# Titanium Dioxide Nanoparticles Aggravate Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice

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**Titanium dioxide ($TiO_2$) nanoparticles are produced abundantly and used ubiquitously in various cosmetic products. However, it remains to be determined whether transdermal exposure to $TiO_2$ nanoparticles affects atopic dermatitis (AD), which has been increasing in developed countries. We investigated the effects of different sized $TiO_2$ nanoparticles on AD-like skin lesions induced to mite allergen in NC/Nga mice assumed to show skin barrier dysfunction/defect. Male mice were injected intradermally with $TiO_2$ nanoparticles of three sizes (15, 50, or 100 nm) and/or mite allergen into their right ears. We evaluated clinical scores, ear thickening, histological findings and the protein expression of T helper (Th) 1 and Th2 cytokines in the ear, and the levels of Ig and histamine in serum. $TiO_2$ nanoparticles aggravated AD-like skin lesions related to mite allergen in NC/Nga mice. The enhancing effects are paralleled by the overproduction of IL-4 in the skin, the levels of total IgE and histamine in serum regarding the overall trend. In contrast, $TiO_2$ nanoparticles decreased the local expression of IFN-$\gamma$ in the presence of allergen. Additionally, $TiO_2$ nanoparticles alone significantly increased histamine levels in serum and IL-13 expression in the ear. However, different effects related to the size differences of $TiO_2$ nanoparticles were not observed. In conclusion, exposure to $TiO_2$ nanoparticles under skin barrier dysfunction/defect can exacerbate AD symptoms through Th2-biased immune responses. Furthermore, $TiO_2$ nanoparticles can play a significant role in the initiation and/or progression of skin diseases following the barrier dysfunction/defect by histamine release even in the absence of allergen.** Exp Biol Med 234:314–322, 2009

**Key words:** atopic dermatitis; titanium dioxide; eosinophils; mast cells; histamine; IL-4

## Introduction

Over the past several decades, the prevalence of atopic dermatitis (AD) has been increasing consecutively in developed countries (1–4). AD is a multifactorial allergic inflammatory skin disorder characterized by chronic pruritic and eczematous lesions, elevated IgE hyperproduction, and infiltrating inflammatory cells, and is generally thought to be a genetic disorder of the immune system, e.g., stratum corneum structural protein and filaggrin (5–9). On the other hand, epidemiological studies have suggested that environmental factors including allergen load, mental stress, and environmental toxicants including chemicals can contribute to the initiation and/or progression of allergic diseases including AD (10, 11). Many animal studies have shown that exposure to various environmental chemicals can aggravate allergic diseases (12–15). Furthermore, environmental chemicals may increase the potency of allergens, and therefore play a crucial role in the enhancement of allergic diseases (16). Consistent with these findings, our recent study has shown that systemic exposure to di-(2-ethylhexyl) phthalate (DEHP), a typical environmental chemical, the most abundant phthalate plasticizer in polyvinylchloride, aggravates AD-like skin lesions related to mite allergen in mice (17). Thus, it is worthwhile to assess whether exposure to environmental chemicals is associated with the increasing prevalence of AD.

Titanium dioxide ($TiO_2$) is a noncombustible and odorless white powder, which is frequently used as a white pigment in a wide range of paints, paper, ceramics, plastics and food colorants. In particular, nano-sized $TiO_2$ particles

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(<100 nm) are increasingly used in industrial products including cosmetics and pharmaceuticals. The widespread use of $TiO_2$ nanoparticles may lead to potential exposure to the engineered nanoparticles in the general environment via respiratory, gastrointestinal, and/or dermal systems.

Generally, nanoparticles, 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). Several studies have demonstrated that exposure to $TiO_2$ nanoparticles via the respiratory system affects a variety of respiratory diseases such as lung inflammation, pulmonary fibrosis and lung tumors *in vivo* (18–21). We have also reported that $TiO_2$ nanoparticles exacerbate lung inflammation related to lipopolysaccharide (LPS) (22). Furthermore, our and some other reports have suggested that exposure to carbon nanoparticles contributes to the exacerbation of allergic airway inflammation (23–27). However, there are few reports regarding the effects of exposure to $TiO_2$ (nano) particles on allergic diseases. De Haar *et al.* reported that intranasal exposure to $TiO_2$ nanoparticles causes airway inflammation through immune adjuvant activity and Th2 type cytokine production (23, 28). With respect to intradermal exposure, another major exposure route of nanoparticles, $TiO_2$ nanoparticles do not readily penetrate through the intact epidermal barrier (29–31). However, once AD patients have the barrier dysfunction/defect, $TiO_2$ nanoparticles may penetrate into the skin, and then affect the cutaneous function. A recent report has shown that a significant increase of *Staphylococcus aureus*, which is thought to play an important role in the exacerbation of AD, can accelerate skin barrier dysfunction induced by anatase-typed $TiO_2$ nanoparticles (7 nm) and UV irradiation (32). However, most $TiO_2$ (nano) particles contained in cosmetics are rutile-typed particles which generally occupy 5–10% of sunscreen agent. In addition, $TiO_2$ nanoparticles in cosmetics can directly contact with the stratum corneum. Recent *in vitro* studies have suggested that $TiO_2$ particles with different crystal structure show different toxicological responses (33, 34). However, it remains unclear whether transdermal exposure to $TiO_2$ nanoparticles, especially rutile-typed ones, affects AD with or without skin barrier dysfunction.

The aim of our study was to investigate the effects of rutile-typed $TiO_2$ nanoparticles with different sizes on AD-like skin lesions related to mite allergen in NC/Nga mice assumed to show skin barrier dysfunction or defect. We also examined the effects following intradermal exposure to $TiO_2$ nanoparticles alone.

## Materials and Methods

**Animals.** Seven-week-old male NC/NgaTndCrj (NC/Nga) mice were purchased from Charles River Japan (Osaka, Japan). They were fed a commercial diet (Japan Clea Co., Tokyo, Japan) and water ad libitum. Mice were housed in an animal facility that was maintained at 22–26°C with 40–69% humidity and a 12 h/12 h light/dark cycle. All animals used in the research were treated humanely according to guidelines of the National Institute for Environmental Studies for animal experiments, with due consideration to the alleviation of distress and discomfort. All protocols were approved by the Institutional Review Board.

