SIZE EFFECTS OF POLYSTYRENE NANOPARTICLES ON ATOPIC DERMATITIS-LIKE SKIN LESIONS IN NC/NGA MICE

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Nano-sized particles are diffusing in the environment with the development of nanotechnology. Polystyrene (PS) nanoparticles are modified industrial products and pharmaceutical agents, however, adverse effects of PS nanoparticles remain to be elucidated. In the present study, we investigated the effects of PS nanoparticles with different sizes on the atopic dermatitis (AD)-like skin lesions in NC/Nga mice assumed to show the skin barrier defect/dysfunction in the presence or absence of mite allergen. Male NC/Nga mice were injected intradermally with three different-sized PS nanoparticles (25, 50, or 100 nm) and/or mite allergen into their right ears. We evaluated clinical scores, ear thickening, histological findings and the local protein expression of inflammatory molecules in the ear and Ig production in serum. PS nanoparticles aggravated AD-like skin lesions related to mite allergen, which was paralleled by the local protein levels of interleukin-4, CCL2/monocyte chemotactic protein-1, CCL3/macrophage inflammatory protein-1 alpha, and CCL4/macrophage inflammatory protein-1 beta. In contrast, PS nanoparticles decreased interferon-γ expression. Furthermore, exposure to PS nanoparticles induced ear swelling and CC-chemokine expression in the absence of allergen. These effects were greater with the smaller PS nanoparticles than with the larger ones regarding overall trend. These results suggest that exposure to PS nanoparticles under skin barrier defect/dysfunction can exacerbate AD-like skin lesions related to mite allergen in a size-dependent manner. The enhancing effects may be accounted for by T helper 2-biased immune responses. Furthermore, PS nanoparticles can evoke skin inflammation via the overexpression of CC-chemokines even in the absence of allergen in atopic subjects.

Atopic dermatitis (AD) is a pruritic inflammatory skin disease and its prevalence has been increasing progressively in industrialized countries (1-2). The underlying immunological milieu of human atopic dermatitis (AD) is characterized by up-regulation of inflammatory cytokines, IgE overproduction in serum and the accumulation of inflammatory cells. In most cases, various factors including immunological abnormalities, skin barrier dysfunction, exposure to allergen such as mite, environmental toxicants and psychotic factors (3-4) can contribute to the initiation and/or progression of AD. In fact, many animal studies have shown that environmental toxicants including chemicals can be closely related to the increasing prevalence of allergic diseases including AD. Recently, we have reported that systemic exposure to di-(2-ethylhexyl) phthalate (DEHP), a typical environmental chemical as the most

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abundant phthalate plasticizer in polyvinylchloride, aggravates AD-like skin lesions related to mite allergen in mice (5-6). Thus, it is worthwhile to assess whether exposure to environmental toxicants is associated with the increasing prevalence of AD and to elucidate the underlying mechanisms.

Nanoparticles include ambient and engineered nano-sized spherical particles. In particular, the rapidl development of nanotechnology may bring about potential human exposure to the engineered nanoparticles via several routes such as respiratory tract, gastrointestinal tract, and skin (7-8). Nano-sized particles (< 100 nm), which are characterized by their small size and large surface area, can exhibit more physical and chemical properties than larger ones (i.e., fine or coarse particles). Previous reports have shown that exposure to nanoparticles (carbon black, TiO_2, and carbon nanotubes) contributes to the exacerbation of allergic airway inflammation (9-12). These reports have also suggested that the smaller particles have more potent toxicity than the larger ones. In a recent study, we reported that intradermal exposure to nano-sized titanium dioxide aggravates AD-like skin lesions related to mite allergen (13), however, our results and other previous reports have indicated that the toxicity of nanoparticles can be independent of the size (13-15). Thus, the toxicity of nanoparticles can depend on a variety of the properties, such as size, shape, bulk, surface area, or chemical modification and thereby they may exert unexpected toxicity/activity.

