

Induction of Thymic Stromal Lymphopoietin Production by Nonanoic Acid and Exacerbation of Allergic Inflammation in Mice

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ABSTRACT

Background: Thymic stromal lymphopoietin (TSLP) plays critical roles in the induction and exacerbation of allergic diseases. We tested various chemicals in the environment and found that xylene and 1,2,4-trimethylbenzene induced the production of TSLP *in vivo*. These findings prompted us to search for additional chemicals that induce TSLP production. In this study, we examined whether fatty acids could induce the production of TSLP *in vivo* and exacerbate allergic inflammation.

Methods: Various fatty acids and related compounds were painted on the ear lobes of mice and the amount of TSLP in the homogenate of ear lobe tissue was determined. The effects of nonanoic acid on allergic inflammation were also examined.

Results: Octanoic acid, nonanoic acid, and decanoic acid markedly induced TSLP production, while a medium-chain aldehyde and alcohol showed only weak activity. Nonanoic acid induced the production of TSLP with a maximum at 24 h. TSLP production was even observed in nonanoic acid-treated C3H/HeJ mice that lacked functional toll-like receptor 4. The aryl hydrocarbon receptor agonist β -naphthoflavone did not induce TSLP production. Nonanoic acid promoted sensitization to ovalbumin, resulting in an enhancement in the cutaneous anaphylactic response. In addition, painting of nonanoic acid after the sensitization augmented picryl chloride-induced thickening of the ear, which was reversed in TSLP receptor-deficient mice.

Conclusions: Nonanoic acid and certain fatty acids induced TSLP production, resulting in the exacerbation of allergic inflammation. We propose that TSLP-inducing chemical compounds such as nonanoic acid be recognized as chemical allerge-accelerators.

KEY WORDS

fatty acid, nonanoic acid, ovalbumin, picryl chloride, thymic stromal lymphopoietin

INTRODUCTION

Thymic stromal lymphopoietin (TSLP) was recently identified as a master cytokine regulating the induction of allergies.^{1,2} TSLP is an IL-7-like cytokine³ that is produced mainly by epithelial cells, and plays important roles in the regulation of immune responses by inducing the maturation and activation of dendritic cells,^{4,6} lymphocytes, basophil precursors, and fibro-

cytes.⁷ The production of TSLP is increased at inflamed sites in patients with severe asthma,⁵ atopic dermatitis,⁸ and allergic rhinitis.⁹ The crucial roles of TSLP in allergic inflammation were demonstrated using transgenic mice. Allergic inflammation in an animal model of asthma and the exacerbation of allergic dermatitis were significantly suppressed in TSLP receptor-deficient mice.^{10,11}

Various environmental factors trigger the produc-

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tion of TSLP.¹² For example, stimulants of toll-like receptor 2 or 4 in the presence of tumor necrosis factor (TNF)- α and Th2 cytokines such as interleukin (IL)-4 induced the production of TSLP in synovial fibroblasts¹³ and skin keratinocytes *in vitro*.¹⁴ In addition, several chemical compounds induced TSLP production. Phthalate ester was shown to induce TSLP production *in vivo*.^{15,16} Having examined the activity of various chemical compounds in the environment, we found that xylene and 1,2,4-trimethylbenzene strongly induced TSLP production *in vivo*.¹¹ These compounds were not allergens, but exacerbated the effects of contact sensitizers, picryl chloride (PiCl),¹¹ and fluorescein isothiocyanate (FITC).^{15,16} Thus, the production of TSLP caused by chemical compounds may be important in promoting sensitization to antigens. These findings prompted us to search for additional chemical compounds that induce TSLP production. Various fatty acids are contained in soaps and plants, and sometimes cause contact dermatitis. Therefore, in this study, we investigated whether fatty acids could induce the production of TSLP *in vivo* and exacerbate allergic inflammation.

METHODS

ANIMALS

Male BALB/c, C57BL/6, C3H/HeN, and C3H/HeJ mice (5 weeks old) were purchased from SLC (Shizuoka, Japan). The generation of TSLP receptor-knockout mice (C57BL/6 background) was described previously.¹⁷ Mice were treated in accordance with procedures approved by the Animal Ethics Committee of Tohoku University, Sendai, Japan.

