

RESEARCH ARTICLE

Airborne Asian sand dust enhances murine lung eosinophilia

Miao He¹, Takamichi Ichinose², Seiichi Yoshida², Masataka Nishikawa³, Ikuko Mori³, Rie Yanagisawa⁴, Hirohisa Takano⁴, Ken-ichiro Inoue⁴, Guifan Sun¹, and Takayuki Shibamoto⁵

¹Department of Environmental and Occupational Health, College of Public Health, China Medical University, Shenyang, China, ²Department of Health Sciences, Oita University of Nursing and Health Sciences, Oita, Japan, ³Environmental Chemistry Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan, ⁴Pathophysiology Research Team, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan, and ⁵Department of Environmental Toxicology, University of California, Davis, California, USA

Abstract

There is no experimental study demonstrating the effects of airborne Asian sand dust (AASD) on allergic lung eosinophilia. The organic substances adsorbed onto AASD collected from the atmosphere of Iki-island in Japan were excluded by heat treatment at 360°C for 30 min. The effects of AASD or heated-AASD (H-AASD) towards allergic lung inflammation were compared in murine lungs to investigate the role of organic substances. ICR mice were administered with the two kinds of AASD and/or ovalbumin (OVA) intratracheally four times at 2-week intervals. AASD and H-AASD enhanced eosinophil recruitment induced by OVA in the alveoli and in the submucosa of the airway, which has a goblet cell proliferation in the bronchial epithelium. AASD and H-AASD synergistically increased Th2 cytokines—interleukin-13 (IL-13), eosinophil-relevant cytokine and chemokine, such as IL-5, and monocyte chemotactic protein-3 (MCP-3) induced by OVA in whole lung lavage fluid. The enhancing effects were much greater in AASD than in H-AASD. AASD induced adjuvant effects on OVA-specific immunoglobulin E (IgE) and IgG1 production. In an *in vitro* study using RAW264.7 cells, AASD increased the expression of Toll-like receptors 2 (TLR2) mRNA, but not TLR4 mRNA. AASD increased mRNA expression of NALP3, ASC, and IL-1 β compared with the control. H-AASD caused no expression of either mRNA. These results suggest that the aggravated lung eosinophilia in AASD is due to activation of a Th2-associated immune response and that the activation of TLR2 and NALP3 inflammasome by microbial materials could be participating in this phenomenon.

Keywords: Airborne Asian sand dust; murine lung eosinophilia; Toll-like receptors; NALP3 inflammasome

Introduction

Asian sand dust (ASD) storms, which occur during the spring, arise in arid and semi-arid areas of mid- and North Western China (the Gobi Desert and the Ocher Plateau). ASD aerosol spreads over large areas, including East China, the Korean Peninsula, and Japan (Duce et al., 1980; Husar et al., 2001; Kim et al., 2001). Recent epidemiologic studies have shown that Asian dust storm events coincided with an increase in daily admissions and clinic visits for asthma (Yang et al., 2005), allergic rhinitis (Chang et al., 2006) or conjunctivitis (Yang, 2006) in Taipei, Taiwan. In Japan, the deterioration of Japanese cedar pollinosis and seasonal allergic rhinitis during a dust storm event has been reported (Sato, 2009). Heavy Asian dust events also increased the risk of hospitalization

of children ages 1–15 years with asthma exacerbation in Toyama, Japan (Kanatani et al., 2010). Therefore, experimental studies to confirm the epidemiological results, suggesting that airborne ASD (AASD) causes the deterioration of allergic diseases during a dust storm event, are in order.

In results compatible to those of these epidemiological investigations, we have experimentally demonstrated the ASD, collected from surface soil in the China's Shapotou Desert enhances antigen-related lung eosinophilia in mice (Ichinose et al., 2006), and aggravates allergic rhinitis in guinea pigs by Japanese cedar pollen (Ichinose et al., 2009). However, at present, there is no experimental evidence showing the aggravating effects of AASD on allergic lung eosinophilia.

Address for Correspondence: Takayuki Shibamoto, Department of Environmental Toxicology, University of California, Davis, California, 95616, USA. E-mail: tshibamoto@ucdavis.edu.

(Received 28 March 2010; revised 15 July 2010; accepted 19 July 2010)

ISSN 0895-8378 print/ISSN 1091-7691 online © 2010 Informa Healthcare USA, Inc.
DOI: 10.3109/08958378.2010.510151

<http://www.informahealthcare.com/ih>

RIGHTS LINK
Copyright Clearance Center

In the present study, we used AASD collected from the atmosphere of Iki-island in Japan. Comparison of allergic inflammation between aggravating effects of heated-AASD (H-AASD) that had been excluded of toxic materials (microbial materials, sulfate, etc.) by heating and crude AASD in murine lungs were performed to investigate the role of organic substances.

On the other hand, Toll-like receptors (TLRs) that belong to the pattern recognition receptors (PRRs) family in antigen presenting cells are well known to lead to the induction of adaptive immune responses through the recognition of microbial components (Beutler, 2004). NALP3 (NACHT domain, leucine-rich repeat, and pyrin domain-containing protein 3) that belongs to the Nod-like receptor (NLR) family in antigen presenting cells is an essential component of inflammasomes triggered by microbial ligands like peptidoglycan, danger-associated molecular patterns, and crystals, to form NALP3 inflammasome with adaptor molecule ASC (apoptosis-associated speck-like protein containing a caspase activating and recruitment domain) (Pétrilli et al., 2007). The NALP3 inflammasome activates caspase-1 (Agostini et al., 2004) for processing and secretion of the cytokines interleukin-1 β (IL-1 β) and IL-18 (Fritz et al., 2006; Mayor et al., 2007). This NALP3 inflammasome is a crucial element in adjuvant effect of the innate immune response in aluminium adjuvants (Eisenbarth et al., 2008).

In this *in vitro* study, the gene expression of TLR2, TLR4, NALP3, ASC, caspase-1 and IL-1 β in RAW264.7 cells was measured in the presence of H-AASD or AASD to investigate the role of TLRs and NALP3 inflammasome in the enhancement by AASD effects. The cytokine levels in the nutrient medium released from RAW264.7 cells were also measured. This is the first experimental study on molecular, biochemical and pathologic evidences of AASD exacerbating lung eosinophilia caused by ovalbumin (OVA).

Materials and methods

Animals

A total of 96 male ICR mice (5 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). After 1 week of screening out sick mice, mice with abnormal body weight and mice stressed from different environmental breeding, the remaining mice were fed a commercial diet CE-2 (CLEA Japan, Inc., Tokyo, Japan) and given water *ad libitum*. Mice were housed in plastic cages lined with soft wood chips. The cages were placed in a conventional room, which was air conditioned at 23°C and 55–70% humidity with a light/dark (12h/12h) cycle. The study adhered to the U.S. National Institutes of Health guidelines for the use of experimental animals. The animal care method was approved by the Animal Care and Use Committee at Oita University of Nursing and Health Sciences in Oita, Japan.

Preparation of particles

The present study used AASD previously collected from Iki-island, Japan on March 21–22 2002, after a massive 3-day

dust storm event occurred in East Asia. The average density of the ambient particulate matter (Total suspended particle: TSP) in March 21–22 was 672 $\mu\text{g}/\text{m}^3$ in Iki-island. AASD was collected using a high-volume air sampler with a quartz fiber filter (2500QT-UP, Pallflex, CT). The particle diameter of the samples was measured under a scanning electron microscope (JSM-5800 JEOL Ltd., Tokyo, Japan). The mass size distribution of the aerosol collected from Iki-island during the dust event had one major peak in the coarse particle size ranging from 2.1 to 7.0 μm . Sixty-eight percent of the total mass originating from coarse particles was larger than 2.1 μm . Nineteen percent of the total mass originating from coarse particles was larger than 7.0 μm (Mori et al., 2003). The size distribution peak of particles was at 4.7 μm . As shown in Figure 1, the shape of the collected particles is almost all pebble, but rock-shaped particles were also occasionally found. The collected AASD was stored at -30°C in a germfree case with a desiccant until use.

The concentrations of chemical elements were measured using an ICP-AES (61E Trace and ICP-750, Thermo Jarrell-Ash, Franklin, MA). The samples were heated at 360°C for 30 min in an electric heater to exclude toxic materials (microbiological materials, sulfate, nitrate, etc.) adhering to them.

Detection of fungi in particles

The fungi of the AASD was detected by an all-in-one fluorescence microscope (BZ-9000, BIOREVO, Keyence, Japan) and fluorescent dye Fungiflora Y (Technicon International, Inc., Tokyo, Japan), which specifically bound to β -linked polysaccharides such as β -glucan. This apparatus is a useful tool for demonstrating the fungal cell wall composition (Nishiyama et al., 1995). According to the manufacture's protocol, in brief, $\sim 2.5 \mu\text{g}$ of each particle sample was suspended in 40 μl Fungiflora Y, and after 5 min slides of the particles were completed. Then the slides were observed with the all-in-one fluorescence microscope with an excitation wavelength of 472.5 nm, and an emission wavelength of 520.0 nm.

Analysis of water soluble components, lipopolysaccharide (LPS), and β -glucan in particles

The concentration of water soluble components, such as sulfate (SO_4^{2-}) and nitrate (NO_3^-), in the samples was determined using an ion chromatograph (DX-100, Dionex, Sunnyvale, CA) and ICP-AES (61E Trace, Thermo Jarrell-Ash). Each particle sample was measured by kinetic assay using Endospec ES test MK (Seikagaku Cop., Tokyo, Japan) for LPS activity and by Fungitec G test MK (Seikagaku Cop., Tokyo, Japan) for β -glucan activity. According to the manufacture's protocol, in brief, approximately 2.5 mg of each particle sample was suspended in 1 ml water (LPS- and β -glucan-free; Otsuka Co., Kyoto, Japan) for 1 h and then the sample was allowed to stand on a bench top at room temperature for 2 h. Then the supernatants were recovered and measured for LPS and β -glucan concentration. The limits of detection in the LPS and β -glucan assays were $<0.001 \text{ EU/ml}$ and 2 pg/ml , respectively.

Study protocol

ICR mice were divided into six groups ($n=16$, each group) according to the treatment with particles: normal saline (control), H-AASD, crude AASD, OVA, OVA + H-AASD, and OVA + AASD. ICR male mice were used because of their moderate responsiveness to airway inflammation caused by OVA or mite allergen treatment in this method (Ichinose et al., 1997; Ichinose et al., 2003).

The particles were suspended in normal saline (0.9 % NaCl) for instillation (Otsuka Co., Kyoto, Japan). This suspension was sonicated for 5 min with an ultrasonic disrupter,

UD-201 type with micro tip (Tomy, Tokyo, Japan), under a cooling condition. OVA was dissolved in the same saline. The instillation dose of particles was 0.1 mg per mouse according to a previous report (Ichinose et al., 2008a,b; He et al., 2010). This instillation dose of ASD can, remarkably, elicit a slight allergic reaction caused by OVA or mite allergen of 1 μ g per mouse (Hiyoshi et al., 2005; Ichinose et al., 2006, 2008a,b).