Polystyrene (PS) particles are widely used in the manufacture of light dispersion, cosmetics, plastics, modified rubber, resins, and insulation. Generally, PS particles are considered to be relatively 'inert' materials, thus they are often used as negative control for the toxicological assessment of nanoparticles. However, several reports have suggested that pulmonary exposure to PS nanoparticles causes lung inflammation (16-17). Also, our recent study has shown that pulmonary exposure to PS nanoparticles enhances LPS-related lung inflammation (18). Furthermore, PS nanoparticles have exerted an adjuvant effect on immuno/inflammatory responses (19-21). With respect to dermal exposure, Edwards et al. have reported that PS products cause contact dermatitis including acute exudative vesicular eruptions in healthy individuals (22). However, previous studies have not clearly defined whether exposure to PS nanoparticles affects skin inflammation such as AD. Furthermore, the size effects of PS nanoparticles have never been indentified.

The aim of our study is to investigate the effects of PS nanoparticles of different sizes on the AD-like skin lesions related to mite allergen in NC/Nga mice assumed to show the skin barrier defect/dysfunction. NC/Nga mice are considered to be a reliable experimental model for human AD from the clinical, pathological, and immunological point of view. In addition, we also examined the effects following exposure to PS nanoparticles alone in the atopic mice.

MATERIALS AND METHODS

Animals

We purchased 7-week-old male NC/NgaTndCrj (NC/Nga) mice from Charles River Japan (Osaka, Japan). They were fed a commercial diet (CE-2; Japan Clea Co., Tokyo, Japan) and water *ad libitum*. Mice were housed in an animal facility that was maintained at 22-26 °C with 40 to 69% humidity and a 12 h/12 h light/dark cycle. All mice were treated humanely with regard for alleviation of suffering in accordance with guidelines of the National Institute for Environmental Studies for animal experiments. All protocols involving mice were approved by the institutional review board.

Study Protocol

PS nanoparticles, which are monodispersed particles from polystyrene, were purchased from Micromod (micromer®-F, Rostock, Germany). The sizes of the PS nanoparticles were 25, 50, and 100 nm. Their shape was spherical and plain. Eight-week old mice (20-23g) were divided into 8 experimental groups. The vehicle group received 10 μL of saline (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). The PS groups received 20 μg of PS nanoparticles with each size. The Dp group received 5 μg of mite allergen extract (*Dermatophagoides pteronyssinus*; Dp, Cosmo Bio LSL, Tokyo, Japan) dissolved in the vehicle. The Dp + PS 25 nm, Dp + PS 50 nm, or Dp + PS 100 nm groups received combined administration of Dp and 25 nm, 50 nm, or 100 nm PS nanoparticles, respectively. The PS nanoparticle solutions were diluted with distilled water (Otsuka Pharmaceutical Factory, Inc.) and then mixed together with mite allergen. Mice were injected intradermally with PS nanoparticles and/or mite allergen or vehicle on the

ventral side of their right ears on days 1, 3, 5, 8, 10, 12, 15, and 17 under anesthesia with 4% halothane (Takeda Chemical Industries, ltd., Osaka, Japan). Twenty-four hours after each intradermal injection, we measured ear thickness using a gauge (OZAKI MFG, Osaka, Japan) and evaluated clinical scores by skin dryness, eruption, edema, and wound graded from 0 to 3 (no symptoms, 0; mild, 1; moderate, 2; and severe, 3). The clinical scores were estimated as the sum of these values.

Histological evaluation

The animals were sacrificed by etherization 24 h after the last intradermal injection. The right ears of mice were removed and fixed in 10% phosphate buffered formalin (pH 7.2), embedded in paraffin, cut into 3-μm sections, and stained with hematoxylin and eosin (H&E) and toluidine blue (pH 4.0). We performed histological analyses using an Olympus AX80 microscope (Olympus Corp., Tokyo, Japan) and measured the length of the cartilage in each specimen using an Olympus VM-30 video micrometer. The infiltration of eosinophils (H&E) and mast cells (toluidine blue) were morphometrically evaluated as the number of cells per millimeter of cartilage. We counted the number of cells between the dorsal and ventral epidermis per 100 μm approx. of cartilage for eosinophils and 300 μm of cartilage for mast cells in 2 high power fields which were selected using Olympus VIDEO MICRO METER VM-30 (Olympus Corp.). We also evaluated the degranulation of mast cells as not degranulated (0%), mildly degranulated (0-50%), and severely degranulated (> 50%) (5).

