Research Article

Biological factor related to Asian sand dust particles contributes to the exacerbation of asthma

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Running title: Biological factor related to Asian sand dust particles affects asthma

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Abstract

Epidemiologic studies have revealed that Asian sand dust particles (ASDs) can affect respiratory and immune health represented by asthma. The factors that are responsible for the exacerbation of asthma remain unclear. The fungus Bjerkandera adusta (B.ad) and polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP) have been identified in ASDs collected from the atmosphere when an ASD event occurred. We investigated the effects of B.ad and BaP related to ASDs on respiratory and immune systems. Bone marrow-derived antigen presenting cells (APCs) and splenocytes from atopic prone NC/Nga mice and human airway epithelial cells were exposed to the B.ad or to BaP in the presence and absence of heated-ASDs (H-ASDs). Both B.ad and BaP in both the presence and absence of H-ASDs increased the expression of cell surface molecules on APCs. H-ASDs alone slightly activated APCs. The expressions induced by B.ad were higher than those induced by BaP in the presence and absence of H-ASDs. There were no remarkable effects on the activation of splenocytes or the pro-inflammatory responses in airway epithelial cells. These results suggest that B.ad rather than BaP contributes to the exacerbation of asthma regardless of the presence or absence of sand particles, especially by the activation of the immune system via APCs.

Key Words: Asian sand dust particles, Bjerkandera adusta, Benzo[a]pyrene, Bone-marrow-derived antigen presenting cells, Asthma

Short Abstract for the Table of Contents

Bjerkandera adusta (B.ad) and benzo[a]pyrene (BaP) each activated antigen presenting cells (APCs) in the presence and the absence of heated Asian sand dust particles (H-ASDs). H-ASDs alone slightly activated APCs. The activation induced by B.ad was more apparent than that by BaP in the presence and absence of H-ASDs. B.ad rather than BaP contributes to the exacerbation of asthma regardless of the presence or absence of sand particles, especially by the activation of the immune system via APCs.

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Introduction

Sand dust storms have adverse health effects on a global scale because they can be transported over a long distance [Esmaeil et al. 2014]. For example, Asian sand dust particles (ASDs) have spread over large areas, including East China, the Korean peninsula, Taiwan, and Japan. Several epidemiological studies suggested that exposure to ASDs can increase daily mortality, cardiovascular and respiratory diseases [Chen et al. 2004; Kwon et al. 2002; Hashizume et al. 2010; Ueda et al. 2012].

The exacerbation of respiratory diseases such as asthma by ASDs is a public health problem. It has been reported that ASD events have been associated with an increased risk in hospitalizations for asthma and the worsening of asthma [Kanatani et al. 2010; Watanabe et al. 2011]. Experimental studies demonstrated that repeated airway exposure of mice to ASDs contributed to the exacerbation of lung inflammation in the presence of an allergen such as ovalbumin (OVA) and dermatophagoides farinae [Hiyoshi et al. 2005; Ichinose et al. 2006]. ASDs can also affect respiratory and immune systems. In brief, ASDs caused a pro-inflammatory response via interleukin (IL)-6 and IL-8 production from airway epithelial cells, induced the maturation and activation of bone marrow-derived antigen presenting cells (APCs), and increased the proliferation of splenocytes [Honda et al. 2014].

We have suggested that not only dust particles themselves but also components related to ASDs may cause adverse health effects [Honda et al. 2014]. However, the responsible factors

that lead to the exacerbation of respiratory and immune diseases remain unclear.

ASDs are composed mainly of silicon, aluminum, calcium, and iron. They also contain various biological and chemical materials such as fungi and polycyclic aromatic hydrocarbons (PAHs) [Ho et al. 2005; Lee et al. 2009; Maki et al. 2008, 2010; Mori et al. 2002, 2003; Tamamura et al. 2007; Wu et al. 2004; Yeo and Kim 2002]. In Japan, the fungus Bjerkandera adusta (B.ad) has been identified in ASDs collected from the atmosphere when an ASD event occurred [Kobayashi et al. 2010]. It was reported that B.ad induces respiratory problems such as allergic cough [Ogawa et al. 2009, 2011]. He et al. [2012, 2016] reported that co-exposure to heated-ASDs (H-ASDs) and B.ad aggravated lung eosinophilia via marked increases in pro-inflammatory mediators in both the presence and absence of OVA. It was also reported that H-ASDs with organic chemicals containing PAH exacerbated OVA-induced lung eosinophilia via increases in Th2-mediated cytokines [Ren et al. 2014]. However, the degree of the contributions of biological and chemical factors and the underlying mechanisms by which these factors contribute to respiratory diseases have not been clarified.

In the present study, we compared the effects of biological and chemical factors related to ASDs on immune cells from atopy-prone NC/Nga mice and human airway epithelial cells. We focused on B.ad as a biological factor and benzo[a]pyrene (BaP) as a chemical factor. Our purpose was to investigate the difference of contributions of B.ad and BaP to the respiratory and immune health degraded by ASDs. Our ultimate goals are to identify the responsible

factors that lead to the exacerbation of respiratory and immune diseases represented by asthma and to determine the underlying mechanisms.