ASSAY OF TSLP, IL-4, AND TNF- α PRODUCTION BY CHEMICALS

β -naphthoflavone was purchased from Sigma-Aldrich (St. Louis, MO, USA). Pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, n-nonane, 1-nonanol, 1-nonanal, 1-decanal, 1-octanal, 2-nonanone, and picryl chloride were purchased from Wako Pure Chemical Industries (Osaka, Japan). Decanoic acid, dodecanoic acid, tetradecanoic acid, and β -naphthoflavone were dissolved in acetone. Acetone was used to dilute nonanoic acid. Twenty microliters of each solution or liquid chemical was painted on the ear lobes of mice. Ear lobe tissue was then punched out (diameter 5 mm) at a specified time and weighed. Tissue samples were homogenized at 4°C in a 10 vol of phosphate-buffered saline by a Beads Cell Disrupter (Precellys 24, Bertin Technologies, France). The concentrations of TSLP, IL-4, and TNF- α in the supernatant of the homogenate were determined by ELISA (TSLP; R & D Systems, Minneapolis, MN, USA, and IL-4 and TNF- α ; eBioscience, San Diego, CA, USA).

HISTOCHEMICAL ANALYSIS

Ear lobes were excised 24 hours after painting, and paraffin-embedded sections were prepared. Sections were stained with hematoxylin-eosin.

DETERMINATION OF THE EXPRESSION OF RECEPTORS AND CYP1B1

Ear lobe tissue was punched out (diameter 5 mm) 24 h after painting and weighed. Total RNA was extracted using a GenElute Mammalian total RNA kit (Sigma-Aldrich) according to the manufacturer's instructions. The extracted RNA (0.5 μ g) was reverse transcribed using PrimeScript reverse transcriptase (Takara Bio, Tokyo, Japan). The expression of mRNA for G protein-coupled receptor 84 (GPR84), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptor α (PPAR α), cytochrome P450 (Cyp) 1B1, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (housekeeping gene) was quantified by real-time PCR (Takara Bio). The sequences of the primers used were as follows: GPR84; (forward) 5'-CTGGCTCAACAGCTGCATCAA-3' and (reverse) 5'-ATGGAACCGGCGGAAACTC-3', AhR; (forward) 5'-ATGGAGGCCAGGACCAGTGTAG-3' and (reverse) 5'-TGCTGTGACAACCAGCACAAAG-3', PPAR α ; (forward) 5'-ACTGTCCCTTGGTGCCATCCTC-3' and (reverse) 5'-GCCCTGTATCCACAACAAGCTG-3', Cyp1B1; (forward) 5'-GCTCTGTTTCATTAGGCTTC-3' and (reverse) 5'-ACATTCAAGGGGTTCTGTTG-3'; and GAPDH; (forward) 5'-TGTGTCCGTCGTGGATC TGA-3' and (reverse) 5'-TTGCTGTTGAAGTCGCAG GAG-3'.

INDUCTION OF ALLERGIC RESPONSES TO OVALBUMIN

Nonanoic acid (20 μ l) was painted on the shaved back of mice. Twenty-four hours later, ovalbumin (OVA, 10 mg/ml saline, 100 μ l) or vehicle was injected i.d. at the same site. Ten days later, 0.1% (w/v) Evans blue (200 μ l) in saline was injected i.v. into the tail, and 50 μ l of OVA (10 mg/ml Hanks balanced salt solution) or vehicle was then injected into the shaven back i.d. Mice were sacrificed 30 min later, and tissue (14 mm in diameter) including skin, the cutaneous muscle layer, and subcutaneous tissue was excised. Evans blue leaked in the tissue was extracted in 0.5 ml of an extraction solution (acetone : 0.5% Na₂SO₄ solution = 7 : 3) and the absorbance of the supernatant at 595 nm was determined. The amount of Evans blue in the tissue was used as an index of plasma leakage. OVA-specific and total IgE levels in the serum were determined with the corresponding ELISA kit (Chondrex, Redmond, WA, USA).

INDUCTION OF PICRYL CHLORIDE (PiCl)-INDUCED ALLERGIC INFLAMMATION

The effects of nonanoic acid on PiCl-induced contact dermatitis were examined according to our previous

