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Effect and mechanism of lipopolysaccharide on allergen-induced interleukin-5 and eotaxins production by whole blood cultures of atopic asthmatics

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Summary

Interleukin (IL)-5 and eotaxin families regulate the development of eosinophilic inflammation of asthma in a co-operative manner. The exposure to airborne lipopolysaccharide (LPS) induces varying degrees of airflow obstruction and neutrophilic airway inflammation. Production of IL-5 and eotaxin subfamily chemokines was analysed in response to *Dermatophagoides pteronyssinus* allergen (D.p.) according to the presence of specific IgE to D.p., and investigated the mechanism underlying their LPS-mediated regulation of these cytokines in response to the specific allergen. Peripheral blood cells (PBCs) from asthmatics with (group 1) or without (group 2) specific IgE to D.p. and from non-asthmatics with (group 3) or without (group 4) were stimulated with D.p. or LPS. For LPS-mediated inhibition of IL-5 and eotaxin-2 production, LPS-induced cytokines were added to the D.p.-stimulated PBCs. IL-5 and eotaxin-2, but not eotaxin-1 and 3, were significantly increased by D.p.-stimulated-PBCs from group 1, while only eotaxin-2 was elevated in group 3. Eotaxin-2 production was found in monocytes and correlated with the level of specific IgE to D.p. LPS treatment resulted in the decrease in eotaxin-2 and IL-5 production by the D.p.-stimulated PBCs. LPS-induced IL-10 completely inhibited D.p.-stimulated production of eotaxin-2 and IL-5. The differential responses of the eotaxin family to specific antigens suggest that the predominant role of eotaxin-2 and LPS may attenuate eosinophilic inflammation by inhibiting IL-5 and eotaxin-2 synthesis through IL-10 production.

Keywords: asthma, D.p. antigen, eotaxin-2, interleukin-10, interleukin-5, LPS

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Introduction

Allergic asthma has been regarded as an atopic disease involving allergen exposure, allergic (IgE-mediated) sensitization with a Th2 $CD4^+$ lymphocyte response and subsequent interleukin (IL)-5-mediated eosinophilic airways inflammation, resulting in enhanced bronchial reactivity and reversible airflow obstruction [1]. In this process, antigen-sensitized T helper 2 (Th2) cells play a key role in development of the manifestations through their production and release of specific cytokines, such as IL-4, IL-5 and IL-13 [2]. The eotaxin subfamily, a member of CC chemokines, also participates in the development of asthma and other allergic disorders through the mobilization of inflammatory cells bearing CCR3, especially eosinophils. The potent effects of eotaxins on eosinophils in concert with IL-5 are explained largely by their ability to signal through the CCR3 [3]. Three members of this family have been identified: eotaxin-1 [4], eotaxin-2 [5] and eotaxin-3 [6], and the three eotaxins share the same CCR3 [7,8]. While limited studies have demonstrated their differential expression and their roles in regulating the kinetics of eosinophil recruitment during allergic inflammation [9–12], the eotaxins/CCR3 pathway evidently plays a fundamental role in eosinophil recruitment in experimental allergic asthma [10,13]. In allergen-sensitized atopic asthmatic subjects, *in vitro* allergen stimulation induces IL-5 production by peripheral blood mononuclear cells (PBMC) [14]; however, it has not been evaluated whether the synthesis of eotaxins depends on antigen sensitization.

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The exposure to airborne lipopolysaccharide (LPS) induces varying degrees of airflow obstruction and neutrophil inflammation and is often associated with an exacerbation of established asthma in children and adults [15,16]. However, emerging evidence suggests that exposure to endotoxin in early life prevents the development of atopy and, potentially, allergic asthma [17–19]. The inhibitory effect of LPS is mediated presumably by the induction of Th1 cytokines such as interferon (IFN)-ã and IL-12 secretion [18,20,21] or regulatory cytokines such as IL-10 [22]. However, the effect and mechanisms of LPS on antigen-sensitized IL-5 and eotaxins production has not yet been evaluated. In this study, we employed an *ex vivo* stimulation of peripheral whole blood cells (PBCs) that were obtained from four groups of asthmatics and non-asthmatics with or without specific IgE to mite *Dermatophagoides pteronyssinus* (D.p.). The production of cytokines and eotaxin subfamily chemokines in response to the mite antigen and the mechanism(s) underlying their LPS-mediated regulation were analysed.