Materials and methods

Mice and preparation of bone marrow cells and splenocytes

Ten-week-old SPF NC/NgaTndCrlj male mice were purchased from Charles River Japan (Osaka, Japan). They were housed in an animal facility that was maintained at 24°–26°C and a 12-h light/dark cycle under conventional conditions. The procedures of all animal studies were approved by the Animal Research Committee at Kyoto University. Mice were sacrificed by cervical dislocation and exsanguinated from the cut abdominal aorta and vein. After the surrounding muscle tissue was removed, the bones were left in 70% ethanol for 3 min and washed with RPMI1640 medium (Life Technologies, Carlsbad, CA). Both ends of the bones were cut, and the marrow was then flushed with RPMI1640 using a syringe with a 24G needle.

The marrow suspension was passed through sterile 250 nylon mesh to remove small pieces of bone and debris, and the red blood cells were lysed with BD PharmLyse (Becton Dickinson, Lincoln Park, NJ). The spleen was pushed through a sterile 200-mesh stainless steel sheet, and red blood cells were also lysed with BD PharmLyse. The cells were centrifuged at 400*g* for 5 min at 20°C. After being washed with RPMI 1640, the cells were resuspended in culture medium R10, which was RPMI 1640 supplemented with 10% heat-inactivated fetal

bovine serum (FBS; MP Biomedicals, Eschwege, Germany), 100 U/mL penicillin, 100 μg/mL streptomycin (Sigma, St. Louis, MO), and 50 μM 2-mercaptoethanol (Life Technologies). The numbers of viable cells were determined by the trypan blue (Life Technologies) exclusion method.

Differentiation of bone marrow-derived APCs

APCs were differentiated using a modification of the protocol of Lutz et al. [1999]. In brief, bone marrow cells (4×10^5 /mL) were cultured in R10 medium (10 mL) containing 20 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF). On day 3, the same volume of the medium containing 20 ng/mL GM-CSF was added to the culture. On day 6, half the culture medium was replaced with fresh medium. On day 8, non-adherent and loosely adherent cells were collected by gentle pipetting. The differentiated APCs were centrifuged at 400*g* for 5 min at 20°C and then resuspended in fresh medium. The numbers of viable cells were determined by the trypan blue exclusion method.

Cell culture for airway epithelial cells

The BEAS-2B cell line, derived from human bronchial epithelial cells transformed by an adenovirus 12-SV40 hybrid virus, was purchased from the European Collection of Cell

Cultures (Salisbury, Wiltshire, UK). The cells (7.5×10^4 cells/mL) were seeded in 96-well (0.07 mL) or 12-well (0.7 mL) collagen I-coated plates and incubated for 72 h to reach semi-confluence in the serum free-medium LHC-9 (Life Technologies) at 37°C in a humidified atmosphere of 5% CO_2.

Preparation of ASDs, B.ad, and BaP

ASDs were collected from the Gobi Desert in north-east Asia (https://www.nies.go.jp/labo/crm-e/gobikosadust.html) and were certified as reference material by the National Institute for Environmental Studies, Japan (CRM No. 30). ASDs were treated by heating at 360°C for 30 min (H-ASDs) to exclude substances that are sensitive to heat. H-ASDs were diluted to give a final concentration of 50 μg/mL in media. Our previous study showed that flying ASDs at the doses of 30-90 μg/mL caused interleukin (IL)-6 and IL-8 releases from airway epithelial cells, induced the maturation and activation of APCs, and increased the proliferation of splenocytes [Honda et al.2014]. About 10% of flying ASDs was materials adhered to flying ASD. Therefore, H-ASDs at a dose of 50 μg/mL were used.

B.ad was prepared as described by He et al. [2016]. In brief, the B.ad was inactivated with 1% formalin for 1 day at 4°C and dissolved in saline. The suspension was sonicated for 1 min with a UD-201 type ultrasonic disrupter with a micro-tip (Tomy, Tokyo) under cooling conditions. Then the suspension was diluted in media (final concentration 2.5% saline).

BaP (CAS.No 50-32-8, purity 98.0%) was purchased from Sigma. BaP was dissolved in dimethyl sulfoxide (DMSO) and then diluted in media (final concentration 0.1% DMSO).

Experimental protocol

APCs (1×10^6 cells/mL) from the NC/Nga mice were exposed to B.ad (0, 1, 5, or 25 μg/mL) or BaP (0, 0.1, 1, or 10 μM) in the presence and absence of H-ASDs (50 μg/mL) for 24 h. The final media volume was 1.2 mL (12-well plates) and 0.2 mL (96-well plates). The cluster of differentiation (CD) 86 and dendritic and epithelial cells (DEC)205 protein expressions on the cell surface were evaluated by a fluorescence-activated cell sorter (FACS).

Splenocytes (1×10^6 cells/mL) from the NC/Nga mice were exposed to B.ad (0, 1, 5, or 25 μg/mL) or BaP (0, 0.1, 1, or 10 μM) in the presence and absence of H-ASDs (50 μg/mL) for 24 or 72 h. The final media volume was 1.2 mL (12-well plates) and 0.2 mL (96-well plates). The cell proliferation and the expression of T cell receptor (TCR) protein on the cell surface were evaluated by a 5-bromo-2'-deoxyuridine (BrdU) enzyme-linked immunosorbent assay (ELISA) and FACS, respectively.

