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LPS priming in early life decreases antigen uptake of dendritic cells via NO production

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ABSTRACT

Immunological mechanisms of hygiene hypothesis are expected to develop a novel strategy for allergy prevention. Although a large number of studies has investigated the relation between allergies and infection, little is known about the influence of the exposure to infections on antigen uptake by dendritic cells (DCs). In this study, we examined the effect of lipopolysaccharide (LPS) priming in early life on the antigen uptake ability of DCs by using an original mouse model. LPS priming in juvenile mice decreased the migration of antigen-capturing $CD11c^+$ cells in the lymph nodes, but not in aged mice. Besides, the bone marrow-derived DCs (BMDCs) from juvenile LPS-primed mice had the poor antigen uptake ability, and constitutively produced NO through the inducible nitric oxide synthase (iNOS). Interestingly, the LPS priming-induced poor antigen uptake of BMDCs was mimicked by the NO donor, and recovered by the iNOS inhibitor. Additionally, LPS priming in juvenile mice prevented the allergic reactions, but not in aged mice.

Our results suggested that an exposure to infections in early life prevents allergy through the alteration of the BM cells fate that is to induce the differentiation of BM cells into inhibitory DCs such as NO-producing DCs.

1. Introduction

The number of patients suffering from allergies has been increasing in recent years; this is often explained by the hygiene hypothesis (Strachan, 1989), which postulates that exposure to infections in early life helps prevent allergic diseases. The mechanisms most accepted by consensus to underlie the hygiene hypothesis involve a disruption of the balance of T helper cells (Gao et al., 2011; Kramer et al., 2013; Le Bert et al., 2011; Versini et al., 2015) and an alteration in the function of antigen-presenting cells (APCs), influencing T cell development (Kuo et al., 2013; Wang et al., 2015, 2016; Yang and Gao, 2011). The most potent APCs are dendritic cells (DCs), which mature into professional APCs through the uptake of antigens. Immature DCs exhibit high antigen uptake ability, while mature DCs are characterized by poor uptake of antigens and high expression of co-stimulator molecules such as MHCII, CD80, and CD86 (Banchereau and Steinman, 1998). Previous studies have indicated that the blockade of antigen presentation by DCs in the challenge phase prevents allergic responses (Wang et al., 2016).

Nitric oxide (NO) is a small autocrine and paracrine signaling molecule, which prevents the decrease in the antigen uptake ability of mature DCs (Paolucci et al., 2000) and inhibits antigen presentation by these cells (Lee et al., 2011; Lu et al., 1996). NO is produced by DCs via the action of nitric oxide synthase (NOS), which has three isoforms-neuronal NOS, endothelial NOS, and inducible NOS (iNOS). The expression of iNOS is often used as a marker for DC subtype as this enzyme plays an important role in the function of these cells (Gabrilovich and Nagaraj, 2009; Serbina et al., 2003).

The activation of Toll-like receptors (TLRs), whose function involves pathogen recognition, influences the development of allergic diseases (Haapakoski et al., 2013; Lin et al., 2016; Sel et al., 2007). Lipopolysaccharide (LPS), a major component of gram-negative bacterial cell membranes, is recognized by TLR4, and LPS stimulation results in the downregulation of antigen uptake *in vitro* (Platt et al., 2010; Sallusto et al., 1995; Sallusto and Lanzavecchia, 1994; West et al., 2004) and *in vivo* (Wilson et al., 2006), inducing the development of the mature DC phenotype. Additionally, LPS is often used as an infectious stimulus: in

Abbreviations: APC, antigen presenting cell; BM, bone marrow; BSA, bovine serum albumin; CCR7, C C chemokine receptor type 7; CD, cluster of differentiation; DC, dendritic cell; F-BEADS, fluorescein isothiocyanate labeled latex beads; F-BSA, fluorescein isothiocyanate conjugated BSA; FBS, fetal bovine serum; FITC, luorescein isothiocyanate; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IgE, immunoglobulin E; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NO, nitric oxide; OVA, ovalbumin; TLR, toll-like-receptoe; TNF, tumor necrosis factor; iNOS, inducible nitric oxide synthase

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animal experiments, treatment with LPS before the sensitization phase results in weaker allergic reactions (Romagnani, 2004; Tulić et al., 2000; Velasco et al., 2005; Von Mutius et al., 2000). In contrast, it also is known that LPS enhances allergic reactions (Eisenbarth et al., 2002; Iwasaki et al., 2015; Kumari et al., 2015; Murakami et al., 2007; Tulić et al., 2000); therefore, the association between LPS and allergy is determined by timing, concentration, and dosing method (Gómez-Casado and Díaz-Perales, 2016).

Although elucidation of the immunological mechanisms underlying the hygiene hypothesis is expected to enable development of novel strategies for allergy prevention and these mechanisms have been the focus of study for a considerable period, the effects of exposure to infections during early life on DC antigen uptake ability has never been examined. Therefore, in this study, we investigated the effect of iNOS on the antigen uptake ability of bone marrow-derived DCs (BMDCs) by using an original mouse model, in which mice were primed with low LPS concentrations.

2. Material and methods

2.1. Mice

Male BALB/c mice were purchased from SLC (Shizuoka, Japan), and were maintained in accordance with procedures approved by the Animal Ethics Committee of Tohoku University, Sendai, Japan.

