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SHORT COMMUNICATION



Physiological effects of brominated flame retardants on NC/Nga mice

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ABSTRACT

Purpose: Brominated flame retardants (BFRs) are used as an additive or reactive components in various materials. Regarding their health concerns, their immunotoxicity have not been clarified yet.

Materials and methods: In the current study, we examined the effects of systemic exposure to two types of BFRs, DE71 and DE79, on pathophysiologic traits of murine atopic dermatitis (AD). Male NC/Nga mice were repeatedly injected intraperitoneally with DE71 and DE79 and/or mite allergen (*Dermatophagoides pteronyssinus*: Dp) into their right ears. Thereafter, clinical scores, macroscopic findings of inflammatory foci, and Ig values in serum were examined.

Results: Both DEs significantly aggravated clinical scores induced by mite allergen including skin dryness and edema. Total IgE titer was significantly greater in the Dp + DE79 group than in the Dp group.

Conclusions: Taken together, exposure to BFRs can exacerbate AD-like skin lesions related to mite allergen in mice. The accentuating effects may be mediated, at least in part, through hyperproduction of IgE.

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KEYWORDS

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Introduction

Flame retardants are used in various industrial and consumer products to prevent fire-related injury and property damage. The most common brominated flame retardants (BFRs) such as brominated diphenyl ethers, cyclododecanes and bisphenols have the highest production volumes. BFRs are used as an additive or reactive component in plastics, polyurethane foams, textiles, televisions, computers, furniture, curtains and building materials¹. The five major BFRs include polybrominated diphenyl ethers (PBDEs) such as pentabromodiphenyl ether (Penta-BDE), octabromodiphenyl ether (Octa-BDE) and decabromodiphenyl ether (Deca-BDE); hexabromocyclododecane (HBCD); and tetrabromobisphenol A (TBBPA)¹. Reactive BFRs including TBBPA are chemically bonded into polymer products, whereas additive BFRs, including PBDEs and HBCD, are simply blended with polymers and easily leach out². BFRs can be released into the environment through volatilization or dust formation from BFR-containing products in homes^{3,4}, offices^{5,6}, hotels⁷ and car interiors⁸, and from emissions during manufacture, waste disposal and BFR-containing product recycling^{9–11}.

As for their health concerns when exposed, BFRs have been reported to possess endocrine-disrupting effects and developmental neurotoxicity^{12,13}. Recently, BDE have been proved to increase the risk of diabetes in animals¹⁴. Nonetheless, their immunotoxic effects and their underlying molecular mechanisms have not been completely elucidated. We have previously investigated the *in vitro* effects of BFRs on phenotypes and the function of splenocytes and

bone marrow-derived dendritic cells (BMDCs) from atopy prone mice¹⁵. As results, some BFRs including DE71 and DE79 increased MHC class II and CD86 expression on splenocytes and accompanying interleukin (IL)-4 production from them¹⁵. DE71, HBCD and TBBPA increased T cell receptor (TCR) expression on splenocytes. Further, in both experiments, all BFRs except TBBPA increased DEC205 expression on BMDCs. BMDCs that differentiated in the presence of HBCD showed enhanced MHC class II, CD80, CD86 and CD11c expression. These results implicate that some BFRs may aberrantly stimulate immune cells, resulting to facilitating impacts on allergic pathology. Nevertheless, these proceeding studies did not verify immunological disrupting impacts of BFRs on allergic subjects. To develop the research, in the present study, we examined the effects of systemic exposure to two types of representative BFRs, DE71 and DE79, also proved to activate dendritic cells and lymphocytes *in vitro*, on pathophysiologic traits of murine atopic dermatitis (AD).

Materials and methods

Chemicals

Figure 1 shows the structures of Penta-BDE mixture (DE71; Wellington Laboratories Inc., ON, Canada), Octa-BDE mixture (DE79; Wellington Laboratories). Each BFR were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO) and diluted with corn oil. The final concentration of DMSO was 0.1% in all experiments.

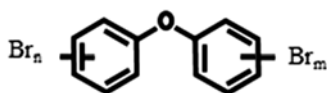


Figure 1. Chemical structures of polybrominated diphenyl ethers (PBDEs).