Methods

Subjects

The study subjects comprised four groups: asthmatics with (group 1) or without (group 2) D.p.-specific IgE, normal controls with (group 3) or without (group 4). The asthmatics had clinical symptoms and physical characteristics compatible with the Global Initiative for Asthma (GINA) guidelines [23]. Asthmatics showed airway reversibility, as documented by an inhalant bronchodilator-induced improvement of more than 15% of forced expiratory volume in 1 second (FEV_1) and/or an airway hyper-responsiveness (AHR) to < 10 mg methacholine/ml [24]. Allergy skin prick tests were performed using 24 commercial inhalant allergens, which included dust mites (*Dermatophagoides farinae* and *D. pteronyssinus*, Bencard, West Sussex, UK) and histamine (1 mg/ml). IgE specific to D.p. was measured using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) and was presented as specific IgE class (1–6) according to UniCap-specific IgE Unites (kU_A/l). All subjects gave informed consent to participate in the study, and the protocols were approved by the local ethics committee of Soonchunhyang University Hospital.

Cell culture and cytokine/chemokine production

Peripheral blood was diluted at a 1 : 1 ratio with tissue culture medium containing RPMI-1640, 2 mM L-glutamine, 25 mM HEPES, 100 U penicillin/ml and 100 µg streptomycin/ml (JBI, Daegu, Korea). PBCs were stimulated with various concentrations of D.p., which was generously gifted by Professor Hong [25], and LPS (*Escherichia coli* 0111:B4, L-2630) (Sigma, St. Louis, MO, USA) for different lengths of time. The culture supernatants were harvested by centrifugation and were stored at −20°C until assayed. The potency of the D.p. was measured by specific IgE inhibition test with the pooled sera of 10 asthmatics having specific IgE (score > 4), as described previously [26]. Fifty per cent inhibition was obtained by preincubation of the pooled serum with 10 µg D.p. extract/ml. The endotoxin concentration of the mixture containing 10 µg D.p./ml was < 0·283 EU/ml (equivalent to 28·3 pg/ml), as determined by a limulus amoebocyte lysate kit (Bio-Whittaker, Walkersville, MD, USA).

Measurement of cytokine and chemokine concentrations

Cytokine and eotaxin concentrations were determined by enzyme-linked immunosorbent assay (ELISA), using kits from R&D Systems (Minneapolis, MN, USA) for eotaxin-2, and eotaxin-3 and kits from BD Biosciences (San Diego, CA, USA) for eotaxin-1, IL-5, IFN-γ, IL-12 and IL-10. The detection limits for eotaxin-1, eotaxin-2, eotaxin-3, IL-5, IFN-γ, IL-12 and IL-10 were 6·3, 15·6, 62·5, 3·9, 18·7, 31·3 and 15·6 pg/ml, respectively. All concentrations below these limits were considered as the detection limit values above for the statistical analysis. The inter- and intra-assay coefficients of variance were below 10%.

Immunocytochemical detection of intracellular eotaxin-2

Peripheral blood leucocytes were isolated from the venous blood of D.p.-specific IgE-positive asthmatics using a Percoll gradient solution. A total of 1×10^7 cells were cultured for 72 h in the presence of autologous serum (10% v/v) and 10 µg D.p./ml, with 3 µM monensin (Sigma, M5273) added 6 h before the termination of culture. The cultured cells were cytocentrifuged and fixed with 1% paraformaldehyde and 0·1% saponin. Eotaxin-2-positive cells were identified by immunostaining with anti-human eotaxin-2 (R&D Systems) and biotinylated goat-anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA). The negative control was incubated with isotype-matched antibody. The cells were then counterstained with Wright–Giemsa.

Inhibition and blocking of cytokine production in PBCs stimulated with D.p.

For inhibition studies, various concentrations of IL-10, IL-12 (R&D Systems) and IFN-γ (BD Bioscience) were added to PBCs in the presence of D.p. (10 µg/ml). For blocking, PBCs were pretreated for 30 min with various concentrations of mouse anti-human IL-10Rα antibody (R&D Systems) or mouse anti-human TLR4 antibody (BD Bioscience) and then for 72 h with D.p. (10 µg/ml) or LPS (10 ng/ml).

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Table 1. Clinical profiles of the study subjects.

