

Effects of Lipopolysaccharide on T helper 2 Cytokine Expression in Adult Atopic Patients

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Abstract

While lipopolysaccharide (LPS) is known to have a preventive effect on allergic inflammation, little is known about its effect on T helper (Th) 2 cytokine expression in human. We investigated the effect of LPS on the expression of Th2 cytokine as well as toll-like receptor (TLR) 4 in peripheral blood mononuclear cells (PBMCs) of adult atopic patients. PBMCs isolated from adult atopic patients and non-atopic controls were incubated with allergen or LPS or both. The mRNA expression of IL-4, IL-5, IL-9, IL-10, IL-13 and TLR4 were measured by quantitative real-time PCR. Allergen stimulation induced the expression of Th2 cytokines in PBMCs of adult atopic patients, but not in non-atopic controls. LPS stimulation induced the expression of IL-5 and IL-10 in atopic adults, IL-5 and TLR4 in non-atopic controls. The expression of TLR4 to LPS was significantly lower in atopic adults than non-atopic controls. When PBMCs were stimulated with both allergen and LPS simultaneously, LPS didn't show the preventive effect on Th2 cytokine expression triggered by allergen in adult atopic patients. The low responsiveness to LPS compared to non-atopic controls may contribute to atopic mechanism in adult atopic patients, further evaluations are needed.

Key Words : Adult Atopy, LPS, Th2 cytokine expression, TLR4

Introduction

The 'hygiene hypothesis' is the idea that decreasing exposure to immunomodulatory products of bacteria is the cause of the development of atopy. Several epidemiologic studies have consistently shown a potential

association between exposure to bacterial product and decreased prevalence of atopic disorders [1-3].

Lipopolysaccharide (LPS), a major component of the cell wall of gram negative bacteria, has been suggested as a potential mediator of immunomodulatory effects [1-3].

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In several mouse model studies, administration of LPS before or short after allergen sensitization have been shown to suppress the development of allergen specific IgE, production of Th2 cytokine or infiltration of airway eosinophilia [4–6]. Similar findings were also found in human studies. In nasal explant model of atopic children, LPS was shown to have a preventive effect to allergen-induced inflammation, and this effect was provided by enhanced Th1 immune reaction by LPS [7]. Besides, LPS was shown to significantly upregulate the IL-10 expression, which was mediated by Toll-like receptor 4 (TLR4). In contrast to atopic children, the immunomodulatory effect of LPS was not observed in adult atopic patients in that study [7]. Although it was suggested that this difference was caused by the different responsiveness to LPS between atopic children and atopic adults, the underlying mechanism is unclear yet.

Accordingly, the aim of our study was to further investigate the LPS effect on allergic reaction in adult atopic patients, especially in the level of mRNA expression of Th2 cytokine. For this study, we separated peripheral blood mononuclear cells (PBMCs) from adult atopic patients and stimulated those cells with LPS, allergen or both. Then we analyzed mRNA expression of Th2 cytokine, such as IL-4, IL-5, IL-9, IL-10, and IL-13 as well as TLR4 in adult atopic patients.

Materials and Methods

Study Subjects

Twenty atopic adults (13 females and 7

males, mean age 32.9 ± 11.4 years) and seven non-atopic adults (3 female and 4 males, 39.8 ± 11.9 years) were included. Adult atopic individuals were identified on the basis of specific symptoms of allergic rhinitis or atopic dermatitis with positive result of skin prick test or serum allergen specific IgE antibody. All the atopic individuals were positive in skin prick test or had value above 3+ of specific IgE antibody against antigen *D. farinae* and/or *D. pteronyssinus*. Seven non-atopic adults who had no history of atopy and had negative for specific IgE antibody, especially for both *D. farinae* and *D. pteronyssinus* antigen were included. This study was approved by the institutional review board of Keimyung University Dongsan Hospital, and informed consents were taken from the all study subjects.

Cell preparation and culture

Venous blood was drawn into heparin treated tubes and PBMCs were separated with Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden) density gradient centrifugation. Separated PBMCs were cultured into four tubes in amount of 1×10^6 cells with 1 mL of RPMI 1640 medium (Invitrogen, Grand Island, New York, USA) supplemented with 10% fetal calf serum, L-glutamine and antibiotics containing penicillin and streptomycin. Each four tubes were incubated with: medium alone or 0.1 mg/L LPS (Sigma-Aldrich, Saint Louis, Missouri, USA) or 10 $\mu\text{g/mL}$ specific allergen of mixture of *D. farinae* and *D. pteronyssinus* (Bencard Allergie, Munich, Germany) or both allergen and LPS. The dose of LPS used (0.1 mg/L) was the optimum dose used in other previous study [7]. Then the tubes were incubated in

37°C CO₂ incubator (Forma Scientific Inc, Ohio, USA) for 3 days.