**Study Protocol.** Eight-week old mice (22–25 g) were divided into 8 experimental groups. $TiO_2$ nanoparticles were purchased from TAYCA Co., Ltd. (Rutile crystal phase, Osaka, Japan). The size of $TiO_2$ nanoparticles was 15, 50, or 100 nm (surface area: 110, 20–25, and 10–15 $m^2$/g, respectively). The vehicle group received 10 μL of saline (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). The $TiO_2$ (15, 50, or 100 nm) groups received 20 μg of $TiO_2$ nanoparticles of each size in the vehicle. The Dp group received 5 μg of mite allergen extract (*Dermatophagoides pteronyssinus*; Dp, Cosmo Bio LSL, Tokyo, Japan) dissolved in the vehicle. The Dp + $TiO_2$ 15 nm, Dp + $TiO_2$ 50 nm, or Dp + $TiO_2$ 100 nm groups received a combined administration of Dp and 15, 50, or 100 nm $TiO_2$ nanoparticles, respectively. The $TiO_2$ nanoparticle suspensions were sonicated for 2 mins with an ultrasonic disruptor (UD-201; Tomy Seiko, Tokyo, Japan) and then mixed together with mite allergen. Mice were injected intradermally with $TiO_2$ nanoparticles and/or mite allergen 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) and received no treatment on day 0, as previously described (17, 35–37). We measured ear thickness 24 h after each intradermal injection, as previously described (17).

**Blood Sampling.** Mice were anesthetized with diethyl ether 24 h after the last intradermal injection. The chest and abdominal walls were opened, and blood was sampled by cardiac puncture. Serum was stored at –80°C until being assayed for total IgE, Dp-specific IgG1, and histamine.

**Histological Evaluation.** The right ears of mice were removed 24 h after the last intradermal injection. Ears were fixed in 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, cut into 3-μm slices, and stained with hematoxylin and eosin or toluidine blue (pH 4.0). Histological analyses were performed using a microscope (AX80; Olympus, Tokyo, Japan). The length of the cartilages in each specimen was measured by a video micrometer (VM-30; Olympus). The numbers of eosinophils and mast cells in each sample were counted with the micrometer. The infiltration of eosinophils and mast cells were morphometrically evaluated as the number of cells per millimeter of cartilage in a blind fashion. We also evaluated the degranulation of mast cells as: non-degranulated (0%), mildly degranulated (0–50%), and severely degranulated (more than 50%), as previously reported (17).

**ELISA.** The right ears of mice were removed 24 h after the last intradermal injection. They were homogenized and centrifuged as previously described (38). ELISA for IL-

4 (Amersham, Buckinghamshire, UK), IL-5 (Endogen, Cambridge, MA), IL-13 (R&D Systems, Minneapolis, MN), IFN-γ (Endogen), and IL-12/IL-23 p40 (R&D Systems) in the ear tissue supernatants were conducted according to the manufacturers' instructions. The detection limits of these assays were <5, 5, 1.5, 10, and 4 pg/ml, respectively.

Dp-specific IgG1 antibodies in serum were measured by ELISA with solid-phase antigen, as previously described (39). Total IgE antibodies and histamine in serum were measured by OptEIA™ Set Mouse IgE (BD Biosciences, San Diego, CA, USA) and HISTAMINE ENZYME IMUUNOASSAY KIT (SPI-BIO, Montigny le Bretonneux, France) according to the manufacturers' instructions, 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 experimental group and control group were determined by Dunnett'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

**Effects of $TiO_2$ Nanoparticles on AD-Like Skin Lesions.** To evaluate the effects of $TiO_2$ nanoparticles on the skin in the presence or absence of mite allergen, we examined ear thickening and clinical scores 24 h after each intradermal injection. The intradermal administration of vehicle or $TiO_2$ nanoparticles did not affect the ear thickening (Fig. 1) nor clinical scores (data not shown). Treatment with mite allergen significantly enhanced ear thickening as compared with vehicle treatment from day 3 after the first intradermal injection ($P < 0.01$). Furthermore, the combined administration of mite allergen and $TiO_2$ nanoparticles significantly enhanced ear thickening as compared with mite allergen administration alone ($P < 0.05$). This result was paralleled by the clinical scores including dryness, wound severity, and edema. However, the size differences of $TiO_2$ nanoparticles did not affect ear thickening and clinical scores in the presence of mite allergen.

**Effects of $TiO_2$ Nanoparticles on Histological Changes in the Skin.** To evaluate histological changes, we performed hematoxylin and eosin (Fig. 2A–D) and toluidine blue staining (Fig. 2E–H) 24 h after the last intradermal injection. Exposure to vehicle (Fig. 2B, F) or $TiO_2$ nanoparticles did not lead to significant pathologic alterations. Intradermal injection of mite allergen caused eosinophilic infiltration into the skin as compared with vehicle injection (Fig. 2A, C: $P < 0.05$). Furthermore, the combined administration of mite allergen and $TiO_2$ nanoparticles increased the number of eosinophils as compared with mite allergen administrated alone (Fig. 2A, D: $P < 0.05$ for Dp + $TiO_2$ treated groups vs. Dp group). A similar trend was observed regarding the severity of mast cell degranulation (Fig. 2E–H). However, the size differences in $TiO_2$ nanoparticles did not affect histological changes in mite allergen presence in our model (Fig. 2A, E).

**Effects of $TiO_2$ Nanoparticles on the Protein Expression of Proinflammatory Molecules in the Skin.** We investigated the effects of $TiO_2$ nanoparticles on the protein expression of Th1 and Th2 cytokines in the ear 24 h after the last intradermal injection (Table 1). The protein levels of IL-4, IL-5, and IL-13 in the Dp groups were significantly greater than those in the vehicle group (IL-4, $P < 0.05$; IL-5, $P < 0.01$; IL-13, $P < 0.01$, respectively). Additionally, the administration of 15 and 50 nm $TiO_2$ nanoparticles combined with mite allergen led to a further significant increase in IL-4 as compared with mite allergen administration alone ($P < 0.05$). Furthermore, intradermal exposure to $TiO_2$ nanoparticles significantly elevated the expression of IL-13 in the absence of mite allergen as compared with vehicle exposure ($P < 0.05$). On the other hand, mite allergen treatment in the presence and absence of $TiO_2$ nanoparticles significantly decreased the expression of IFN-γ as compared with vehicle treatment ($P < 0.05$). In addition, the administration of 15 or 50 nm $TiO_2$ nanoparticles combined with mite allergen further decreased IFN-γ production compared with mite allergen administration alone ($P < 0.05$). IL-12/IL-23 p40 was not detectable among all experimental groups.