TSLP Production by Nonanoic Acid

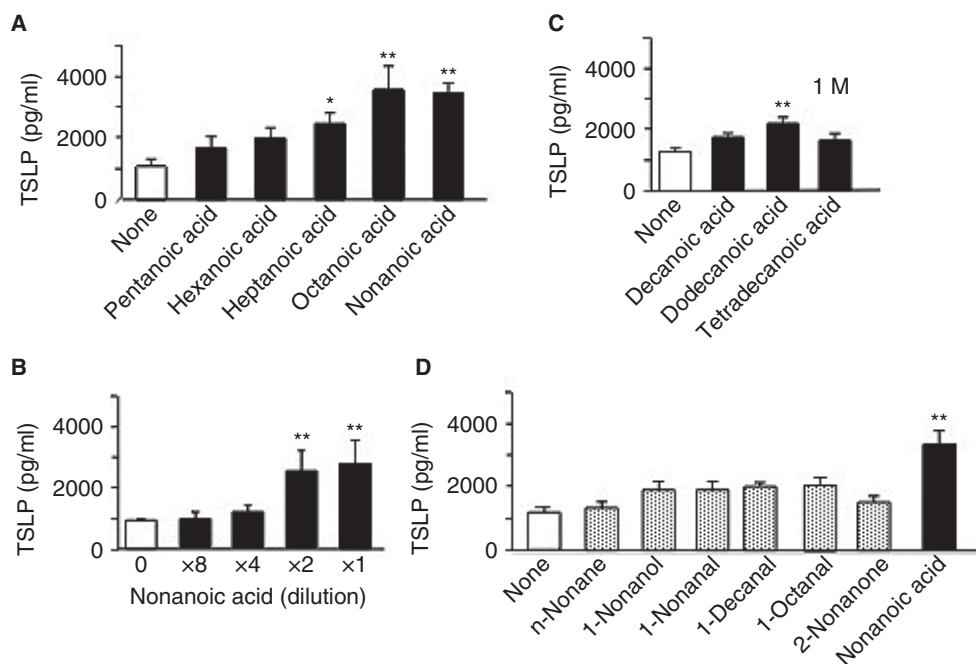


Fig. 1 Induction of TSLP production in ear lobe tissue by fatty acids. Short-chain and medium-chain fatty acids (20 μ l) (**A**), diluted nonanoic acid with acetone as indicated (**B**), decanoic acid, dodecanoic acid, and tetradecanoic acid dissolved in acetone to 1 M (**C**), and related compounds (20 μ l) (**D**) were painted on the ear lobes of BALB/c mice. Ear lobe tissue was excised 24 h later and the level of TSLP in the tissue homogenate was determined by ELISA. Data are shown as the mean $\pm$ S. E. M. for 6 samples. Statistical significance; * p < 0.05 and ** p < 0.01 vs. none.

studies, showing that this model was useful to assess the effects of TSLP-inducers on contact dermatitis.^{11,18} Briefly, cyclophosphamide (Sigma-Aldrich) was dissolved in saline and injected subcutaneously in the abdomens of C57BL/6 mice and TSLP receptor-knockout mice at a dose of 150 mg/kg to induce eosinophilia.¹⁹ Two days later, mice were sensitized with 50 μ l of a 7% (w/v) PiCl (Nacalai Tesque, Kyoto, Japan) solution (3 : 1 in acetone : ethanol) by painting it on the right ear lobe (day 0). Thereafter, 20 μ l of nonanoic acid was applied twice (day 5 and day 10) to the same site. On day 12, mice were challenged with 20 μ l of 1% (w/v) PiCl by painting it on the same ear lobe. Ear thickness was measured with a dial thickness gauge (Peacock, Ozaki, Tokyo, Japan) at a specified time, and the ear thickness before the challenge was subtracted from the data.

STATISTICAL SIGNIFICANCE

The significance of the results was analyzed with Dunnett's test or the Student-Newman-Keuls test for multiple comparisons.

RESULTS

ACTIVITY OF NONANOIC ACID AND RELATED COMPOUNDS TO INDUCE TSLP PRODUCTION

Pentanoic acid, hexanoic acid, heptanoic acid, oc-

tanoic acid, and nonanoic acid, all liquids at room temperature, were painted without dilution on ear lobes and TSLP levels in the homogenates of ear lobes were determined 24 h later. As shown in Figure 1A, heptanoic acid, octanoic acid, and nonanoic acid caused a significant increase in TSLP levels. Nonanoic acid induced TSLP production in a concentration-dependent manner, while only high concentrations (1-fold [5.2 M] and 2-fold dilution [2.6 M]) induced TSLP production (Fig. 1B). Decanoic acid, dodecanoic acid, and tetradecanoic acid, which are solids at room temperature, were dissolved in acetone at 1 M and painted on ear lobes. Among these compounds, dodecanoic acid significantly increased of TSLP levels (Fig. 1C). In addition, compounds related to nonanoic acid; n-nonane, 1-nonanol, 1-nonanal, 1-decanal, 1-octanal, and 2-nonanone, all liquids at room temperature, induced little if any TSLP production (Fig. 1D), indicating that the carboxyl group was required for activity. None of these compounds, except for nonanoic acid, induced significant increases in ear thickness. Since nonanoic acid is the most analyzed medium chain fatty acid as an irritant,²⁰ we decided to use nonanoic acid in the subsequent experiments.