After airway epithelial cells grew to semi-confluence in LHC-9, the cells were exposed to B.ad (0, 1, 5, or 25 μg/mL) or BaP (0, 0.1, 1, or 10 μM) in the presence and absence of H-ASDs (50 μg/mL) for 24 h. The final media volume was 0.7 mL (12-well plates) and 0.07 mL (96-well plates). The releases of IL-6 and IL-8 in the culture supernatants (50 μL) were

evaluated by ELISA.

Quantitation of inflammatory proteins in the culture supernatants

After exposure, the medium was harvested and centrifuged at 400*g* for 5 min to remove floating cells. The final supernatants were stored at −80°C until analysis. The levels of IL-6 and IL-8 (Thermo Scientific, Waltham, MA) in the culture medium were measured by ELISA according to the manufacturer's instructions. Absorbance was measured on an iMark Microplate Absorbance Reader (Bio-Rad Laboratories, Hercules, CA) with the wavelength set at 450 nm and a reference wavelength at 550 or 630 nm. The detection limits of IL-6 and IL-8 were <1 pg/mL and <2 pg/mL, respectively.

FACS analysis

For the FACS analysis, the following monoclonal antibodies were used: Mouse BD Fc Block™ purified anti-mouse CD16/CD32 (Becton Dickinson), CD86 (GL-1, PE-conjugated, Becton Dickinson), Rat IgG2a, κ Isotype Control (R35-95, PE-conjugated, Becton Dickinson), DEC205 (NLDC-145, PE-conjugated, BioLegend, San Diego, CA), Rat IgG2a, κ Isotype Control (RTK2758, PE-conjugated, BioLegend), Hamster Anti-Mouse TCR-βChain (H57-597, FITC-conjugated, Becton Dickinson), and Hamster IgG2,λ1 Isotype Control (Ha4/8, FITC-conjugated, Becton Dickinson).

After exposure, the cells were resuspended in 50 μL phosphate-buffered saline with 0.3% bovine serum albumin and 0.05% sodium azide (Wako Pure Chemical Industries, Osaka, Japan) and then incubated with a 0.1–1 μg amount of each antibody for 45 min at 4°C. After incubation, the cells were washed, and the fluorescence was measured by a FACS Calibur (Becton Dickinson). For each sample, fluorescence data from approx. 10,000 cells were collected, and the number of positive cells expressed as % events.

Cell proliferation

The cell proliferation was measured with a Cell-Proliferation-ELISA Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. This technique is based on the incorporation of the pyrimidine analogue BrdU instead of thymidine into the DNA of proliferating cells. BrdU incorporated into DNA is measured by a sandwich-type enzyme immunoassay using monoclonal anti-BrdU antibodies. After exposure, the cell proliferation was measured by adding BrdU to each well 20 h before the measurement. Absorbance was measured on an iMark Microplate Absorbance Reader with the wavelength set at 450 nm and a reference wavelength at 630 nm.

Statistical analysis

Data are presented as the mean ± standard error of the mean (S.E.M.) for each

experimental group (n=4). Differences among groups were analyzed using the Tukey multiple comparison test (Excel Statistics 2010, Social Survey Research Information, Tokyo). A *p*-value <0.05 was considered significant.

Results

Effects of B.ad and BaP in the presence and absence of H-ASDs on APCs

To determine the effects of exposure to B.ad and BaP in the presence and absence of H-ASDs on the maturation and activation of APCs, we analyzed the expression patterns of CD86 (Fig. 1) and DEC205 (Fig. 2) on APCs at 24 h after exposure.

B.ad in both the presence and absence of H-ASDs significantly increased the expression of CD86 in a dose-dependent manner compared to the corresponding control ($p<0.05$ vs. B.ad 0 μg/mL and $p<0.05$ vs. B.ad 0 μg/mL with H-ASDs). H-ASDs alone slightly increased the expression of CD86 compared to the controls ($p<0.05$ vs. B.ad 0 μg/mL). B.ad at a dose of 1 μg/mL with H-ASDs elevated the CD86 expression at the same dose compared to B.ad alone ($p<0.05$).

BaP alone at the dose of 1 or 10 μM significantly increased the expression of CD86 compared to the control ($p<0.05$ vs. BaP 0 μM). BaP at the dose of 0.1, 1 or 10 μM with H-ASDs also significantly increased the expression of CD86 compared to BaP 0 μM with H-ASDs ($p<0.05$). BaP at the dose of 0.1 μM with H-ASDs elevated the CD86 expression at the

same dose compared to BaP alone (p<0.05).

The increases in CD86 expression induced by B.ad were higher than those induced by BaP in both the presence and absence of H-ASDs.

B.ad in both the presence and absence of H-ASDs significantly increased the expression of DEC205 in a dose-dependent manner compared to the corresponding control (p<0.05 vs. B.ad 0 μg/mL and p<0.05 vs. B.ad 0 μg/mL with H-ASDs). H-ASDs alone slightly increased the expression of DEC205 compared to the control (p<0.05 vs. B.ad 0 μg/mL).

In contrast, the BaP treatment resulted in no significant elevation of DEC205 in the presence or absence of H-ASDs compared to the corresponding control. H-ASDs alone slightly increased the expression of DEC205 compared to the control (p<0.05 vs. BaP 0 μM). BaP at the dose of 0.1 or 10 μM with H-ASDs increased the DEC205 expression at the same dose compared to BaP alone (p<0.05).

