

Effects of Tributyltin on the Development of Atopic Dermatitis-Like Eruptions in DS-Nh Mice

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Key Words

Th1/Th2 balance • Atopic dermatitis • *Staphylococcus aureus* • Endocrine disruptor

Abstract

Background: In the last few decades, numerous chemical compounds have been produced as a result of industrial development. At the same time, the number of atopic dermatitis (AD) patients has been increasing. It has been reported that tributyltin (TBT) compounds have effects not only on the reproductive system but also on the immune system.

Objective: To investigate whether TBT has an effect on AD, we fed a diet containing TBT to DS-Nh mice, which spontaneously developed dermatitis under conventional conditions.

Methods: DS-Nh mice fed TBT or a control diet were examined for skin changes, number of *Staphylococcus aureus* on the skin and serum IgE levels. To determine Th1/Th2 cytokine production by lymphocytes, lymphocytes of DS-Nh mice fed TBT and of controls were cultured with staphylococcal enterotoxin B and cytokine levels in the supernatants were measured by ELISA. We observed not only spontaneous dermatitis but also dermatitis induced by sensitization with 2,4,6-trinitrochlorobenzene (TNCB). **Results and Con-**

clusion: The AD-like lesions induced by TNCB sensitization were more severe in the mice fed TBT than in those fed the control diet. A greater increase in *S. aureus* on the skin was observed in the mice fed TBT than in the mice fed the control diet. A decrease in IFN- γ production and an increase in IL-5 and IL-13 production were observed in the mice fed the TBT diet and treated with TNCB. These findings suggest that the increase in *S. aureus* and the enhancement of Th2 response induced by TBT exacerbate the AD-like lesions in mice treated with TNCB.

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Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory disease that exhibits fluctuation with remissions and progressions. The cause of AD is multifunctional, involving immunological abnormalities as well as physiological and biochemical defects of the skin barrier structures [1–5]. There are several reports that suggest a Th1/Th2 imbalance in AD [6, 7]. Based on an analysis of cytokine production stimulated with specific antigens, it has also been reported that peripheral blood mononucle-

ar cells from AD patients are Th2 predominant [6]. In acute AD lesions, the IL-4/IFN- γ ratio is increased and mononuclear cells produce larger amounts of IL-4, IL-5 and IL-13 than those of healthy skin [7, 8]. On the other hand, chronic AD skin lesions have significantly fewer IL-4 and IL-13 mRNA-expressing cells but increased numbers of IL-5, IL-12 and IFN- γ mRNA-expressing cells than acute AD skin lesions. It is thought that IL-12 production by infiltrating macrophages results in switching to a Th1-type milieu associated with increased IFN- γ expression [8].

Many new chemical compounds have been produced as a result of industrial development. Tributyltin (TBT) is one such chemical compound and it is used in a variety of industrial, agricultural and domestic products because of its strong biocidal activity against a large spectrum of organisms such as bacteria, fungi, algae, mollusks and crustaceans. For example, TBT is used in antifouling paints for boats and ships, wood preservation, disinfection of circulating industrial cooling water and slime control in paper mills [9]. TBT is known to be an endocrine disrupter [10–12]. It has also been reported that TBT compounds affect not only the reproductive system but also the immune system. In short-term feeding studies with TBT compounds in rats, thymic atrophy, decreased numbers of lymphocytes in spleen and lymph nodes, increased serum IgM level and decreased serum IgG levels were observed [13, 14]. In mice, TBT compounds reduced spleen weight and decreased the number of leukocytes [15]. In our unpublished data, enhancement of Th2 cytokine production and suppression of Th1 cytokine production was observed in C57BL/6 mice fed a TBT diet for 4 weeks. To investigate the effects of TBT on allergic disease, we used the murine model of asthma. As a result, airway inflammation in the mice fed the TBT diet was more severe than in the mice fed a control diet.

DS-*Nh* mice housed under conventional conditions, but not under specific pathogen-free (SPF) conditions, spontaneously developed AD-like dermatitis [16, 17]. The DS-*Nh* mouse strain originated from a mutant male mouse with deficient hair growth in an inbred DS strain colony at Aburahi Laboratories (Shionogi Co. Ltd., Shiga, Japan) in 1976. In DS-*Nh* mice, elevation of serum IgE was detected from around 17 weeks of age [16]. Increases in eosinophils and mast cells are also observed in the skin lesions [17]. Over the course of time, the affected mice developed severe lesions including splenomegaly and lymphadenopathy [16]. An increase in *Staphylococcus aureus* on the skin surface was observed under conventional conditions even before the appearance of eruptions on

these mice. This increase in skin *S. aureus* is thus believed to play an important role in the induction of dermatitis in these mice. This is supported by the finding that the application of *S. aureus* to the skin of mice raised under SPF conditions induces dermatitis [16, 17].