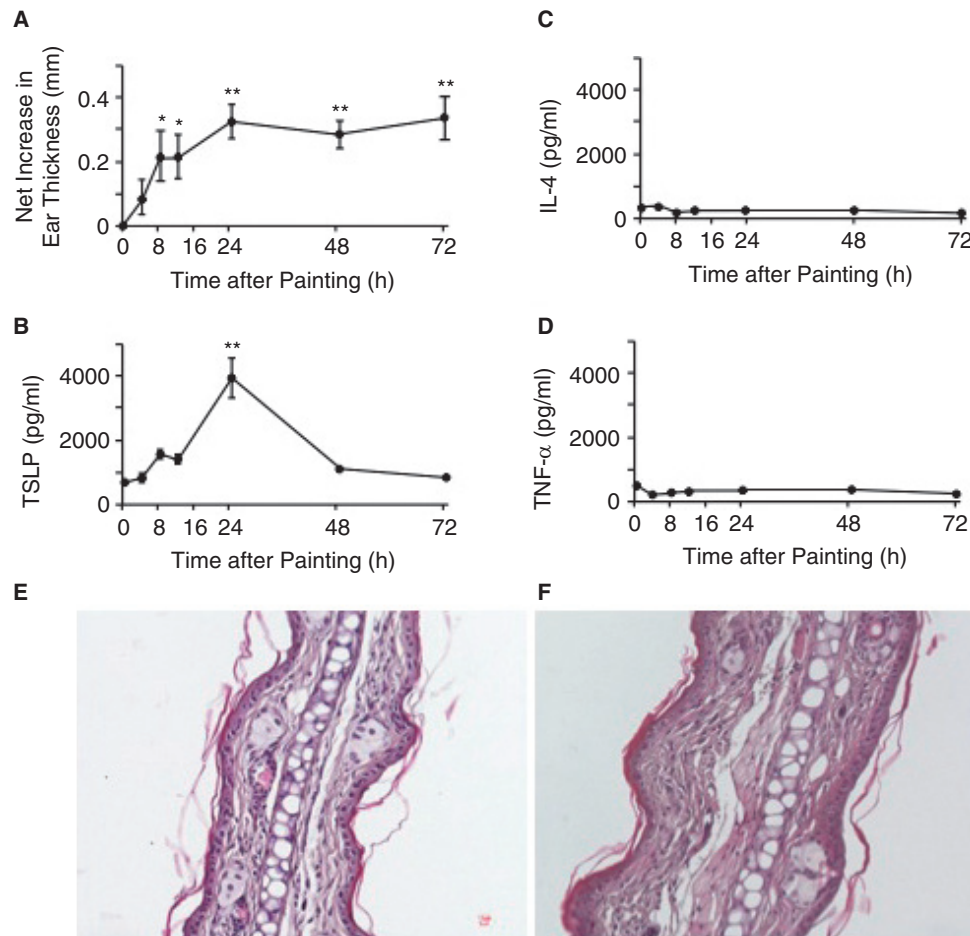


Fig. 2 Inflammatory responses and cytokine production induced by nonanoic acid. Ear lobe thickness was measured 0-72 h after the painting of nonanoic acid (A). Ear thickness before the challenge was subtracted from the data. The levels of TSLP (B), IL-4 (C), and TNF- α (D) levels were determined in the homogenates of ear lobe tissues collected at the indicated time points after the application of nonanoic acid (20 μ l). Data are shown as the mean $\pm$ S. E. M. for 6 samples. Statistical significance; ** p < 0.01 vs. none. Histological analysis was carried out of the ear lobes of control mice (E) and nonanoic acid-treated mice (24 h) (F).

TIME CHANGE IN TSLP PRODUCTION INDUCED BY NONANOIC ACID

Painting of nonanoic acid significantly increased ear thickness (Fig. 2A). TSLP levels in the tissue homogenate slowly increased and reached a maximum at 24 h (Fig. 2B). However, IL-4 (Fig. 2C) and TNF- α (Fig. 2D) levels did not increase. Histologically, edema, but no obvious leukocyte infiltration, was observed in tissue treated with nonanoic acid (Fig. 2E, F).

TSLP PRODUCTION IN MICE LACKING FUNCTIONAL TLR4

Lipopolysaccharide is known to be an inducer of TSLP in synovial fibroblasts,¹³ and the receptor TLR4 responds to saturated long chain fatty acids such as palmitic acid in INS-1 beta cells.²¹ Therefore, we

firstly examined whether TLR4 was involved in the nonanoic acid-induced production of TSLP using C3H/HeJ mice, which have a point mutation of TLR4 and are known to be completely resistant to lipopolysaccharide (LPS).²² Nonanoic acid was painted on the ears of C3H/HeJ mice and C3H/HeN mice, the corresponding wild-type mice, and the production of TSLP was determined. TSLP levels were lower in C3H/HeJ mice than in C3H/HeN mice, but were significantly higher than those in untreated mice (Fig. 3). The increase in the ear thickness was slightly lower C3H/HeJ mice (0.27 ± 0.05 mm) than in C3H/HeN mice (0.38 ± 0.04 mm).