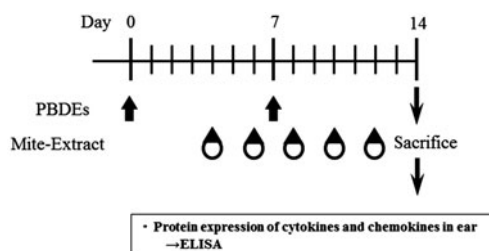


Figure 2. Experimental protocol.

Animals

Five-week-old NC/Nga male mice were purchased from SLC Japan Inc. (Shizuoka, Japan); experiments were performed when mice were 9 weeks old (weighing 24–27 g). Mice were given sterile distilled water and a commercial diet (Labo MR Stock; Nosan Corporation Life-Tech Department, Yokohama, Japan) *ad libitum*, and housed in an animal facility maintained at 24–26 °C with 55–75% humidity and a 12-h light/dark cycle under conventional conditions. All animal studies were approved by The Institutional Review Board of the International University of Health and Welfare. Animals were treated humanely and with regard for alleviation of suffering.

Experimental design

The mice were divided into four groups as follows: (1) saline + vehicle (control group), (2) mite extract [*Dermatophagoides pteronyssinus* (Dp); Cosmo Bio LSL, Tokyo, Japan] + vehicle, (3) Dp + DE71, and (4) Dp + DE79. As shown in Figure 2, the animals in the experimental groups were exposed to the allergen through the subcutaneous injection of 5 µg of mite extract Dp dissolved in 10 µl of saline in the ventral side of the right ear 2–3 d a week (a total of five times) using animal fixing implement. We administered PBDEs dissolved in DMSO (Sigma-Aldrich Co.) at a dose of 1 µg diluted in 0.1 ml corn oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan) by intraperitoneal injection to four groups on days 0 and 7 (a total of two times) as we have previously examined^{16,17}. The final concentration of DMSO was 0.1% in all experiments. All animals were sacrificed on the last day of the experiment (day 14). In another experiment, we set 'only BDE groups' to which each BDE was administered on days 0 and 7.

Evaluation of skin disease

Twenty-four hours after each subcutaneous injection, the clinical score for ADSLs was determined using a slight modification of a previously described method¹⁶. The total clinical severity score was defined as the sum of the individual scores for the symptoms measured. The symptoms of skin

dryness and eruption were scored as follows: 0, no symptoms; 0.5, mild symptoms; 1, moderate symptoms; and 2, severe symptoms. The presence of edema was scored as follows: 0, no symptoms; 0.5, very mild symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. Crusting and erosion were scored as follows: 0, no symptoms; 0.5, very mild symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms.

Quantitation of cytokines and chemokines in ear tissues

Right ears of mice were removed after sacrifice and then homogenized and centrifuged as previously described¹⁸. After centrifuging, stored at –80 °C until assayed for cytokines profiles and other parameters ($n=5$ or 6 in each group). The cytokine and chemokine protein levels in the supernatants were determined using enzyme-linked immunosorbent assays (ELISA). Interleukin (IL)-5, IL-13, interferon (IFN)- γ , eotaxin, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , were measured using an ELISA kit from R&D Systems Inc. (Minneapolis, MN).

Total IgE antibodies

Total IgE antibodies in the serum were measured using a Mouse IgE ELISA kit (Shibayagi Co., Shibukawa, Japan; $n=5-6$ in each group).

Statistical analysis

All data are expressed as the mean $\pm$ standard errors (SE). Statistical comparisons were made using unpaired Student's *t*-test. Significance was assigned to *p* values smaller than .05.

Results

BDE enhance symptoms of atopic dermatitis-like skin lesions

To evaluate the effects of BDE on AD-like skin lesions induced by Dp, we examined clinical scores and ear thickening. Treatment with Dp enhanced clinical scores (Figure 3(A)), including dryness, eruption, wound, edema, and ear thickening compared with no treatment from day 10. DE79 and DE71 significantly increased clinical scores and ear thickening compared with vehicle exposure in the presence of an allergen. For macroscopic findings, DE79 (Figure 3(B)) or DE71 (Figure 3(C)) and Dp caused marked wound compared with exposure to vehicle and Dp (Figure 3(D)). Measurement of ear thickness revealed that the value was significantly greater in the Dp $\pm$ DE71 ($0.30 \pm 0.0 \mu\text{m}$) or Dp $\pm$ DE79 ($0.31 \pm 0.0 \mu\text{m}$) group than in the Dp ($0.27 \pm 0.0 \mu\text{m}$) group. No change was observed in the control group (Figure 3(E)).