Reverse transcription and real time PCR

After 3 days of culture, cells were separated and RNA was extracted using High Pure RNA isolation kit (Roche, Penzberg, Germany) and 5 µg of RNA was converted to complementary DNA (cDNA) by reverse transcription (RT) PCR. For the relative quantification of cytokine expression, the real time PCR reactions were used. β -actin was used as an internal control. The master PCR reaction mixture contained 2 µl of 10X LightCycler® FastStart DNA master SYBR green I (Roche Molecular Biochemicals, Mannheim, Germany), 2.5 µl of MgCl₂ in final concentration of 4 mM, 10 pmol of each of sense and antisense primers, 2 µl of cDNA and distilled water in final volume of 20 µl. The reaction mixture was loaded into the capillary tubes and centrifuged to spin down before PCR. The quantitative real time PCR was performed using LightCycler® system (Roche Molecular Biochemical, Mannheim, Germany). The PCR thermal conditions and primers used are described in Table 1. The amount of cytokines was calculated relative to the amount of β -actin present in each sample and described as cytokine/ β -actin ratio. All the analysis was performed in duplicate.

Statistical analysis

The comparison of the mRNA expression of Th2 cytokine or TLR4 between atopic adults and non-atopic adults, or among cells of different stimulation status was analyzed using independent t-test. All the statistical analysis was performed with SPSS program (version 12.0, SPSS Inc, USA) and the *p*

value of < 0.05 was considered as statistically significant.

Results

In un-stimulated PBMCs cultured with medium alone, we could detect constitutive mRNA expression of Th2 (IL-4, 5, 9, 10, and 13) cytokines and TLR4 in both atopic and non-atopic adults. The expression level of Th2 cytokines and TLR4 was not statistically different between atopic and non-atopic adults (*p*>0.05).

After stimulation with allergen alone, the mRNA expression of Th2 cytokine but not TLR4 was significantly increased compared to un-stimulated PBMCs in atopic adults. In non-atopic adults, the mRNA expression of Th2 cytokines was not increased after stimulation with allergen alone. However, the mRNA expression of the TLR4 was significantly increased compared with un-stimulated PBMCs in non-atopic adults (*p*<0.05) (Fig.1).

Stimulation of PBMCs with LPS alone induced increment of the mRNA expression of IL-5 and IL-10 in atopic adults, IL-5 and TLR4 in non-atopic adults. When the increasing level of IL-10 and TLR4 expression was compared between atopic and non-atopic adults, the TLR4 expression was significantly lower in atopic adults than non-atopic adults, but not IL-10 (Fig.2).

When PBMCs were stimulated with both allergen and LPS simultaneously, the Th2 cytokine expression was shown increasing pattern compared to those after stimulation with allergen alone. However, statistically significant increment was not found, except

Table 1. Sequences of Primers Used in Real-time PCR

Targets	Primer sequences	Amplicon size (bps)	Amplification condition*
β -actin	for 5'-AGATCAAGATCATTGCTCCTCCTG-3'	145	95°C 10 sec
	rev 5'-CATTTGCGGTGGACGATGGA-3'		66°C 5 sec
IL-4	for 5'-CGAGTTGACCGTAACAGACAT-3'	281	72°C 6 sec
	rev 5'-CGTCTTTAGCCTTTCCAAGAAG-3'		95°C 10 sec
IL-5	for 5'-GACTGGTGGCAGAGACCTTGA-3'	158	56°C 5 sec
	rev 5'-CGTGGGCAGTTTGTTCTTC-3'		72°C 13 sec
IL-9	for 5'-GATCCAGCTTCCAAGTGCCA-3'	76	95°C 10 sec
	rev 5'-TGCAGTTGTCAGAGGGAATGC-3'		53°C 5 sec
IL-10	for 5'-GCTGGAGGACTTTAAGGGTTACCT-3'	108	72°C 7 sec
	rev 5'-CTTGATGTCTGGGTCTTGGTTCT-3'		95°C 10 sec
IL-13	for 5'-ACAGCCCTCAGGGAGCTCAT-3'	96	57°C 5 sec
	rev 5'-TCAGGTTGATGCTCCATACCAT-3'		72°C 5 sec
TLR4	for 5'-TGGAAGTTGAACGAATGGAATGTG-3'	147	95°C 10 sec
	rev 5'-ACCAGAACTGCTACAAAGATACT-3'		55°C 5 sec
			72°C 6 sec

for, forward; rev, reverse. *forty amplification cycles

IL-5 and TLR4 in atopic adults (Fig.1).

Discussion

The aim of present study is to investigate the LPS effect on atopic immune reaction, especially effect on Th2 cytokine expression in PBMCs of atopic adults. Our result showed that PBMCs from atopic adults who sensitized

to particular allergen expressed Th2 cytokine after simulation with that allergen, and LPS co-exposure could not reduce the Th2 cytokine expression induced by the allergen in those adults.