The DEC205 expression induced by B.ad was higher than that induced by BaP in both the presence and absence of H-ASDs,

Effects of B.ad and BaP in the presence and absence of H-ASDs on splenocytes

To determine the effects of exposure to B.ad or BaP in the presence and absence of H-ASDs on the activation of T cells, we analyzed the proliferation and TCR expression of splenocytes at 24 and 72 h after exposure (Tables 1, 2). B.ad resulted in no significant elevation

of the proliferation in the presence or absence of H-ASDs compared to the corresponding control. H-ASDs alone significantly decreased the proliferation compared to the control (p<0.05 vs. B.ad 0 μg/mL). B.ad at the dose of 5 or 25 μg/mL with H-ASDs decreased the proliferation at the same dose compared to B.ad alone (p<0.05). B.ad in both the presence and absence of H-ASDs resulted in no apparent increase in TCR expression.

No change was caused by BaP in the presence or absence of H-ASDs (Table 2).

Effects of B.ad and BaP in the presence and absence of H-ASDs on airway epithelial cells

To evaluate whether B.ad or BaP in the presence or absence of H-ASDs induce pro-inflammatory responses via IL-6 and IL-8 on airway epithelial cells, we examined the production of IL-6 and IL-8 at 24 h after exposure (Tables 3, 4).

B.ad in both the presence and absence of H-ASDs did not cause a pro-inflammatory response via IL-6 (Table 3). B.ad in the presence of H-ASDs partially decreased the IL-6 release compared to B.ad 0 μg/mL with H-ASDs (p<0.05). In the IL-8 analysis, no change was caused by B.ad in the presence or absence of H-ASDs.

On the other hand, BaP alone did not cause pro-inflammatory responses via IL-6 (Table 4), but it partially decreased the IL-6 release compared to the control (p<0.05 vs. BaP 0 μM). BaP at the dose of 1 or 10 μM with H-ASDs increased the IL-6 release compared to BaP at 0 μM with H-ASDs (p<0.05). However, the pro-inflammatory impact was very small. H-ASDs

alone decreased the IL-6 release compared to the control (p<0.05 vs. BaP 0 μM). BaP at the dose of 0.1 or 1 μM with H-ASDs increased the IL-6 release at the same dose compared to BaP alone (p<0.05).

BaP in the presence or absence of H-ASDs did not cause pro-inflammatory responses via IL-8. Both exposed groups showed a partially decreased IL-8 release compared to the corresponding control (p<0.05 vs. BaP 0 μM or BaP 0 μM with H-ASDs). H-ASDs alone increased the IL-8 release compared to the control (p<0.05 vs. BaP 0 μM). BaP at 10 μM with H-ASDs decreased the IL-8 release at the same dose compared to BaP alone (p<0.05).

Discussion

We observed that the exposure of APCs to B.ad in both the presence and absence of H-ASDs increased the expression of both CD86 and DEC205. The exposure of APCs to BaP in the presence and the absence of H-ASDs increased the expression of CD86. B.ad showed greater effects than BaP on APCs in both the presence and absence of H-ASDs. There were no remarkable effects on the activation of splenocytes or the pro-inflammatory responses in the airway epithelial cells.

CD86 on APCs is the ligand for CD28 on the cell surface of T cells. The binding of CD86 with CD28 is a costimulatory signal for the activation of T cells [Chen 2004]. It has been shown that elevated CD86 expression is correlated with the development of allergic disease

and asthma [Lombardi et al. 2010]. In our present experiments, both B.ad and BaP in both the presence and the absence of H-ASDs increased the CD86 expression on APCs. Our findings are the first to reveal that B.ad alone and co-exposure to B.ad and H-ASDs activated CD86 expression.

It was also suggested that the other types of fungi such as Candida albicans and Aspergillus fumigatus increase the CD86 expression on APCs [Grazziutti et al. 2001]. BaP in the presence of rat liver S9 homogenate and tetrachlorodibenzo-para-dioxin (TCDD) as representative PAHs were reported to induce CD86 expression on APCs [Chipinda et al. 2011; Vogel et al. 2013]. Therefore, B.ad as a fungus or BaP as a PAH can enhance the antigen-presenting ability via CD86 expression on APCs. In addition, the expression of CD86 on APCs has been reported to be regulated by NF-κB [Janeway and Medzhitov 2002].

Lin et al. [2009] indicated that the protein derived from fungi induces CD86 on human monocyte-derived dendritic cells by the NF-κB and MAPK pathways. Vogel et al. [2013] reported that the enhanced maturation of APCs by TCDD is associated with elevated levels of CD86 and an increased aryl hydrocarbon receptor-dependent nuclear accumulation of NF-κB RelB. Therefore, the increased expression of CD86 caused by exposure to B.ad or BaP may be related to intracellular mechanisms such as the involvement of NF-kB signal transduction pathways.

DEC205 is a member of the C-type lectin receptor (CLR) family. This molecule is

known to mediate the capture and internalization of ligands for subsequent processing and presentation by APCs [Jiang et al. 1995]. We previously demonstrated that flying Asian sand dust particles elevated the DEC205 expression on APCs [Honda et al. 2014]. In brief, that study obtained evidence that components of ASDs could contribute to the maturation and activation of APCs. In the present study, we observed for the first time that B.ad in the presence or absence of H-ASDs activated APCs via DEC205. These present findings are in accord with those of our previous study from the point of view that components of ASDs activate APCs.