Effects of BDE on the protein expression of Th cytokines and chemokines related to Dp in the skin

To elucidate the impacts of BDEs on the local inflammatory status, we examined protein levels of cytokines and

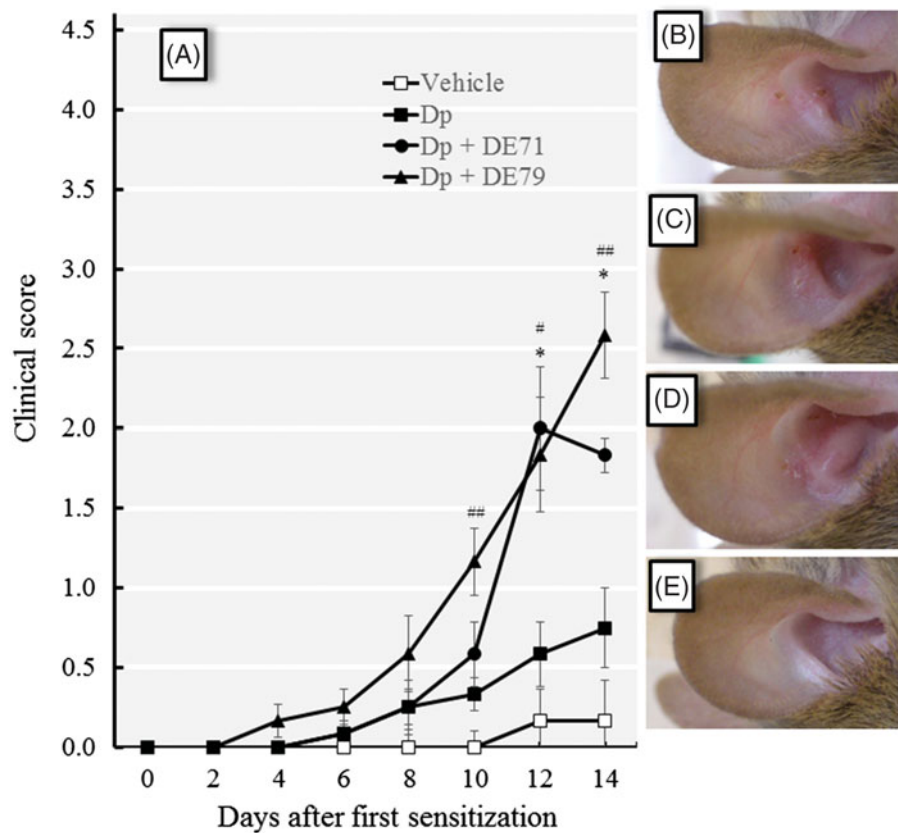


Figure 3. (A) Skin severity scores and (B–E) macroscopic findings for the ears of NC/Nga mice. The total clinical severity score was defined as the sum of the individual scores for the symptoms measured as described in the section ‘Materials and methods’. Open square; saline ± vehicle; solid square; Dp; solid circle; Dp ± DE71; solid triangle; Dp ± DE79. All data are the mean ± standard errors (SE) values of 5–6 mice. * $p < .01$, Dp + DE71 vs. Dp; # $p < .05$, ## $p < .01$, Dp + DE79 vs. Dp. Macroscopic features: (B) Dp ± DE79, (C) Dp ± DE71, (D) Dp ± vehicle, and (E) saline ± vehicle (control).

chemokines related to allergy in the homogenates of the ear. Treatment with Dp increased the expression of IL-5 and IL-13 as compared with saline-treated groups ($p < .01$). In the presence of an allergen, however, exposure to DE71 decreased and DE79 increased the expression of IL-5 as compared with the vehicle without statistical significance. On the other hand, both BDE decreased IL-13 expression without significance. Treatment with Dp decreased the expression of IFN- γ compared with saline-treated groups, and both BDE further decreased the level in the presence of an allergen. Treatment with Dp increased the expression of eotaxin, MCP-1 and MIP-1 α as compared with saline-treated groups ($p < .05$). In the presence of an allergen, exposure to DE79 further increased these expressions without statistical significance (Figure 4).