2.2. LPS priming and sensitization

To prime mice with LPS, juvenile (4 weeks old) and aged (10 weeks old) mice were injected intraperitoneally (i.p.) with LPS (1 μg/kg; Wako Pure Chemical, Osaka, Japan) in saline three times at 1-week intervals (Days 0, 7, and 14). As control, only saline was injected.

For sensitization, at 7 days after the previous LPS treatment (Day 21), mice were sensitized with 10 μg ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO, USA) precipitated with 4 mg of aluminum hydroxide (Alum; Wako Pure Chemical) in 200 μl of saline, i.p., two times at 1-week intervals.

2.3. Serum antibody production

OVA-specific serum IgE and total IgE levels were determined using the corresponding ELISA kit (Chondrex, Redmond, WA, USA).

2.4. Cell isolation and culture

BMDCs were isolated from LPS-primed mice and control mice, as described previously (Yamashita et al., 2013). To examine antigen uptake, cells were treated with 1 mg/mL FITC-labeled BSA (F-BSA), and stimulated with/without DEA NONOate (50 μM, Cayman Chemical, Ann Arbor, MI, USA) in culture medium (RPMI1640 with 10% FBS) for 3 h. Cells were pre-incubated with 1400W dihydrochloride (1400W, 100 μM, Wako Pure Chemical) for 30 min. To examine phagocytosis, cells were treated with 10 beads/cell fluorescence-labeled latex beads (F-BEADS, 1-μm diameter, Sigma-Aldrich) for 3 h. To examine the response to LPS, BMDCs were treated with LPS (10 ng/mL) for 24 h, and the supernatant and cell lysates were collected.

2.5. Flow cytometric analysis

To analyze antigen trafficking, draining lymph nodes were collected at the indicated time after F-BSA injection (1 mg in 200 μl of saline) i.p., and lymphocytes were isolated. To detect surface molecule expression, the following antibodies were used: FITC anti-mouse CD80, FITC anti-mouse CD86, FITC or PE anti-mouse CD11c, and FITC anti-mouse MHC Class II (TONBO Biosciences, San Diego, CA, USA). Data were acquired using FACSCalibur (BD Bioscience, San Diego, CA, USA) with the dedicated software CellQuest (BD Bioscience).

2.6. Western blots

Western blots were performed as described previously (Mizuno et al., 2012). Briefly, BMDC lysates were generated using the Laemmli sample buffer and denaturized. Samples were loaded on a 10% SDS-polyacrylamide gel for SDS-PAGE and transferred to a PVDF membrane; the membranes were blocked with Block Ace (DS Pharma Biomedical, Osaka, Japan). iNOS andα-tubulin were detected using an anti-iNOS antibody (1:1000, #2977, Cell Signaling Technology, Danvers, MA, USA) and anti-α-tubulin antibody (1:1000, sc-5386, Santa Cruz Biotechnology, Santa Cruz, CA, USA) as primary antibodies along with HRP-conjugated anti-rabbit IgG (1:2000, NA934 V, GE Healthcare Life Sciences, Buckinghamshire, England) and anti-mouse IgG (1:2000, #7076, Cell Signaling Technology) as secondary antibodies. Blots were detected with ECL™ Western blotting detection reagents. Western blot band corresponding to iNOS were quantified using ImageJ software (http://rsb.info.nih.gov/ij/index.html) and normalized against α-tubulin.

2.7. Measurement of NOx

NOx, nitrite and nitrate, as indexes of NO production, in the LPS-treated BMDC supernatant were determined using the Griess reagent system. Supernatants (100 μl) were incubated with 50 μl reagent 1 (1% sulfanilamide in 5% phosphoric acid) for 5 min. Furthermore, 50 μl reagent 2 (0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid) was added and incubated for 5 min. NOx concentrations were determined by measuring absorbance at 540 nm.

2.8. ELISA

Cytokine concentrations in the peritoneal lavage fluid collected on Day 21 with ice-cold PBS and in the BMDC culture supernatant were measured by ELISA kits specific for IL-1β (R & D Systems, Minneapolis, MN, USA), IL-6 (R & D Systems), TNFα (eBioscience, San Diego, CA, USA), and IL-10 (R & D Systems).

2.9. Statistical analysis

Data are presented as the mean ± the standard error of the mean (SEM). Statistical significance was analyzed using the unpaired Student's *t*-test or the Mann-Whitney *U* test, and was defined as a *P* value of less than 0.05.

3. Results

3.1. LPS priming in juvenile mice suppressed the trafficking of antigen-capturing $CD11c^+$ cells into draining lymph nodes

A mouse experimental model, in which mice were primed with LPS as the infectious stimulant. In order to determine whether LPS priming affects antigen-capturing cell migration to the lymph nodes, we performed a time-course experiment to study antigen trafficking using F-BSA as an antigen marker. Accumulation of F-BSA-capturing ($F\text{-}BSA^+$) cells in the lymph nodes of primed mice was significantly lower than that in control mice at 24 h post-injection (Fig. 1A). In particular, $CD11c^+$ $F\text{-}BSA^+$ cells in the lymph nodes decreased as a result of LPS priming (Fig. 1B, Juvenile). In contrast, decrease in $CD11c^+$ $F\text{-}BSA^+$ cells was not observed in aged mice (Fig. 1B, Aged). In addition, the LPS priming schedule in our model reduced the trafficking of $F\text{-}BSA^+$ cells into lymph nodes more effectively compared to that by single LPS priming of 4- or 6-week-old-mice (Fig. 1C).