ELISA

The right ears of mice were removed 24 h after the last intradermal injection. They were homogenized using PHYSCOTORON NS-310E (MICROTEC Co., Ltd., Chiba, Japan) and centrifuged as previously described (23). ELISA for interleukin (IL)-4 (Amersham, Buckinghamshire, UK), IL-5 (Endogen, Cambridge, MA), IL-13 (R&D systems, Minneapolis, MN), interferon-gamma (IFN-γ) (Endogen), CCL2/monocyte chemotactic protein-1 (CCL2/MCP-1) (R&D systems), CCL3/macrophage inflammatory protein-1α (CCL3/MIP-1α) (R&D systems), and CCL4/macrophage inflammatory protein-1beta (CCL4/MIP-1β) (R&D systems) in the ear tissue supernatants were conducted according to the manufacturer's instructions. The detection limits of these assays were <5 pg/ml, 5 pg/ml, 1.5 pg/ml, 10 pg/m, 2 pg/ml, 1.5 pg/ml, and 3 pg/ml, respectively. Blood was retrieved by cardiac puncture 24 h after the last intradermal injection. Serum was stored at –80°C until assayed. Dp-specific IgG1 antibodies in serum were measured by ELISA with solid-phase antigen as previously described (24). Total IgE antibodies in serum were measured by OptEIA™ Set Mouse IgE (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instruction, respectively.

Statistical analysis

Data are reported as the mean ± SE. The significance of variation among different groups was determined by one-way ANOVA or Kruskal-Wallis analysis. Differences between the experimental group and the control group were determined by Dunnet's or steel multiple comparison test. *P*-value of less than 0.05 was considered to be significant. We used statistical software (Stat View version 5.0; Abacus Concepts, Inc., Berkeley, CA).

RESULTS

PS nanoparticles aggravate symptoms of AD-like skin lesions

To evaluate the effects of PS nanoparticles on the skin in atopic mice, we examined ear thickening and clinical scores 24 h after each intradermal exposure. Administration of vehicle had no effect on the ear thickening (Fig. 1). Treatment with mite allergen enhanced ear thickening as compared with vehicle treatment ($p<0.01$). The combined exposure to mite allergen and PS nanoparticles significantly enhanced ear thickening as compared with mite allergen exposed alone ($p<0.01$). Surprisingly, exposure to PS nanoparticles increased ear thickening from day 9 as compared with vehicle exposure, in spite of the absence of mite allergen. Furthermore, these results were more potent with the smaller PS nanoparticles than with the larger ones in the presence or absence of mite allergen (25 nm≥50 nm>100nm). In addition, clinical scores including dryness, wound, and edema showed similar results as ear swelling (data not shown).

PS nanoparticles enhance histological changes in the skin

To evaluate histological changes, we performed hematoxylin-eosin (Fig. 2A-E) and toluidine-blue staining (Fig. 2F-I) 24 h after the last intradermal exposure. Treatment with vehicle (Fig. 2B and 2G) did not show significant pathologic alterations. PS nanoparticle exposure slightly enhanced the infiltration of eosinophils into the skin (Fig. 2E). Administration of mite allergen caused eosinophilic infiltration into the skin as compared with vehicle administration (Fig. 2A, E: $p<0.01$). In addition, the

Group	pg/mg total protein			
	IFN-γ	IL-4	IL-5	IL-13
Vehicle	2614 ± 351	17.4 ± 3.56	2.36 ± 0.19	0.33 ± 0.21
PS 25nm	2210 ± 172	10.5 ± 1.77	2.00 ± 032	0.65 ± 0.45
PS 50nm	1740 ± 213	14.4 ± 3.17	1.66 ± 0.15	0.37 ± 0.25
PS 100nm	1974 ± 181	38.2 ± 4.36	1.34 ± 0.08	1.31 ± 0.58
Dp	1247 ± 137**	58.9 ± 8.98**	4.43 ± 0.73*	5.05 ± 0.56**
Dp+PS 25 nm	585 ± 121**,#	130.6 ± 26.9**,#	3.33 ± 0.88	5.33 ± 0.76**
Dp+PS 50 nm	557 ± 119**,#	186.8 ± 38.0**,#	5.66 ± 1.38	5.77 ± 0.62**
Dp+PS 100 nm	902 ± 118**	120.3 ± 17.2**,#	3.06 ± 0.53	4.71 ± 0.54**