	Asthma		Normal control	
	Group 1	Group 2	Group 3	Group 4
Subject (M/F)	20 (10/10)	12 (7/5)	7 (5/2)	10 (4/6)
Age (years)	30·9 ± 2·6	33·83 ± 1·2	29·71 ± 2·4	32·0 ± 3·6
Current smoker (%)	38	33·3	32·5	30
FEV1%, predicted	81·1 ± 2·2†‡	97·2 ± 4·8	101·7 ± 4·2	107·8 ± 6·3
Methacholine PC_{20} (mg/ml)	2·1 ± 1·4†‡	2·7 ± 1·1§¶	> 25	> 25
Blood eosinophil (%)	5·1 ± 0·7†‡	6·2 ± 1·5¶	2·1 ± 0·2	1·7 ± 0·4
Skin test to D.p. (%)	100*‡	0 ££	100**	0
Total IgE (U/ml)	571·5 ± 127·6*‡¶	203·9 ± 76·4¶	185·1 ± 37·6**	22·8 ± 6·2
Specific IgE, to D.p. antigen	3·7 ± 0·2*†‡	0 ± 0§	2·7 ± 0·3**	0 ± 0

Specific IgE is presented as specific IgE class (grades 1–6) according to Unicap-specific IgE Unites (kU_A/l). Values are the means ± s.e.m. *P*-values were obtained using the Mann–Whitney *U*-test or the χ^2 test. The following symbols represent significant differences ($P < 0·05$) between two groups: *between groups 1 and 2, †between groups 1 and 3, ‡between groups 1 and 4, §between groups 2 and 3, ¶between groups 2 and 4 and **between groups 3 and 4.

Reverse transcriptase–polymerase chain reaction (RT–PCR)

PBCs from group 1 were stimulated for 72 h with D.p. in the presence or absence of LPS and/or various concentrations of anti-IL-10Rα. PBMCs were then isolated from the cultured PBCs. Total RNA was extracted and reverse-transcribed by incubation with 200 U SuperScript RT (Invitrogen Life Technologies, Grand Island, NY, USA) at 42°C for 50 min. The resulting cDNA were placed into tubes containing specific primer pairs for human eotaxin-2, IL-5 or glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) gene, and were amplified for 30 cycles (one cycle: 1 min at 95°C, 1 min at 55°C and 1 min at 72°C). The PCR products were resolved by agarose (1%) gel electrophoresis. The primers used were follows; eotaxin-2 forward primer 5′-GCTCTGTGGTCATCCCCTC TCCCTG-3′, reverse primer 5′-GCAGGTGGTTT GGTTC CAGGATAT-3′; IL-5 forward primer 5′-GAG GATGCTTC TGCATTTGAGTTTG-3′, reverse primer 5′-GTCAATGT ATTTCTTTATTAAGGACAAG-3′; GAPDH forward primer 5′-GGCATTGCTCTC AATGACAA-3′, reverse primer 5′-AGGGCCTC TCT CTTGCTCTC-3′.

Statistical analysis

Data were expressed as mean ± s.e.m. Statistical analysis was carried out using the SPSS program (version 11·0; SPSS Inc., Chicago, IL, USA). Differences between independent groups or samples were compared using the non-parametric Kruskal–Wallis *H*-test for continuous data. If differences were found to be significant, the Mann–Whitney *U*-test was applied to compare differences between two samples. The Wilcoxon signed rank test was applied for time-dependent changes in the parameters. Spearman's rank correlation was calculated to assess correlations between data.

Results

Subject details

Asthmatics (groups 1 and 2) had significantly lower FEV_1 and methacholine PC_{20} values than did non-asthmatics (groups 3 and 4), while the former exhibited higher blood eosinophil levels than the latter. Specific and total IgE were significantly higher in the subjects allergic to D.p. (groups 1 and 3) than in the non-allergic groups (groups 2 and 4) (Table 1). These findings are consistent with previously described criteria [27].

Production of cytokines and chemokines by PBCs in response to D.p.

Throughout this study, a bulk whole blood culture system was employed in which peripheral blood was diluted at a 1 : 1 ratio with culture medium and used without fractionation. To optimize IL-5 and eotaxin subfamilies production, PBCs from group 1 ($n = 7$) were stimulated with various doses of D.p. for different periods of time. Eotaxin-2 and IL-5 increased continuously until 120 h after stimulation with a dose of 10 μg/ml D.p. and was elevated significantly compared with those of unstimulated PBC, whereas eotaxin-1 was decreased. No significant differences were found in eotaxin-3 production. Increased IL-5 and eotaxin-2 production by PBCs for 72-h stimulation with D.p. antigen reached a plateau at a dose of 10 μg/ml D.p and eotaxin-1 was significantly down-regulated upon exposure of PBCs in a dose-dependent manner to D.p. antigen (Fig. 1a).