Atopic diseases are characterized with specific symptoms with raised levels of allergen-specific IgE and eosinophils. These manifestations are modulated by the Th2 cytokines, such as IL-4, IL-5, IL-9, IL-10

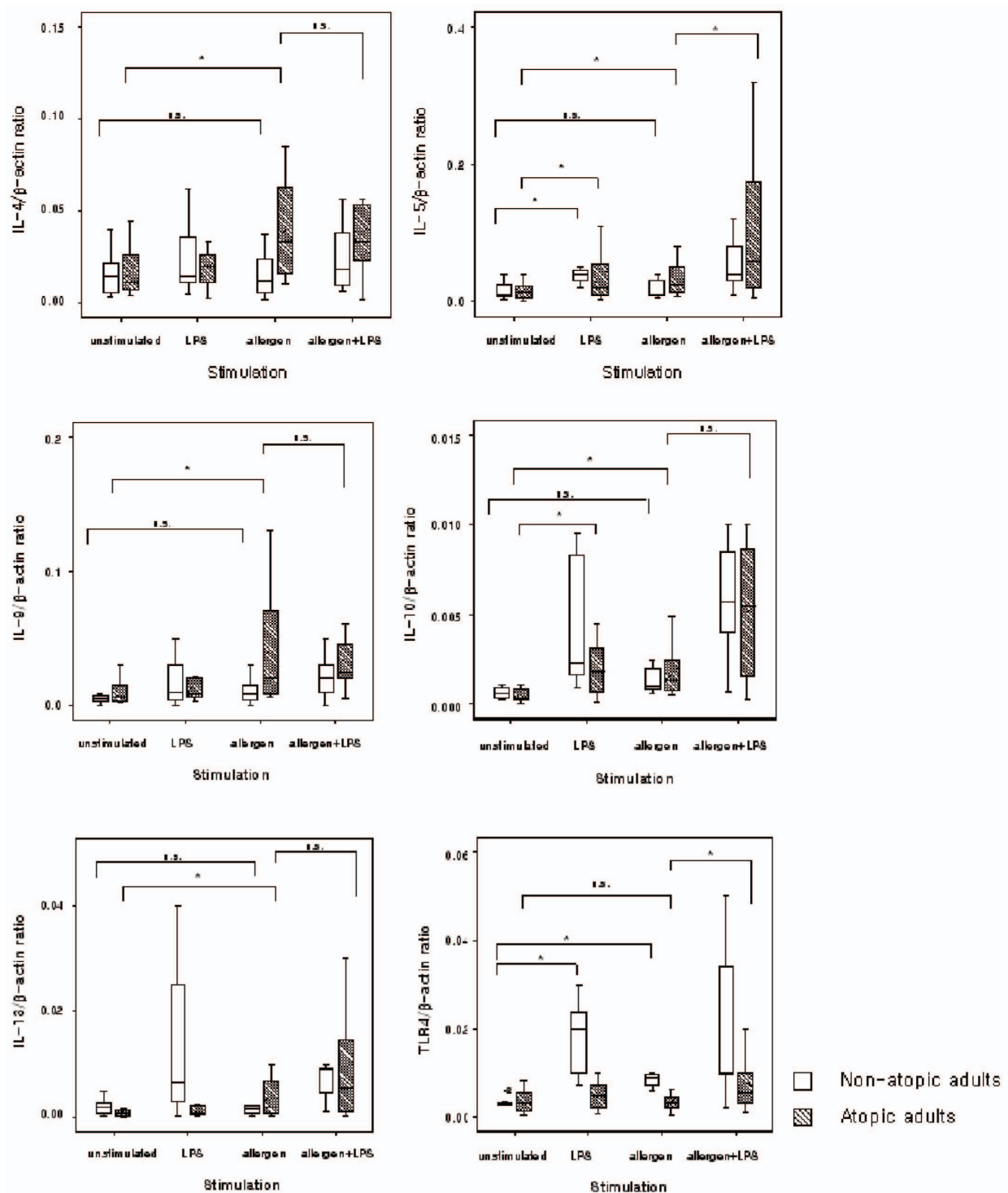


Fig. 1. The mRNA expression of Th2 cytokine (IL-4, 5, 9, 10, 13) and TLR4 after stimulation with LPS, allergen and LPS with allergen in PBMCs of atopic and non atopic adults. When compared the mRNA expression between allergen and LPS with allergen stimulation, significant differences were found in the expression of IL-5 and TLR4 in atopic adults. The mRNA expression was described as cytokine/β-actin ratio. * $p < 0.05$; n.s., not significant.