EFFECT OF AN AHR STIMULANT ON TSLP PRODUCTION

The expression of the medium-chain fatty acid recep-

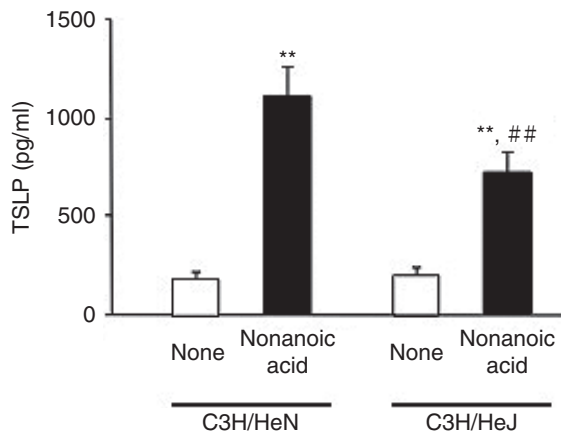


Fig. 3 Nonanoic acid-induced TSLP production in C3H/HeN and C3H/HeJ mice. The right ear lobes were painted with nonanoic acid and the left ear lobes were left untreated. Twenty-four hours later, both ear lobes were excised and TSLP levels were determined. Data are shown as the mean $\pm$ S. E. M. for 6 samples. Statistical significance; ** $p < 0.01$ vs. none and ## $p < 0.01$ vs. nonanoic acid in C3H/HeN mice.

tor GPR84,²³ the aryl hydrocarbon receptor AhR,²⁴ and the nuclear fatty acid receptor PPAR α ²⁵ in mouse ear tissue was determined by real-time PCR. GPR84 and PPAR α levels were very low, while AhR was highly expressed (Fig. 4A). Therefore, we examined the involvement of AhR in nonanoic acid-induced TSLP production. The ligand of AhR, β -naphthoflavone,²⁵ was painted on the ear, and the expression of TSLP protein and Cyp1B1 mRNA, as an index of the activation of AhR,²⁶ was determined. Although β -naphthoflavone induced the expression of Cyp1B1 mRNA, it did not induce that of TSLP protein or edema formation in the ear. In addition, nonanoic acid did not induce the expression of Cyp1B1 mRNA (Fig. 4B, C).

PROMOTION OF SENSITIZATION TO OVA

We examined whether or not nonanoic acid promoted sensitization to OVA, a protein antigen. The efficiency of sensitization was assessed by the amount of plasma leakage induced by a second injection of OVA. The injection of OVA alone caused only a weak response to the second injection of OVA (Fig. 5C, E). Interestingly, when nonanoic acid was applied 24 h before the first injection of OVA, the anaphylactic response induced by the second injection of OVA was apparently increased (Fig. 5D, E), indicating that nonanoic acid promoted sensitization to OVA. No sensitization (Fig. 5A) and the painting of nonanoic acid alone (Fig. 5B) did not induce any plasma leakage following the injection of the antigen. Although no OVA-specific IgE was detected in serum collected 10 days after sensitization in any of the groups (data