Many studies have shown that fungi activate members of the CLR family on APCs. For example, CLR family members Dectin-1, Dectin-2 and macrophage mannose receptor (MMR) recognize β-glucans, high-mannose structures and branched N‐linked mannans of fungi, respectively [Romani 2011; Dambuza and Brown 2015]. These CLRs are important for fungal recognition and for the induction of immune responses. It has also been reported that Dectin-2 promotes house dust mite-induced T-helper type 2 and type 17 cell differentiation and allergic airway inflammation in mice [Norimoto et al. 2014]. The CLR family member DEC205 may thus also contribute to the enhancing effects of B.ad in the presence or absence of H-ASDs on allergic responses or diseases.

BaP in the presence and the absence of H-ASDs did not cause a significant increase in the DEC205 expression in the present experiments compared to the corresponding control (BaP 0 μM or BaP 0 μM with H-ASDs). However, BaP in the presence of H-ASDs partially elevated

the DEC205 expression compared to BaP alone. It was reported that carbon black nanoparticles promoted the maturation, activation and function of DEC205 on APCs [Koike et al. 2008]. In present experiment, sand particles themselves may weakly affect DEC205 expression, and the immunological responses via DEC205 by BaP with H-ASDs mainly reflect the effects of sand particles, i.e., H-ASDs.

We observed herein that the maturation and activation of APCs induced by B.ad was higher than that induced by BaP. It is thought that B.ad can act as not only a positive adjuvant but also as an allergen. He et al. [2012, 2015, 2016] suggested that co-exposure to H-ASDs and B.ad affects lung eosinophilia via marked increases in pro-inflammatory mediators in the presence or absence of OVA as an allergen. Indeed, our present results also showed that B.ad with H-ASDs contributes to the expression of DEC205, which mediates the capture and internalization of ligands.

However, it was reported that BaP exacerbates OVA-, mite-, and Japanese cedar pollen-induced allergic responses [Kanoh et al. 1996; Kadkhoda et al. 2004, 2005; Mizutani et al. 2007]. BaP alone has not elevated allergen-specific IgE [Kadkhoda et al. 2004, 2005]. There is thus a possibility that B.ad has significant direct and indirect effects on the immune response as an adjuvant and an allergen, respectively, whereas BaP can function mainly as an adjuvant. Therefore, B.ad may have greater effects than BaP.

In the present study, materials related to ASDs other than sand particles exaggerated

immune responses through the activation of APCs. Regarding diesel exhaust particles (DEPs), organic chemicals rather than the carbonaceous nuclei of DEPs were reported to exaggerate allergic airway inflammation, possibly through the enhancement of Th2 responses *in vivo* [Yanagisawa et al. 2006]. In immune cells, DEPs act on immature APCs and enhance their antigen-presenting activity, and the action may be mediated chiefly by the organic compounds rather than by the residual carbonaceous particles of DEPs [Koike and Kobayashi 2005]. These results are in accord with our findings regarding ASDs; that is, that materials related to the particles have greater effects than the particles themselves. The organic chemical components on/in DEPs and biological components on/in ASDs may be critical for the exacerbation of respiratory and immune diseases.

Although flying ASDs caused a pro-inflammatory response via IL-6 and IL-8 production from airway epithelial cells, induced the maturation and activation of APCs, and elevated the proliferation of splenocytes [Honda et al. 2014], we observed no remarkable effects of B.ad or BaP in the presence or absence of H-ASDs on the activation and pro-inflammatory responses in splenocytes and airway epithelial cells respectively in the present investigation. Many studies have reported that ASDs provide various biological and chemical materials [Ho et al. 2005; Lee et al. 2009; Maki et al. 2008, 2010; Mori et al. 2002, 2003; Tamamura et al. 2007; Wu et al. 2004; Yeo and Kim 2002]. Endotoxin is one of the components related to ASDs and the outer membrane of gram-negative bacteria, which are associated with an increase of

respiratory diseases [Douwes et al. 2003]. Moreover, it was indicated that co-exposure to SO_4^{2-} and ASDs enhanced inflammatory responses [Ichinose et al. 2005] and that organic chemicals containing various PAHs with H-ASDs in the presence of OVA increased eosinophils, lymphocytes and goblet cells in the airway compared to H-ASD exposure [Ren et al. 2014]. Materials other than B.ad and BaP may thus influence airway epithelial cells and splenocytes.

In conclusion, exposure to B.ad or BaP induced the maturation and activation of APCs via CD86 or DEC205 regardless of the presence or absence of sand particles (Fig.3). B.ad showed greater effects than BaP. These results suggest that B.ad rather than BaP related to ASDs contributes to the exacerbation of respiratory and immune diseases such as asthma, especially by the activation of costimulatory molecules and recognition of xenobiotics by APCs. On the other hand, there were no remarkable effects on the activation of splenocytes or IL-6 and IL-8 release from airway epithelial cells. Because frying ASDs promoted these events, further investigations are needed to understand the contribution of other components.