Table I. *Effects of PS nanoparticles on the protein expression of cytokines in the skin. Rig ears of mice were removed 24 h after the last intradermal exposure. Protein levels of inflammatory molecules in the ear tissue were analyzed using ELISA. *;p<0.05 vs. vehicle group, **;p<0.01 vs. vehicle group, #; p<0.05 vs. Dp group. Data are the means ± SEM of 8 animals per group. IFN; interferon, IL; interleukin, PS; polystyrene, Dp; Dermatophagoides pteronyssimus.*

Group	pg/mg total protein		
	CCL2/MCP-1	CCL3/MIP-1α	CCL4/MIP-1β
Vehicle	1.19 ± 1.19	0	0.42 ± 0.30
PS 25nm	37.3 ± 8.96**	17.5 ± 5.84**	24.7 ± 5.45*
PS 50nm	44.1 ± 8.69**	33.2 ± 16.4*	10.4 ± 7.59
PS 100nm	13.6 ± 3.00**	4.41 ± 2.11*	23.5 ± 4.70**
Dp	32.8 ± 14.9**	90.4 ± 18.6**	104.6 ± 26.8**
Dp+PS 25 nm	136.4 ± 20.9**,##	269.3 ± 40.2**,#	397.6 ± 69.6**,#
Dp+PS 50 nm	97.5 ± 15.7**,##	242.4 ± 52.2**	375.7 ± 93.4**
Dp+PS 100 nm	68.8 ± 17.5**	106.9 ± 17.7**	204.0 ± 77.7**

Table II. *Effects of PS nanoparticles on the protein expression of chmokines in the skin. Right ears of mice were removed 24 h after the last intradermal exposure. Protein levels of inflammatory molecules in the ear tissue were analyzed using ELISA. *;p<0.05 vs. vehicle group, **; p<0.01 vs. vehicle group, #, p<0.05 vs. Dp group. Data are the means ± SEM of 6-8 animals per gropup. MCP-1; monocyte chemotactic protein-1. MIP+1 ; macrophage inflammatory protein-1 alpha, MIP-1β; Dermatophagoides pteronyssinus*

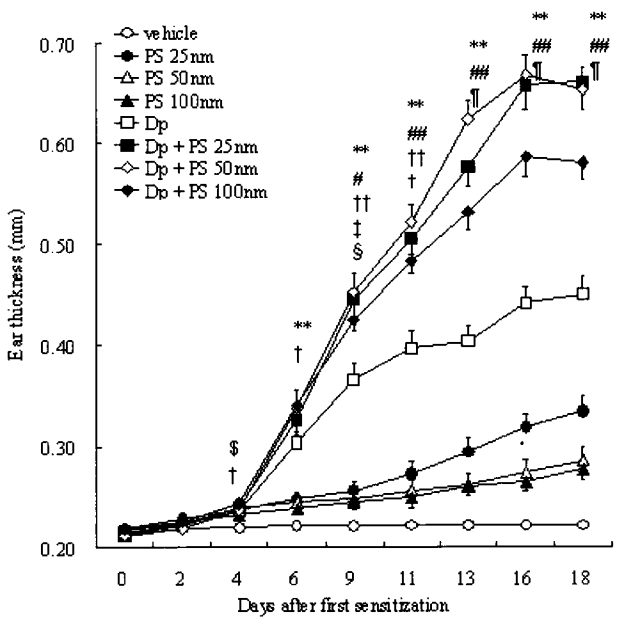

Fig. 1. *Effects of PS nanoparticles on ear thickening of AD-like skin lesions induced by Dp. We measured ear thickening 24 h after each Dp intradermal injection. Data are the means ± SE of 12-14 animals per group. **; p<0.01, Dp treated groups vs vehicle group; #; p<0.05, Dp + PS treated groups vs. Dp group; ##; p<0.01, Dp + PS treated groups vs Dp group; ¶; p<0.01, PS treated groups vs. vehicle group; †; p<0.05, PS 25 nm group vs vehicle group; ††; p<0.01, PS 25 nm group vs. vehicle group; ‡; p<0.05, PS 50 nm group vs vehicle group; §; p<0.05, PS 100 nm group vs. vehicle group; $; p<0.05 Dp + PS treated groups vs vehicle group*

combined exposure to mite allergen and 25 nm PS nanoparticles increased the number of eosinophils in the ear as compared with mite allergen exposed alone (Fig. 2C, D, and E: p<0.05 for Dp+ PS 25 nm group vs Dp group). A similar trend was observed in the total cell number of mast cells and the severity of mast cell degranulation (Fig. 2F-I). The effects of smaller PS nanoparticles were more prominent than those of larger ones (100 nm) for the accumulation of eosinophils and mast cells and the severity of mast cell degranulation.