Next, we stimulated PBCs from subjects of four groups. To minimize the effect derived from both different absolute and relative numbers of leucocytes in each group and in each individual within the group, the results were expressed as a fold increase of D.p. stimulation *versus* D.p. non-stimulation. PBCs were obtained from four groups (group 1, $n = 20$;

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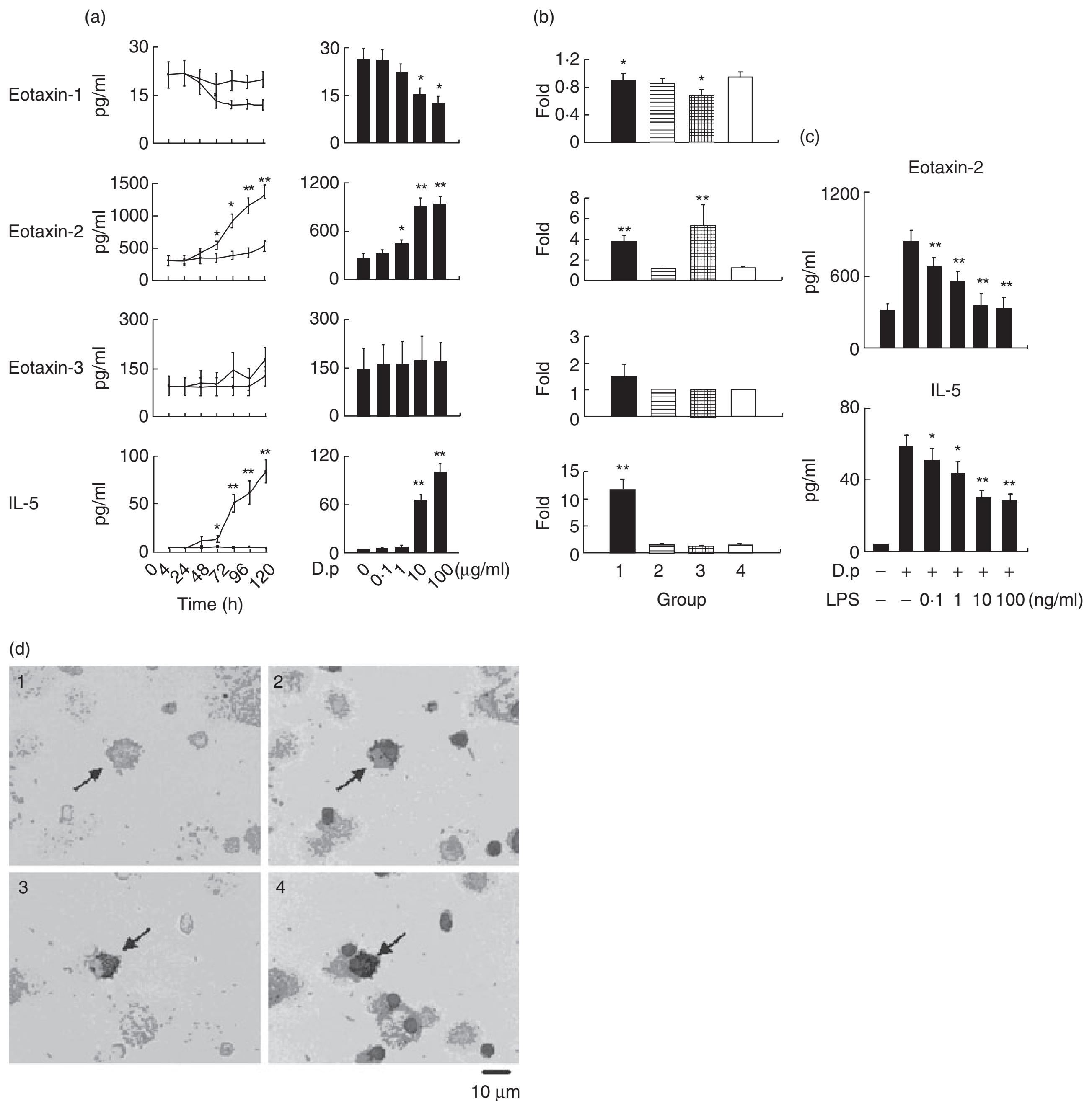

