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Effects of two Asian sand dusts transported from the dust source regions of Inner Mongolia and northeast China on murine lung eosinophilia

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ABSTRACT

The quality and quantity of toxic materials adsorbed onto Asian sand dust (ASD) are different based on dust source regions and passage routes. The aggravating effects of two ASDs (ASD1 and ASD2) transported from the source regions of Inner Mongolia and northeast China on lung eosinophilia were compared to clarify the role of toxic materials in ASD. The ASDs contained different amounts of lipopolysaccharides (LPS) and β-glucan (ASD1 < ASD2) and SiO_2 (ASD1 > ASD2). CD-1 mice were instilled intratracheally with ASD1, ASD2 and/or ovalbumin (OVA) four times at 2-week intervals. ASD1 and ASD2 enhanced eosinophil recruitment induced by OVA in the submucosa of the airway, with goblet cell proliferation in the bronchial epithelium. ASD1 and ASD2 synergistically increased OVA-induced eosinophil-relevant cytokines interleukin-5 (IL-5), IL-13 (ASD1 < ASD2) and chemokine eotaxin (ASD1 > ASD2) in bronchoalveolar lavage fluid. ASD2 aggravating effects on lung eosinophilia were greater than ASD1. The role of LPS and β-glucan in ASD2 on the production of pro-inflammatory mediators was assessed using in vitro bone marrow-derived macrophages (BMDMs) from wild type, Toll-like receptor 2-deficient (TLR2 −/−), TLR4 −/−, and MyD88 −/− mice (on Balb/c background). ASD2-stimulated TLR2 −/− BMDMs enhanced IL-6, IL-12, TNF-α, MCP-1 and MIP-1α secretion compared with ASD2-stimulated TLR4 −/− BMDMs. Protein expression from ASD2-stimulated MyD88 −/− BMDM were very low or undetectable. The in vitro results indicate that lung eosinophilia caused by ASD is TLR4 dependent. Therefore, the aggravation of OVA-related lung eosinophilia by ASD may be dependent on toxic substances derived from microbes, such as LPS, rather than SiO_2.

Introduction

Asian sand dust (ASD) storms arise from the dry and semi-arid areas of southern Mongolia and northeast China, and ASD is transported to East China, the Korean Peninsula, and Japan as well as across the Pacific Ocean to the United States (Duce et al., 1980; Husar et al., 2001; Kim et al., 2001).

A major public concern of ASD is its potential hazardous effects on respiratory diseases in Eastern Asian countries. Recently ASD attracted attention as an aerosol affecting patients with allergies. Epidemiologic studies demonstrated that ASD events coincided with an increase in child and adult asthma (Kanatani et al., 2010; Watanabe et al., 2011), allergic rhinitis (Chang et al., 2006; Sato, 2009), and an increase of hospitalization for pneumonia (Meng and Lu, 2007). We previously demonstrated that virgin desert ASD and wind-borne ASD aggravated antigen-related lung eosinophilia in a murine model of asthma (He et al., 2010; Ichinose et al., 2008). The characteristics of ASD can change during its long-range transport. For example, ASD passing through an industrial area contains large amounts of by-product materials derived from air-pollutants including polycyclic aromatic hydrocarbons (PAHs), sulfates (SO_4^{2-}), and nitrates (NO_3^-) (Mori et al., 2003). Microbe species and the amounts of these toxic materials adsorbed onto ASD differ in each Asian dust event (Lee et al., 2009). The quality and quantity of these components adsorbed onto ASD are different based on dust source regions and passage routes.

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Therefore, it is important to perform a comparative study on the respiratory health effects of ASD with different components.

An Asian dust event occurred during the period from April 28th to May 4th 2011, and a second dust event occurred during the period from May 11th to May 14th 2011, in East Asia. The first dust event was large and very severe and originated from the Badanjilin desert in Inner Mongolia, China. The second dust event was middle-scale and originated from the Hunshandake desert in northeast China (Global Environment Issues Division, 2013). We collected ASD using a high-volume air sampler from the atmosphere in Kitakyushu, Fukuoka, Japan for each 3-day dust storm event.

In this study, the aggravating effects of the two kinds of windborne ASDs on OVA-induced lung eosinophilia were compared to clarify the role of toxic materials adsorbed onto ASD. Pathologic changes in airway, cytological alterations in bronchoalveolar lavage fluids (BALF), and changes of inflammatory cytokines and chemokines in BALF were investigated in CD-1 mice.

Toll-like receptors (TLRs) are pathogen-associated recognition receptors that recognize bacteria, fungi and virus structures. For example, TLR2 is a receptor for β-glucan or peptidoglycan of Gram-positive bacteria (Schwandner et al., 1999) and TLR4 is a receptor for lipopolysaccharide (LPS) (Beutler, 2004). Myeloid differentiation factor 88 (MyD88), a downstream signaling adapter molecule, is a cytoplasmic TLR-associated adapter protein and essential for cytokine production in response to TLR ligands including LPS or β-glucan (Kaisho et al., 2002; Schnare et al., 2001). The cytokine production from LPS-stimulated MyD88 −/− antigen presenting cells is blocked. Therefore, the MyD88 −/− cells are predominantly used in order to investigate the function of the TLR2 or TLR4/MyD88 signaling pathway to induce pro-inflammatory cytokines (Brown et al., 2011). An in vitro study was performed to clarify the role of LPS and β-glucan in ASD on the production of pro-inflammatory molecules using bone marrow-derived macrophages (BMDMs) from wild-type (WT) TLR2-deficient (TLR2 −/−), TLR4 −/− and MyD88 −/− BALB/c mice. Cytokines and chemokines secreted into culture medium from BMDMs treated with ASD were measured.