PS nanoparticles affect the protein expression of cytokines in the skin

We investigated the effects of PS nanoparticles on the protein expression of T helper (Th) 1 and Th2 cytokines in the ear 24 h after the last intradermal exposure (Table I). PS nanoparticle exposure did not significantly change the local expression of IL-4, IL-5, IL-13, and IFN-γ as compared with vehicle exposure. The protein levels of IL-4 and IL-13 in the Dp-treated groups were greater than those in the vehicle group (IL-4; p<0.01 and IL-13; p<0.01). Mite allergen treatment elevated IL-5 levels as compared with vehicle treatment (p<0.05). Furthermore, exposure to PS nanoparticles and mite allergen showed a further increase in IL-4 as compared with mite allergen exposed alone (p<0.05). On the other hand, administration of mite allergen significantly decreased the expression of IFN-γ as compared with vehicle administration (p<0.01). In addition, exposure to 25 nm or 50 nm PS nanoparticles with mite allergen further decreased IFN-γ expression as compared with mite allergen exposed alone (p<0.05). These Th2-dominant effects were more prominent with the smaller PS nanoparticles than with the larger ones.

PS nanoparticles affect the protein expression of chemokines in the skin

We investigated the effects of PS nanoparticles on the protein expression of chemokines in the ear 24 h after the last intradermal exposure (Table II). Exposure to PS nanoparticles alone increased CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β in the ear as compared with vehicle exposure (CCL2/MCP-1; p<0.01 for PS groups *vs* vehicle group, CCL3/MIP-1α; p<0.01 for PS 25 nm group *vs*. vehicle group, p<0.05 for PS 50 nm and 100 nm groups *vs* vehicle group, CCL4/MIP-1β; p<0.01 for PS 25 nm group *vs* vehicle group, p<0.05 for PS 100 nm group *vs* vehicle group). The protein levels of CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β in the Dp-treated groups were significantly greater than those in the vehicle group (p<0.01). Furthermore, exposure to PS nanoparticles combined with mite allergen showed a further increase in CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β as compared with mite allergen exposed alone, which was more prominent with the smaller PS nanoparticles than with the larger ones (CCL2/MCP-1; p<0.01 for Dp + PS 25 nm and Dp + PS 50 nm groups *vs* Dp group, CCL3/MIP-1α; p<0.05 for Dp + PS 25 nm group *vs* Dp group, CCL4/MIP-1β; p<0.05 for Dp + PS 25 nm