Fig. 1. (a) Time kinetics and dose–responses of IL-5 and eotaxin family chemokine production by D.p.-stimulated PBCs. PBCs were prepared from group 1 ($n = 7$) and were stimulated with various concentrations of D.p. for different lengths of time. Data are expressed as the means ± SEM. The statistical analysis was carried out using the Wilcoxon signed rank test. $^{*}P < 0.05$, $^{**}P < 0.01$. (b) Production of IL-5 and eotaxin subfamilies by D.p.-stimulated PBCs. PBCs were stimulated with either medium or 10 μg D.p./ml for 72 h. Groups 1, 2, 3, and 4 included 20, 12, 7, and 10 individual samples, respectively. The amounts of cytokines were expressed as a fold increase in which cytokine levels in the presence of D.p. were divided by those in its absence. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. cytokine levels in the absence of D.p. (c) Inhibition of D.p.-induced production of eotaxin-2 and IL-5 by LPS. PBCs from group 1 ($n = 7$) were treated with increasing concentrations of LPS in the presence of D.p. (10 μg/ml) for 72 h, and the eotaxin-2 and IL-5 levels in the culture supernatants were determined. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. D.p.-induced production of eotaxin-2 and IL-5. Cytokine production was determined by ELISA. (d) Percoll gradient-isolated leukocytes from group 1 were stimulated with medium (panels 1, 2) or D.p. antigen (panels 3, 4), cytospin, and either incubated with anti-human eotaxin-2 antibody (panels 1, 3) or stained with Wright-Giemsa solution (panels 2, 4). Bar =10 μm.

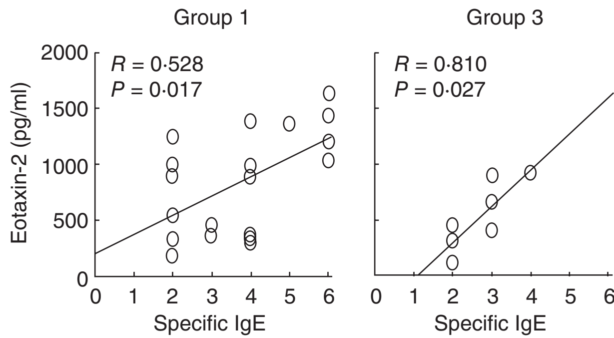

Fig. 2. Correlation of eotaxin-2 production with plasma IgE specific to D.p. and with production of eotaxin-2. Eotaxin-2 production in D.p.-stimulated PBCs from asthmatics positive to D.p. (group 1, $n = 20$; group 3, $n = 7$) was plotted against the level of plasma IgE specific to it. The statistical analysis was carried out using Spearman's rank test.

group 2, $n = 12$; group 3, $n = 7$; and group 4, $n = 10$) and were stimulated with D.p. (10 μg/ml) for 72 h. The significantly increased production of IL-5 was observed only in group 1, while eotaxin-2 was elevated in groups 1 and 3 (Fig. 1b). In contrast, eotaxin-1 production decreased significantly in groups 1 and 3. Eotaxin-3 was not changed in the four groups (Fig. 1b). Eotaxin-2 production in groups 1 and 3 correlated strongly with the respective levels of specific serum IgE to D.p. ($r = 0{\cdot}528$, $P = 0{\cdot}017$ for group 1 and $r = 0{\cdot}810$, $P = 0{\cdot}027$ for group 3) (Fig. 2). Immunocytochemical and Wright–Giemsa staining of PBCs from group 1 showed that monocytes were the eotaxin-2-producing cells (Fig. 1d).