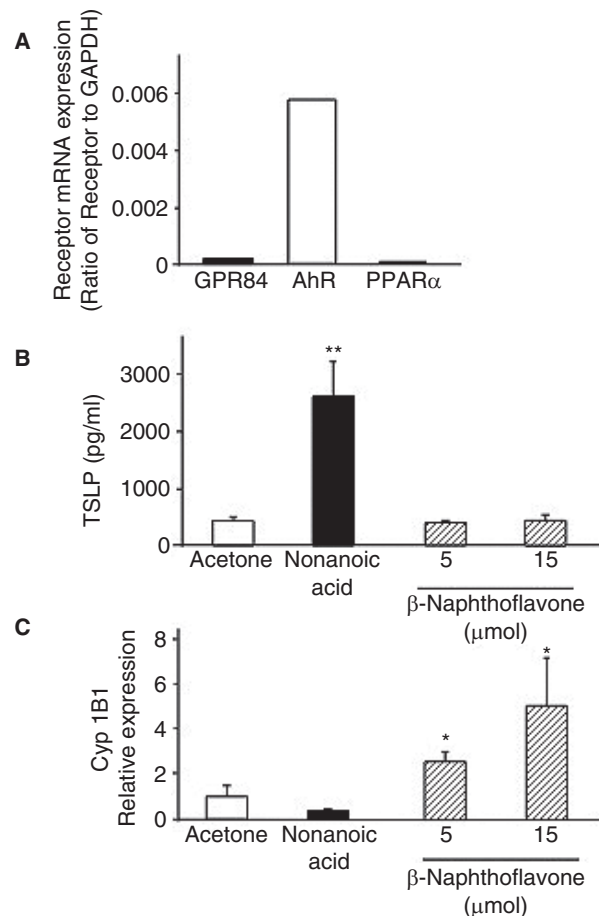


Fig. 4 No involvement of AhR in nonanoic acid-induced TSLP production. (A) The expression of mRNA for GPR84, AhR, and PPAR α in ear lobe tissues. Total RNA was extracted from untreated ear lobes and mRNA levels were determined by real-time PCR. The ratio of mRNA for each receptor to mRNA for GAPDH was calculated. (B and C) Nonanoic acid and β -naphthoflavone were painted on ear lobes and TSLP levels in ear lobes (B) and mRNA for Cyp1B1 (C) were determined. Data are shown as the mean $\pm$ S. E. M. for 6 samples. Statistical significance; * $P < 0.05$, and ** $p < 0.01$ vs. none.

not shown), total IgE levels were increased by sensitization with OVA alone (untreated mice, 72 ± 17 ng/ml; sensitized mice, 101 ± 11 ng/ml) and further increased by the pretreatment with nonanoic acid (119 ± 4 ng/ml).

EXACERBATION OF PICL-INDUCED ALLERGIC INFLAMMATION BY NONANOIC ACID AND THE INVOLVEMENT OF TSLP

We investigated the possibility that nonanoic acid exacerbated PICl-induced allergic dermatitis via TSLP production. Painting of nonanoic acid 5 and 10 days after sensitization enhanced PICl-induced swelling of the ear on day 12 (Fig. 6A). This enhancement by

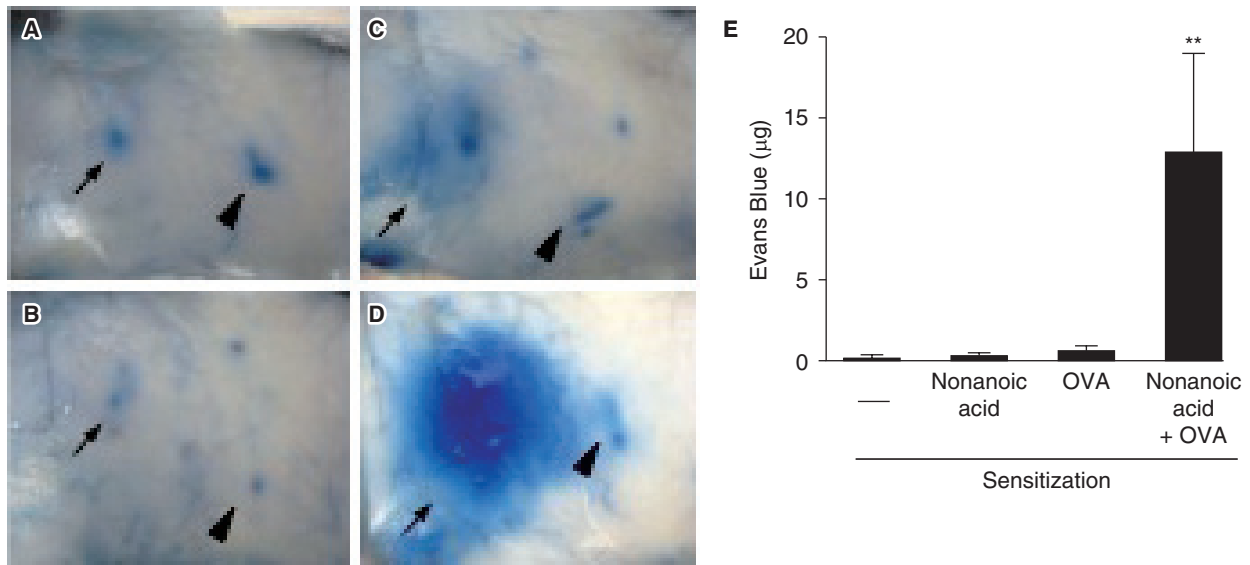


Fig. 5 Enhancement of ovalbumin-induced cutaneous anaphylaxis by nonanoic acid. BALB/c mice were treated with nonanoic acid painted on the shaven back (**B** and **D**) or left untreated (**A** and **C**), and were sensitized 24 h later by injecting 100 μ l of a 1% (w/v) ovalbumin (OVA)-saline solution i.d. at the same site (**C** and **D**). Ten days later, 200 μ l of a 0.05% (w/v) Evans Blue-saline solution was injected intravenously into the tail, and mice were then challenged by injecting 50 μ l of a 1% (w/v) OVA-Hanks' balanced salt solution (arrows) and vehicle (arrow heads) into the shaven back i.d. Mice were sacrificed 30 min after the injection and the skin was dissected (**A-D**). Evans blue in the tissue (diameter: 14 mm) was extracted and quantified by colorimetric analysis and the average at the site injected with vehicle was subtracted from the data (**E**). Values are means for five mice with the S. E. M. shown by vertical bars. Statistical significance; ** $P < 0.01$ vs. untreated, nonanoic acid alone, and ovalbumin alone groups.