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Table 1. Effects of B.ad in the presence and absence of H-ASDs on the proliferation and TCR expression of splenocytes from NC/Nga mice

H-ASDs (50 μg/mL)	B.ad. (μg/mL)	Cell proliferation (%)				TCR-positive cells (%)					
						24 h			72 h		
−	0	100.0	±	8.4		28.6	±	2.6	39.3	±	3.8
	1	83.8	±	3.7		28.9	±	2.3	39.9	±	3.4
	5	95.0	±	5.5		29.0	±	2.6	40.8	±	3.8
	25	91.5	±	2.8		29.5	±	2.5	41.4	±	4.0
+	0	76.6	±	4.6	#	29.6	±	2.8	40.9	±	3.9
	1	70.1	±	4.5		29.4	±	2.7	40.6	±	4.6
	5	70.3	±	5.1	#	29.9	±	2.7	40.8	±	4.4
	25	70.6	±	5.8	#	29.6	±	2.3	42.3	±	4.3

Cells were treated with the indicated concentrations for 24 h or 72 h. Control was exposed to 2.5% saline. The data presented as the percentage of the proliferation of the control and as the positive cells expressed as the % events are the mean ± SEM of four individual cultures. $^{\#}p<0.05$ vs. B.ad-exposed group at the same concentration.

Table 2. Effects of BaP in the presence and absence of H-ASDs on the proliferation and TCR expression of splenocytes from NC/Nga mice

H-ASDs (50 μg/mL)	BaP (μM)	Cell proliferation (%)	TCR-positive cells (%)	
			24 h	72 h
−	0	100.0 ± 20.6	30.2 ± 0.7	34.8 ± 1.5
	0.1	63.7 ± 13.1	30.2 ± 0.9	35.4 ± 2.1
	1	76.1 ± 12.4	29.6 ± 0.5	37.3 ± 1.6
	10	67.3 ± 10.9	30.7 ± 1.1	40.5 ± 2.0
+	0	89.5 ± 13.4	28.8 ± 0.8	34.0 ± 1.6
	0.1	62.7 ± 5.4	28.6 ± 0.8	35.2 ± 1.3
	1	61.9 ± 7.8	29.0 ± 0.9	35.5 ± 1.4
	10	64.5 ± 10.5	29.6 ± 0.8	39.7 ± 1.1

Cells were treated with the indicated concentrations for 24 h or 72 h. Control was exposed to 0.1% DMSO. The data presented as the percentage of the proliferation of the control and as the positive cells expressed as the % events are the mean ± SEM of four individual cultures.

Table 3. Effects of B.ad in the presence and absence of H-ASDs on the IL-6 and IL-8 released from human airway epithelial cells

H-ASDs (50 μg/mL)	B.ad (μg/mL)	IL-6 (pg/mL)		IL-8 (pg/mL)
−	0	46.0 ± 2.8		475.9 ± 16.8
	1	45.9 ± 2.3		488.0 ± 60.7
	5	49.2 ± 1.0		432.0 ± 46.4
	25	46.8 ± 1.7		444.0 ± 74.0
+	0	51.3 ± 0.7		482.6 ± 103.7
	1	43.4 ± 1.4	*	472.6 ± 101.8
	5	49.3 ± 0.8		498.7 ± 116.7
	25	42.9 ± 2.5	*	395.2 ± 100.9

Cells were treated with the indicated concentrations for 24 h. Control was exposed to 2.5% saline. The data are the mean ± SEM of four individual cultures. *p<0.05 vs. B.ad 0 μg/mL.

Table 4. Effects of BaP in the presence and absence of H-ASDs on the release of IL-6 and IL-8 from human airway epithelial cells

H-ASDs (50 μg/mL)	BaP (μM)	IL-6 (pg/mL)		IL-8 (pg/mL)	
−	0.0	41.5 ± 3.5		345.7 ± 7.4	
	0.1	21.9 ± 0.8	*	255.2 ± 11.8	*
	1.0	37.6 ± 2.1		239.6 ± 17.2	*
	10.0	47.7 ± 3.5		309.5 ± 32.4	
+	0.0	34.1 ± 2.3	#	451.1 ± 28.4	#
	0.1	36.8 ± 1.3	#	283.5 ± 4.7	*
	1.0	51.3 ± 2.5	*,#	200.6 ± 16.2	*
	10.0	49.4 ± 2.8	*	213.6 ± 4.8	*,#

Cells were treated with the indicated concentrations for 24 h. Control was exposed to 0.1% DMSO. The data are the mean ± SEM of four individual cultures. *p<0.05 vs. each control (BaP 0 μM or BaP 0 μM with H-ASDs). $^{\#}p$<0.05 vs. BaP-exposed group at the same concentration.

Figure legends

Figure 1. Effects of B.ad and BaP in the presence and absence of H-ASDs on the CD86 expression of APCs. APCs from NC/Nga mice were treated with the indicated concentrations for 24 h. Controls for B.ad and BaP were exposed to 2.5% saline and 0.1% DMSO, respectively. The data are presented as positive cells expressed as % events, and they are the mean ± S.E.M. of four individual cultures. $^{*}p<0.05$ vs. each control. $^{\#}p<0.05$ vs. the B.ad- or BaP-exposed group at the same concentration.