The instillation dose of OVA was set to 1 μ g per mouse according to a previous report (Hiyoshi et al., 2005). Mice were intratracheally instilled with these particles through a polyethylene tube under anesthesia with 4% halothane (Takeda Chemical, Osaka, Japan) four times at 2-week intervals. The control mice were instilled intratracheally with 0.1 ml of normal saline (Otsuka Co., Kyoto, Japan) per mouse. An intratracheal instillation of OVA repeated four times at 2-week intervals is needed for the induction of allergic lung inflammation (Ichinose et al., 2008a,b; He et al., 2010). Therefore, this repeated instillation method was chosen to investigate the aggravating effect of AASD on allergen-related lung eosinophilia. One day after the last intratracheal administration, the mice from all groups were killed by exsanguination under deep anesthesia by intraperitoneal injection of pentobarbital at 12 weeks of age.

Pathological evaluation

Eight of the 16 mice from each group were used for a pathologic examination. The lungs were fixed by 10% neutral phosphate-buffered formalin. After separation of the lobes, 2 mm thick blocks were taken for paraffin embedding. Embedded blocks were sectioned at a thickness of 3 μ m, and then were

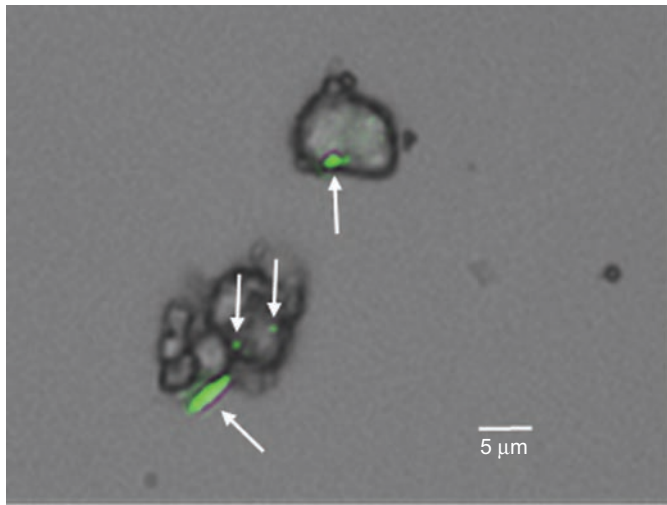


Figure 1. Fluorescent images of the particles stained with Fungiflora Y. Bar = 5 μ m. Arrows show β -glucan of fungi. (See colour version of this figure online at www.informahealthcare.com/ihf)

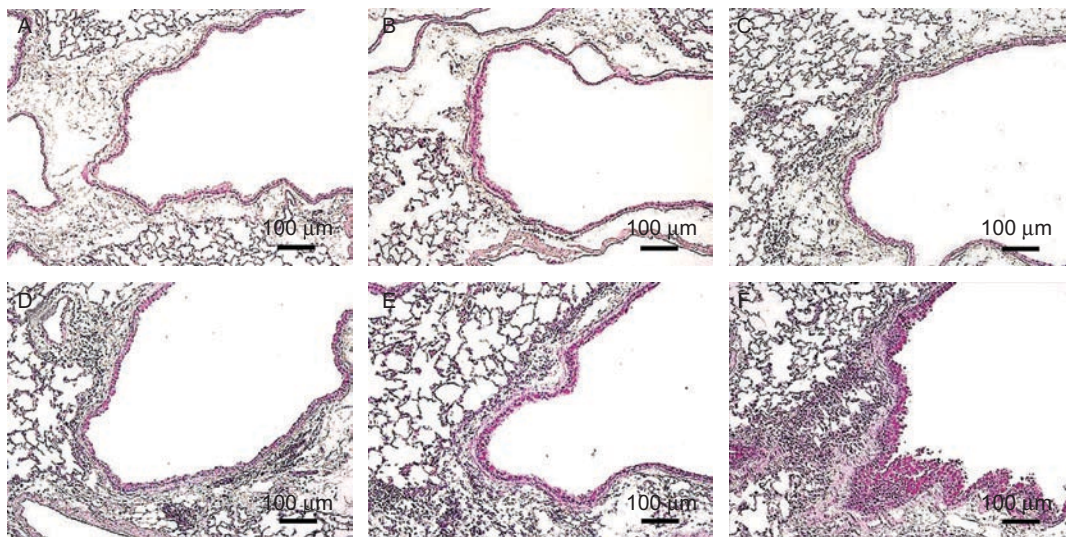


Figure 2. Effects of AASD (with or without heat treatment) on pathological changes in the lungs. The size distribution peak of AASD was observed at 4.7 μ m. (A) No pathological changes in lungs treated with saline. (B) No significant pathological changes in the airway and slight alveolitis in the lung treated with H-AASD. (C) Accumulation of inflammatory cells (neutrophils and lymphocytes) in the airway, and proliferation of alveolar cells and alveolitis in the lung treated with AASD. (D) Slight proliferation of goblet cells that have mucus stained pink with PAS solution in the airway epithelium and slight infiltration of inflammatory cells into connective tissue around the airway treated with OVA alone. (E) Moderate proliferation of goblet cells in the airway and moderate infiltration of inflammatory cells into connective tissue around the airway treated with OVA + H-AASD. (F) Marked proliferation of goblet cells and numerous inflammatory cells in the airway and accumulation of inflammatory cells in the alveoli treated with OVA + AASD. A-F: PAS stain; Bar = 100 μ m. (See colour version of this figure online at www.informahealthcare.com/ihf)

stained with periodic acid-schiff (PAS) to evaluate the degree of proliferation of goblet cells in the bronchial epithelium. A pathological analysis of the inflammatory cells and epithelial cells in the airway of each lung lobe on the slides was performed using a Nikon ECLIPSE light microscope (Nikon Co., Tokyo, Japan). The degree of proliferation of goblet cells in the bronchial epithelium was graded on the following scale: 0, not present; 1, slight; 2, mild; 3, moderate; 4, moderate to marked; and 5, marked. 'Slight' was defined as less than 10% of the airway with goblet cells stained with PAS; 'mild' as 20–30%; 'moderate' as 40–50%; 'moderate to marked' as 60–70%; and 'marked' as more than 80% (Ichinose et al., 2006; 2008a,b; He et al., 2010). Pathological changes were assessed on one slide stained with PAS per mouse. This evaluation procedure was performed by two pathologists who cross-checked the data in blinded specimens. All values were expressed as mean $\pm$ SD ($n=8$).

Whole lung lavage fluids (WLLF)

The remaining eight mice were used for an examination of the free cell contents from whole lung lavage fluids (WLLF). WLLF and cell counts were conducted using a previously reported method (Ichinose et al., 2006; 2008a,b; He et al., 2010). In brief, the tracheas were cannulated after the collection of blood. The lungs were lavaged with two injections of 0.8 ml of sterile saline at 37°C by syringe. The lavaged fluid was harvested by gentle aspiration. The average volume retrieved was 90% of the amount instilled (1.6 ml). The fluids from the two lavages were combined, cooled to 4°C, and centrifuged at 1500 rpm for 10 min. The total amount of lavages collected from individual mice was used in order to measure the protein levels of cytokines and chemokines in the WLLF. The total cell count of a fresh fluid specimen was determined using a hemocytometer. Differential cell counts were assessed on cytologic preparations. Slides were prepared using a Cytospin (Sakura Co., Ltd., Tokyo, Japan) and stained with Diff-Quik (International Reagents Co., Kobe, Japan) to identify eosinophils with red granules. A total of 300 cells were counted under oil immersion microscopy. The WLLF supernatants were stored at -80°C until analyzed for cytokines and chemokines.

Quantitation of cytokines and chemokines in WLLF

The cytokine protein levels in the WLLF were determined using enzyme-linked immunosorbent assays (ELISA). IL-5 and IL-12 were measured using an ELISA kit from Endogen, Inc. (Cambridge, MA). Interferon (IFN)- γ , IL-1 β , IL-6, keratinocyte chemoattractant (KC), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein-1 α (MIP-1 α), regulated on activation normal T-cell expressed and presumably secreted (RANTES), IL-13, IL-4, and eotaxin were measured using an ELISA kit from R&D Systems Inc. (Minneapolis, MN). IL-18 was measured using an ELISA kit from Medical & Biological Laboratories Co., LTD (Nagoya, Japan). MCP-3 was measured using an ELISA kit from Bender MedSystems Inc. (Burlingame, CA).

Antigen-specific IgE and IgG1 antibodies

OVA-specific immunoglobulin E (IgE) and IgG1 antibodies were measured using a Mouse OVA-IgE ELISA kit and a Mouse OVA-IgG1 ELISA kit (Shibayagi Co., Shibukawa, Japan). According to the manufacturer's protocol, 1 U of the anti-OVA IgE is defined as 1.3 ng of the antibody, and 1 U of the anti-OVA IgG1 as 160 ng of the antibody. The absorption at 450 nm (sub-wave length, 620 nm) for OVA-specific IgE and IgG1 antibody was measured using a microplate reader (Spectrafluor, Tecan, Salzburg, Austria).

Cell culture

RAW264.7 cells, which are macrophage-like cells derived from BALB/c male mice, were cultured at 37°C in a humidified atmosphere of 5% CO₂–95% air and maintained in Dulbecco's modified Eagle's medium with 10% heat inactivated fetal bovine serum. For gene expression analysis, they were plated at a concentration of 4×10^5 cells per 60-mm dish, and then AASD with or without heating treatment was added to cells to give a final concentration of 3 $\mu\text{g}/\text{ml}$ or LPS was added to cells to give a final concentration of 100 ng/ml. Cells were then incubated for 1 h and 3 h (3 h only in the case of LPS). For ELISA analysis of cytokine in nutrient medium, RAW264.7 cells were seeded at a concentration of 1×10^5 cells/ml in 96-well culture plate of 200 $\mu\text{l}/\text{well}$. Cells were then incubated with 3 $\mu\text{g}/\text{ml}$ AASD with or without heating treatment or 100 ng/ml LPS for 0, 1, 3, 6, 12, and 24 h.

Gene expression analysis

Total RNA was extracted by standard procedures using 0.5 ml of Isogen (Nippon Gene, Tokyo, Japan) per dish. After DNase treatment of the total RNA, cDNA was synthesized by reverse transcription using M-MLV. Quantitative PCR was performed using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Inc., Foster City, CA) under the same conditions as in our previous study (Yoshida et al., 2006; He et al., 2010). Two wells were used for each sample. The relative expression of each sample was calculated as the mean value divided by the mean value for GAPDH. The primers and probes used in this *in vitro* study are shown in Table 1.

Assay for cytokines in nutrient medium

The protein levels of cytokines in the nutrient medium were determined using ELISA. IL-1 β and IL-6 were measured using an ELISA kit from R&D Systems Inc. IL-18 was measured using an ELISA kit from Medical & Biological Laboratories Co., Ltd.

Statistical analysis

Statistical analyses on the pathologic evaluation in the airway, cytokine and chemokine proteins in WLLF, and gene expression in RAW264.7 cells and protein levels of cytokines in the nutrient medium were conducted using Tukey for Pairwise Comparisons (KyPlot Ver.5, Kyens Lab Inc., Tokyo, Japan). Differences among groups were determined as statistically significant at a level of $p < 0.05$.

Results

The detection of fungi in AASD particles

Figure 1 shows the fluorescence of particles after being stained with Fungiflora Y Bright fluorescence was clearly observed in AASD.