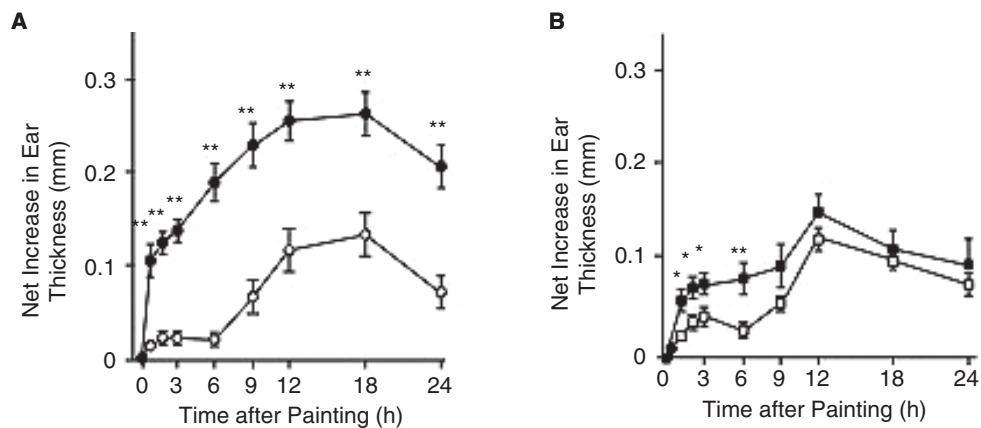


Fig. 6 Exacerbation of PiCl-induced allergic inflammation by nonanoic acid. C57BL/6 wild-type (**A**) and TSLP receptor-deficient (**B**) mice were pretreated with cyclophosphamide (150 mg/kg) on day -2 and 50 μ l of a 7% (w/v) PiCl solution was painted on the right ear lobe on day 0. Sensitized mice were treated with nonanoic acid (20 μ l) at the same site on days 5 and 10 (closed symbols) or left untreated (open symbols). On day 12, mice were challenged with 20 μ l of a 1% (w/v) PiCl solution by painting it on the right ear lobe. Ear lobe thickness was measured 0-24 h after the PiCl challenge. Ear thickness before the challenge was subtracted from the data. Data are shown as the mean $\pm$ S.E.M. for 4 mice. Statistical significance: * $P < 0.05$, and ** $P < 0.01$ vs. the corresponding nonanoic acid-untreated group.

nonanoic acid was apparently weakened in TSLP receptor-deficient mice (Fig. 6B), which indicates that the effect was mediated by TSLP and was not induced by the irritancy.

DISCUSSION

While searching for chemical compounds that induce the production of TSLP *in vivo*, we found that medium chain fatty acids such as nonanoic acid, heptanoic acid, octanoic acid, and dodecanoic acid markedly induced the production of TSLP (Fig. 1A, C). In contrast, a medium-chain aldehyde, alcohol, and ketone caused little TSLP production (Fig. 1D), indicating that only saturated fatty acids with 7-12 carbons possess this activity. One of them, nonanoic acid, is known as a chemical irritant and its proinflammatory actions have been well studied.^{20,28} For example, patch tests in healthy male volunteers show that 40-80% (v/v) nonanoic acid caused irritancy.²⁹ In the present model, nonanoic acid induced TSLP production at concentrations of more than 50% (v/v) (Fig. 1B), indicating that the concentrations needed to cause contact irritancy and to induce TSLP production were almost the same.

Nonanoic acid induced TSLP production with a maximum 24 h after painting (Fig. 2B). Changes in TSLP production were similar to those induced by xylene¹¹ and dibutyl phthalate.¹⁵ Although contact sensitizers such as 1-chloro-2,4-dinitrobenzene, toluene 2,4-diisocyanate, and 2-phenyl-4-ethoxymethylene-5-oxalane induce the production of many cytokines by themselves, including IL-1, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, IL-17, granulocyte macrophage-colony stimulating factor, TNF- α , and interferon- γ , in draining lymph nodes when painted on the ears of mice, nonanoic acid induced the production of IL-1 β only weakly and the production of other cytokines almost not at all.^{28,29} Consistent with these studies, TNF- α and IL-4 levels in the homogenate of ear lobes collected 4 to 72 h after the painting of nonanoic acid were not higher than those of compared with the control (Fig. 2C, D). In addition, nonanoic acid did not elicit leukocyte infiltration. These results suggested that the target cells of nonanoic acid were mainly epithelial cells, which produce TSLP, but little TNF- α and IL-4.