Kitagaki et al. [18] reported that repeated application of 2,4,6-trinitrochlorobenzene (TNCB) to the same skin site shifted cytokine expression from the Th1 to the Th2 profile in lymph nodes. In our previous study, repeated application of TNCB to the dorsal skin of conventional DS-*Nh* mice induced inflammatory skin eruptions with *S. aureus* colonization, not only on the dorsal skin but also on the face, neck and forelegs [19]. These skin lesions clinically and pathologically mimic AD and are associated with an increase in serum IgE level. These changes were more severe in TNCB-treated mice than in untreated mice. Furthermore, IFN- γ and IL-13 production by splenocytes stimulated with staphylococcal enterotoxin B (SEB) was higher in DS-*Nh* mice treated with TNCB than in mice treated with vehicle alone. Since repeated exposure to TNCB facilitates the stable occurrence of AD-like lesions in the DS-*Nh* mouse, we used this model to investigate the effect of TBT on AD.

Although it is believed that the onset of allergic diseases is influenced by genetic factors, environmental factors are considered one of the most important factors influencing the onset of such diseases [20]. Because worldwide environmental contamination with TBT has persisted, humans have been exposed to TBT compounds via TBT-contaminated meat and fish products [21–23]. In this study, we examined the effects of TBT on AD in the DS-*Nh* mouse.

Material and Methods

Animals and Diet

Five-week-old female DS-*Nh* mice raised under SPF conditions were provided by Aburahi Laboratories. The DS-*Nh* mice used for this study were obtained as F1(*Nh*/+) from male DS-*Nh*(*Nh*/*Nh*) $\times$ female DS(+ / +) crosses. They were kept under conventional conditions throughout this study.

TBT chloride was purchased from Wako Pure Chemical Industries (Osaka, Japan). The mice were fed a solid diet containing 100 or 0 ppm of TBT (TBT diet or control diet) from 6 to 18 weeks of age ($n = 5$ per group). Three independent experiments were performed for each group. This study was conducted in accordance with the Guidelines for Animal Experiments of the Yokohama City University Graduate School of Medicine.

Sensitization and Repeated Application of TNCB

TNCB was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). DS-*Nh* mice were treated with 7% TNCB dissolved in ac-

etone:olive oil (4:1) which was applied epicutaneously to the abdomen (50 µl) at 10 weeks of age after 4 weeks of feeding with the TBT or control diet. Starting 1 week later, 1% TNCB was applied epicutaneously 3 times a week to the dorsal skin for 8 weeks.

Evaluation of Skin Eruption

Skin eruptions, which were defined as erythema, edema, excoriation/erosion and dry skin, were observed macroscopically. Each of these 4 changes was graded from 0 to 3, with grade 0 representing no visible skin change, grade 1 slight skin change, grade 2 moderate skin change and grade 3 severe skin change. The total score for skin eruptions was the sum of the individual scores as indicated above. At 10 or 18 weeks of age, the dorsal skin was resected, fixed with 10% phosphate-buffered formalin, stained with hematoxylin and eosin (HE) solution and examined for histological changes by light microscopy. The inflammatory cells in the sections were observed in 10–20 high-power fields.

Isolation of S. aureus on Skin Surface

DD checker Seiken MSEY agar (Denka Seiken, Tokyo, Japan), a medium for the isolation and identification of *S. aureus*, was used to evaluate the colonization of *S. aureus* on the skin surface. Dorsal skin areas were selected for sampling. The plates were applied for 10 s to the skin for sampling and then cultured in an incubator at 37°C for 24 h. After incubation, the numbers of colonies on the medium were counted. Identification was performed according to the manufacturer's instructions.

Measurement of Serum Immunoglobulins

Serum samples were collected and stored at –80°C until measurement. Serum IgM, IgG1, IgG2a and IgE levels were determined by ELISA. For the detection of IgM, IgG1 and IgG2a, 96-well plates were coated with rabbit anti-mouse IgM (Zymed Laboratories Inc., San Francisco, Calif., USA) or rabbit anti-mouse IgG (Zymed) antibodies. After blocking with BSA, standards or samples were added. As a second antibody, affinity-purified rabbit anti-mouse IgM-peroxidase (Zymed), affinity-purified rabbit anti-mouse IgG1-peroxidase (Zymed) or affinity-purified rabbit anti-mouse IgG2a-peroxidase (Zymed) was used. Serum IgE levels were measured using a mouse IgE ELISA kit (Yamasa, Tokyo, Japan). A 100-µl portion of 25-fold-diluted serum was applied to each well and further procedures were performed according to the manufacturer's recommendations. The total IgE concentration in each serum sample was estimated using a standard sample from the kit.