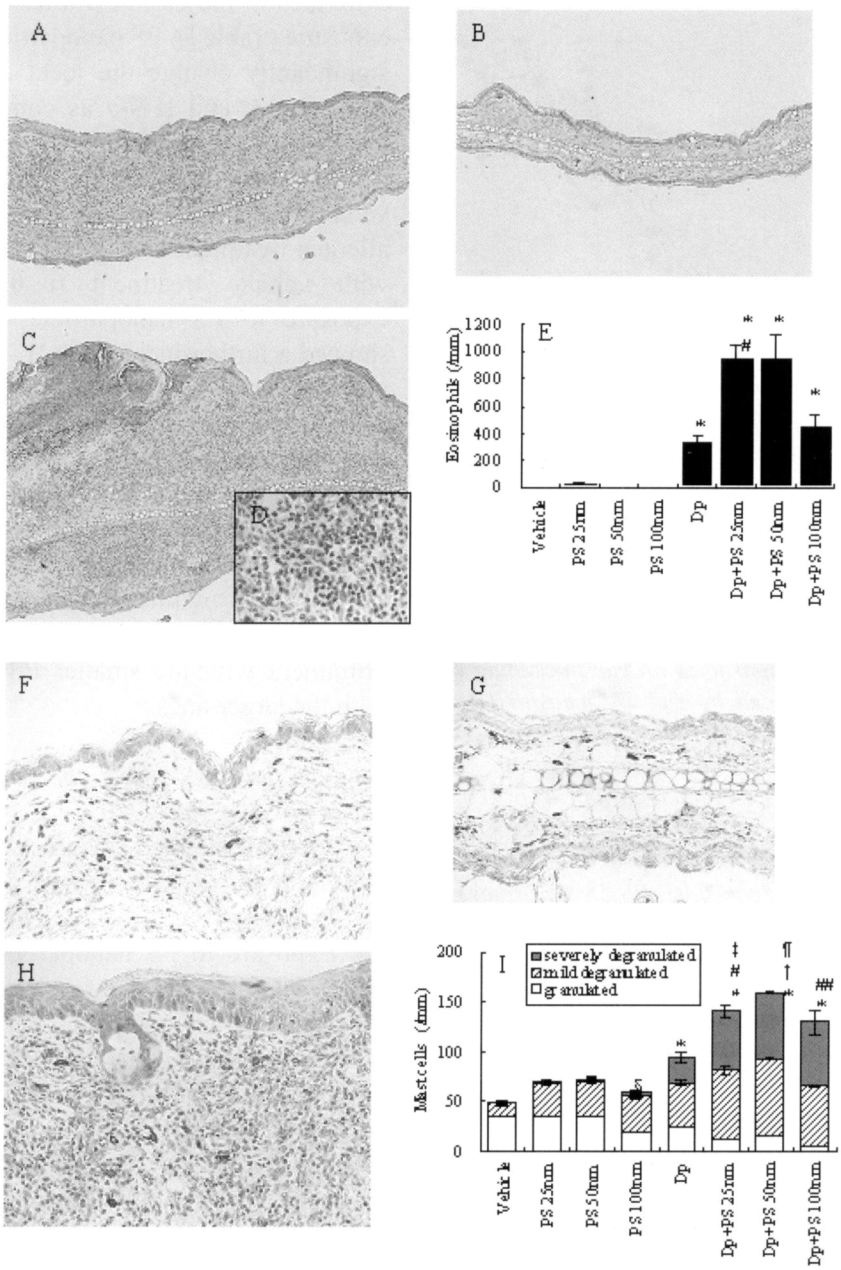

Fig. 2. *Effects of PS nanoparticles on histological changes in the ear. Right ears of mice were removed 24 h after the last intradermal injection. The infiltration of eosinophils (**A-D**) and the degranulation of mast cells (**F-H**) were morphometrically evaluated as the number of cells between the dorsal and ventral epidermis per 100 μm approx. of cartilage for eosinophils and 300 μm of cartilage for mast cells in 2 high power fields. Histological findings of the vehicle group (**B, G**), Dp group (**A, F**), or Dp+ PS 25 nm group (**C, D, H**) were shown with hematoxylin-eosin staining (**A-D**) or toluidine blue staining (**F-H**). Data are the mean ± SE of 4 animals per group. Sections were observed at a magnification of ×100, ×200 and × 400.*

**: $p<0.05$, vs cell numbers of all types (granulated, mild degranulated, severely degranulated, and total mast cells) in the vehicle group; §: $p<0.05$, vs cell numbers of granulated mast cells in the vehicle group; #; $p<0.05$, vs cell numbers of granulated mast cells in the Dp group; †: $p<0.05$, vs cell numbers of mild degranulated mast cells in the Dp group; ‡: $p<0.05$, vs cell numbers of severely degranulated mast cells in the Dp group; ¶: $p<0.05$, vs cell numbers of total mast cells in the Dp group*