Inhibitory effect of LPS on D.p.-induced eotaxin-2 and IL-5 production

As eotaxin-2 and IL-5 levels increased following stimulation with D.p., the production of these two cytokines in response to LPS was examined. PBCs from group 1 ($n = 7$) were stimulated with increasing concentrations of LPS in the presence of D.p. (10 μg/ml). The results showed that eotaxin-2 and IL-5 production declined in a dose-dependent fashion. Eotaxin-2 was inhibited completely ($P = 0{\cdot}01$) and IL-5 was inhibited by 80% ($P = 0{\cdot}01$) at 10 ng/ml LPS (Fig. 1c). To examine whether the inhibitory effect of LPS was mediated through Toll-like receptor 4 (TLR4), a neutralization antibody to TLR4 was added to PBC cultures from group 1 ($n = 6$) in the presence of D.p. (10 μg/ml) and LPS (10 ng/ml). Eotaxin-2 and IL-5 production were partly restored by neutralization with anti-TLR4 in a dose-dependent manner (Fig. 3a), suggesting an inhibitory mechanism via TLR4. To identify which factor(s) mediate the inhibitory effect of LPS on the D.p.-induced production of eotaxin-2 and IL-5, production of

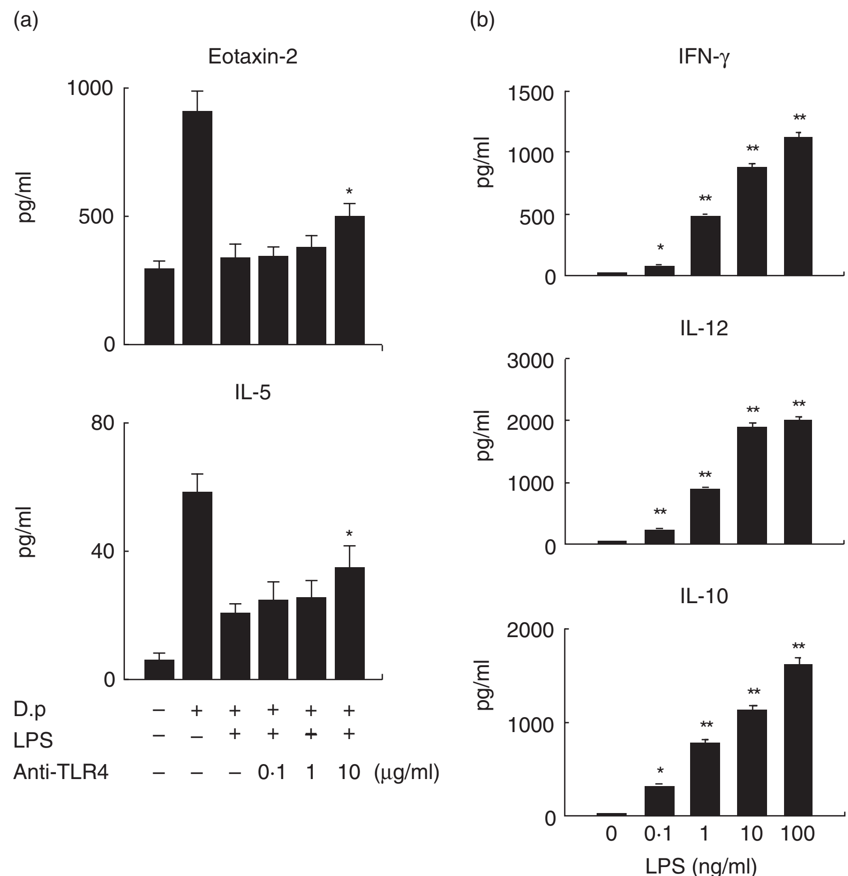

Fig. 3. (a) The effect of anti-TLR4 on LPS-induced inhibition of eotaxin-2 and IL-5 production. PBCs were prepared from group 1 ($n = 6$) and were incubated with different concentrations of anti-TLR4 in the presence of LPS (10 ng/ml) and D.p. antigen (10 μg/ml) for 72 h. (b) IFN-γ, IL-12, and IL-10 production by PBCs stimulated with LPS. Group 1 PBCs ($n = 7$) were stimulated with LPS (0·1–100 ng/ml) for 72 h. The amounts of cytokine were measured by ELISA. Data are expressed as the means ± SEM. The statistical analysis was carried out using the Wilcoxon signed rank test. $^{*}P < 0.05$, $^{**}P < 0.01$.