Cell Preparation and Flow-Cytometric Analysis

DS-Nh mice fed the TBT or control diet were sacrificed at 18 weeks of age. Their axillary and cervical lymph nodes were excised and single-cell suspensions were prepared in an RPMI 1640 medium. The cells were incubated with anti-CD4 and anti-CD8 mAb on ice for 30 min. After 2 washes with PBS, the cells were analyzed with a FACScan flow cytometer and CellQuest software.

Cytokine ELISA Assays

The single-cell suspensions (1.25×10^6 cells/0.5 ml) prepared from axillary and cervical lymph nodes were cultured in an RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo., USA) with 10% fetal calf serum supplemented with optimal stimulating con-

centrations of SEB (10 µg/ml) at 37°C in 5% CO₂ for 48 h. The supernatant samples were collected and stored at –80°C until use and assayed for IL-4, IFN-γ, IL-5 and IL-13, employing commercial ELISA kits (BioSource International Inc., Camarillo, Calif., USA and Genzyme Techné, Minneapolis, Minn., USA).

Data Analysis

Statistical analysis was performed using the two-tailed Student t test. Values are presented as mean ± SE.

Results

Effects of TBT on DS-Nh Mice prior to the Development of Dermatitis

First, 6-week-old DS-Nh mice were fed the TBT or control diet for 4 weeks to examine the effects of TBT on DS-Nh mice prior to the development of dermatitis. At 10 weeks of age, the number of colonies of *S. aureus* in the mice fed the TBT diet was significantly higher than in the mice fed the control diet (fig. 1a). The numbers of lymphocytes in the lymph nodes of the mice fed the TBT diet were significantly lower than those in the mice fed the control diet (fig. 1b). Production of IL-4, IL-5, IL-13 and IFN-γ by lymph node cells was significantly lower in mice given the TBT diet than in those given the control diet (fig. 1c). Serum IgE was detected in neither of the groups (data not shown). TBT has immunosuppressive effects on DS-Nh mice.

Exacerbation of AD-Like Lesions Induced by Treatment with TNCB in DS-Nh Mice Fed TBT

To investigate the effects of TBT on AD-like skin lesions, the mice were observed until 18 weeks of age with or without TBT diet. There was no significant difference in skin score between these groups (fig. 2). In our previous study, repeated TNCB application facilitated the development of AD-like lesions in DS-Nh mice [16]. Some mice were repeatedly treated with TNCB at 10 weeks of age after 4 weeks of feeding with the TBT diet. First elicitation of TNCB hypersensitivity induced erythema and edema that appeared on the dorsal skin in both the group with the TBT diet and the control group. Between 11 and 13 weeks of age, dry skin, erythema and erosion appeared not only on the dorsal skin areas where TNCB had been applied, but also on the face and forelegs (data not shown). After 14 weeks of age the skin lesions on the mice fed the TBT diet became wider and more severe than those on the controls (fig. 2, 3). TBT exacerbated AD-like lesions induced by repeated TNCB application.

Fig. 1. The mice were fed the TBT or control diet for 4 weeks and sacrificed at 10 weeks of age. Number of *S. aureus* on their skin (a), total cell numbers in axillary and cervical lymph nodes (b), and cytokine production (IL-4, IL-5, IL-13 and IFN- γ) with SEB stimulation by lymph node cells (c) of DS-*Nh* mice fed the TBT or control diet. Numbers of colonies of *S. aureus* and lymph node cells were increased significantly and total numbers of cells of axillary and cervical lymph nodes and cytokine production were significantly decreased with TBT diet compared with control diet. Values are presented as mean $\pm$ SE. # $p < 0.05$, ## $p < 0.01$ vs. control diet.