Effects of BDE on the IgE production in the serum

To evaluate the adjuvant capacity of BDE for Ig production, we measured the levels of total IgE in serum. Intradermal injection of Dp increased the levels of total IgE. The value was significantly greater in the Dp ± DE79 group than in the Dp group ($p < .05$; Figure 5).

Effects of only BDE on NC/Nga mice

In ‘only BDE groups’, there was no significant change in clinical scores, ear histology and values of proinflammatory

cytokines, and IgE titer as compared to those in the control group (data not shown).

Discussion

In our previous *in vitro* study, we first have shown that some BDE including DE71 and DE79 aberrantly stimulate immune cells in terms of enhanced surface expression of molecules related to phagocyte and antigen-presenting functions on dendritic cells and hyperproduction of Th cytokines from splenocytes. In the present cutting-edge study, we further showed evidence that exposure to two types of BDE, at least in part, facilitates pathophysiology of the immune disease.

In our previous model using NC/Nga mice, mite allergen treatment significantly elevated the production of total IgE and allergen-specific IgG₁ in serum, increased the number of submandibular lymph node cells, and enhanced cell proliferation of submandibular lymph node cells in the presence of allergen stimulation *ex vivo*¹⁷. Nonetheless, in the present study, Dp allergen mildly aggravate atopic-like dermatitis pathology as compared to vehicle treatment (not significant). This difference might be raised from the difference in season, lot of Dp, environment, and etc. Since NC/Nga mouse has been established as an animal model for human AD^{19,20}, which are hypersensitive to some kind of allergens such as mite allergen, ovalbumin, and hapten to develop dermatitis in association with IgE overproduction under conventional

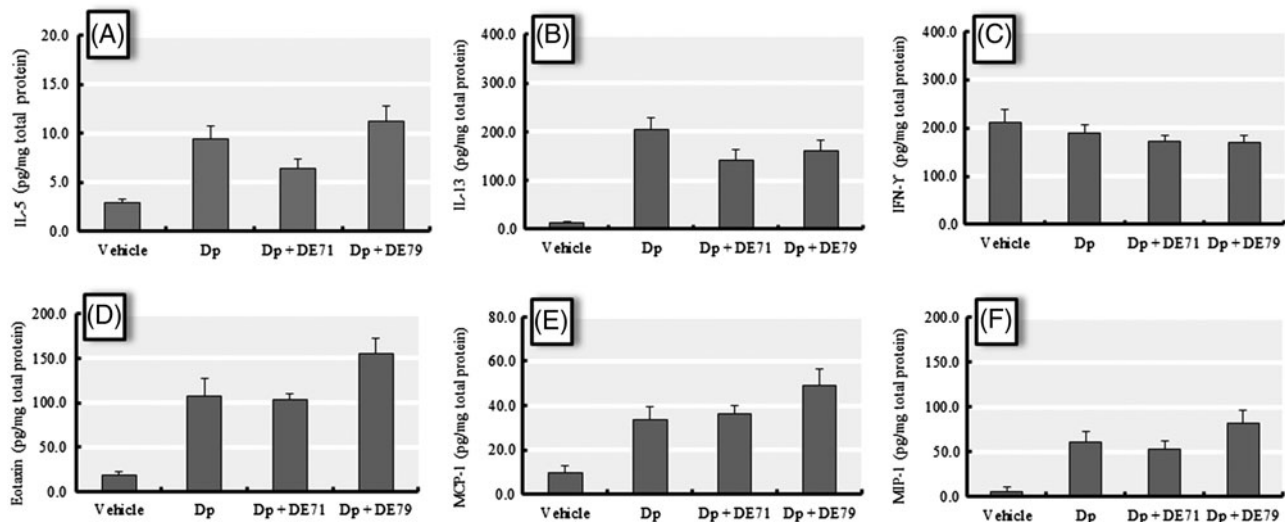


Figure 4. Protein levels of cytokines and chemokines in the ear tissue supernatants. Ears from mice in each group were obtained at day 14 and homogenized as described in the section 'Materials and methods'. Thereafter, interleukin (A) (IL)-5, (B) IL-13, (C) interferon (IFN)- γ , (D) eotaxin, (E) MCP-1 and (F) MIP-1 α levels in the ear tissue supernatants were measured by ELISA. Results are shown as mean $\pm$ SE values of 5–6 mice.