Contents of chemical elements, water soluble components, LPS and β -glucan in AASD

To clarify the relationship between toxic substances and the aggravation of allergic inflammatory responses by AASD, the contents of chemical elements, water soluble components, LPS and β -glucan in AASD were measured. As shown in Table 2, the chemical elements in the AASD sample were 61.8% SiO₂, 13.6% Al₂O₃, 5.7% Fe₂O₃, 5.4% CaO, 3.3% MgO, 0.01% TiO₂, and 2.6% K₂O. The concentration of SO₄²⁻ was 15,000 μ g/g in AASD and non-detected in the H-AASD sample. The concentration of NO₃⁻ was 5000 μ g/g in AASD and also non-detected in the H-AASD sample. The amounts of LPS and β -glucan in the both samples were consistent (Table 3).

Table 1. Primers and probes used in the present *in vitro* study.

Primers and probes	Gene sequence
GAPDH sense	TGCACCACCACTGCTTAG
GAPDH antisense	GGATGCAGGGATGATGTT
GAPDH probe	CAGAAGACTGTGGATGGCCCTC
TLR2 sense	GAATTGCATCAC CGTCAGAA
TLR2 antisense	CTGAGCAGA ACAGCGTTTGC
TLR2 probe	CGTCAAATCTCAGAGGATGCTACGAGC
TLR4 sense	AGGACTCTGATCA TGGCACTGTT
TLR4 antisense	GAGTTTCTGATC CATGCATTGG
TLR4 probe	CCAGGAAGCTTGAATCCCTGCATAGAGG
IL-1 β sense	TCCTGAACCTCAACTGTGAAATGC
IL-1 β antisense	AGCCCAGGTCAAAGGTTTGG
IL-1 β probe	AGCAGCCCTTCATCTTTTGGGGTCCG
Nalp3 sense	TGGAGAAAATGCCTTGGGAGAC
Nalp3 antisense	GGTCAGAGCTGAACAACAGATTG
Nalp3 probe	AGGCCGGAATTCACCAACCCAGCT
CARD sense	AGCTGCAAACGACTAAAGAAGAG
CARD antisense	ACCCTGGCAATGAGTGCTTG
CARD probe	CCACAAAGTGTCTGTCTGGCTGTACTCT
Casp1 sense	TCACGCCCTGTTGGAAAGG
Casp1 antisense	CTCACTGAGTTGATAAATTGCTTCC
Casp1 probe	CTTGCCCTCAGGATCTTGTACGCCATG

Table 2. Concentration of chemical elements in sand dust.

Chemical element compositions	AASD ^a (%)	ASD ^b (%)
SiO ₂	61.8	60
Al ₂ O ₃	13.6	11.1
Fe ₂ O ₃	5.7	4.1
Na ₂ O	1.2	1.8
CaO	5.4	9.0
MgO	3.3	2.5
TiO ₂	0.01	0.7
K ₂ O	2.6	2.2

^aAirborne Asian sand dust (AASD) collected from the atmosphere in Iki-Island of Japan.

^bASD was collected from the desert of Inner Mongolia where dust storms occur frequently.

Enhancement of pathologic changes in the airway by AASD

To evaluate the effects of AASD on airway inflammation and goblet cell proliferation caused by OVA, lung pathology in the eight groups of mice was investigated. Table 4 shows the pathologic changes caused by the AASD in the murine airway, and Figure 2 illustrates the effects of the AASD on pathological changes in the lungs. No pathologic alterations were found in the lungs of the control group (Figure 2A). Proliferation of epithelial cells in the airway was very slight in H-AASD (Figure 2B). AASD alone caused bronchitis and alveolitis along with proliferation of alveolar cells and neutrophilic inflammation (Figure 2C). The treatment with OVA alone caused slight increased infiltration of eosinophils in the submucosa of the airway with slight goblet cell proliferation in the bronchial epithelium (Figure 2D). However, OVA + H-AASD (Figure 2E) and OVA + AASD (Figure 2F) caused increasing proliferation of goblet cells ($p < 0.001$) in the airway compared with the single treatment (Table 4). The degree of proliferation was higher in OVA + AASD than in OVA + H-AASD (Table 4). Both combination treatments caused more prominent infiltration of eosinophils into connective tissue in the airway. The changes of OVA + H-AASD ($p < 0.001$) and OVA + AASD ($p < 0.001$) were significantly different from that of OVA alone (Table 4). The magnitude of eosinophils infiltration was higher in OVA + AASD than in OVA + H-AASD. OVA + H-AASD and OVA + AASD also caused moderate to marked accumulation of lymphocytes

Table 3. Concentration of water soluble components, LPS, and β -glucan in particles.

Particles	SO ₄ ²⁻ (Pg/g)	NO ₃ ⁻ (Pg/g)	Cl ⁻ (Pg/g)	LPS (EU/mg)	β -Glucan (pg/mg)
AASD ^a	15,000	5000	7000	1.06	76.0
H-AASD ^b	ND	ND	ND	ND	ND

ND, not detected.

^aAASD, crude AASD collected from the atmosphere in Iki-Island.

^bH-AASD, AASD were heated at 360°C for 30 min in an electric heater.

Table 4. Evaluation of pathological changes in the murine airway.

Group*	Animals (n)	Pathological changes		
		Lymphocytes	Eosinophils	Proliferation of goblet cells
Control	8	ND	ND	ND
H-AASD	8	0.5 $\pm$ 0.27	ND	0.25 $\pm$ 0.27
AASD	8	1.06 $\pm$ 0.5 [‡]	0.13 $\pm$ 0.23	0.5 $\pm$ 0.65
OVA	8	0.88 $\pm$ 0.58 [†]	0.44 $\pm$ 0.42	1.19 $\pm$ 0.80 [‡]
OVA + H-AASD	8	2.56 $\pm$ 0.78 ^{§ **}	2.13 $\pm$ 0.95 ^{§ **}	2.94 $\pm$ 0.56 ^{§ **}
OVA + AASD	8	3.44 $\pm$ 0.42 ^{§ ††}	3.38 $\pm$ 0.64 ^{§ ††}	3.56 $\pm$ 0.56 ^{§ ††}

*Six groups of mice were treated intratracheally with normal saline (Control), H-AASD (heated-AASD), AASD (crude AASD), ovalbumin (OVA), OVA + H-AASD, and OVA + AASD, four times at 2-week intervals.

The degree of pathological changes in the airway was estimated: (0) none; (1) slight; (2) mild; (3) moderate; (4) moderate to marked; (5) marked. All values were expressed as mean $\pm$ SD ($n = 8$). Statistical analyses were conducted using Tukey for pairwise comparisons.

ND, not detectable.

[†] $p < 0.05$ vs. Control, [‡] $p < 0.01$ vs. Control, [§] $p < 0.001$ vs. Control, ^{||} $p < 0.001$ vs. OVA, ^{**} $p < 0.001$ vs. H-AASD, ^{††} $p < 0.001$ vs. AASD.

in the airway. The degree of the accumulation was higher in OVA + AASD than in OVA + H-AASD (Table 4).

Enhancement of cell numbers in WLLF by AASD

To evaluate the effects of AASD on airway inflammation and goblet cell proliferation caused by OVA, lung pathology in the eight groups of mice was investigated. Figure 3 shows the cellular profile in WLLF. AASD alone increased the number of macrophages and neutrophils compared with the control ($p < 0.05$), but H-AASD did not. OVA alone caused no significant increases of free cells in WLLF compared with the control. OVA + H-AASD significantly increased the number of macrophages, neutrophils, eosinophils, and lymphocytes compared with the control, OVA and H-AASD alone. Furthermore, OVA + AASD resulted in a remarkable elevation of macrophages, eosinophils, and lymphocytes compared with the control, OVA and AASD alone ($p < 0.001$). OVA + AASD also significantly increased neutrophils compared with control and OVA ($p < 0.001$), but not AASD alone.

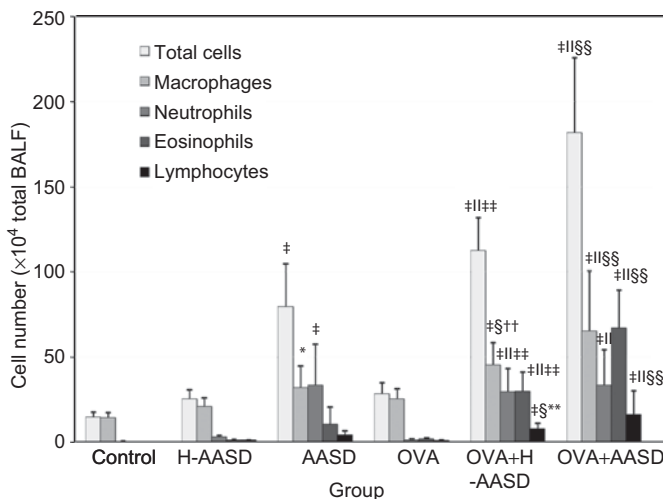


Figure 3. Cellular profile in whole lung lavage fluids (WLLF). All values expressed as mean $\pm$ SE. * $p < 0.05$ compared to Control, $^{\dagger}p < 0.01$ compared to Control, $^{\ddagger}p < 0.001$ compared to Control, $^{\S}p < 0.05$ compared to OVA, $^{\parallel}p < 0.001$ compared to OVA, $^{**}p < 0.05$ compared to H-AASD, $^{++}p < 0.01$ compared to H-AASD, $^{**}p < 0.001$ compared to H-AASD, $^{\S\S}p < 0.001$ compared to AASD.

Enhancement of cytokines and chemokines in WLLF by AASD

To determine which cytokines or chemokines were involved in the AASD-mediated enhancement of OVA-induced lung eosinophilia, we measured protein levels of IL-1 β , IFN- γ , IL-4, IL-5, IL-6, IL-12, IL-13, IL-18, RANTES, KC, MIP-1 α , Eotaxin, MCP-1, and MCP-3.

AASD alone significantly increased protein levels of IL-1 β ($p < 0.001$), IL-12 ($p < 0.01$), IL-6 ($p < 0.001$) and IFN- γ ($p < 0.001$) compared with the control, but H-AASD did not (Table 5 and 6). OVA + H-AASD and OVA + AASD significantly increased the expression of IL-1 β and IL-6 compared with the control and OVA. However, OVA + AASD decreased IL-1 β , IL-6, and IFN- γ compared with AASD alone (Tables 5 and 6). OVA + AASD significantly increased the expression of IL-12 compared with the control ($p < 0.001$) and OVA ($p < 0.001$). IL-18 was not detected in the all groups. H-AASD and AASD alone increased protein levels of KC and MIP-1 α compared with the control ($p < 0.001$) (Table 7). OVA + H-AASD and OVA + AASD also increased the protein levels of KC compared with the control and OVA ($p < 0.001$). However, the increased levels were similar as compared with H-AASD and AASD alone. AASD alone increased protein level of RANTES (Table 7) compared with the control ($p < 0.01$). However, OVA + AASD did not show further increase of RANTES compared with AASD alone. OVA + AASD tended to increase the Th2 cytokine, IL-4 (Table 5), but OVA + AASD synergistically increased the Th2 cytokine, IL-13 (Table 5) compared with the each single treatment. OVA + H-AASD and OVA + AASD synergistically increased protein levels of eosinophil-relevant cytokines, such as IL-5, and MCP-1 in WLLF (Tables 5 and 7) compared with the each single treatment. OVA + AASD also synergistically increased eosinophil-relevant chemokines like Eotaxin and MCP-3 (Table 7) compared with the each single treatment ($p < 0.001$). OVA + AASD caused increases of 1.80-fold by IL-5; 1.44-fold by IL-13, 3.83-fold by Eotaxin, 1.56-fold by MCP-1, and 2.56-fold by MCP-3 compared with OVA + H-AASD.