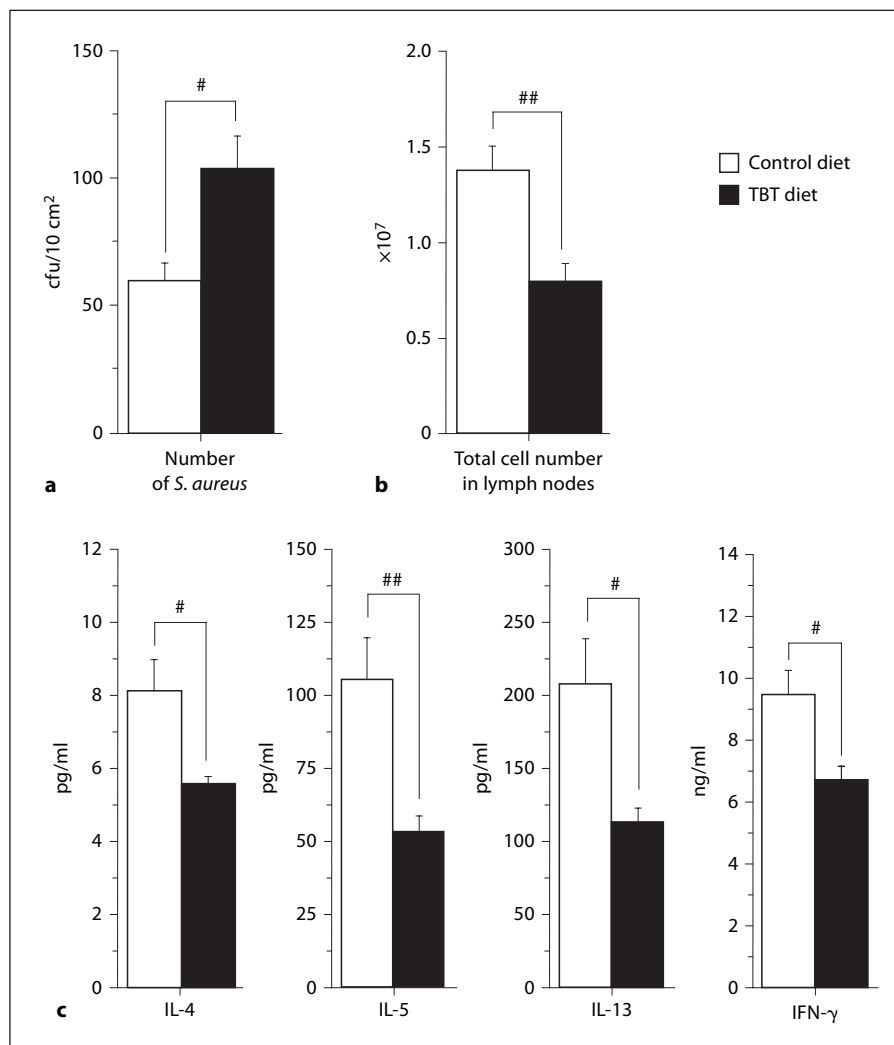
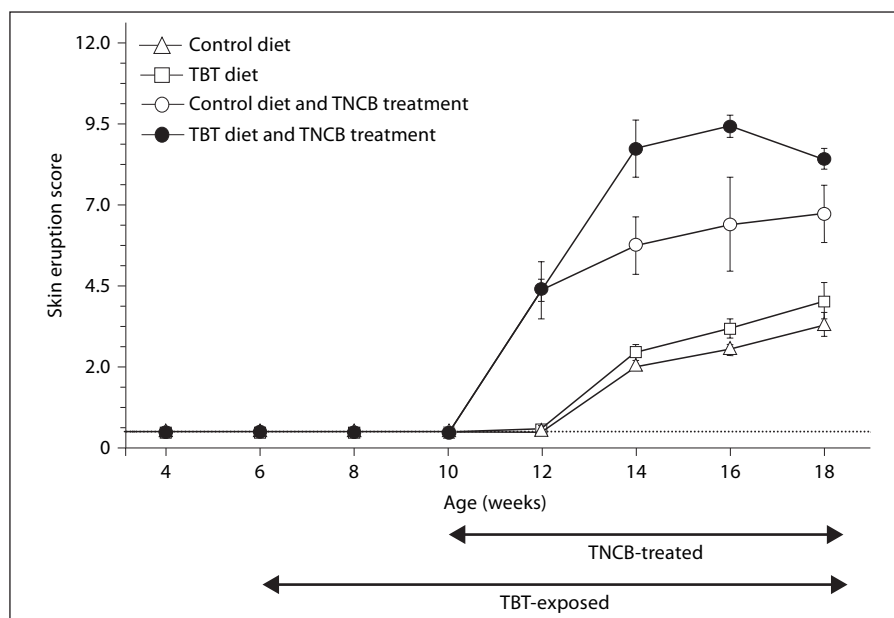


Fig. 2. Skin eruption score in DS-*Nh* mice. Six-week-old DS-*Nh* mice were fed the TBT or control diet for 12 weeks. In some mice, TNCB was applied repeatedly for 8 weeks after 10 weeks of age with the TBT or control diet. Skin eruption score was highest for the mice with TNCB treatment and TBT diet at 14 weeks of age. Each group consisted of 5 mice. Values are presented as mean $\pm$ SE.



Severe Cellular Infiltration into Dermis Induced by Treatment with TNCB in DS-Nh Mice Fed TBT

To determine the histological differences between AD-like skin lesions of DS-Nh mice fed TBT or the control diet, dorsal skin was resected and stained with HE (fig. 4). At 10 weeks of age, no significant histopathological changes were observed in DS-Nh mice fed the TBT or control diet (fig. 4a, b). In TNCB-treated DS-Nh mice, the lesions exhibited significant thickness of the dermis and epidermis, and cellular infiltrates into dermis at 18 weeks of age (fig. 4c, d). The infiltrating cells were mainly lymphocytes, with some eosinophils. More inflammatory cells were observed in the dermis of mice fed TBT than in that of mice fed the control diet. TBT promoted the infiltration of lymphocytes and eosinophils into the dermis of TNCB-treated mice.