Materials and methods

Animals. A total of 96 male CD-1 mice (5 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Mice were checked for abnormal body weight or sickness for 1 week, and then 96 mice were used at 6 weeks of age. Homozygous TLR2, TLR4 and MyD88 knockout (KO) mice and WT mice (BALB/c parental strain, males) were purchased from Oriental BioService Japan, Inc. (Kyoto, Japan). They were fed a commercial diet CE-2 (CLEA Japan, Inc., Tokyo) and given water ad libitum. Mice were housed in plastic cages lined with soft wood chips. The cages were placed in an air conditioned room at 23 °C with 55–70% humidity and a light/dark (12 h/12 h) cycle. CD-1 male mice were used because of their moderate responsiveness to airway inflammation caused by OVA (Ichinose et al., 2003). 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.

Asian sand dust particles. The present study used two kinds of ASD collected using a high-volume air sampler (Sibata Scientific Technology, Japan) with a Teflon filter from the atmosphere at the University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan on 1–3 May, 2011 (ASD1) and 12–14 May, 2011 (ASD2), after massive dust storm events in East Asia. When ASD1 and ASD2 were collected, the humidity was 84–43% and 31–36%, respectively and the temperature was 19.6–21 °C and 18–21 °C, respectively. The density ranges of the ambient particulate matter by LIDAR (Light Detection And Ranging) on 1–3 May and 12–14 May was 350–550 μg/m^3 and 150–250 μg/m^3 in Nagasaki (Nagasaki Prefectural Institute of Public Health and Environmental Science), Japan. The collection flow was 770 l/min and the instrumental classification size (defined as the 50% cut-off size of the aerodynamic diameter) was 1st stage (5.9 μm), 2nd stage (2.8 μm), 3rd stage (1.7 μm), 4th stage (0.91 μm) and back-up (<0.91 μm), respectively. The top peak in the mass size distribution for ASD1 and ASD2 existed and ranged from 1.7 to 2.8 μm, respectively, although the peak profile of ASD2 was clearly sharper than that of ASD1. The size distribution peak of ASD1 and ASD2 was at 3.8 and 2.3 μm, respectively. Particles were removed from each stage Teflon filter except a backup filter using a sterilized stainless steel spatula. The particle weight collected from each Teflon filter was measured. Then they were pooled to one sterilized dry bottle. The collected ASD (multiple sample) was stored at −30 °C in a germ free case with a desiccant until use for all the experiments and analysis.

The concentration of each element (Si, Na, Mg, Al, P, Ca, Ti, Cr, Mn, Fe, Cu, Ba and Sr) was determined by inductively-coupled plasma atomic emission spectroscopy (ICP-AES, 61E Trace and ICP-750, Thermo Jarrell-Ash, MA) after acid digestion with mixed acids (68% nitric, 38% hydrofluoric, and 70% perchloric = 5:1:1) was performed a sample at 180 °C for 3 h.

Analysis of water soluble components, LPS, β-glucan and polyaromatic compounds 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. In brief, approximately 5 mg of ASD1 or ASD2 was suspended in 1 ml water (LPS and β-glucan free; Seikagaku Corp., Tokyo, Japan) for 1 h and placed on the bench top at room temperature for 2 h. Supernatants then were recovered and tested for LPS and β-glucan concentrations using Pyro Color-MP: Chromogenic Diazo-Coupling Kit (Associates of Cape Cod. Inc., MA, USA) for endpoint-colorimetry. The detection limits for LPS and β-glucan were 0.001 EU/ml and 2 pg/ml, respectively.

Analysis of polycyclic aromatic hydrocarbons (PAHs) in particles. The Teflon filter collected ASD was cut into 1/8 of all filters for analysis of polycyclic aromatic hydrocarbons (PAHs). Nine PAHs were identified including strongly carcinogenic Benzo(a)pyrene. The analysis of PAHs in filter samples was performed using ultrasonic extraction with dichloromethane and high-performance liquid chromatography (Hitachi 600, Japan) equipped with a fluorescence spectrophotometer. PAHs from the cut filter were extracted with 20 ml of dichloromethane for 15 min at 15 °C using ultrasonic extraction. This operation was repeated twice. The extracted solution was filtered with NO 5 filter paper, evaporated using natural drying in the dark, and the residue was dissolved in 0.5 ml of acetonitrile. This solution was used for the determination of PAHs by HPLC.

Study protocol. A total of 96 male CD-1 mice were divided into six groups (n = 16 per group) according to the treatment with particles: normal saline (control), ASD1, ASD2, OVA, OVA + ASD1, and OVA + ASD2. ASD 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 cooling conditions. OVA was dissolved in saline. The instillation dose of ASD was 0.1 mg per mouse and the treatment dose of OVA was 1 μg per mouse (He et al., 2010, 2013; Ichinose et al., 2008). Mice were intratracheally instilled with particles through a polyethylene tube under anesthesia with 4% halothane (Takeda Chemical, Osaka, Japan). One day after the last intratracheal administration, mice from all groups (age = 12 weeks) were euthanized by exsanguination under deep anesthesia by intraperitoneal injection of pentobarbital.