Enhancement of OVA-specific IgE and IgG1 in serum by AASD

To examine whether AASD has adjuvant activity on antigen-specific Ig production, OVA-specific IgE and IgG1 antibodies

Table 5. Expression of cytokines in whole lung lavage fluids (WLLF).

Group*	Animals (n)	Cytokines (pg protein/total WLLF)					
		IL-1 β	IL-4	IL-5	IL-6	IL-12	IL-13
Control	8	11.1 $\pm$ 7.64	ND	ND	2.94 $\pm$ 1.81	6.71 $\pm$ 6.71	ND
H-AASD	8	50.7 $\pm$ 12.2	ND	2.39 $\pm$ 2.39	57.5 $\pm$ 19.1	311 $\pm$ 35.5	ND
AASD	8	245 $\pm$ 32.6 [§]	ND	6.76 $\pm$ 6.76	176 $\pm$ 21.4 [§]	693 $\pm$ 59.0 [§]	3.38 $\pm$ 3.38
OVA	8	ND	ND	22.3 $\pm$ 6.67	1.82 $\pm$ 1.10	27.0 $\pm$ 14.3	ND
OVA + H-AASD	8	111 $\pm$ 34.9 ^{†‡}	ND	152 $\pm$ 20.1 ^{‡‡‡}	70.1 $\pm$ 18.4 ^{†‡}	467 $\pm$ 41.1	74.8 $\pm$ 17.2 ^{‡‡‡§§}
OVA + AASD	8	160 $\pm$ 21.8 ^{§††}	4.67 $\pm$ 4.67	273 $\pm$ 69.3 ^{§††‡‡‡}	92.8 $\pm$ 11.8 ^{§††‡‡‡‡}	1061 $\pm$ 300 ^{§††}	108 $\pm$ 29.1 ^{§††‡‡‡}

*Six groups of mice were treated intratracheally with normal saline (Control), H-AASD (heated-AASD), AASD (crude AASD), ovalbumin (OVA), OVA + H-AASD and OVA + AASD, four times at 2-week intervals. Whole lung lavage was conducted 24 h after the last intratracheal instillation. All values were expressed as mean $\pm$ SE ($n = 8$). Statistical analyses were conducted using Tukey for pairwise comparisons.

ND, not detectable.

[†] $p < 0.05$ vs. Control, [‡] $p < 0.01$ vs. Control, [§] $p < 0.001$ vs. Control, ^{||} $p < 0.05$ vs. OVA, ^{**} $p < 0.01$ vs. OVA, ^{††} $p < 0.001$ vs. OVA, ^{§§} $p < 0.05$ vs. H-AASD, ^{§§} $p < 0.01$ vs. H-AASD, ^{‡‡} $p < 0.01$ vs. AASD, ^{‡‡‡} $p < 0.001$ vs. AASD

were measured. As shown in Figure 4, OVA + H-AASD tended to increase OVA-specific IgE compared with the single treatment. OVA + AASD markedly elevated the production of

Table 6. Expression of cytokines in whole lung lavage fluids (WLLF).

Group*	Animals(n)	Cytokines (pg protein/total WLLF)	
		IL-18	IFN- γ
Control	8	ND	ND
H-AASD	8	ND	8.75 $\pm$ 8.75
AASD	8	ND	81.9 $\pm$ 27.6 [†]
OVA	8	ND	ND
OVA + H-AASD	8	ND	ND
OVA + AASD	8	ND	12.3 $\pm$ 12.3 [‡]

*Six groups of mice were treated intratracheally with normal saline (Control), H-AASD (heated-AASD), AASD (crude AASD), ovalbmin (OVA), OVA + H-AASD and OVA + AASD, four times at 2-week intervals. Whole lung lavage was conducted 24 h after the last intratracheal instillation. All values were expressed as mean $\pm$ SE ($n=8$). Statistical analyses were conducted using Tukey for pairwise comparisons.

ND, not detectable.

[†] $p < 0.001$ vs. Control, [‡] $p < 0.01$ vs. AASD.

OVA-specific IgE compared with the control, AASD and OVA ($p < 0.05$). OVA + H-AASD significantly increased OVA-specific IgG1 compared with the control ($p < 0.05$), H-AASD ($p < 0.001$) and OVA ($p < 0.001$). OVA + AASD also increased the antibody compared with the control, AASD and OVA ($p < 0.001$). These antibodies were higher in the OVA + AASD group than in the OVA + H-AASD group.

In vitro analysis of mRNA expression in RAW264.7 cells

To investigate the effects of AASD on sensors for leading inflammation in the antigen presenting cells, the gene expression of TLR2, TLR4, NALP3 inflammasome (NALP3, ASC, caspase-1) and IL-1 β using RAW264.7 cells derived from macrophages of mice were measured. We also examined the effects of LPS on them.

Figure 5A) shows the expression of TLR2 mRNA in RAW264.7 cells after 1 h and 3 h. H-AASD caused no expression of TLR2 mRNA. AASD significantly increased the expression of TLR2 mRNA compared with the control ($p < 0.001$) after 1 h and 3 h. Figure 5B) shows the expression of TLR4

Table 7. Expression of chemokines in whole lung lavage fluids (WLLF).

Group*	Animals (n)	Chemokines (pg protein/total WLLF)					
		RANTES	KC	MIP-1 α	Eotaxin	MCP-1	MCP-3
Control	8	ND	31.6 $\pm$ 12.5	ND	ND	ND	ND
H-AASD	8	13.5 $\pm$ 9.75	593 $\pm$ 89.0 [‡]	62.1 $\pm$ 7.29 [‡]	ND	267 $\pm$ 58.5	3.98 $\pm$ 3.98
AASD	8	73.0 $\pm$ 16.2 [†]	517 $\pm$ 63.1 [‡]	62.9 $\pm$ 4.31 [‡]	ND	342 $\pm$ 83.8	47.2 $\pm$ 24.6
OVA	8	ND	80.8 $\pm$ 18.7	ND	ND	ND	4.13 $\pm$ 4.13
OVA + H-AASD	8	16.9 $\pm$ 6.03	419 $\pm$ 32.9 ^{‡II}	13.5 $\pm$ 4.11 ^{††}	12.0 $\pm$ 6.11	1132 $\pm$ 302 ^{†§**}	396 $\pm$ 165
OVA + AASD	8	77.1 $\pm$ 23.8 ^{†§}	583 $\pm$ 56.2 ^{‡II}	21.7 $\pm$ 2.61 ^{†§**‡}	45.9 $\pm$ 15.4 ^{‡III‡‡}	1771 $\pm$ 340 ^{‡III‡‡}	1012 $\pm$ 224 ^{‡III‡‡}

*Six groups of mice were treated intratracheally with normal saline (Control), H-AASD (heated-AASD), AASD (crude AASD), ovalbmin (OVA), OVA + H-AASD, and OVA + AASD, four times at 2-week intervals. Whole lung lavage was conducted 24 h after the last intratracheal instillation. All values were expressed as mean $\pm$ SE ($n=8$). Statistical analyses were conducted using Tukey for pairwise comparisons.

ND, not detectable.

[†] $p < 0.01$ vs. Control, [‡] $p < 0.001$ vs. Control, [§] $p < 0.01$ vs. OVA, ^{II} $p < 0.001$ vs. OVA, ^{**} $p < 0.05$ vs. H-AASD, ^{††} $p < 0.001$ vs. H-AASD, ^{‡‡} $p < 0.001$ vs. AASD.

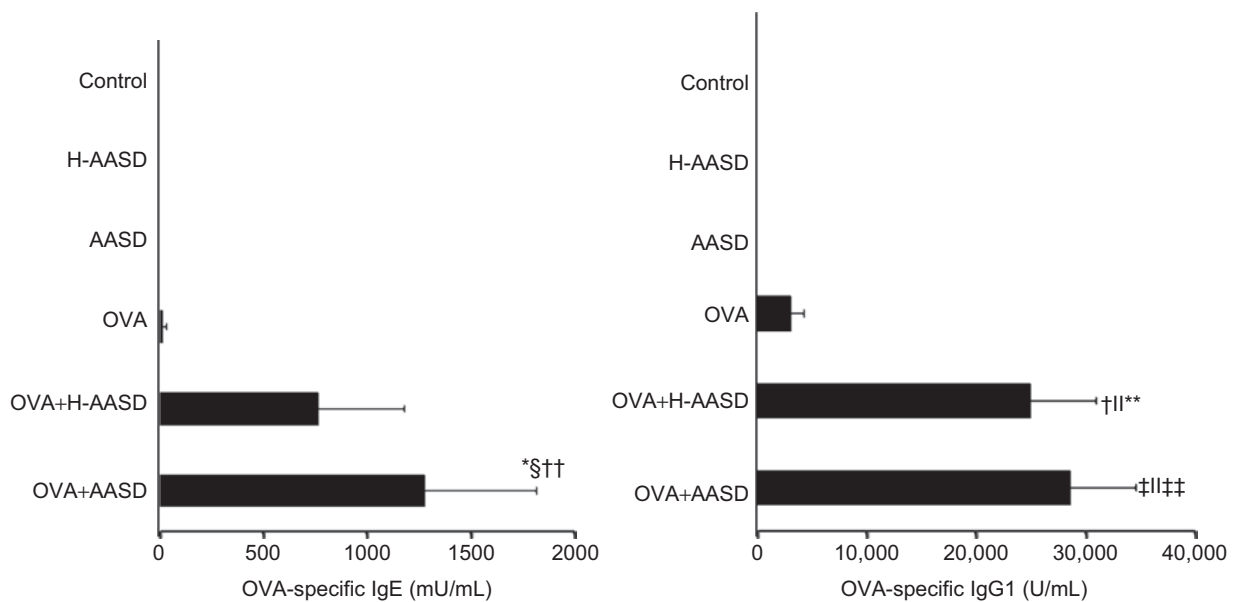


Figure 4. Effect of AASD (with or without heat treatment) on OVA-specific IgE and IgG1 production in serum. According to the manufacturer's protocol, 1 U of the anti-OVA IgE is defined as 1.3 ng of the antibody, and 1 U of the anti-OVA IgG1 as 160 ng of the antibody. Results are expressed as mean $\pm$ SE. * $p < 0.05$ compared Control, [†] $p < 0.01$ compared Control, [‡] $p < 0.001$ compared Control, [§] $p < 0.05$ compared OVA, ^{II} $p < 0.001$ compared OVA, ^{**} $p < 0.001$ compared H-AASD, ^{††} $p < 0.05$ compared AASD, ^{‡‡} $p < 0.001$ compared AASD.