Effects of TBT on *S. aureus* on the Skin, Regional Lymphadenopathy and Serum Antibody Levels in DS-Nh Mice after the Development of Skin Lesions

To investigate the cause of exacerbation by TBT, colonies of *S. aureus* on the skin, lymphadenopathy and serum antibody levels were examined at 18 weeks of age. Colonies of *S. aureus* on the skin were counted in TNCB-treated mice fed the TBT or control diet. The number of colonies of *S. aureus* increased significantly with the TBT diet compared with the control diet (fig. 5a).

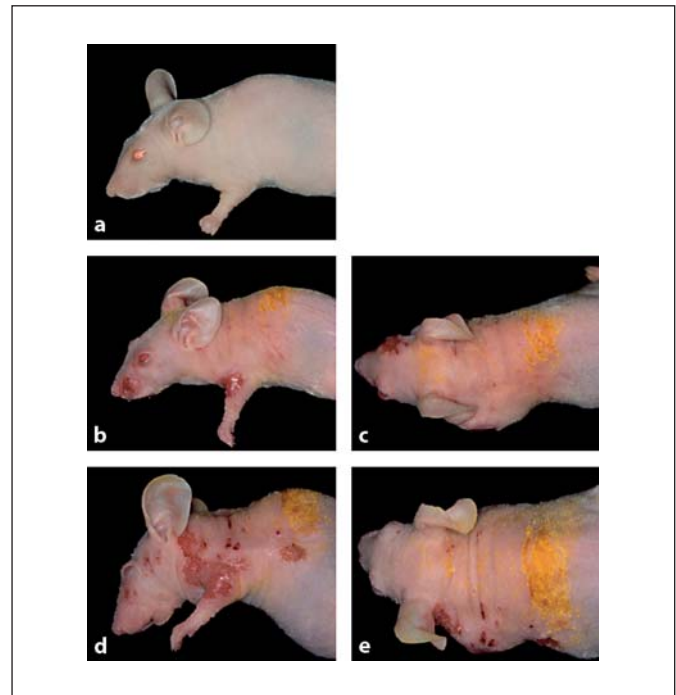


Fig. 3. Clinical features of DS-Nh mice fed the TBT or control diet. **a** DS-Nh mouse fed the TBT diet for 4 weeks at 10 weeks of age. No skin lesions were observed. **b, c** TNCB-treated DS-Nh mouse fed the control diet. Erythema, dry skin, erosions and edema were observed on the dorsal skin, face and forelegs at 18 weeks of age. **d, e** TNCB-treated DS-Nh mouse fed the TBT diet. At 18 weeks of age, skin lesions were more severe than in TNCB-treated DS-Nh mice fed the control diet.

Fig. 4. Histological analysis of skin from DS-Nh mice. These sections were obtained from dorsal skin of DS-Nh mice fed the control diet for 4 weeks with examination at 10 weeks of age (**a**); the TBT diet for 4 weeks with examination at 10 weeks of age (**b**); the control diet and TNCB treatment with examination at 18 weeks of age (**c**); the TBT diet and TNCB treatment with examination at 18 weeks of age (**d**). The infiltrating cells were mainly lymphocytes, with some eosinophils. More inflammatory cells were observed in the dermis of mice fed the TBT diet than in the dermis of mice fed the control diet. Sections were stained by HE. $\times 100$.