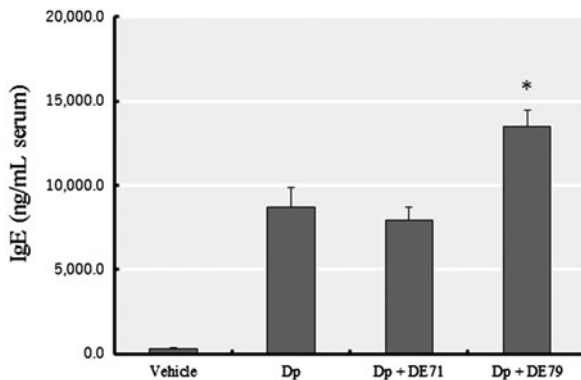


Figure 5. Total immunoglobulin E levels in the serum of NC/Nga mice. Sera from mice in each group were collected by cardiac puncture at day 14, and total IgE level was assayed by ELISA. Results are mean $\pm$ SE values of 5–6 mice. * $p < .05$, Dp + DE79 vs. Dp.

conditions²¹, otherwise, these BDE used may have potential to precipitate dermatitis in AD-prone subjects.

AD is often associated with activation of IL-4, IL-5, and GM-CSF gene cluster, a pattern compatible with predominant activation of Th2-like T-lymphocyte population. Also among chemokines, eotaxin is essential for eosinophil recruitment in allergen-related airway inflammation^{22,23}. regulated on activation normal T expressed and secreted (RANTES) is a strong chemotactic and activating factor for eosinophils and can modulate eosinophil adhesion^{24,25}. On the other hand, IL-2 has been reported to increase allergen-specific IgG and airway inflammation²⁶. In fact, our previous studies have confirmed that the exaggerated allergic airway inflammation induced by DEP paralleled the local elevation of the inflammatory proteins^{18,27}. Also, we have observed that exaggerated dermatitis symptoms induced by di-(2-ethylhexyl) phthalate (DEHP) were concomitant with an amplified local expression of IL-5 in the same animal model as the current one¹⁶. Therefore, we measured the local expression of the cytokines and chemokines also in the present study. However, BDE did not

significantly enhance the expression of these proteins in the presence of allergen (Figure 3 and data not shown). These results indicate that the enhancing effects of BDE on exacerbation of AD may not be mediated via the enhanced local expression of these cytokines and chemokines examined. Otherwise, there might be kinetic differences between pathological change and protein synthesis in the inflammatory sites. Therefore, future studies focusing on other biological molecules such as IL-33, IL-18, and Thymic stromal lymphopoietin (TSLP), than those in the present one and/or kinetic analysis of these important cytokines and chemokines will resolve the puzzle.

Alternatively, exacerbating effects of BDE (especially DE79) on AD pathology can be, at least in part, through the IgE-dependent pathway. A Japanese group has observed that exposure to HBCD through diet increases the number of circulatory B cells in rats²⁸. On the other hand, worsening of the AD on allergen challenge supports the hypothesis that specific IgE might play a critical role in cutaneous inflammation in the activation of mast cells and DCs through high-affinity Fc receptors²⁹. Furthermore, IgE-mediated histamine release from cutaneous mast cells can aggravate AD through the itch-scratch cycle. However, to date, some data indicate a less clear relationship between allergen-specific IgE and AD, since comparing extrinsic and intrinsic AD demonstrated a similar disease pathomechanism independent of IgE³⁰. On the other hand, our previous *in vitro* study has shown that DE71 dominantly exaggerate TCR expression on splenocytes, implying that the BDE has a high affinity for activating T cells¹⁵. As well, some BDE including DE71 and DE79 can stimulate BMDC, a headstream of immunity, *in vitro*¹⁵. In any case, further detailed studies are required to investigate the impact of BDE on several types of players in allergy including dendritic cells, B and T lymphocytes, basophils/mast cells, and related cascade and pathology.

In conclusion, two types of BDE (DE71 and DE79) aggravated AD-like pathology in atopy-prone mice. The aggravation may be partly concomitant with an elevation of the

circulatory level of IgE. The experimental study illustrates BFRs as risk chemicals for exacerbation of patients with AD.

Disclosure statement

There are no conflicts of interest in this work.

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