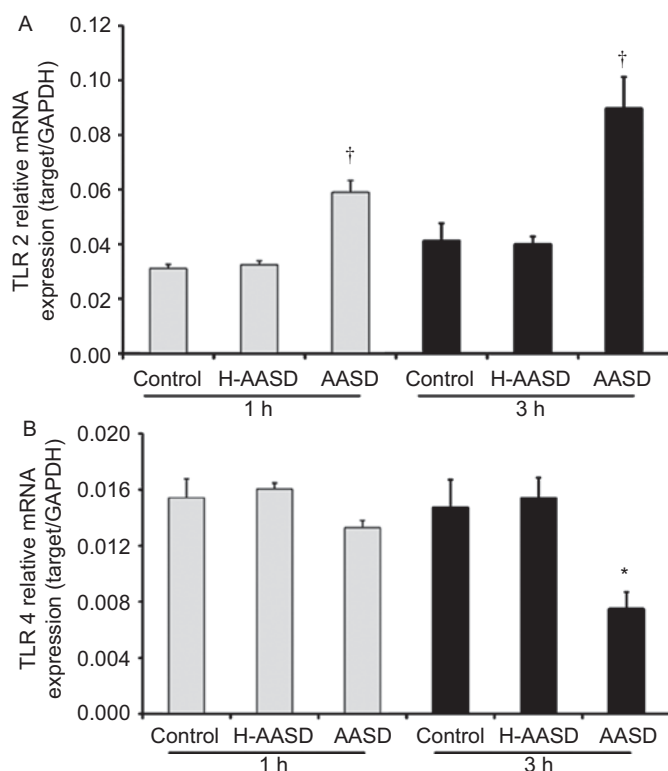


Figure 5. Effects of AASD (with or without heat treatment) on TLR2 and TLR4 mRNA (relative to GAPDH mRNA) in RAW264.7 mouse macrophage cells. RAW264.7 cells were plated at a concentration of 4×10^5 cells per 60-mm dish, and AASD with or without heating treatment was added to cells to give a final concentration of $3 \mu\text{g/ml}$. Cells were then incubated for 1 h and 3 h. Results are expressed as mean $\pm$ SE. * $p < 0.05$ compared to Control, † $p < 0.001$ compared to Control.

mRNA in RAW264.7 cells after 1 h and 3 h. H-AASD and AASD caused no change of TLR4 mRNA after 1 h. However, AASD significantly decreased TLR4 mRNA ($p < 0.001$) after 3 h compared with the control. In Figure 6 AASD significantly increased the expression of NALP3 ($p < 0.001$) after 1 h and 3 h, and in ASC after 3 h compared with the control. However, Caspase-1 exhibited no significant changes (Figure 7A). H-AASD caused no expression of either mRNA. AASD significantly increased the expression of IL-1 β mRNA after 1 h and 3 h compared with the control ($p < 0.001$) (Figure 7B). In Figure 8 LPS significantly increased the gene expression of IL-1 β ($p < 0.001$), NALP3 ($p < 0.05$) and caspase-1 ($p < 0.001$) after 3 h compared with the control. ASC tended to increase compared with the control.

Cytokine secretion from RAW264.7 cells by AASD

To evaluate secretion of pro-inflammatory cytokines from RAW264.7 cells by AASD treatment, the protein levels of IL-1 β , IL-6, and IL-18 in the nutrient medium were measured.

Control and H-AASD caused no expression of IL-1 β , IL-6 and IL-18 at any time points (data not shown). AASD significantly increased the expressions of IL-1 β at 24 h ($p < 0.001$), and in IL-6 at 12 h ($p < 0.05$) and 24 h ($p < 0.01$) compared with 0 h, but not IL-18 (Figure 9A). LPS significantly increased the expression of IL-1 β protein at 12 h and 24 h ($p < 0.001$), and

in IL-6 at 6 h, 12 h, and 24 h ($p < 0.001$) compared with 0 h (Figure 9B). LPS did not induce IL-18 at the all time points (Figure 8B). It is well known the RAW264.7 cell cannot induce IL-18 by LPS.

Discussion

Epidemiological studies have shown that ASD events are associated with respiratory problems, cardiovascular and an increase in daily mortality in Seoul, Korea (Yoo et al., 2008; Kwon et al., 2002), and Taipei, Taiwan (Chen et al., 2004; Chen and Yang, 2005). AASD contained sulfate (SO_4^{2-}), nitrate (NO_3^-) derived from air pollutants, and various microbial materials such as LPS and β -glucan. In our previous study we found that the amounts of SO_4^{2-} , NO_3^- , LPS, and β -glucan in ASD collected from surface soils in the Shapotou Desert located in north central China were $900 \mu\text{g/g}$, $500 \mu\text{g/g}$, 1.9 EU/mg , and 12.0 pg/mg , respectively (Ichinose et al., 2005). However, in the present study the amounts of SO_4^{2-} , NO_3^- , and β -glucan in AASD were higher than those of ASD (Table 3). On the other hand, the contents of mineral elements in the AASD used in this study were almost similar to the ASD used before (Table 2).

This study demonstrated that H-AASD alone, from which toxic materials (microbiological materials, SO_4^{2-} , etc.) had been excluded by heating, caused considerably less severe pathological changes. However, while crude AASD alone caused bronchitis and alveolitis, and clearly increased neutrophils along with its relevant chemokines MIP-1 α (Standiford et al., 1995), KC (Akahoshi et al., 1994), and RANTES (Pan et al., 2000), and Th1 relevant cytokines IL-12 (Hsieh et al., 1993) and IFN- γ (Adamthwaite and Cooley, 1994) in WLLF, H-AASD did not have the same effects. Neutrophilic lung inflammation accompanying these cytokines and chemokines was induced by LPS (Standiford et al., 1995) or β -glucan (1 $\rightarrow$ 3)-glucans (Young et al., 2001). The IL-6 and IL-1 β by treatment of crude AASD were detected in the present *in vivo* and *in vitro* studies. IL-6 is the myeloid differentiation factor (MyD88)-dependent cytokine released from antigen presenting cells (Takeda and Akira 2004). These pro-inflammatory cytokines are induced by LPS and β (1 $\rightarrow$ 3)-glucan (Wouters et al., 2002). LPS and β -glucan contained in crude AASD may contribute to the production of IL-6 and IL-1 β , and lead to subsequent lung inflammation *in vivo*.

Crude AASD enhanced allergic airway inflammation and the proliferation of goblet cells in the airway epithelium in OVA-stimulated mice, which was evidenced by cellular profiles of WLLF and histological examination. The exacerbated antigen-related airway inflammation with eosinophils was concomitant with amplified expression of pro-inflammatory molecules in WLLF as well as the production of antigen-specific antibodies. The enhancing effects were more remarkable in the OVA + AASD group than in the OVA + H-AASD group. OVA causes a slight proliferation of goblet cells and an infiltration of eosinophils and lymphocytes in the airway, which are the pathology correlatives seen in human asthma. The toxic-released mediators from eosinophils,

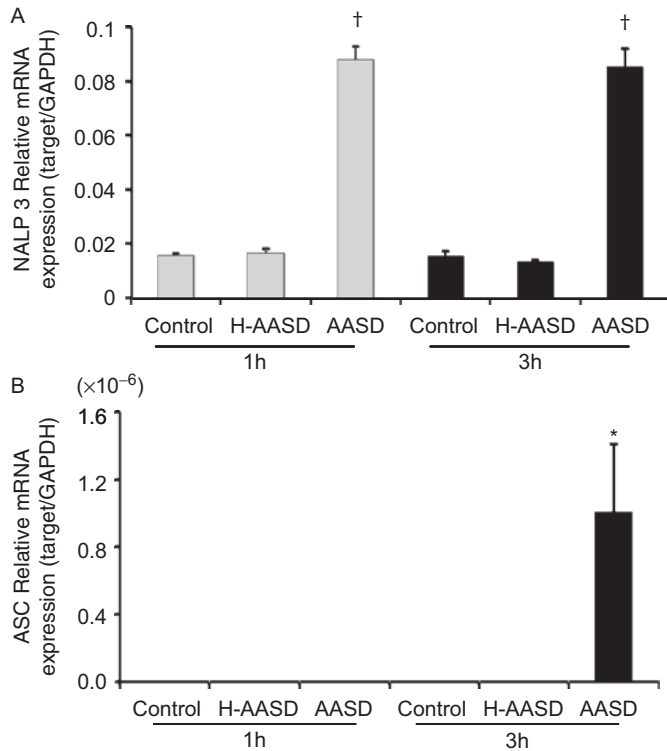


Figure 6. Effects of AASD (with or without heat treatment) on NALP3 and ASC mRNA (relative to GAPDH mRNA) in RAW264.7 mouse macrophage cells. RAW264.7 cells were plated at a concentration of 4×10^5 cells per 60-mm dish, and AASD with or without heating treatment was added to cells to give a final concentration of 3 $\mu\text{g}/\text{ml}$. Cells were then incubated for 1 h and 3 h. Results are expressed as mean $\pm$ SE. * $p < 0.05$ compared to Control, [†] $p < 0.001$ compared to Control.

such as platelet-activating factors, reactive oxygen radicals, leukotrienes, and major basic proteins, play an important role in inducing bronchial mucosal injury (Gleich, 1990). Eosinophils also contribute to bronchial smooth muscle cell death in asthma (Solarewicz-Madejek et al., 2009). The transformation of airway epithelial cells to goblet cells by OVA in the crude AASD group and the H-AASD group correlated with the amount of eosinophils introduced into the submucosa of the airway. Therefore, the injury to airway epithelial cells along with the proliferation of goblet cells may be induced by the activated eosinophils.

Eosinophils, Th2 lymphocytes and their released inflammatory mediators play a crucial role in human allergic asthma (Pearlman, 1999). H-AASD and AASD alone showed no increase of asthma-related mediators in WLLF. However, OVA + AASD especially caused synergistic increases of eosinophil-relevant cytokines and chemokines, such as IL-5 (Robinson et al., 1993; Foster et al., 1996), MCP-1 (Dunzendorfer et al., 2001), MCP-3 (Nagase et al., 1999), Eotaxin (Rothenberg et al., 1997), and Th2 cytokines IL-13 (D'Andrea et al., 1995) in WLLF, compared with H-AASD, AASD or OVA alone. The expressions of these cytokines and chemokines in OVA + AASD were much greater than those in OVA + H-AASD, suggesting that organic substances, such as microbial materials or chemical substances, excluded by heating are at least partly related to the enhancement of eosinophil recruitment

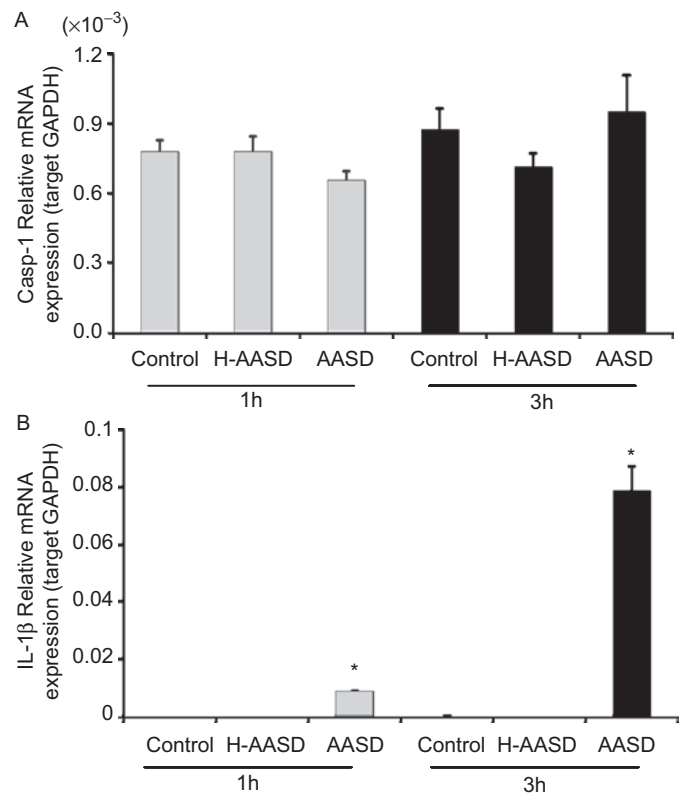


Figure 7. Effects of AASD (with or without heat treatment) on caspase-1 and IL-1 β mRNA (relative to GAPDH mRNA) in RAW264.7 mouse macrophage cells. RAW264.7 cells were plated at a concentration of 4×10^5 cells per 60-mm dish, and AASD with or without heating treatment was added to cells to give a final concentration of 3 $\mu\text{g}/\text{ml}$. Cells were then incubated for 1 h and 3 h. Results are expressed as mean $\pm$ SE. The asterisk indicates significance at $p < 0.001$ compared to Control.

via these chemical mediators in the alveoli and airway. Thus, the changes of the biological components in WLLF were consistent with the pathological changes in the lungs.