The recognition mechanism for nonanoic acid remains to be elucidated. Although a long fatty acid, palmitic acid, activates TLR4,²¹ TLR4 was not responsible for the nonanoic acid-induced production of TSLP because it occurred even in C3H/HeJ mice, which were completely nonresponsive to LPS^{21,30} (Fig. 3). This finding was supported by the observation that nonanoic acid did not induce apparent inflammation such as leukocyte infiltration (Fig. 2). It was unlikely that AhR, the receptor for external chemical compounds, played an important role in TSLP production (Fig. 4). Although the involvement of GPR84, which is the receptor for medium chain

fatty acids, in TSLP production has not been ruled out, the expression of GPR84 mRNA in ear lobes was very low. PPAR α , which prefers unsaturated fatty acids and oxidized phospholipids as endogenous ligands,³¹ is unlikely to work as a receptor for saturated medium fatty acids. Because nonanoic acid is a chemical irritant, as indicated by the formation of edema (Fig. 2), another possibility exists that a receptor-independent danger signal to epithelial cells caused TSLP production. We found that certain fatty acids also induced TSLP production in a murine keratinocyte cell line (data not shown), the molecular mechanisms of which are currently under investigation *in vitro*.

We confirmed that production of TSLP induced by nonanoic acid attained levels high enough to affect immune responses using two models: sensitization and allergic contact dermatitis. Pretreatment with nonanoic acid before the injection of OVA promoted sensitization to OVA, resulting in an increase in anaphylactic plasma leakage (Fig. 5). Although OVA-specific IgE in serum was undetectable by ELISA, the increase in total IgE was observed. Typically, antigen-specific IgE was much less than 1% of total IgE. The antigen-induced cutaneous reaction is more sensitive to detect antigen-specific IgE than ELISA and there is a strong consistent relationship between total IgE and antigen-specific IgE.³² Therefore, the enhancement of OVA-induced plasma leakage by nonanoic acid was probably due to the increase in IgE production. However, it remains to be elucidated that the enhancement by nonanoic acid was mediated by TSLP.

In a PiCl-induced allergic dermatitis model,^{11,18} the application of nonanoic acid to ear lobes twice after sensitization with PiCl aggravated the PiCl-induced contact dermatitis (Fig. 6). This exacerbating effect was mediated via the production of TSLP, but not by non-specific irritancy because it was reduced in TSLP receptor-deficient mice (Fig. 6B). In this model, we demonstrated that 12-*O*-tetradecanoylphorbol-13-acetate and xylene, which were painted in the same manner as nonanoic acid, shifted the cytokine milieu from Th1 to Th2 via TSLP production and augmented edema formation in the immediate and early phases.^{11,18} Cyclophosphamide was pretreated because it induces eosinophilia probably via shifting the immune balance to Th2.¹⁹ The timing of 12-*O*-tetradecanoylphorbol-13-acetate treatment was selected to minimize the effects of non-specific irritancy.¹⁸ Using this model, we found that xylene treatment enhanced PiCl-induced contact dermatitis via TSLP-dependent manner,¹¹ indicating that this model is suitable to assess the effects of chemical compound-induced TSLP on allergic contact dermatitis.

Shigeno *et al.*¹⁵ and Larson *et al.*¹⁶ also reported that a phthalate ester, such as dibutyl phthalate, induced TSLP production, which resulted in FITC-

induced contact hypersensitivity. In addition, Koike *et al.*³³ recently reported that diisononyl phthalate aggravated atopic dermatitis-like inflammation via TSLP production. Therefore, compounds inducing TSLP production can enhance sensitization and exacerbate allergic inflammation.

Various types of cells including epithelial cells such as keratinocytes and fibroblasts were shown to produce TSLP when stimulated with antigens, cytokines, and toll-like receptor stimulants.^{7,12} As described above, evidence has accumulated that several chemical compounds induce TSLP production. Here we have indicated that such chemicals exist in the environment and may cause allergic diseases. Since nonanoic acid is easily formed in the air from nonanal, a component of perfume, we may be exposed to nonanoic acid in our daily life, although its concentration in the environment is too low to induce TSLP production. On other hand, several natural soaps include medium-chain fatty acids, and their excessive use may lead to allergies via TSLP production.

In conclusion, we found that nonanoic acid and certain fatty acids induced TSLP production and exacerbated allergic inflammation. This is the first report to show that nonanoic acid triggered TSLP production and, as a result, enhanced sensitization to an antigen and worsened allergic inflammation *in vivo*. Therefore, we propose a new category of chemical compounds, chemical allergeo-accelerators, that augment Th2-type immune responses to antigens via the production of TSLP.

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