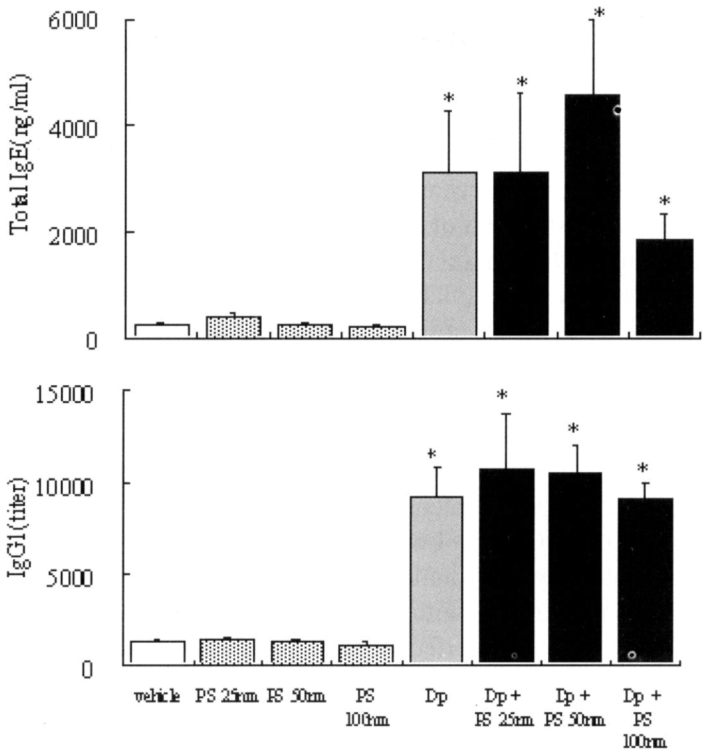

Fig. 3. *Effects of PS nanoparticles on Ig production in serum. To evaluate adjuvant activity of PS nanoparticles, we measured total IgE and Dp-specific IgG1 in serum 24 h after the last intradermal injection using ELISA. Data are the mean ± SE of 6-8 animals per group. *: p<0.01, vs vehicle group; #: p<0.05, vs Dp group.*

group *vs* Dp group).

PS nanoparticles did not affect Ig levels in serum

To evaluate the adjuvant activity of PS nanoparticles for Ig production, we measured total IgE and allergen-specific IgG1 in serum 24 h after the last intradermal exposure. Mite allergen administration significantly increased the levels of total IgE and allergen-specific IgG1 as compared with vehicle administration (Fig. 3; $p<0.01$). However, there were no significant differences between the Dp group and the Dp + PS treated groups in Ig production.

DISCUSSION

The present study shows that intradermal exposure to PS nanoparticles aggravated AD-like skin lesions related to mite allergen in NC/Nga mice. The aggravation was paralleled by the infiltration of eosinophil and mast cells into the skin, the severity of mast cell degranulation, and the local protein expression of IL-4, CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β regarding overall trend. In contrast, exposure to PS nanoparticles plus mite allergen decreased the expression of IFN-γ as compared with mite allergen exposed alone. On the other hand, PS nanoparticles induced ear swelling and the local expression of CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β in spite of the absence of allergen. Furthermore, these effects were greater with the smaller PS nanoparticles than with the larger ones.

Nano-sized particles are diffusing in the environment with the development of nanotechnology. The small size and large surface area can contribute to intrinsic toxicity. However, the potential toxicity issues regarding PS nanoparticles are often ignored (25). In particular, PS particles are considered to be inert and have low toxicity. They have the are capable of chemical modification and thus are well applied to cosmetics, sunscreen,

and even to drug delivery (26). On the other hand, several in vivo analyses have suggested that pulmonary exposure to PS nanoparticles enhances lung inflammation (16-17). In addition, the enhancing effects have been greater with the smaller PS nanoparticles than with the larger ones. Recently, we have reported that intratracheal instillation of PS nanoparticles enhanced lung inflammation related to LPS, which is more prominent with the smaller nanoparticles than with the larger ones regarding overall trend (18). Nygaard et al. have shown that the adjuvant effects on IgE response were initiated by subcutaneous injection of PS particles into the footpad (20-21). In addition, the smaller PS particles (0.0588 and 0.202 μm) have exerted more intense adjuvant effects than the larger ones (1.053, 4.64, and 11.34 μm) (21). In the present study, the smaller PS nanoparticles, especially PS nanoparticles with a diameter of less than 50 nm, had more potent effects than the larger ones (100 nm) for the aggravation of AD-like skin lesions related to mite allergen. Furthermore, exposure to 25 nm PS nanoparticles increased ear thickening in spite of the absence of mite allergen. These findings suggest that exposure to the smaller PS nanoparticles under skin barrier defect/dysfunction can enhance skin inflammation including AD-like skin lesions.

Several studies have demonstrated that skin barrier dysfunction can be responsible for the pathogenesis of AD in a murine model and patients (27-28). Previous in vivo analyses have shown that nanoparticles do not readily penetrate through the intact epidermal barrier and remain in the stratum corneum (29-31). However, Mahe et al. have reported that percutaneous exposure to solid fluorescent 40 or 200 nm PS nanoparticles with virus particles penetrates deeply into hair follicles in mice (32). Besides, they are uptaken by perifollicular antigen-presenting cells and then transported to the draining lymph nodes. As the first step of risk assessment of PS nanoparticles, we exposed via intradermal route in the present study to aim the "risk identification" of PS nanoparticles on AD. Thus, in the next stage, we should evaluate the effects of PS nanoparticles using other exposure methods which assume percutaneous penetration/absorption, i.e., the 'real world'. Nonetheless, once PS nanoparticles penetrate into the skin they can thereby affect biological and/or immune responses involved in AD.