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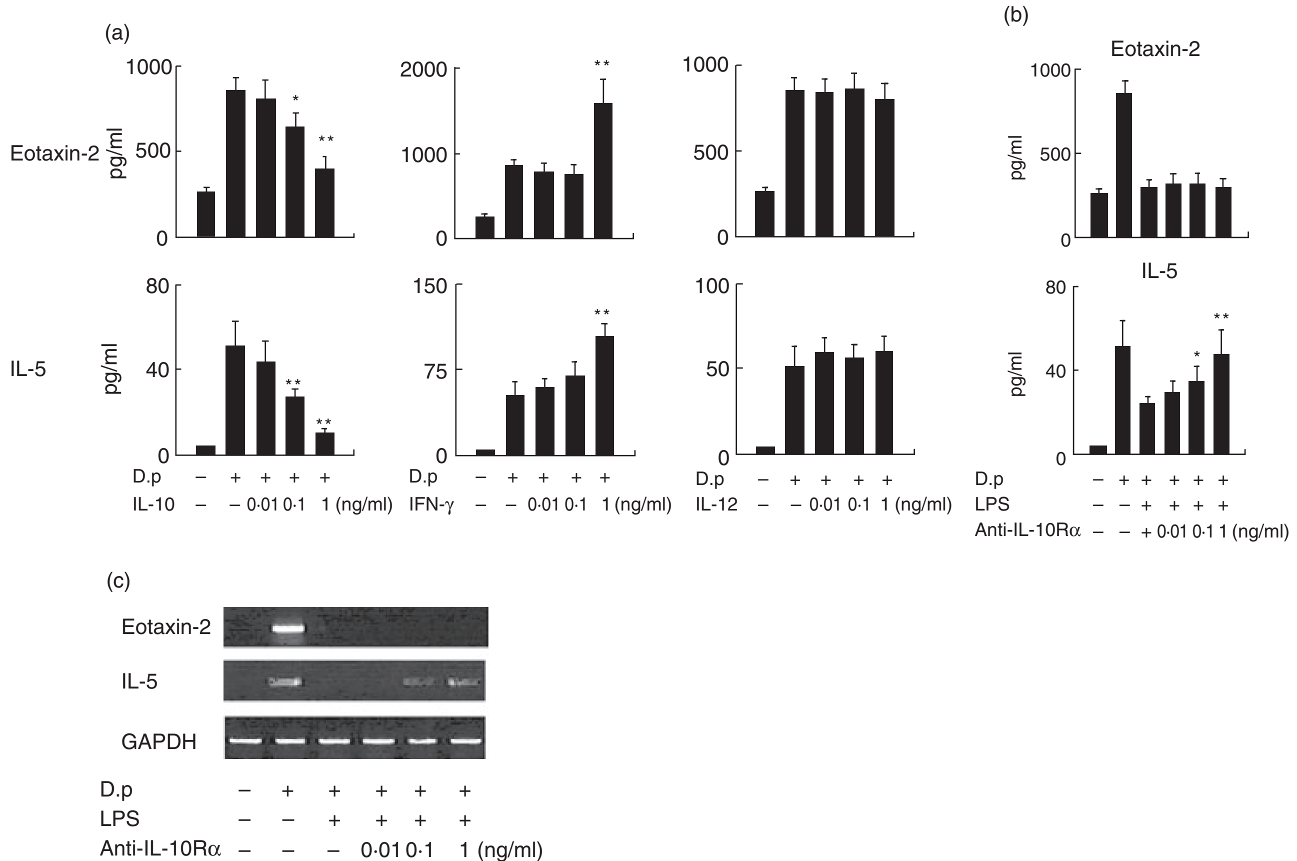

Fig. 4. The inhibitory effects of IL-10 on D.p. antigen-stimulated production of eotaxin-2 and IL-5. PBCs were prepared from group 1 ($n = 8$). (a) PBCs were stimulated with D.p. (10 μg/ml) for 72 h in the absence or presence of recombinant IL-10, IFN-γ, or IL-12 (each 0.01–1 ng/ml). (b) PBCs were stimulated with D.p. (10 μg/ml) and LPS (10 ng/ml) in the presence of increasing concentrations of anti-IL-10Rα (0.01–1 μg/ml) for 72 h, and eotaxin-2 and IL-5 production was evaluated. The statistical analysis was carried out using the Wilcoxon signed rank test. *$P < 0.05$, **$P < 0.01$. (c) PBMCs from group 1 ($n = 8$) and were stimulated under the indicated conditions for 72 h.

cytokines (IL-5, IFN-γ, IL-12 and IL-10) and eotaxins was analysed in group 1 PBCs ($n = 7$) stimulated with LPS alone. Among these, IFN-γ, IL-12 and IL-10 were significantly up-regulated upon the exposure of PBCs in a dose-dependent manner of LPS (Fig. 3b).