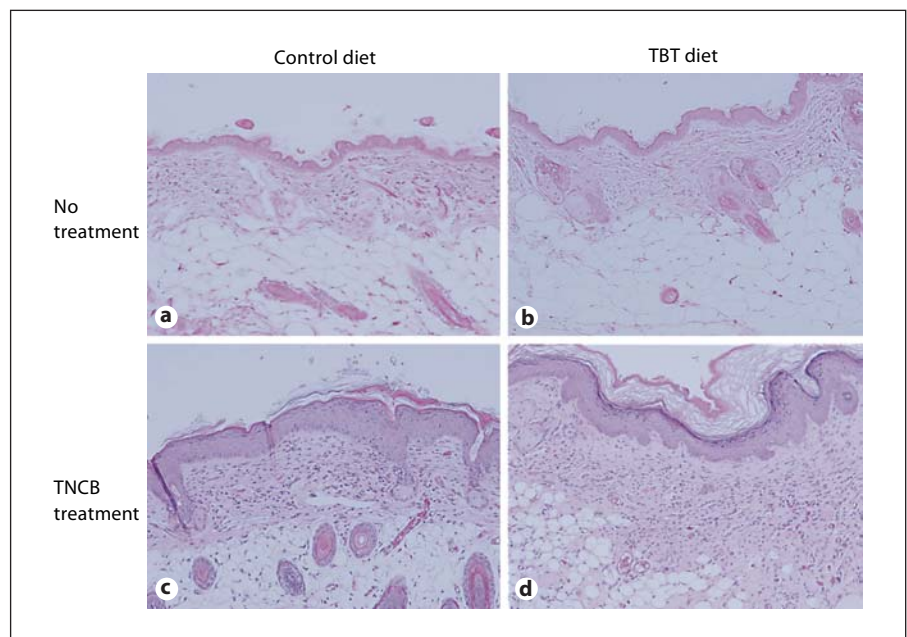
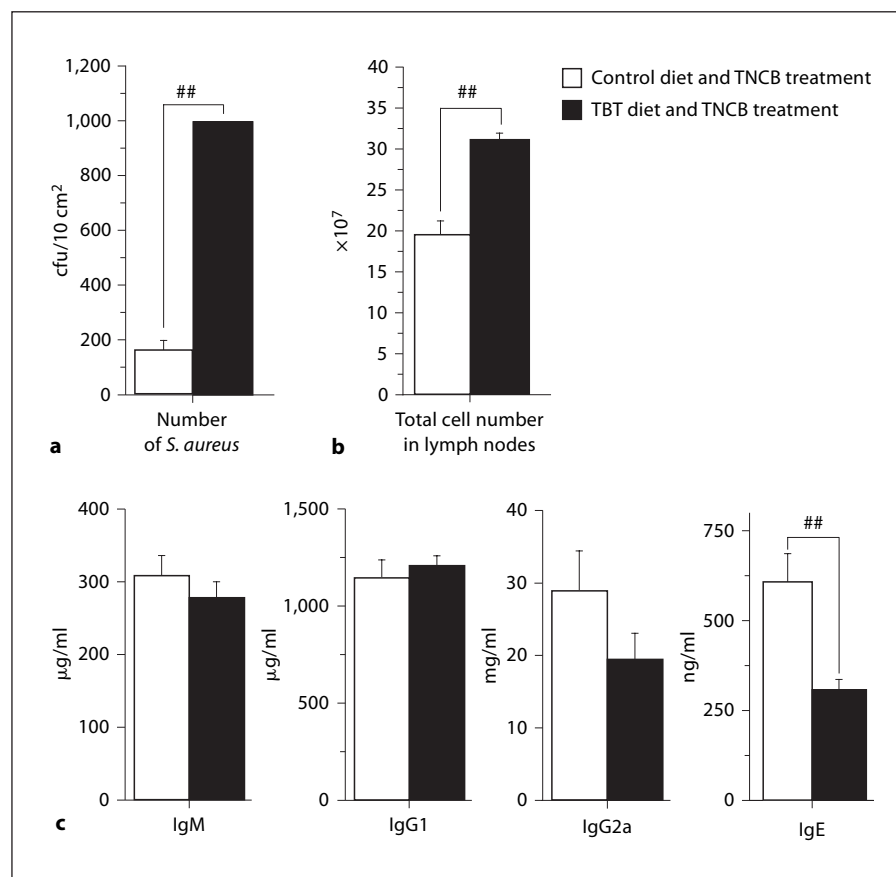
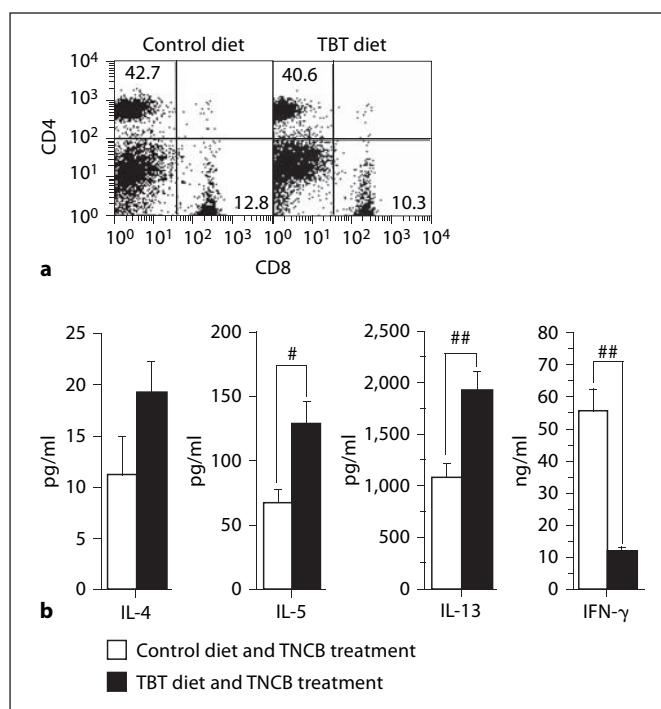


Fig. 5. Numbers of colonies of *S. aureus* on skin, total numbers of axillary and cervical lymph node cells and total serum antibody levels in DS-*Nh* mice treated with TNCB. The mice were fed the TBT or control diet from 6 weeks of age. **a** Numbers of *S. aureus* on the skin. **b** Total IgM, IgG1, IgG2a and IgE levels in serum. **c** Total numbers of axillary and cervical lymph node cells. The numbers of colonies of *S. aureus* and lymph node cells were significantly increased and the level of serum IgE significantly decreased with TBT diet compared with the control diet. Values are presented as mean $\pm$ SE. ## $p < 0.01$ vs. control diet.