Figure 2. Effects of B.ad and BaP in the presence and absence of H-ASDs on the DEC205 expression of APCs. APCs from NC/Nga mice were treated with the indicated concentrations for 24 h. Controls for B.ad and BaP were exposed to 2.5% saline and 0.1% DMSO, respectively. The data are presented as positive cells expressed as % events, and they are the mean ± S.E.M. of four individual cultures. $^{*}p<0.05$ vs. each control. $^{\#}p<0.05$ vs. the B.ad- or BaP-exposed group at the same concentration.

Figure 3. The scheme of the mechanism by which B.ad and BaP related to Asian sand dust particles contributes to the exacerbation of asthma

Fig.1

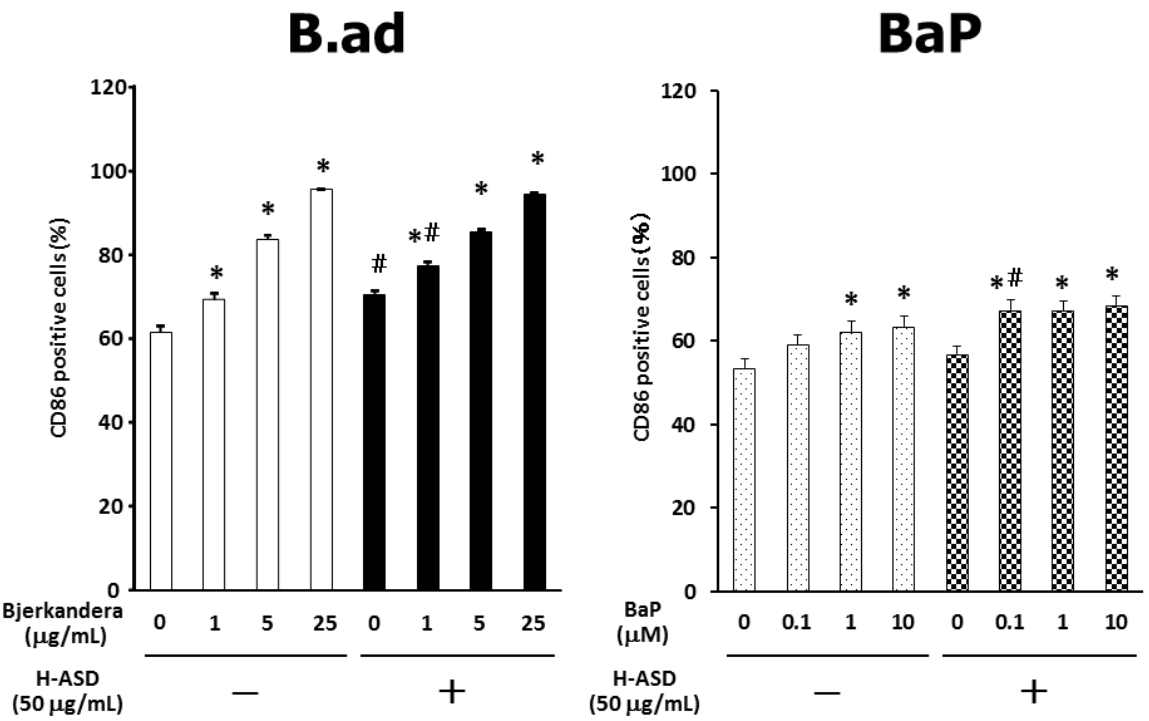

B.ad
BaP
CD86 positive cells (%)
120
100
80
60
40
20
0
Bjerkandera (μg/mL)
0 1 5 25 0 1 5 25
H-ASD (50 μg/mL)
−
+
BaP (μM)
0 0.1 1 10 0 0.1 1 10
H-ASD (50 μg/mL)
−
+