Several epidemiologic studies have reported that the levels of IgG and IgE antibodies related to inhaled allergens increased significantly both in children and adults with asthma (Soliman and Rosenstreich, 1986; Rizzo et al., 1993). Allergen-specific antibodies may contribute to the occurrence of inflammatory cell accumulation after an allergen challenge via IgE-mediated degranulation of mast cells (Zweiman, 1993). A chemical mediator, such as histamine, released from mast cells by antigen-antibody reactions, induces epithelial permeability, edematous changes, and allergic inflammation (White, 1990). The IgE-activated mast cells can produce eosinophil-relevant cytokines IL-3 and IL-5 (Gordon et al., 1990). On the other hand, antigen-specific IgG1 antibody contributes to the occurrence of antigen-specific eosinophil degradation via Fc γ RII on the eosinophil surface (Kaneko et al., 1995). Furthermore, late asthmatic reactions are associated with IgG antibodies (Durham et al., 1984). In the present study, an adjuvant effect of AASD toward the OVA-specific IgE and IgG₁ production was detected. IL-4, IL-5, IL-6, IL-10, and IL-13 released from Th2 lymphocytes are well known to stimulate B cells to lead to production of these antibodies (Mosmann and Coffman, 1989;

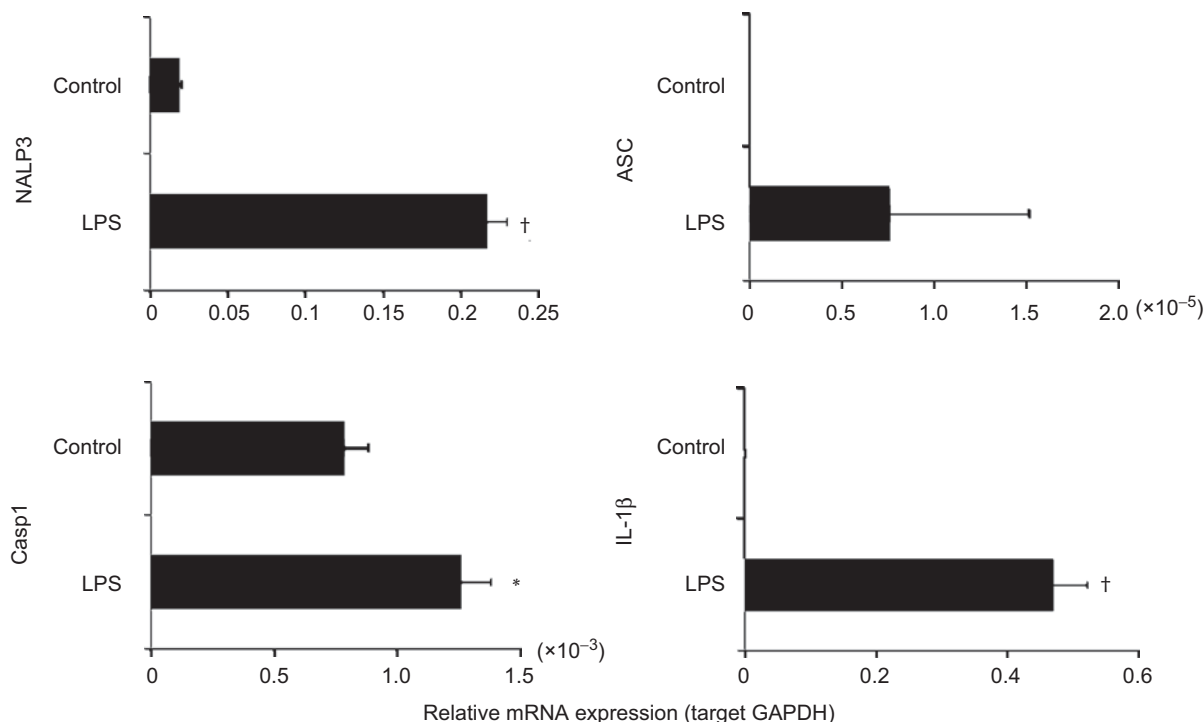


Figure 8. Effects of LPS on NALP3, ASC, caspase-1, and IL-1β mRNA (relative to GAPDH mRNA) in RAW264.7 mouse macrophage cells. Results are expressed as mean ± SE. * $p < 0.05$ compared to Control, † $p < 0.001$ compared to Control. RAW264.7 cells were plated at a concentration of 4×10^5 cells per 60-mm dish, and LPS was added to cells to give a final concentration of 100 ng/ml. Cells were then incubated for 3 h.

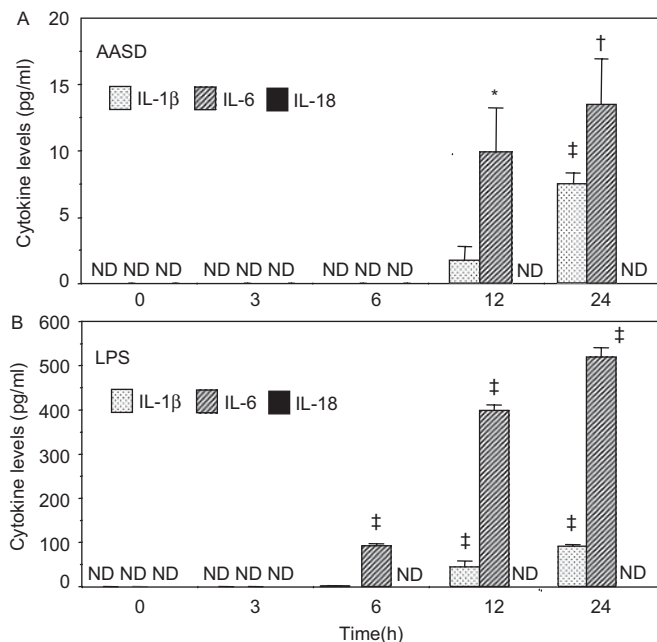


Figure 9. Cytokines (IL-1β, IL-6, and IL-18) secretion from RAW264.7 mouse macrophage cells by AASD and LPS treatment. For ELISA analysis of cytokine in nutrient medium, RAW264.7 cells were seeded at a concentration of 1×10^5 cells/ml in 96-well culture plate of 200 μl/well. Cells were then incubated with 3 μg/ml AASD with or without heating treatment or 100 ng/ml LPS for 0, 1, 3, 6, 12, and 24 h. Results are expressed as mean ± SE. * $p < 0.05$ compared to 0 h, † $p < 0.01$ compared to 0 h, ‡ $p < 0.001$ compared to 0 h. ND, not detectable. Control and H-AASD caused no expression of IL-1β, IL-6, and IL-18 at any time points (data not shown).

Kühn et al., 1991). In the present study, increased expressions of IL-4, IL-5, and IL-13 were detected in the OVA + AASD group compared to the OVA + H-AASD group, suggesting that increases of these cytokines may be related to the adjuvant activity of OVA-specific IgE and IgG₁ production, and these antibodies may play an important role in the aggravation of allergic inflammation caused by the AASD.

As one of the organic substances excluded by heating, a large amount of SO_4^{2-} derived from seawater and the sulfur oxide (SO_2) in air pollutants was contained in the crude AASD used in the present study. Previous reports have shown that either ASD or ASD + SO_4^{2-} enhanced eosinophils recruitment induced by OVA in the alveoli and in the submucosa of the airway. However, a further increase in eosinophils by the addition of SO_4^{2-} was not observed (Hiyoshi et al., 2005). We concluded that extra sulfate did not contribute to the increase of eosinophils in the airway and WLLF (Hiyoshi et al., 2005). Therefore, it seems that sulfate is not involved in the aggravating effects of lung eosinophilia and allergy diseases.

On the other hand, LPS is a major constituent of the outer cell wall of gram-negative bacteria and the predominant inducer of inflammatory responses to these bacteria (Beutler, 2002; Opal and Glück, 2003; Knapp et al., 2006). β-glucan is a unique component of the fungal cell wall (Hearn and Sietsma, 1994). The reduced effects of H-AASD on the pathological changes could be due to the exclusion of the toxic organic substances by heat treatment. Therefore, it seems that microbial materials adsorbed on AASD contribute to the development of bronchitis and alveolitis.

TLRs that belong to PRRs family have been the molecules involved in the recognition of pathogen-associated molecular patterns (PAMP) that lead to inflammatory process (Mariathasan and Monack, 2007). TLR2 is a receptor for zymosan (β -glucan), peptidoglycan of gram-positive bacteria (Schwandner et al., 1999) and TLR4 is a receptor for LPS (Beutler, 2002). TLR2-ligands can activate an OVA-associated Th2-biased immune response, such as the induction of Th2-associated effector molecules like IL-13, IL-5, and OVA-specific IgG₁ and IgE. TLR2-ligands can have an effect on the development of Th2-associated diseases such as experimental asthma (Redecke et al., 2004). The AASD tested in this study also increased the expression of TLR2 mRNA as well as ASD used in previous study (Ichinose et al., 2008b). Therefore, microbial materials such as β -glucan or peptidoglycan adsorbed onto AASD may contribute to the enhancement of lung eosinophilia related to allergen *via* the activation of TLR2.

MyD88 is a common TLRs adaptor molecule found to be essential for induction of adaptive Th1 immunity (Medzhitov et al., 1998; Schnare et al., 2001). Kaisho et al. (2002) have shown that LPS-stimulated MyD88-deficient (MyD88^{-/-}) dendritic cells (DC) lacked Th1 cell-supporting ability, but enhance instead Th2 cell-supporting ability and LPS-stimulated the wild-type (WT) DC augmented Th1 cell-supporting ability. On the other hand, LPS-stimulated TLR4-deficient (TLR4^{-/-}) DC did not enhance either Th1- or Th2-supporting ability. They suggested that the MyD88-dependent pathway through TLR4 is predominant over the MyD88-independent one in wild-type DC, but that the MyD88-independent pathway (like TIRAP/MAL) through TLR4 can confer on DC the ability to support Th2-immune responses. It has been reported that OVA exposure in the presence of high dose LPS fails to induce Th2 cells, but instead induces both IL-12 production and a Th1 response. However, a low dose of LPS is not sufficient to induce Th1 cells but is required to induce Th2 inflammation (Eisenbarth et al., 2008). Although the AASD caused no increase of TLR4 mRNA (Figure 5B) in this *in vitro* study, the AASD tested in this study contained small amounts of LPS. From these reports, we speculate that the low dose LPS signaling through TLR4 and MyD88-independent pathway in macrophages could be important in the enhancement of OVA-associated Th2-immune responses by AASD.