**Effects of $TiO_2$ Nanoparticles on Ig Levels in Serum.** To evaluate the adjuvant activity of exposure to $TiO_2$ nanoparticles for Ig production, we measured total IgE and allergen-specific IgG1 in serum 24 h after the last intradermal injection. Mite allergen administration significantly increased the levels of total IgE and allergen-specific IgG1 as compared with vehicle administration (Fig. 3; $P < 0.01$). There was a trend toward higher levels of Ig production in the Dp + $TiO_2$ groups than in the Dp group. In particular, combined treatment with mite allergen and 50 nm $TiO_2$ nanoparticles resulted in a further significant increase in total IgE as compared to treatment with mite allergen alone ($P < 0.05$).

**Effects of $TiO_2$ Nanoparticles on Histamine Levels in Serum.** To evaluate the effects of exposure to $TiO_2$ nanoparticles on histamine release in the presence or absence of allergen, we measured histamine levels in serum 24 h after the last intradermal injection. Mite allergen or $TiO_2$ treatment elevated the levels of histamine in serum as compared with vehicle treatment (Fig. 4, $P < 0.01$). Additionally, combined administration of mite allergen and $TiO_2$ nanoparticles showed a further significant increase in histamine release as compared with mite allergen administration alone ($P < 0.01$).

## Discussion

The present study showed that intradermal injection of $TiO_2$ nanoparticles could aggravate AD-like skin lesions

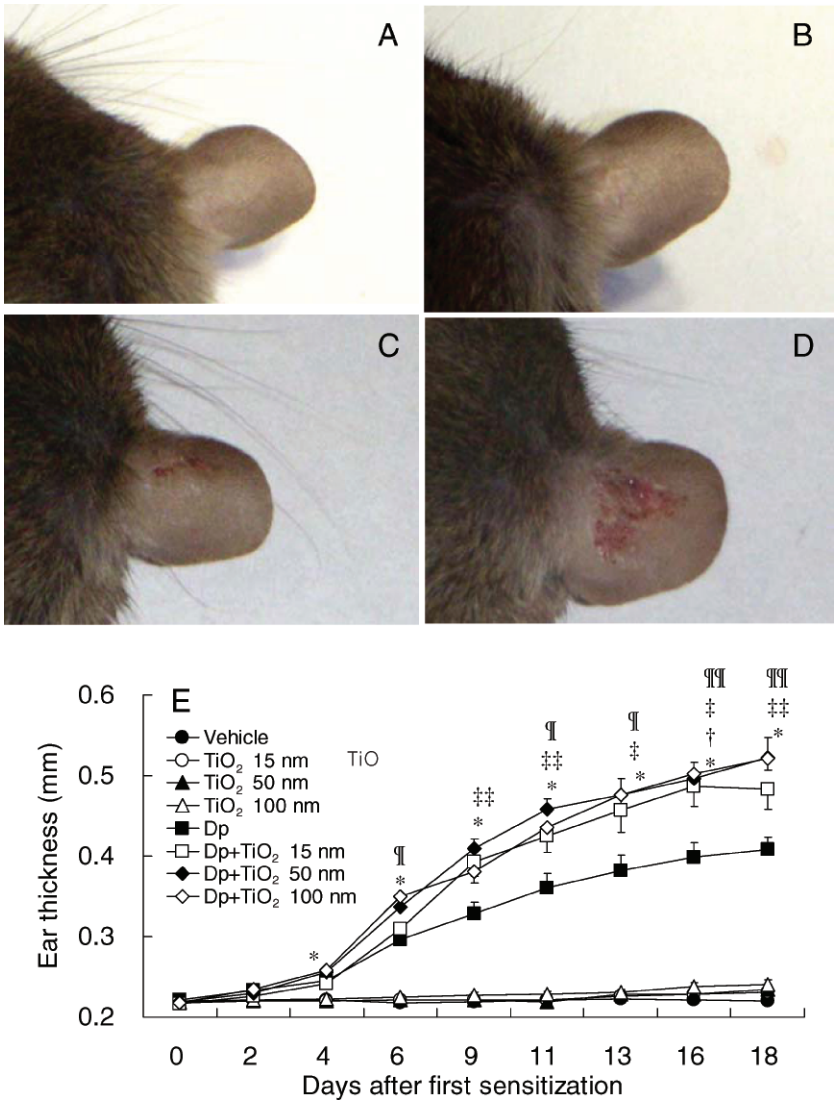


**Figure 1.** Effects of $TiO_2$ nanoparticles on ear thickening of AD-like skin lesions induced by mite allergen (Dp). We showed macroscopic features (A–D) and measured ear thickening (E) 24 h after each intradermal Dp injection. (A): vehicle group, (B): $TiO_2$ 50nm group, (C): Dp group, (D): Dp+$TiO_2$ 50 nm group. Data are the means ± SE of 11 animals per group. * $P < 0.01$ Dp-treated groups vs. vehicle group. † $P < 0.05$ Dp+$TiO_2$ 15 nm group vs. Dp group. †† $P < 0.01$ Dp+$TiO_2$ 15 nm group vs. Dp group. ‡ $P < 0.05$ Dp+$TiO_2$ 50 nm group vs. Dp group. ‡‡ $P < 0.01$ Dp+$TiO_2$ 50 nm group vs. Dp group. ¶ $P < 0.05$ Dp+$TiO_2$ 100 nm group vs. Dp group. ¶¶ $P < 0.01$ Dp+$TiO_2$ 100 nm group vs. Dp group.

related to mite allergen in NC/Nga mice assumed to show skin barrier dysfunction/defect. These results were paralleled by the overproduction of IL-4 in the skin, the levels of total IgE and histamine in serum regarding the overall trend. In contrast, the administration of $TiO_2$ nanoparticles combined with mite allergen decreased the local expression of IFN-γ.

$TiO_2$ nanoparticles are produced abundantly and used ubiquitously because of their marked stability, as well as activities as anticorrosion and photocatalysis. However, the potential toxicity remains unclear. Several studies have demonstrated that exposure to $TiO_2$ nanoparticles affects a variety of respiratory diseases including pulmonary inflammation, pulmonary fibrosis, and lung tumors *in vivo* (18–21). Recently, we have also reported that intratracheal exposure to $TiO_2$ nanoparticles exacerbates acute lung inflammation related to LPS (22). On the other hand, the effects of $TiO_2$ nanoparticles on the skin, another major route of exposure other than the respiratory system, remains poorly determined, although they can serve directly as a physical photoprotective reagent in sunscreen and are widely used in various cosmetic products. In particular, sunscreens generally include about 5–10% of $TiO_2$ (nano) particles and we routinely use 100 μL of sunscreen agent (equivalent to 5–10 mg $TiO_2$ (nano) particles) on the face each use.