Fig.2

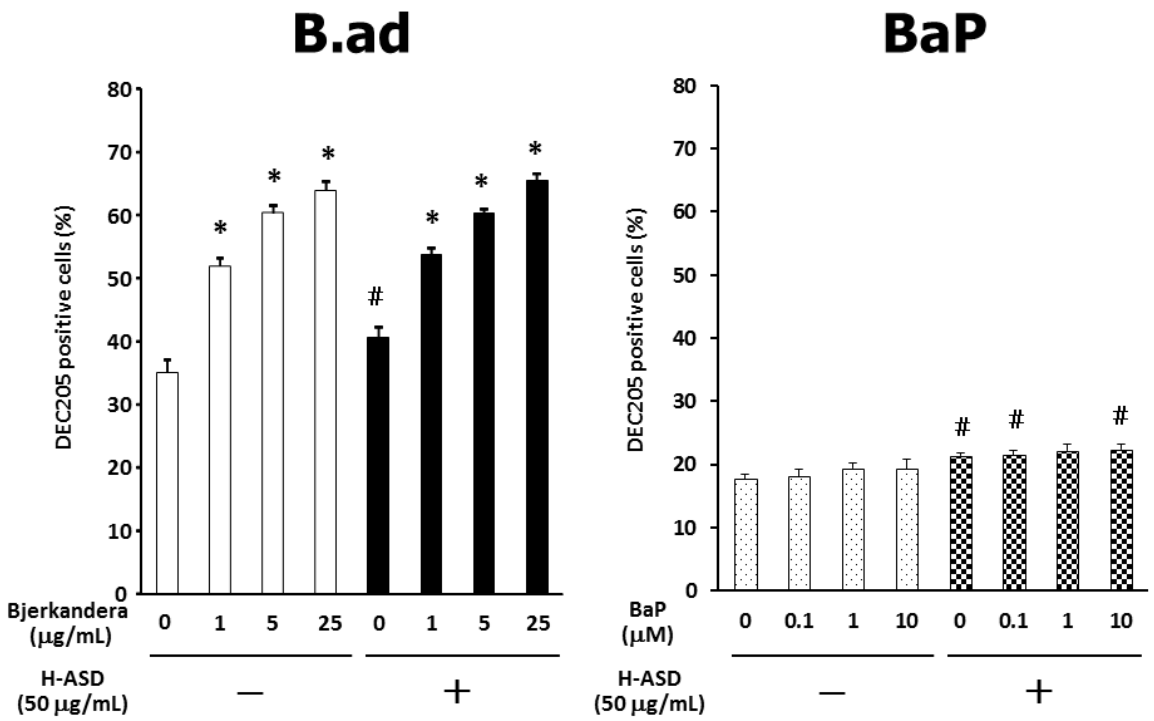
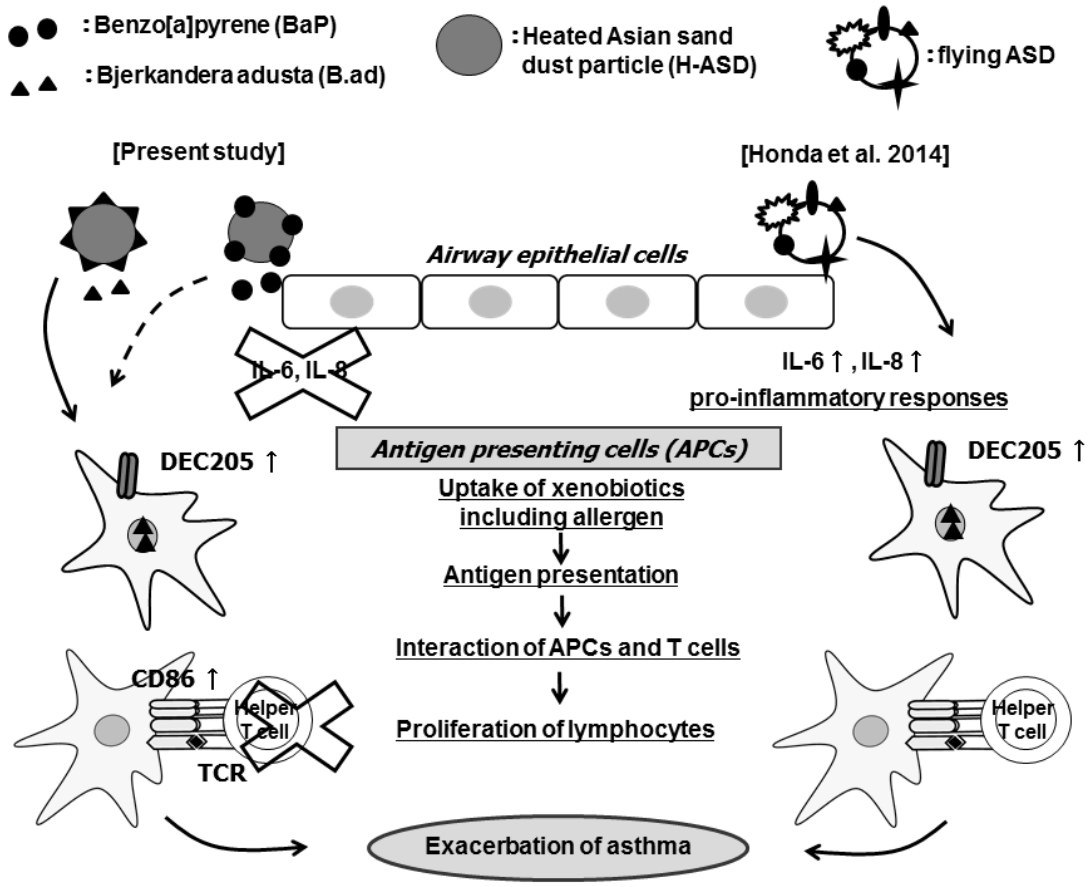

B.ad
BaP
DEC205 positive cells (%)
80
70
60
50
40
30
20
10
0
Bjerkandera (μg/mL)
0 1 5 25 0 1 5 25
H-ASD (50 μg/mL)
−
+
BaP (μM)
0 0.1 1 10 0 0.1 1 10
H-ASD (50 μg/mL)
−
+

Fig.3


: Benzo[a]pyrene (BaP)
: Bjerkandera adusta (B.ad)
: Heated Asian sand dust particle (H-ASD)
: flying ASD
[Present study]
[Honda et al. 2014]
Airway epithelial cells
IL-6, IL-8
IL-6 ↑, IL-8 ↑
pro-inflammatory responses
Antigen presenting cells (APCs)
DEC205 ↑
DEC205 ↑
Uptake of xenobiotics including allergen
Antigen presentation
Interaction of APCs and T cells
Proliferation of lymphocytes
CD86 ↑
Helper T cell
TCR
Helper T cell
Exacerbation of asthma