the clinical severity score.

To analyze the hyper-IgE production and fatty acid composition, peripheral blood was collected from the tails (300 μl/mouse). After centrifugation at 400 g for 20 min, the separated blood cells and plasma were stored at −20°C until needed for use. Blood cells were washed twice with phosphate-buffered saline (PBS) before storage. The plasma IgE level was quantified by a sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies (R35-72 and R35-92) and instructions from Pharmingen (San Diego, CA, U.S.A.). For fatty acid analysis, total blood cells (100 μl vol.) were mixed with 1 ml 2,2-dimethoxypropane (DMF, used for direct dehydrazt) containing 1% (v/v) of hydrochloride, and were incubated at room temperature for 10 min. After evaporating the DMF layer under N2-purging, total intracellular lipid was extracted by a mixture of chloroform/methanol (2:1, v/v). The lipid fraction was then methyl-esterified with 10% (v/v) hydrochloride in methanol. The resulting fatty acid methyl esters were applied to gas-liquid chromatography (GC; model GC-17A, Shimadzu, Kyoto, Japan), using a GC-70 capillary column (GL Science, Tokyo, Japan) and a flame ionization detector. The conditions for the GC analysis have been described in a previous paper.10

The leukotriene B4 (LTB4)-release assay used freshly isolated peripheral blood cells (80 μl) that were incubated in 80 μl of PBS supplemented with 1.8 μM calcium ionophore A23187 (Sigma, St. Louis, MO, U.S.A.) for 30 min. After centrifugation, released LTB4 was quantified by a Biobrik LTB4, ELA system (Amersham Pharmacia Biotech, Uppsala, Sweden). All statistical analyses in this study were performed by Student's t-test,11 a confidence level of P<0.05 being evaluated as significant.

Figure 1 shows the AD-like pathogenic manifestation of the NC/Nga mice used in this study. At 22 weeks of age, conv NC/Nga mice exhibited a high clinical severity score, demonstrating AD-like inflammatory skin lesions. In contrast, no such pathogenesis was seen in age-matched SPF NC/Nga mice nor in non-atopic BALB/c mice (Fig. 1(A)). Plasma IgE quantification revealed the conv group to exhibit the typical hyper-IgE syndrome, whereas the SPF group and BALB/c mice did not (Fig. 1(B)). These data confirm that the NC/Nga mice under our experimental conditions exhibited the AD-like phenotype under conventional circumstances, which is in agreement with previous reports.7,12

To examine whether the fatty acid metabolism altered concomitantly with the pathogenesis in NC/Nga mice, we next analyzed the fatty acid composition of their peripheral blood cells by gas chromatography. As shown in Fig. 2, a significant accumulation of AA (C20:4, n-6) was observed in the conv NC/Nga mice (P<0.05, as compared with the BALB/c group). There was also concomitant increases in LA (C18:2, n-6) and docosahexaenoic acid (DHA, C22:6, n-3). Conversely, the contents of palmitic acid (C16:0) and stearic acid (C18:0) was significantly lower than those in BALB/c mice. Unexpectedly, an accumulation of AA, LA and DHA was also seen in blood cells from the SPF group (Fig. 2) which did not exhibit any AD-like phenotypes, including a hyper-IgE titer (see Fig. 1). This suggests that an abnormal PUFA accumulation by itself was not a trigger factor of AD-like pathogenesis, but rather an inherent property in the AD-prone NC/Nga strain. We also analyzed the fatty acid composition in the plasma and skin tissues. However, we found no significant difference in the fatty acid profile within three groups of mice (data not shown).

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**Fig. 1. AD-Like Pathogenic Manifestation of the NC/Nga Mice Used in This Study.**

(A) Clinical skin conditions in conventional and SPF NC/Nga mice (n=8, 8 each, male) at 22 weeks of age. The skin severity score was obtained by the method described previously.7 Age-matched conventional BALB/c mice (n=6, male) were also evaluated as the non-atopic control strain.

(B) Total plasma IgE levels in the three groups of mice at 22 weeks old. IgE was quantified by sandwich ELISA, using the reagents and methods from Pharmingen. Data in (A) and (B) represent the mean ± SE of each group. *Significantly higher value than that for the SPF NC/Nga group or BALB/c group (P<0.05).
AA Accumulation in NC/Nga Mice

Fig. 2. Fatty Acid Composition of Peripheral Blood Cells from NC/Nga Mice (n = 8) and from Conventional BALB/c Mice (n = 6) at 22 Weeks Old.

The total lipid fraction from each blood cell sample was methyl-esterified and then applied to a GC analysis. Data represent the mean ± SE of each group. *Significantly higher in comparison to the BALB/c group (P < 0.05). **Significantly low fatty acid content as compared with that of the BALB/c group (P < 0.05).

suggesting that the change in PUFA composition might have occurred in a blood cell-specific manner.

To test whether AA-rich NC/Nga blood cells were able to overproduce proinflammatory eicosanoid, we stimulated total peripheral blood cells with calcium ionophore A23187 to quantify the release of LTB₄, which has recently been reported as a candidate for the AD-mediator in humans.¹³ As expected, conv NC/Nga blood cells released significantly more LTB₄ (about 1.8-fold, P < 0.05) than the BALB/c group (Fig. 3).

Our data provide the first evidence for the fatty acid metabolism being disturbed in AD-prone NC/Nga mice. We have also shown that AA accumulation per se was not associated with the onset of AD-like pathogenesis, since blood cells from healthy SPF NC/Nga mice also exhibited an increased AA content (Fig. 2). It is therefore plausible that, rather than AA cascade activation, IgE-mediated histamine release via skin mast cell degranulation might be primarily important for their dermatitis. However, our results cannot fully rule out the possibility for AA accumulation being involved in chronic inflammatory dermatitis. It is possible that an AA-rich blood cell lineage might participate in the local inflammatory response via infiltration into skin lesions. Vestergaard et al.¹⁴ have reported that overproduction of T helper type 2 (Th2) chemokines was involved in pathogenic Th2 cell recruitment in the skin of NC/Nga mice. In addition, massive eosinophilia and mast cell infiltration have also been demonstrated in their lesional skin.¹⁵,¹⁶ If these infiltrated cells are rich in AA, local eicosanoid overproduction might be triggered via inflammatory stimuli. In fact, we have provided here in vitro evidence that NC/Nga blood cells were able to release more LTB₄ than those from BALB/c mice (Fig. 3). To test this assumption, a further investigation should be carried out by a PUFA composition analysis of the infiltrating cells, and an assessment of their eicosanoid production in vitro as well as in situ. Another important issue is whether AA accumulation and/or AA cascade activation is crucial in the chronic phase of dermatitis. Generation of AA cascade-impaired congenic NC/Nga mice would be one way of clarifying this question. AA cascade regulator-deficient mice [e.g. mice lacking cytosolic phospholipase A₂ (CLA₂) or 5-lipoxygenase (5-LOX)] might be useful for this purpose.

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