Recent studies have demonstrated that skin barrier dysfunction/defect can be responsible for the pathogenesis of AD in both a murine model and in human patients (40, 41). In our present study, intradermal injection of $TiO_2$

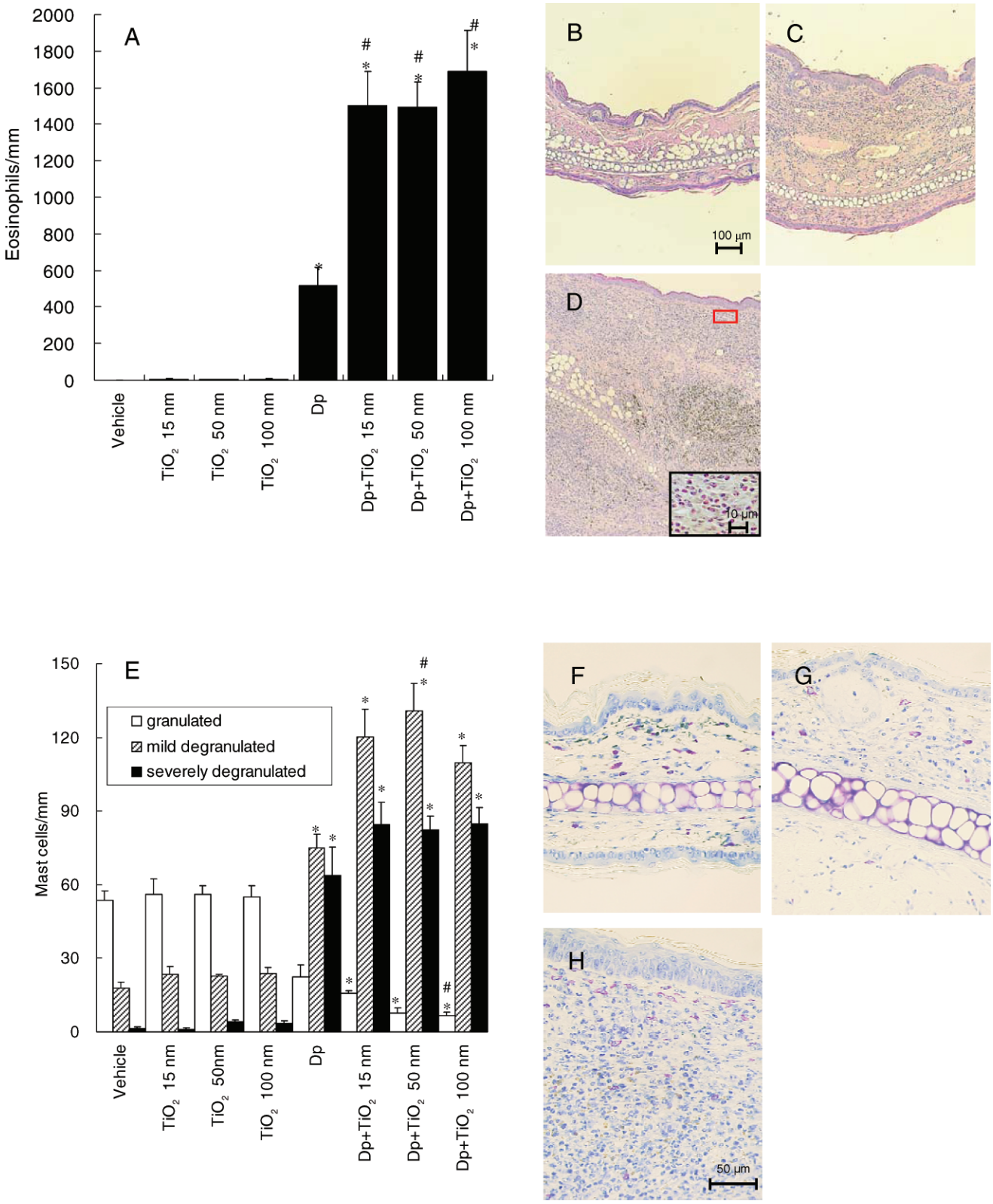


**Figure 2.** Effects of $TiO_2$ nanoparticles on histological changes in the ear. Right ears of mice were removed 24 h after the last intradermal injection. The infiltration of eosinophils (A) and the degranulation of mast cells (E) were morphometrically evaluated as the number of cells per millimeter of cartilage. Histological findings of the vehicle group (B, F), Dp group (C, G), or Dp+$TiO_2$ 50 nm group (D, H) are shown with hematoxylin and eosin staining (B–D) or toluidine blue staining (F–H). Data are the mean ± SE of 4 animals per group. Sections were observed at magnifications of ×100, ×200, and ×400. * $P < 0.05$ vs. vehicle group. # $P < 0.05$ vs. Dp group.

**Table 1.** Effects of $TiO_2$ Nanoparticles on the Protein Expression of Th1 and Th2 Cytokines in the Ear[a]

| | Th1 cytokines | | Th2 cytokines | | |
|---|---|---|---|---|---|
| Group | IFN-$\gamma$[b] | IL-12/IL-23 p40[b] | IL-4[b] | IL-5[b] | IL-13[b] |
| Vehicle | 487 ± 18.2 | | 0 | 0 | 0 |
| $TiO_2$ 15 nm | 432 ± 31.2 | | 0 | 0 | 3.09 ± 0.72** |
| $TiO_2$ 50 nm | 402 ± 51.7 | | 0 | 0 | 2.50 ± 0.95* |
| $TiO_2$ 100 nm | 365 ± 20.4 | ND | 0 | 0 | 2.84 ± 0.52** |
| Dp | 185 ± 19.3* | | 8.48 ± 1.93* | 4.55 ± 1.28** | 8.73 ± 0.64** |
| Dp+$TiO_2$ 15 nm | 80.9 ± 9.67*,# | | 32.9 ± 7.58**,# | 2.15 ± 0.58** | 8.27 ± 0.60** |
| Dp+$TiO_2$ 50 nm | 83.8 ± 16.6*,# | | 39.5 ± 8.21**,# | 3.30 ± 0.95** | 9.07 ± 1.24** |
| Dp+$TiO_2$ 100 nm | 105 ± 17.7* | | 30.3 ± 9.14** | 2.36 ± 0.45** | 7.54 ± 0.77** |

[a] Right ears of mice were removed 24 h after the last intradermal injection. Protein levels of cytokines in the ear tissue were analyzed using ELISA. $TiO_2$, titanium dioxide; Th, T helper; IFN, interferon; IL, interleukin; Dp, *Dermatophagoides pteronyssinus*; ND, not detected.
[b] Results expressed in pg/mg total protein.
* $P < 0.05$ vs. vehicle group.
** $P < 0.01$ vs. vehicle group.
# $P < 0.05$ vs. Dp group. Data are the means ± SEM of 6–7 animals per group.