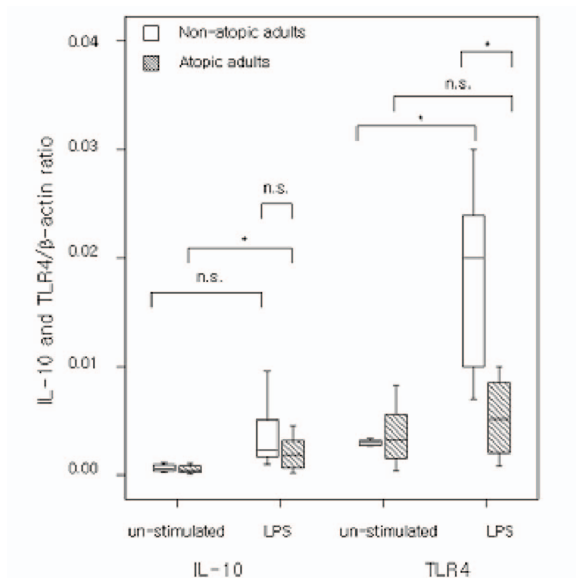


Fig. 2. The mRNA expression of IL-10 and TLR4 after stimulation with LPS in PBMCs of atopic and non-atopic adults. The increment of TLR4 expression was significantly lower in atopic adults compared to non-atopic adults after stimulation of LPS. * $p < 0.05$; n.s., not significant.

and IL-13. IL-4, IL-9 and IL-13 induce IgE production by B cells and IL-5 is a major eosinophil-activating cytokine [8–10]. IL-10 is an anti-inflammatory cytokine that inhibits Th1 and Th2 cells, suppress IgE synthesis and shorten eosinophil survival [11].

In vitro production or mRNA expression of allergen-induced Th2 cytokine from PBMCs of atopic patients has been demonstrated in several studies, and it has been known that Th2 cytokine expression is specific to the sensitized allergen [12–16]. Thus, we stimulated PBMCs of adult atopic patients with allergen of *D. farinae* and *D. pteronyssinus* which specific IgE was positive in those patients. Then we found the increased expression of Th2 cytokines after allergen stimulation compared with that of un-

stimulated cells in atopic adults, but not in non-atopic adults (Fig.1). When we stimulated PBMCs with LPS and allergen simultaneously, the Th2 cytokine expression rather showed increasing pattern compared to that of allergen stimulation alone, although statistically not significant. In IL-5, LPS rather aggravated the expression in atopic adults.

LPS is a cell wall component of gram-negative bacteria and common in the environment. LPS activates T cells through TLR4 and then results in the production of Th1 cytokines, such as IL-1, IL-12, IFN- γ and TNF- α , which induce Th1 immune response. Through activation of Th1 immune reaction, LPS was likely to suppress allergen-induced Th2 inflammation [4]. However, the effect of LPS on Th2 immune reaction has been reported to depend on the timing of exposure and the dose given. LPS exposure during early allergen sensitization reduced allergen specific IgE level, but late exposure of LPS after allergen challenge further exacerbated the allergic inflammation [6]. Moreover, although LPS was exposed early allergen sensitization, low dose of LPS more aggravated the allergic reaction [17]. Therefore, there is possibility that the LPS dose used (0.1 mg/L) in this study might be insufficient to induce the Th1 immune reaction, thus might have aggravated or not changed Th2 cytokine expression. However, Tulic et al. obviously suppressed the Th2 immune reaction with same dose of LPS and observed the immunomodulatory effect of LPS on allergic inflammation in nasal explant model study [7]. Additionally, they also found that IL-10 and TLR4 expression by LPS was higher in atopic children in contrast to atopic

adults. TLR4 is the main receptor for LPS-induced signal transduction, and LPS up-regulates Th1 cytokine reactivity via TLR4. Therefore TLR4 expression has been thought to associate with LPS sensitivity and contribute to the LPS effect on allergic inflammation. Therefore they suggested that expression of IL-10 along with TLR4 by LPS was an important factor to control allergen specific immune response. We also observed low expression of TLR4 by LPS in atopic adults compared to non-atopic adults. However, we could not observe the similar finding in IL-10 expression. The IL-10 expression was rather shown increased pattern after LPS stimulation and the expression level was similar with non-atopic adults.

The different TLR4 expression between atopic children and atopic adults has been explained related to immune maturation process. However, the difference between atopic adults and non-atopic adults could not be explained with that. Therefore it is possible that there might be some different underlying cause which induced low responsiveness of TLR4 to LPS in adult atopic patients.

In conclusion, we observed that LPS did not reveal preventive effect on Th2 cytokine expression in adult atopic patients, which might be contributed by the low expression of TLR4 respond to LPS. For explanation of these findings to be associated with atopic mechanism in adult atopic patients, further evaluations are needed.

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