In order to assess regional lymphadenopathy, the axillary and cervical lymph nodes were excised and the numbers of cells in these lymph nodes counted. After 8 weeks of TNCB treatment, the number of such cells in the mice fed the TBT diet was significantly higher than in the controls (fig. 5b). This data indicated that the proliferation of lymph node cells in TNCB-treated DS-*Nh* mice was enhanced by TBT.

Fig. 6. **a** Flow cytometry analysis of lymphocytes prepared from axillary and cervical lymph nodes of Ds-*Nh* mice treated with TNCB. CD4⁺ and CD8⁺ cell profiles are shown with percentages of cells in each area. The CD4⁺ and CD8⁺ cell ratios in the lymph nodes of mice fed the TBT diet are comparable to those of Ds-*Nh* mice fed the control diet. **b** Production of cytokines (IL-4, IL-5, IL-13 and IFN- γ) with SEB stimulation by lymph node cells of DS-*Nh* mice fed the TBT or control diet. IFN- γ production in DS-*Nh* mice fed the TBT diet was significantly lower but IL-5 and IL-13 production was significantly greater than in mice fed the control diet. Values are presented as mean $\pm$ SE. # $p < 0.05$, ## $p < 0.01$ vs. control diet.



Total antibody levels in the serum of the mice fed TBT or the control diet were examined by ELISA. The serum IgM, IgG1, and IgG2a levels in the mice fed the TBT diet were comparable to those in the mice fed the control diet. However, the serum IgE level in the mice fed the TBT diet was significantly lower than that in the DS-*Nh* mice fed the control diet (fig. 5c).

Enhancement of Th2 Cytokine Production by TBT in TNCB-Treated DS-Nh Mice

The effect of TBT on T cells in the lymph nodes of TNCB-treated DS-*Nh* mice was examined. First, the CD4+ and CD8+ cell ratios in TNCB-treated mice at 18 weeks of age were assessed by flow cytometry. In total, CD4+ and CD8+ cell ratio accounted for half of the lymph node cells in all mice. The CD4+ and CD8+ cell ratio in the mice fed the TBT diet was comparable to that in the mice fed the control diet (fig. 6a). All lymphocytes were cultured with SEB to measure cytokine levels in the supernatants. Next, the effect of TBT on Th1/Th2 cytokine productions by T cells stimulated SEB was examined. Production of IFN- γ was significantly lower in the mice fed the TBT diet than in the mice fed the control diet. In contrast, production of IL-5 and IL-13 was higher in the mice fed the TBT diet than in the controls (fig. 6b). These results indicate that exposure to TBT enhances Th2 cytokine production by T cells in TNCB-treated DS-*Nh* mouse.

Discussion

In this study, we examined the effects of TBT on AD, using the DS-*Nh* mouse as a model of AD. People have been exposed to TBT compounds via ingestion of TBT-contaminated meat and fish products [6, 7]. TBT levels of 21.0 ± 10.7 ng/ml [11] or 8.18 ± 15.4 ng/ml [7] of TBT have been detected in human serum. In the present study, we detected 3.9–4.0 ng/ml TBT in the serum of C57BL/6 mice receiving a 100 ppm TBT diet for 30 days using an inductivity-coupled plasma mass spectrometer; TBT was not detected in the serum of control mice (data not shown). The TBT concentration observed in this study thus appears to be comparable to that in the serum of humans reported previously.

We initially examined the effects of the TBT diet in the mice before they exhibited AD-like dermatitis. An increase in skin *S. aureus* colonization was observed, accompanied by a decrease in lymph node cells in the mice fed the TBT diet. IL-4, IL-5, IL-13 and IFN- γ production by these cells was also decreased. These findings showed

that TBT has immunosuppressive effects on the mouse as reported previously [13, 14].