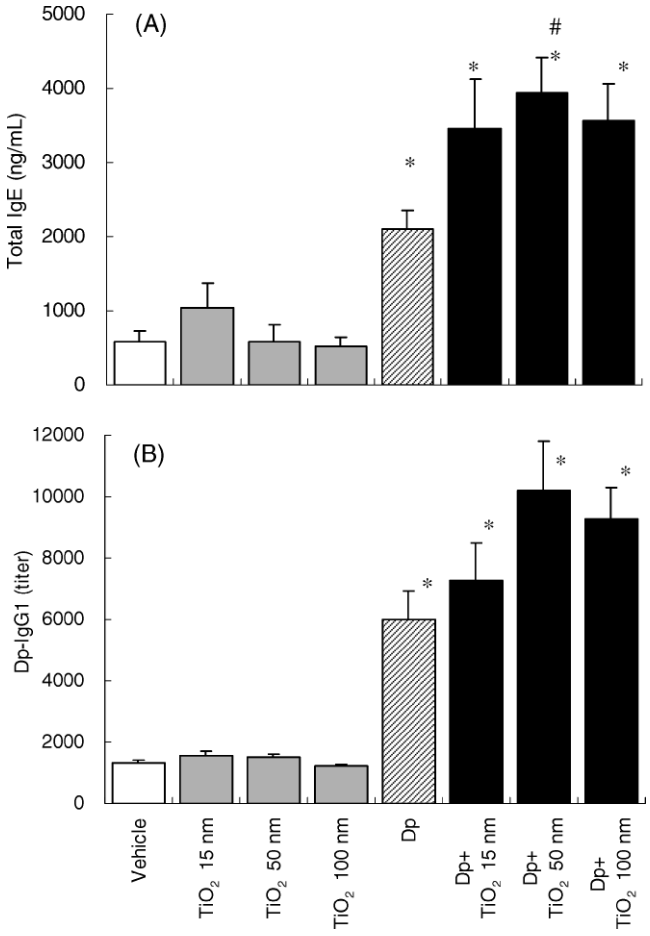


**Figure 3.** Effects of $TiO_2$ nanoparticles on Ig production in serum. To evaluate the adjuvant activity of $TiO_2$ nanoparticles, we measured total IgE and Dp-specific IgG1 in serum 24 h after the last intradermal injection using ELISA. Data are the mean ± SE of 11 animals per group. * $P < 0.01$ vs. vehicle group. # $P < 0.05$ vs. Dp group.

nanoparticles into the skin aggravated AD-like skin lesions induced by mite allergen in a mouse model. Almost all previous studies have demonstrated that $TiO_2$ nanoparticles *in vivo* do not readily penetrate through the intact epidermal barrier and remain in the stratum corneum (29–31). In a minority of subjects, the presence of $TiO_2$ nanoparticles was observed in the hair follicles (42). On the other hand, once $TiO_2$ nanoparticles penetrate into the skin, they can affect the cutaneous function. Kambara *et al*. reported that anatase-typed $TiO_2$ nanoparticles can accelerate skin barrier dysfunction/defect induced by UV irradiation in mice (32). Additionally, fluoresceinated quantum dots following intradermal injection (15–20 nm) have been shown to migrate to local lymph nodes in mice and pigs (43). It is likely that $TiO_2$ nanoparticles after transdermal exposure can transfer to local lymph nodes and thereby affect biological and/or immune responses including AD. When administrated directly to cell culture *in vitro*, $TiO_2$ nanoparticles can exert a variety of adverse effects. Ambient nanoparticles (manufactured $TiO_2$, carbon black, and polystyrene) were capable of inducing cellular ROS production (44). $TiO_2$ treatment catalyzes DNA damage in human cells (45) and induces apoptosis in Syrian hamster embryo fibroblasts (46). In addition, Wottrich *et al*. reported that $TiO_2$ nanoparticles increase inflammatory cytokine release such as IL-6 and IL-8 from epithelial cells (47). Actually, the present *in vivo* study demonstrated that intradermal $TiO_2$ nanoparticles in the presence of allergen could contribute to the aggravation of AD, a multifactorial allergic skin disorder.

Nano-sized particles are diffusing into the environment with the increasing development of nanotechnology. Their small size and large surface area can contribute to intrinsic toxicity. Concerning $TiO_2$ toxicity, intratracheal instillation and inhalation exposure to $TiO_2$ (nano) particles enhance the infiltration of inflammatory cells into the lung and histological changes in animal models (19, 20, 48, 49).

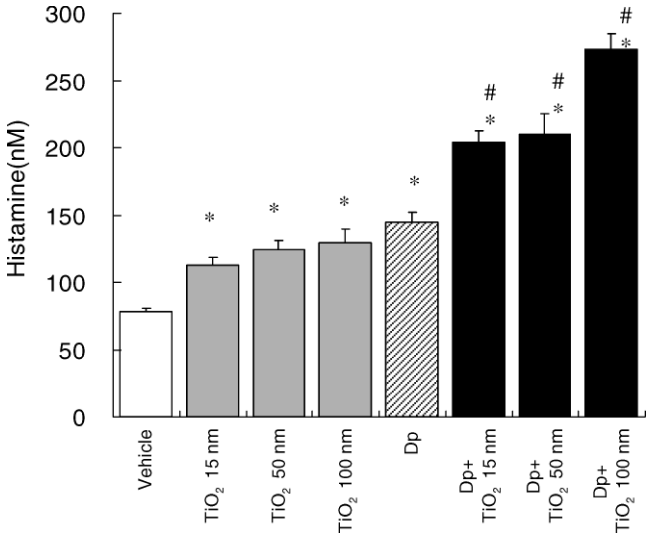


**Figure 4.** Effects of $TiO_2$ nanoparticles on histamine release in serum 24 h after the last intradermal injection. Histamine levels in serum were measured using ELISA. Data are the mean ± SE of 11 animals per group. * $P < 0.01$ vs. vehicle group. # $P < 0.01$ vs. Dp group.