IL-18 (family member of IL-1), which is secreted from antigen presenting cells, enhances both Th1- and Th2-driven immune responses (Pollock et al., 2003). IL-18 can induce IL-4 production from T cells in the absence of IL-12 (Yoshimoto et al., 2000). LPS-stimulated MyD88-deficient (MyD88^{-/-}) Kupffer cells (KC) secrete IL-18 without IL-1 β and IL-12 production in caspase-1-dependent (Seki et al., 2001). Thus, IL-18 has an important role to lead to Th2-immune responses. In the present *in vivo* and *in vitro* studies, however, the expression of IL-18 was not detected. Therefore, the Th2-pathway through IL-18 may be not important in the enhancement of OVA-associated Th2-immune responses by AASD in this study.

On the other hand, other PRRs such as NLR could also be participating in the recognition of PAMP (Mariathasan and Monack, 2007). NALP3 of NLR family members is a cytoplasmic receptor, which is activated by microbial ligands like peptidoglycan as well as endogenous markers of cellular damage (for example ATP or uric acid crystals) (Mariathasan and Monack, 2007; Sutterwala, et al., 2007). The activated NALP3 interacts with adaptor proteins like ASC to form NALP3 inflammasome (Pétrielli et al., 2007). The inflammasome activates caspase-1 (Agostini et al., 2004). NALP3 inflammasome complex with the active caspase-1 can produce active IL-1 β with pro-IL-1 β (Fritz et al., 2006).

NALP3 inflammasome is a crucial element in the adjuvant effect of aluminium adjuvants (Eisenbarth et al., 2008). Crystalline silica (standard quartz DQ12 with particle size less than 5 microns) and amorphous silica also caused adjuvant effects toward the production of OVA-specific IgE and IgG₁ (Mancino and Ovary, 1980; Mancino et al., 1984), as do aluminium adjuvants. Crystalline silica also activates NALP3 inflammasome (Dostert et al., 2008). Moreover, amorphous silica aggravated OVA-induced lung eosinophilia (Ichinose et al., 2008a). Recent research has shown that silica and asbestos are sensed by NALP3 inflammasome, whose subsequent activation leads to IL-1 β production (Dostert et al., 2008).

In this *in vitro* study, AASD increased the mRNA expression of NALP3, ASC, and IL-1 β , but not caspase-1. However it seems that caspase-1 is activated, because increased mRNA expression of IL-1 β (Figure 7B) and IL-1 β secretion (Figure 9A) were detected by AASD treatment. H-AASD caused no expression of either mRNA, and no IL-1 β secretion from RAW264.7 cells, though the 60% SiO₂ was contained. This phenomenon is consistent with the previous *in vitro* study in which it was shown that silica induces no expression of IL-1 β in macrophages from WT mice. However, silica activates NALP3 inflammasome in the LPS-stimulated macrophages, as does aluminium adjuvants (Eisenbarth et al., 2008), and this leads to subsequent IL-1 β secretion (Cassel et al., 2008). Therefore we speculate that NALP3 inflammasome in RAW264.7 cells could be activated by LPS adsorbed on to AASD, and lead to the increased mRNA expression of IL-1 β and IL-1 β secretion. According to our results, LPS only can activate NALP3 inflammasome (Figure 8) and induce subsequent IL-1 β secretion (Figure 9B).

In this *in vivo* study, the increased expression of IL-1 β in WLLF was detected by H-AASD and AASD treatment. The increased expression of IL-1 β was much greater in AASD than in H-AASD. We speculate that the increased expression of IL-1 β in H-AASD could be due to activation of NALP3 inflammasome triggered by danger signals like reactive oxygen species, because the inflammasome activation reportedly is triggered by reactive oxygen species, which are generated by a NADPH oxidase upon silica particle phagocytosis (Dostert et al., 2008). However, the further increase of IL-1 β in AASD could be due to activation of NALP3 inflammasome triggered by pathogen-associated molecules like LPS or peptidoglycan adsorbed on to AASD together with reactive oxygen species.

The increased IL-1 β production by AASD may lead to subsequent inflammatory response as shown by significant increase in neutrophils (Figure 3).

The IL-1 β but not IL-1 α , also is required for antigen-specific T-cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses (Nambu et al., 2006). In the present study, therefore IL-1 β produced by AASD under the OVA existence activates Th2 cell, may lead to allergic inflammation along with Th2 relevant cytokines IL-5, IL-4, and IL-13 production, and subsequent IgE and IgG₁ production.

The microbial materials and silica contained in AASD are important in aggravating lung eosinophilia. However, our findings obtained in the present *in vivo* and *in vitro* study were not enough to explain the aggravating mechanism in lung eosinophilia by AASD. Recently, we separated eight kinds of microorganisms (bacillus and mold) from AASD. In future work, experimental studies to clarify whether these microbial materials adsorbed on to AASD actually enhances an OVA-associated Th2-immune response *via* TLRs, whether low dose LPS signaling through TLR4 and MyD88-independent pathway in macrophages is actually associated with the enhancement of OVA-induced Th2-immune responses by AASD, and whether the activation of NALP3 inflammasome by microbial materials separated from AASD causes and then leads to enhancement of Th2-immune response, are in order. To confirm the aggravating mechanism, it is also necessary to conduct studies on the comparative effects of AASD or microorganisms on TLR2-, TLR4-, MyD88-, ASC-, NALP3-, caspase-1-deficient mice and their WT mice.

Conclusions

This study demonstrates the aggravating effect of airborne ASD on antigen-induced lung eosinophilia. The activations of TLR2 and NALP3 inflammasome by AASD could be participating in this phenomenon. The airborne ASD contains biogenic particles such as bacteria, fungi, virus, pollen, cell debris, etc., and transports long distances together with mineral particles like silica. Camels living in Gansu Province (northwestern China) for 20 years showed silicosis in their lungs. Also, residents living in an area of high wind-sand incidence reportedly exhibited silicotic changes in their chest radiographs (Xu et al., 1993). Desert-dust exposure to 0.3156 mg/m³ for 6 h or 0.6845 mg/m³ for 4 h caused neutrophilic lung inflammation and injury in pulmonary hypertensive rats (Lei et al., 2004). These reports indicate that desert-dust clearly reaches human or animal lungs. The hazardous effects of bioaerosols (biogenic particles) and mineral dust on human respiratory disease are also becoming a public concern. Atmospheric exposure to fungi, bacteria, and silica-carrying particulate matters may affect human health directly through allergic induction of respiratory stress. The experimental findings in the present study may serve as a warning about the ill effects of airborne sand dust on the human respiratory system.

Acknowledgements

We appreciate the vital contribution of students at Oita University of Nursing and Health Sciences in this research.

Declaration of interest

This study was supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the Ministry of Environment in Japan. This work was partly supported by the Global Environment Research Fund (C-091) of the Ministry of Environment, Japan.

References

- Adamthwaite D, Cooley MA. 1994. CD8+ T-cell subsets defined by expression of CD45 isoforms differ in their capacity to produce IL-2, IFN- γ and TNF- β . *Immunology* 81:253–260.
- Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J. 2004. NALP3 forms an IL-1 β -processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 20:319–325.
- Akahoshi T, Endo H, Kondo H, Kashiwazaki S, Kasahara T, Mukaida N, Harada A, Matsushima K. 1994. Essential involvement of interleukin-8 in neutrophil recruitment in rabbits with acute experimental arthritis induced by lipopolysaccharide and interleukin-1. *Lymphokine Cytokine Res* 13:113–116.
- Beutler B. 2002. TLR4 as the mammalian endotoxin sensor. *Curr Top Microbiol Immunol* 270:109–120.
- Beutler B. 2004. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* 430:257–263.
- Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, Tephly LA, Carter AB, Rothman PB, Flavell RA, Sutterwala FS. 2008. The Nalp3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci USA* 105:9035–9040.
- Chang CC, Lee IM, Tsai SS, Yang CY. 2006. Correlation of Asian dust storm events with daily clinic visits for allergic rhinitis in Taipei, Taiwan. *J Toxicol Environ Health Part A* 69:229–235.
- Chen B, Hong C, Kan H. 2004. Exposures and health outcomes from outdoor air pollutants in China. *Toxicology* 198:291–300.
- Chen YS, Yang CY. 2005. Effects of Asian dust storm events on daily hospital admissions for cardiovascular disease in Taipei, Taiwan. *J Toxicol Environ Health Part A* 68:1457–1464.
- D'Andrea A, Ma X, Aste-Amezaga M, Paganin C, Trinchieri G. 1995. Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor α production. *J Exp Med* 181:537–546.
- Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. 2008. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320:674–677.
- Duce RA, Unni CK, Ray BJ, Prospero JM, Merrill JT. 1980. Long-range atmospheric transport of soil dust from Asia to the tropical north pacific: temporal variability. *Science* 209:1522–1524.
- Dunzendorfer S, Kaneider NC, Kaser A, Woell E, Frade JM, Mellado M, Martínez-Alonso C, Wiedermann CJ. 2001. Functional expression of chemokine receptor 2 by normal human eosinophils. *J Allergy Clin Immunol* 108:581–587.
- Durham SR, Lee TH, Cromwell O, Shaw RJ, Merrett TG, Merrett J, Cooper P, Kay AB. 1984. Immunologic studies in allergen-induced late-phase asthmatic reactions. *J Allergy Clin Immunol* 74:49–60.
- Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. 2008. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 453:1122–1126.
- Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 183:195–201.
- Gleich GJ. 1990. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 85:422–436.
- Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. 2006. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol* 7:1250–1257.
- Gleich GJ. 1990. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 85:422–436.