The skin lesions in AD are characterized by recruitment of lymphocytes, monocytes/macrophages, eosinophils and mast cells, and the degranulation of eosinophils and mast cells. The lymphocytes infiltrating into skin lesions of AD are Th2-type T cells, which produce IL-4, IL-5, and IL-13 (33-34). On the other hand, defective IFN-γ (a Th1 cytokine) production is associated with allergen-specific Th2 immune responses in AD-patients (35). Th2 cytokines play an important role in the pathogenesis of AD-like skin lesions in murine models as well as in that of human AD (27-28). Th2 cells are also associated with the proliferation/activation of mast cells. IL-4 promotes the development and/or expansion of Th2 cells, B cell proliferation, and IgE class switching. IL-5 is also the key factor for the activation and/or proliferation of eosinophils. Several studies have reported that subcutaneous exposure to PS particles with allergen elevates the production of allergen-specific IgE and the expression of IL-4 by the draining lymph node cells ex vivo (20-21). In addition, these results were associated with the proliferation/activation of B and T cells in the draining popliteal lymph nodes. However, the local expression of IL-4 in AD-like skin lesion following exposure to PS nanoparticles has not been examined. In the present study, the local protein levels of IL-4, IL-5, and IL-13 in the ear increased following exposure to mite allergen. In addition, exposure to PS nanoparticles combined with mite allergen significantly increased IL-4 production as compared with mite allergen exposed alone, which was concomitant with the accumulation of eosinophils and mast cells into the skin and the severity of mast cell degranulation. On the other hand, administration of mite allergen reduced the expression of IFN-γ, which was more prominent in the Dp+PS group than in the Dp group. These results and previous reports suggest that exposure to PS nanoparticles may accelerate Th2-skewed immune responses via the proliferation/activation of immunocompetent cells and subsequent production of Th2 cytokines.

CC chemokines are potent molecules that directly migrate leukocytes to inflammatory lesions, and thus are considered to play a crucial role in allergic responses. Serum levels of CC

chemokines have been increased in AD patients compared with normal controls (36-37). Gene expression analysis has shown that human mast cells produce and release CC-chemokines such as CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β upon stimulation of FCεRI (38-39). Yamamoto et al. have reported that the mRNA and protein overexpression of CCL3/MIP-1α and CCL4/MIP-1β enhances atopic skin inflammation related to 2, 4, 6-trinitro-1-chlorobenzene (TNCB) in murine model (40). Our recent study reported that combined pulmonary exposure to PS nanoparticles and allergen increases the expression of MIP-1 α, MCP-1, and KC in lung as compared with allergen alone (18). In the current study, exposure to smaller PS nanoparticles plus mite allergen significantly increased the local expression of CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β as compared with mite allergen exposed alone. On the other hand, eotaxin, a potent eosinophil chemoattractant (CC-chemokine), tended to increase by mite allergen treatment, which was not significantly enhanced by the coexistence of PS nanoparticles (data not shown). Noteworthy, PS nanoparticle exposure induced CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β in spite of the absence of allergen, which was paralleled by ear thickening (Fig. 1) and the infiltration of mononuclear leukocytes (data not shown). Taken together, exposure to PS nanoparticles can facilitate the local expression of chemokines and subsequent accumulation of inflammatory cells in the presence or absence of allergen and thereby can aggravate AD-like skin lesions.

In conclusion, intradermal exposure to PS nanoparticles under skin barrier defect/dysfunction can aggravate AD-like skin lesions related to mite allergen in atopic subjects. The aggravation was paralleled by histological changes and the local expression of IL-4, CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β. In contrast, IFN-γ expression was significantly reduced by PS nanoparticle exposure in the presence of allergen. Collectively, PS nanoparticles can aggravate AD symptoms via Th2-skewed immune responses. Furthermore, PS nanoparticles can take part in the initiation and/or progression of skin inflammation by the local infiltration of leukocytes and the induction of chemokines even in the absence of allergen.

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