Oberdorster and his colleagues have shown that a pulmonary inflammatory response occurs only for 20 nm $TiO_2$ particles, but not for 250 nm (50). Ultrafine $TiO_2$ particles (29 nm) were more potent than fine particles (250 nm) regarding airway inflammation related to allergen (28). Furthermore, our recent study showed that intratracheal exposure to $TiO_2$ nanoparticles exacerbates lung inflammation related to LPS, which is more prominent with smaller nanoparticles than with larger ones. On the other hand, several reports have suggested that the toxicity of particles is not correlated with the size (51, 52). In our present murine model, size-related effects were not observed, which could not be considered to be due to differences in the absorption/penetration of nanoparticles because of the injection directly into the skin. Several previous studies have used the skin-stripping methods as a model of skin barrier dysfunction/defect in humans (53, 54) and experimental animals (55, 56). However, it is difficult to generate quantitatively skin barrier dysfunction/defect with identical severity. Thus, we should try to investigate the size-related effects of $TiO_2$ nanoparticles using other exposure methods which assume exposure in the "real world" in the future. Nonetheless, our present study suggests that particle sizes may be independent of the severity of AD-like skin lesions in the presence of intradermal $TiO_2$ nanoparticles of an equal quantity.

Skin lesions in AD are characterized by the recruitment of lymphocytes, monocytes/macrophages, and eosinophils, and the infiltration/degranulation of mast cells. The lymphocytes infiltrating into skin lesions of AD are Th2-type T cells, which produce IL-4, IL-5, and IL-13 (57, 58). On the other hand, defective IFN-γ (a Th1 cytokine) production is considered to be associated with allergen-specific Th2 immune responses in AD patients (59–61). 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 (40, 41). IL-4 is a crucial factor in IgE synthesis, and IL-5 is also a key factor in the activation and/or proliferation of eosinophils. In *ex vivo* analyses, the protein and mRNA levels of IL-4, IL-5, and IL-13 in spleen or lymph node cells are paralleled by AD-like skin legions in NC/Nga mice (62, 63). The intranasal administration of $TiO_2$ combined with allergen increase Th2 cytokines (IL-4, IL-5, and IL-13), and allergen-specific IgE and IgG1 levels in serum (28). In the present study, the local protein expression of IL-4, IL-5, and IL-13 in the ear elevated following exposure to mite allergen. In addition, the administration of $TiO_2$ nanoparticles combined with mite allergen significantly increased IL-4 production as compared with mite allergen administration alone, which was concomitant with the histological changes including the accumulation of eosinophils in the skin and the infiltration/degranulation of mast cells accompanied by histamine release in serum. On the other hand, treatment with mite allergen reduced the expression of IFN-γ, which was more prominent in the Dp + $TiO_2$ group than in the Dp group. These results and the previous reports suggest that intradermal exposure to $TiO_2$ nanoparticles can accelerate AD-like skin lesions, possibly via Th2-skewed immune responses.

The degranulation of mast cells on allergen-specific or nonspecific stimulation induces the release of several chemical mediators including histamine. In patients with AD, increases in histamine levels have been observed (64, 65). Our previous study demonstrated that the degranulation of mast cells in the skin is concomitant with the severity of AD-like skin lesions in a murine model (17). Chymase, which is a specific enzyme of connective tissue mast cells, may contribute to the development of atopic inflammation (66). Mast cell degranulation and consequent histamine release can play an important role in the aggravation of AD-like skin lesions. In the present study, intradermal exposure to nanoparticles enhanced histamine release in serum in the presence of allergen. More interestingly, $TiO_2$ nanoparticles significantly increased histamine release in serum and IL-13 production in the ear in spite of the absence of mite allergen (Table 1, Fig. 4). In addition, the degranulation of mast cells slightly increased following the administration of $TiO_2$ nanoparticles alone (Fig. 2). Th2 cells as well as mast cells generate IL-13. Thus, $TiO_2$ nanoparticles might be an important contributor to cutaneous abnormalities with or without allergen under skin barrier dysfunction/defect through chemical mediators derived from mast cells.

In conclusion, intradermal exposure to $TiO_2$ nanoparticles can aggravate AD-like skin lesions related to mite allergen in our murine model. The effects are paralleled by the enhancement of IL-4 expression in the ear, the levels of total IgE and histamine in serum, and the reduction of IFN-γ expression. Taken together, TiO2 nanoparticles under skin barrier dysfunction/defect may aggravate AD symptoms via Th2-biased immune responses.