- Gordon JR, Burd PR, Galli SJ. 1990. Mast cells as a source of multifunctional cytokines. *Immunol Today* 11:458-464.
- He M, Ichinose T, Yoshida S, Nishikawa M, Mori I, Yanagisawa R, Takano H, Inoue K, Sun G, Shibamoto T. 2010. Urban particulate matter in Beijing, China, enhances allergen-induced murine lung eosinophilia. *Inhal Toxicol* 22:709-718.
- Hearn VM, Sietsma JH. 1994. Chemical and immunological analysis of the *Aspergillus fumigatus* cell wall. *Microbiology (Reading, Engl)* 140 (Pt 4):789-795.
- Hiyoshi K, Ichinose T, Sadakane K, Takano H, Nishikawa M, Mori I, Yanagisawa R, Yoshida S, Kumagai Y, Tomura S, Shibamoto T. 2005. Asian sand dust enhances ovalbumin-induced eosinophil recruitment in the alveoli and airway of mice. *Environ Res* 99:361-368.
- Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. 1993. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260:547-549.
- Husar RB, Tratt DB, Schichtel BA, Falke SR, Li F, Jaffe D, Gasso S, Gill T, Laulainen NS, Lu F, Reheis MC, Chun Y, Westphal D, Holben BN, Gueymard C, McKendry I, Kuring N, Feldman GC, McClain C, Frouin RJ, Merrill J, DuBois D, Vigonola F, Sugimoto N, Malm WC. 2001. Asian dust events of April 1998. *J Geophys Res* 106:18316-18330.
- Ichinose T, Takano H, Miyabara Y, Yanagisawa R, Sagai M. 1997. Murine strain differences in allergic airway inflammation and immunoglobulin production by a combination of antigen and diesel exhaust particles. *Toxicology* 122:183-192.
- Ichinose T, Takano H, Sadakane K, Yanagisawa R, Kawazato H, Sagai M, Shibamoto T. 2003. Differences in airway-inflammation development by house dust mite and diesel exhaust inhalation among mouse strains. *Toxicol Appl Pharmacol* 187:29-37.
- Ichinose T, Nishikawa M, Takano H, Sera N, Sadakane K, Mori I, Yanagisawa R, Oda T, Tamura H, Hiyoshi K, Quan H, Tomura S, Shibamoto T. 2005. Pulmonary toxicity induced by intratracheal instillation of Asian yellow dust (Kosa) in mice. *Environ Toxicol Pharm* 20:48-56.
- Ichinose T, Sadakane K, Takano H, Yanagisawa R, Nishikawa M, Mori I, Kawazato H, Yasuda A, Hiyoshi K, Shibamoto T. 2006. Enhancement of mite allergen-induced eosinophil infiltration in the murine airway and local cytokine/chemokine expression by Asian sand dust. *J Toxicol Environ Health Part A* 69:1571-1585.
- Ichinose T, Yoshida S, Sadakane K, Takano H, Yanagisawa R, Inoue K, Nishikawa M, Mori I, Kawazato H, Yasuda A, Shibamoto T. 2008a. Effects of asian sand dust, Arizona sand dust, amorphous silica and aluminum oxide on allergic inflammation in the murine lung. *Inhal Toxicol* 20:685-694.
- Ichinose T, Yoshida S, Hiyoshi K, Sadakane K, Takano H, Nishikawa M, Mori I, Yanagisawa R, Kawazato H, Yasuda A, Shibamoto T. 2008b. The effects of microbial materials adhered to Asian sand dust on allergic lung inflammation. *Arch Environ Contam Toxicol* 55:348-357.
- Ichinose T, Hiyoshi K, Yoshida S, Takano H, Inoue K, Nishikawa M, Mori I, Kawazato H, Yasuda A, Shibamoto T. 2009. Asian sand dust aggravates allergic rhinitis in guinea pigs induced by Japanese cedar pollen. *Inhal Toxicol* 21:985-993.
- Kaisho T, Hoshino K, Iwabe T, Takeuchi O, Yasui T, Akira S. 2002. Endotoxin can induce MyD88-deficient dendritic cells to support T(h)2 cell differentiation. *Int Immunol* 14:695-700.
- Kanatani KT, Ito I, Al-Delaimy WK, Adachi Y, Mathews WC, Ramsdell JW; Toyama Asian Desert Dust and Asthma Study Team. 2010. Desert-dust Exposure is Associated with Increased Risk of Asthma Hospitalization in Children. *Am J Respir Crit Care Med* [Epub ahead of print].
- Kaneko M, Swanson MC, Gleich GJ, Kita H. 1995. Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. *J Clin Invest* 95:2813-2821.
- Kim BG, Han JS, Park SU. 2001. Transport SO₂ and aerosol over the Yellow Sea. *Atmos Environ* 35:727-737.
- Knapp S, Florquin S, Golenbock DT, van der Poll T. 2006. Pulmonary lipopolysaccharide (LPS)-binding protein inhibits the LPS-induced lung inflammation *in vivo*. *J Immunol* 176:3189-3195.
- Kühn R, Rajewsky K, Müller W. 1991. Generation and analysis of interleukin-4 deficient mice. *Science* 254:707-710.
- Kwon HJ, Cho SH, Chun Y, Lagarde F, Pershagen G. 2002. Effects of the Asian dust events on daily mortality in Seoul, Korea. *Environ Res* 90:1-5.
- Lei YC, Chan CC, Wang PY, Lee CT, Cheng TJ. 2004. Effects of Asian dust event particles on inflammation markers in peripheral blood and bronchoalveolar lavage in pulmonary hypertensive rats. *Environ Res* 95:71-76.
- Mancino D, Ovary Z. 1980. Adjuvant effects of amorphous silica and of aluminum hydroxide on IgE and IgG1 antibody production in different inbred mouse strains. *Int Arch Allergy Appl Immunol* 61:253-258.
- Mancino D, Vuotto ML, Minucci M. 1984. Effects of a crystalline silica on antibody production to T-dependent and T-independent antigens in Balb/c mice. *Int Arch Allergy Appl Immunol* 73:10-13.
- Mariathasan S, Monack DM. 2007. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat Rev Immunol* 7:31-40.
- Mayor A, Martinon F, De Smedt T, Pétrilli V, Tschopp J. 2007. A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol* 8:497-503.
- Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CA Jr. 1998. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 2:253-258.
- Mori I, Nishikawa M, Tanimura T, Quan H. 2003. Change in size distribution and chemical composition of Kosa (Asian dust) aerosol during long-range transport. *Atmospheric Environment* 37:4253-4263.
- Mosmann, Coffman RL. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 7:145-173.
- Nambu A, Nakae S, Iwakura Y. 2006. IL-1beta, but not IL-1alpha, is required for antigen-specific T cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses. *Int Immunol* 18:701-712.
- Nagase H, Yamaguchi M, Jibiki S, Yamada H, Ohta K, Kawasaki H, Yoshie O, Yamamoto K, Morita Y, Hirai K. 1999. Eosinophil chemotaxis by chemokines: a study by a simple photometric assay. *Allergy* 54:944-950.
- Nishiyama Y, Aoki Y, Yamaguchi H. 1995. Morphological aspects of cell wall formation during protoplast regeneration in *Candida albicans*. *J Electron Microsc* (Tokyo) 44:72-78.
- Opal SM, Glück T. 2003. Endotoxin as a drug target. *Crit Care Med* 31:S57-S64.
- Pan ZZ, Parkyn L, Ray A, Ray P. 2000. Inducible lung-specific expression of RANTES: preferential recruitment of neutrophils. *Am J Physiol Lung Cell Mol Physiol* 279:L658-L666.
- Pearlman DS. 1999. Pathophysiology of the inflammatory response. *J Allergy Clin Immunol* 104:S132-S137.
- Pétrilli V, Dostert C, Muruve DA, Tschopp J. 2007. The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol* 19:615-622.
- Pollock KG, Conacher M, Wei XQ, Alexander J, Brewer JM. 2003. Interleukin-18 plays a role in both the alum-induced T helper 2 response and the T helper 1 response induced by alum-adsorbed interleukin-12. *Immunology* 108:137-143.
- Redecke V, Häcker H, Datta SK, Fermin A, Pitha PM, Broide DH, Raz E. 2004. Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* 172:2739-2743.
- Rizzo MC, Arruda LK, Chapman MD, Fernandez-Caldas E, Baggio D, Platts-Mills TA, Naspietz CK. 1993. IgG and IgE antibody responses to dust mite allergens among children with asthma in Brazil. *Ann Allergy* 71:152-158.
- Robinson DS, Ying S, Bentley AM, Meng Q, North J, Durham SR, Kay AB, Hamid Q. 1993. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 92:397-403.
- Rothenberg ME, MacLean JA, Pearlman E, Luster AD, Leder P. 1997. Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J Exp Med* 185:785-790.
- Sato T. 2009. The study on the Change of Symptoms in Allergic Rhinitis during Asian Sand Dust Phenomenon. Jibi Ryensyou (in Japanese) 102:831-839.
- Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. 2001. Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2:947-950.
- Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* 274:17406-17409.
- Seki E, Tsutsui H, Nakano H, Tsuji N, Hoshino K, Adachi O, Adachi K, Futatsugi S, Kuida K, Takeuchi O, Okamura H, Fujimoto J, Akira S, Nakanishi K. 2001. Lipopolysaccharide-induced IL-18 secretion from murine Kupffer cells independently of myeloid differentiation factor 88 that is critically involved in induction of production of IL-12 and IL-1beta. *J Immunol* 166:2651-2657.
- Solarewicz-Madejek K, Basinski TM, Cramer R, Akdis M, Akkaya A, Blaser K, Rabe KE, Akdis CA, Jutel M. 2009. T cells and eosinophils in bronchial smooth muscle cell death in asthma. *Clin Exp Allergy* 39:845-855.

- Soliman MY, Rosenstreich DL. 1986. Natural immunity to dust mites in adults with chronic asthma. I. Mite-specific serum IgG and IgE. *Am Rev Respir Dis* 134:962-968.
- Standiford TJ, Kunkel SL, Lukacs NW, Greenberger MJ, Danforth JM, Kunkel RG, Strieter RM. 1995. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. *J Immunol* 155:1515-1524.
- Sutterwala FS, Ogura Y, Flavell RA. 2007. The inflammasome in pathogen recognition and inflammation. *J Leukoc Biol* 82:259-264.
- Takeda K, Akira S. 2004. TLR signaling pathways. *Semin Immunol* 16:3-9.
- White MV. 1990. The role of histamine in allergic diseases. *J Allergy Clin Immunol* 86:599-605.
- Wouters IM, Douwes J, Thorne PS, Heederik D, Doekes G. 2002. Inter- and intraindividual variation of endotoxin- and beta(1 -> 3)-glucan-induced cytokine responses in a whole blood assay. *Toxicol Ind Health* 18:15-27.
- Xu XZ, Cai XG, Men XS. 1993. [A primitive study on the histological changes in the lungs of residents in the wind-sand area of Gansu Province]. *Zhonghua Jie He He Hu Xi Za Zhi* 16:202-4, 251.
- Yang CY, Tsai SS, Chang CC, Ho SC. 2005. Effects of Asian dust storm events on daily admissions for asthma in Taipei, Taiwan. *Inhal Toxicol* 17:817-821.
- Yang CY. 2006. Effects of Asian dust storm events on daily clinical visits for conjunctivitis in Taipei, Taiwan. *J Toxicol Environ Health Part A* 69:1673-1680.
- Yoshida S, Yoshida M, Sugawara I, Takeda K. 2006. Mice strain differences in effects of fetal exposure to diesel exhaust gas on male gonadal differentiation. *Environ Sci* 13:117-123.
- Yoshimoto T, Mizutani H, Tsutsui H, Noben-Trauth N, Yamanaka K, Tanaka M, Izumi S, Okamura H, Paul WE, Nakanishi K. 2000. IL-18 induction of IgE: dependence on CD4+ T cells, IL-4 and STAT6. *Nat Immunol* 1:132-137.
- Young SH, Robinson VA, Barger M, Porter DW, Frazer DG, Castranova V. 2001. Acute inflammation and recovery in rats after intratracheal instillation of a 1->3-beta-glucan (zymosan A). *J Toxicol Environ Health Part A* 64:311-325.
- Yoo Y, Choung JT, Yu J, Kim do K, Koh YY. 2008. Acute effects of Asian dust events on respiratory symptoms and peak expiratory flow in children with mild asthma. *J Korean Med Sci* 23:66-71.
- Zweiman B. 1993. The late-phase reaction: role of IgE, its receptor and cytokines. *Curr Opin Immunol* 5:950-955.