Next, repeated application of TNCB to the dorsal skin was performed in DS-*Nh* mice fed the TBT or control diet to facilitate the development of AD-like skin eruptions. With the repeated application of TNCB, the regions of dermatitis became wider and more severe and were accompanied by more lymphadenopathy in the mice fed the TBT diet but not in the controls (fig. 2, 3). Th2 cytokine production was increased, while the production of IFN- γ , a Th1 cytokine, was decreased in the mice fed the TBT diet. In previous studies, rats fed TBT oxide for 6 weeks exhibited significantly decreased delayed-type hypersensitivity reaction, which is mediated by Th1 cells, compared to rats fed a normal diet [24, 25]. Further, allograft rejection was significantly delayed by a diet containing di-*n*-butyltin chloride, which is a metabolite of TBT chloride, for 6 weeks [26]. The decreased delayed-type hypersensitivity reaction and the delay of allograft rejection in mice exposed to di-*n*-butyltin appeared to be caused by a decreased Th1 response. These results are consistent with our finding that TBT exposure may have a Th1 suppressive effect in DS-*Nh* mice. The mechanisms by which TBT decreases Th1 responses are still unclear. One possible explanation is that TBT inhibits cytochrome P450 1A1-dependent aromatase, which catalyzes aromatization of androgen to estrogen [12]. Estrogen was shown to increase secretion of IFN- γ from ConA-activated mouse splenocytes, while it had no effect on the secretion of IL-4 in vitro. Furthermore, antigen-presenting cells possessing estrogen receptors are required for the secretion of IFN- γ by splenocytes stimulated by ConA in the presence of estrogen [27]. Exposure of mice to TBT may decrease the estrogen levels and downregulate Th1 responses mediated through antigen-presenting cells, such as dendritic cells or macrophages.

In contrast to the suppression of IFN- γ production in the mice fed the TBT diet, production of IL-5 and IL-13 by lymphocytes was significantly higher with the TBT diet than with the control diet in the mice that underwent repeated TNCB application (fig. 6b). It is well known that the Th1 cytokines, IFN- γ and IL-12, inhibit Th2 responses. It thus appears that TBT exposure enhanced Th2 response through the suppression of IFN- γ production and induced exacerbation of AD-like skin lesion in DS-*Nh* mice treated with TNCB.

However, serum IgE levels were significantly lower in the mice fed the TBT diet than in the mice fed the control diet despite the increase in IL-13 production, which induces IgE synthesis [28] (fig. 5b). IL-4 production was

also increased, though not to a significant extent. Levels of other serum antibodies (IgM, IgG1 and IgG2a) in the mice fed the TBT diet were comparable to those in the mice fed the control diet (fig. 5c). TBT may affect class switching from IgG to IgE in the DS-*Nh* mouse. On histopathological analysis of skin lesions, the cellular infiltrates in the dermis of DS-*Nh* mice fed the TBT diet were found to be more severe than those in the control mice (fig. 4c, d) despite the low level of serum IgE in the mice fed the TBT diet. Some AD patients have serum IgE levels that are within the normal range or not markedly high [29]. Taken together, these findings indicated that the severity of AD-like dermatitis, which was exacerbated by the TBT diet, was not related to the production of IgE in TNCB-treated DS-*Nh* mice. The severity of AD-like dermatitis is thus not related to the production of IgE in the DS-*Nh* mouse.

TBT exposure increased *S. aureus* colonization on the skin in DS-*Nh* mice (fig. 1, 5). One possible explanation for this is that it was an effect of the reduction of the IFN- γ production, which plays an important role in host defenses against bacteria, in mice with TBT exposure. In a previous study, TBT suppressed host resistance to *Listeria monocytogenes* infection in rats [30]. These findings suggest that a Th1-suppressive effect induced by TBT exposure impaired resistance to *S. aureus* in DS-*Nh* mice. Another possibility is the impairment of the innate immune system by TBT exposure. The innate immune system of the human skin includes antimicrobial peptides known as defensins. Significantly lower expression of β -defensin-2 mRNA has been found in atopic lesions [31]. It has not been reported whether TBT affects the antimicrobial peptides in the skin.

The increase in *S. aureus* on the skin surface probably resulted in increased severity of cellular infiltration in the skin lesion, since a clear correlation between the clinical severity of AD-like lesions and the number of colonizing *S. aureus* has been reported [32, 33]. Furthermore, superantigens on *S. aureus* have been shown to upregulate the expression of cutaneous lymphocyte antigen, and to thus enhance the homing of T cells to sites of cutaneous inflammation [34]. It also appeared that the more severe cell infiltration in the dermis was caused not only by the increase in *S. aureus* but also by the increase in production of IL-5 and IL-13 by lymphocytes in DS-*Nh* mice fed the TBT diet. IL-5 is known to attract eosinophils. Recently, it was reported that human keratinocytes stimulated with IL-13 produce CCL22 and attract CD4+ T cells [35]. Increased *S. aureus* infection and increased IL-5 and IL-13 production due to TBT exposure may thus have induced more severe cellular infiltration.

In summary, the impairment of resistance to *S. aureus* and the enhancement of Th2 response by TBT exacerbated AD-like lesions in TNCB-treated DS-*Nh* mice. These findings suggest that TBT exacerbates AD induced by repeated antigen exposure, and thus that TBT exposure is one of the risk factors for AD.

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