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1. Neame RL, Berth-Jones J, Kurinczuk JJ, Graham-Brown RA. Prevalence of atopic dermatitis in Leicester: a study of methodology and examination of possible ethnic variation. Br J Dermatol 132:772–777, 1995.
2. Olesen AB, Ellingsen AR, Larsen FS, Larsen PO, Veien NK, Thestrup-Pedersen K. Atopic dermatitis may be linked to whether a child is first- or second-born and/or the age of the mother. Acta Derm Venereol 76: 457–460, 1996.
3. Williams HC. Is the prevalence of atopic dermatitis increasing? Clin Exp Dermatol 17:385–391, 1992.
4. Yura A, Shimizu T. Trends in the prevalence of atopic dermatitis in school children: longitudinal study in Osaka Prefecture, Japan, from 1985 to 1997. Br J Dermatol 145:966–973, 2001.
5. Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, Gruber C, Lau S, Worm M, Keil T, Kurek M, Zaluga E, Wahn U, Lee YA. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 118: 866–871, 2006.
6. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, Goudie DR, Sandilands A, Campbell LE, Smith FJ, O'Regan GM, Watson RM, Cecil JE, Bale SJ, Compton JG, DiGiovanna JJ, Fleckman P, Lewis-Jones S, Arseculeratne G, Sergeant A, Munro CS, El Houate B, McElreavey K, Halkjaer LB, Bisgaard H, Mukhopadhyay S, McLean WH. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 38:441–446, 2006.
7. Sandilands A, O'Regan GM, Liao H, Zhao Y, Terron-Kwiatkowski A, Watson RM, Cassidy AJ, Goudie DR, Smith FJ, McLean WH, Irvine AD. Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. J Invest Dermatol 126:1770–1775, 2006.
8. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, Liao H, Evans AT, Goudie DR, Lewis-Jones S, Arseculeratne G, Munro CS, Sergeant A, O'Regan G, Bale SJ, Compton JG, DiGiovanna JJ, Presland RB, Fleckman P, McLean WH. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 38:337–342, 2006.
9. Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, Klopp N, Wagenpfeil S, Zhao Y, Liao H, Lee SP, Palmer CN, Jenneck C, Maintz L, Hagemann T, Behrendt H, Ring J, Nothen MM, McLean WH, Novak N. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 118:214–219, 2006.
10. Burney PG, Chinn S, Rona RJ. Has the prevalence of asthma increased in children? Evidence from the national study of health and growth 1973–86. BMJ 300:1306–1310, 1990.
11. Peat JK, Li J. Reversing the trend: reducing the prevalence of asthma. J Allergy Clin Immunol 103:1–10, 1999.
12. Riedl M, Diaz-Sanchez D. Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol 115:221–228; quiz 229, 2005.
13. Takano H, Ichinose T, Miyabara Y, Yoshikawa T, Sagai M. Diesel exhaust particles enhance airway responsiveness following allergen exposure in mice. Immunopharmacol Immunotoxicol 20:329–336, 1998.
14. Yanagisawa R, Takano H, Inoue KI, Ichinose T, Sadakane K, Yoshino S, Yamaki K, Yoshikawa T, Hayakawa K. Components of diesel exhaust particles differentially affect Th1/Th2 response in a murine model of allergic airway inflammation. Clin Exp Allergy 36:386–395, 2006.
15. Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. Immunology 112:489–495, 2004.
16. Casillas AM, Hiura T, Li N, Nel AE. Enhancement of allergic inflammation by diesel exhaust particles: permissive role of reactive oxygen species. Ann Allergy Asthma Immunol 83:624–629, 1999.
17. Takano H, Yanagisawa R, Inoue K, Ichinose T, Sadakane K, Yoshikawa T. Di-(2-ethylhexyl) phthalate enhances atopic dermatitis-like skin lesions in mice. Environ Health Perspect 114:1266–1269, 2006.
18. Renwick LC, Brown D, Clouter A, Donaldson K. Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occup Environ Med 61:442–447, 2004.
19. Bermudez E, Mangum JB, Asgharian B, Wong BA, Reverdy EE, Janszen DB, Hext PM, Warheit DB, Everitt JI. Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol Sci 70:86–97, 2002.
20. Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 77:347–357, 2004.
21. Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years. Toxicol Appl Pharmacol 79:179–192, 1985.
22. Inoue K, Takano H, Ohnuki M, Yanagisawa R, Sakurai M, Shimada A, Mizushima K, Yoshikawa T. Size effects of nanomaterials on lung inflammation and coagulatory disturbance. Int J Immunopathol Pharmacol 21:197–206, 2008.
23. de Haar C, Hassing I, Bol M, Bleumink R, Pieters R. Ultrafine carbon black particles cause early airway inflammation and have adjuvant activity in a mouse allergic airway disease model. Toxicol Sci 87:409–418, 2005.
24. Alessandrini F, Schulz H, Takenaka S, Lentner B, Karg E, Behrendt H, Jakob T. Effects of ultrafine carbon particle inhalation on allergic inflammation of the lung. J Allergy Clin Immunol 117:824–830, 2006.
25. Alessandrini F, Semmler-Behnke M, Jakob T, Schulz H, Behrendt H, Kreyling W. Total and regional deposition of ultrafine particles in a mouse model of allergic inflammation of the lung. Inhal Toxicol 20: 585–593, 2008.
26. Inoue K, Takano H, Yanagisawa R, Sakurai M, Ichinose T, Sadakane K, Yoshikawa T. Effects of nano particles on antigen-related airway inflammation in mice. Respir Res 6:106, 2005.
27. Inoue K, Takano H, Yanagisawa R, Ichinose T, Sakurai M, Yoshikawa T. Effects of nano particles on cytokine expression in murine lung in the absence or presence of allergen. Arch Toxicol 80:614–619, 2006.
28. de Haar C, Hassing I, Bol M, Bleumink R, Pieters R. Ultrafine but not fine particulate matter causes airway inflammation and allergic airway sensitization to co-administered antigen in mice. Clin Exp Allergy 36: 1469–1479, 2006.
29. Pflucker F, Hohenberg H, Holzle E, Will T, Pfeiffer S, Wepf R, Diembeck W, Wenck H, Gers-Barlag H. The outermost stratum corneum layer is an effective barrier against dermal uptake of topically applied micronized titanium dioxide. Int J Cosmet Sci 21:399–411, 1999.
30. Schulz J, Hohenberg H, Pflucker F, Gartner E, Will T, Pfeiffer S, Wepf R, Wendel V, Gers-Barlag H, Wittern KP. Distribution of sunscreens on skin. Adv Drug Deliv Rev 54 Suppl 1:S157–163, 2002.
31. Kiss B, Biro T, Czifra G, Toth BI, Kertesz Z, Szikszai Z, Kiss AZ, Juhasz I, Zouboulis CC, Hunyadi J. Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. Exp Dermatol 17:659–667, 2008.
32. Kambara T, Aihara M, Matsukura S, Sato I, Kubota Y, Hirasawa T, Ikezawa Z. Effects of photocatalytic agent on DS-Nh mice, developing atopic dermatitis-like eruption with an increase of Staphylococcus aureus. Int Arch Allergy Immunol 141:151–157, 2006.

33. Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB, Colvin VL. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci 92:174–185, 2006.
34. Uchino T, Tokunaga H, Ando M, Utsumi H. Quantitative determination of OH radical generation and its cytotoxicity induced by TiO(2)-UVA treatment. Toxicol In Vitro 16:629–635, 2002.
35. Sasakawa T, Higashi Y, Sakuma S, Hirayama Y, Sasakawa Y, Ohkubo Y, Goto T, Matsumoto M, Matsuda H. Atopic dermatitis-like skin lesions induced by topical application of mite antigens in NC/Nga mice. Int Arch Allergy Immunol 126:239–247, 2001.
36. Sasakawa T, Higashi Y, Sakuma S, Hirayama Y, Sasakawa Y, Ohkubo Y, Mutoh S. Topical application of FK506 (tacrolimus) ointment inhibits mite antigen-induced dermatitis by local action in NC/Nga mice. Int Arch Allergy Immunol 133:55–63, 2004.
37. Yanagisawa R, Takano H, Inoue K, Koike E, Sadakane K, Ichinose T. Effects of maternal exposure to di-(2-ethylhexyl) phthalate during fetal and/or neonatal periods on atopic dermatitis in male offspring. Environ Health Perspect 116:1136–1141, 2008.
38. Takano H, Yoshikawa T, Ichinose T, Miyabara Y, Imaoka K, Sagai M. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. Am J Respir Crit Care Med 156: 36–42, 1997.
39. Sadakane K, Takano H, Ichinose T, Yanagisawa R, Shibamoto T. Formaldehyde enhances mite allergen-induced eosinophilic inflammation in the murine airway. J Environ Pathol Toxicol Oncol 21:267–276, 2002.
40. Denda M, Sato J, Tsuchiya T, Elias PM, Feingold KR. Low humidity stimulates epidermal DNA synthesis and amplifies the hyperproliferative response to barrier disruption: implication for seasonal exacerbations of inflammatory dermatoses. J Invest Dermatol 111:873–878, 1998.
41. Tabata N, Tagami H, Kligman AM. A twenty-four-hour occlusive exposure to 1% sodium lauryl sulfate induces a unique histopathologic inflammatory response in the xerotic skin of atopic dermatitis patients. Acta Derm Venereol 78:244–247, 1998.
42. Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G, Sterry W. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol 12:247–256, 1999.
43. Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol 22:93–97, 2004.
44. Xia T, Kovochich M, Brant J, Hotze M, Sempf J, Oberley T, Sioutas C, Yeh JI, Wiesner MR, Nel AE. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett 6:1794–1807, 2006.
45. Dunford R, Salinaro A, Cai L, Serpone N, Horikoshi S, Hidaka H, Knowland J. Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS Lett 418:87–90, 1997.
46. Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss DG, Schiffmann D. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ Health Perspect 110:797–800, 2002.
47. Wottrich R, Diabate S, Krug HF. Biological effects of ultrafine model particles in human macrophages and epithelial cells in mono- and co-culture. Int J Hyg Environ Health 207:353–361, 2004.
48. Driscoll KE, Lindenschmidt RC, Maurer JK, Higgins JM, Ridder G. Pulmonary response to silica or titanium dioxide: inflammatory cells, alveolar macrophage-derived cytokines, and histopathology. Am J Respir Cell Mol Biol 2:381–390, 1990.
49. Driscoll KE, Maurer JK, Lindenschmidt RC, Romberger D, Rennard SI, Crosby L. Respiratory tract responses to dust: relationships between dust burden, lung injury, alveolar macrophage fibronectin release, and the development of pulmonary fibrosis. Toxicol Appl Pharmacol 106: 88–101, 1990.
50. Oberdorster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder AC. Acute pulmonary effects of ultrafine particles in rats and mice. Res Rep Health Eff Inst 5–74; disc 75–86, 2000.
51. Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, in vivo particle persistence, and lung injury. Environ Health Perspect 102 Suppl 5:173–179, 1994.
52. Yamamoto A, Honma R, Sumita M, Hanawa T. Cytotoxicity evaluation of ceramic particles of different sizes and shapes. J Biomed Mater Res A 68:244–256, 2004.
53. Jakasa I, Verberk MM, Esposito M, Bos JD, Kezic S. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. J Invest Dermatol 127:129–134, 2007.
54. Vavrova K, Hrabalek A, Mac-Mary S, Humbert P, Muret P. Ceramide analogue 14S24 selectively recovers perturbed human skin barrier. Br J Dermatol 157:704–712, 2007.
55. Gao XK, Nakamura N, Fuseda K, Tanaka H, Inagaki N, Nagai H. Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. Biol Pharm Bull 27:1376–1381, 2004.
56. Segawa S, Kuroda H, Kaneko T, Watari J. Oral administration of a hop water extract ameliorates the development of dermatitis induced by the periodical topical application of a mite antigen in atopic dermatitis model NC/Nga mice. Biosci Biotechnol Biochem 72:974–981, 2008.
57. Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, Wardlaw AJ, Hamid Q. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 173:775–778, 1991.
58. Rousset F, Robert J, Andary M, Bonnin JP, Souillet G, Chretien I, Briere F, Pene J, de Vries JE. Shifts in interleukin-4 and interferon-gamma production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. J Allergy Clin Immunol 87:58–69, 1991.
59. Reinhold U, Wehrmann W, Kukel S, Kreysel HW. Recombinant interferon-gamma in severe atopic dermatitis. Lancet 335:1282, 1990.
60. Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DY. Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. J Invest Dermatol 99:403–408, 1992.
61. Lester MR, Hofer MF, Gately M, Trumble A, Leung DY. Down-regulating effects of IL-4 and IL-10 on the IFN-gamma response in atopic dermatitis. J Immunol 154:6174–6181, 1995.
62. Takakura M, Takeshita F, Aihara M, Xin KQ, Ichino M, Okuda K, Ikezawa Z. Hyperproduction of IFN-gamma by CpG oligodeoxynucleotide-induced exacerbation of atopic dermatitis-like skin lesion in some NC/Nga mice. J Invest Dermatol 125:1156–1162, 2005.
63. Tenda Y, Yamashita M, Kimura MY, Hasegawa A, Shimizu C, Kitajima M, Onodera A, Suzuki A, Seki N, Nakayama T. Hyper-responsive TH2 cells with enhanced nuclear factor-kappa B activation induce atopic dermatitis-like skin lesions in Nishiki-nezumi Cinnamon/Nagoya mice. J Allergy Clin Immunol 118:725–733, 2006.
64. Ring J. Plasma histamine concentrations in atopic eczema. Clin Allergy 13:545–552, 1983.
65. Ruzicka T, Gluck S. Cutaneous histamine levels and histamine releasability from the skin in atopic dermatitis and hyper-IgE-syndrome. Arch Dermatol Res 275:41–44, 1983.
66. Omoto Y, Tokime K, Yamanaka K, Habe K, Morioka T, Kurokawa I, Tsutsui H, Yamanishi K, Nakanishi K, Mizutani H. Human mast cell chymase cleaves pro-IL-18 and generates a novel and biologically active IL-18 fragment. J Immunol 177:8315–8319, 2006.