LPS inhibits D.p.-induced production of IL-5 and eotaxin-2 via IL-10 production

The three cytokines induced by LPS were examined individually for their inhibitory effect on the production of eotaxin-2 and IL-5 by D.p.-stimulated PBCs from group 1 ($n = 8$). IL-10 inhibited eotaxin-2 and IL-5 production almost completely (Fig. 4a). In contrast, IFN-γ significantly augmented the production of both cytokines, and IL-12 had no effect. Anti-IL-10Rα suppressed dose-dependently the inhibitory effect of LPS on D.p.-stimulated IL-5 production but not eotaxin-2 production (Fig. 4b), suggesting that a signal transmitted through the IL-10R effectively blocks IL-5 production by D.p.-primed Th2 cells, yet is not effective for monocyte eotaxin-2 production. RT–PCR analysis showed that the LPS-mediated inhibition as well as the restoration of IL-5 mRNA expression by anti-IL-10Rα indeed occurred at the transcriptional level (Fig. 4c). The neutralization of eotaxin-2 mRNA expression by anti-IL-10Rα was not observed, indicating that the inhibitory effects of LPS on D.p.-induced IL-5 and eotaxin-2 production are regulated differently.

Discussion

We employed an *ex vivo* stimulation of peripheral blood cells (PBCs) that were obtained from four groups of asthmatics and non-asthmatics with or without specific IgE to mite D.p. PBC contains lymphocytes, monocytes and other leucocytes. It also contains an array of protein and non-protein factors that may influence the availability of the antigen and LPS used. Thus it functionally represents the *in vivo* milieu more accurately than do purified peripheral blood leucocytes or combinations thereof.

Stimulation of PBCs with D.p. resulted in characteristic expression patterns of IL-5 and eotaxin subfamily

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chemokines. We identified increased IL-5 and eotaxin-2 production in D.p.-stimulated PBCs from the specific IgE-positive (group 1) and monocytes as a major producer of eotaxin-2. This result indicates the strict dependence of IL-5 and eotaxin-2 production on sensitization with specific antigens. IL-5 synthesis was observed only in PBCs from group 1. This result is in agreement with a previous study in which allergen-induced IL-5 production by PBMC from sensitized atopic subjects with symptoms, but not subjects without symptoms, is elevated [14].

CD23, a low-affinity receptor of IgE (FcåRII), is expressed at a much higher level in monocytes from allergic asthmatics than in cells from normal individuals [28]. It is therefore speculated that D.p. may form a complex with circulating specific IgE to induce eotaxin-2 production through the engagement of abundant CD23 on monocytes from allergic subjects. As a result, eotaxin-2 production may be related to the presence of specific IgE. Our data demonstrate that eotaxin-1 is down-regulated and eotaxin-2 is up-regulated by D.p., while eotaxin-3 remains unchanged. This may be due to different cell sources of each eotaxin. While monocytes are a major source of eotaxin-2, eotaxin-1 and 3 are produced mainly by epithelial cells [9]. Limited studies have demonstrated the differential expression and roles of eotaxin subfamilies in regulating the kinetics of eosinophil recruitment during allergic inflammation [11,29]. However, experimental asthma models using eotaxin 1 and/or eotaxin 2 knock-out mice showed a dominant role of eotaxin-2 in ovalbumin (OVA)-induced airway eosinophilia [13], in spite of a co-operative role for eotaxin-1 and eotaxin-2 in recruitment of eosinophils to the lung tissue. We have shown that polymorphism in the gene encoding eotaxin-2, but not eotaxin-1, is associated with a risk of asthma [30] and correlates with plasma eotaxin-2 levels [31]. These data, including ours, may suggest a dominant role of eotaxin-2 among the eotaxin subfamily in peripheral circulation of atopic asthma.

The effect of endotoxin exposure in asthma is still controversial. The beneficial effects of LPS are thought to be mediated by enhanced secretion of IFN-γ and IL-12 [32,33], whereas LPS affects asthmatics adversely by enhancing established airway inflammation and airway obstruction [16]. In the present study, we showed that LPS inhibited dose-dependently the production of IL-5 and eotaxin-2 in response to specific antigen; thus only IL-10 almost completely inhibited antigen-induced production of IL-5 and eotaxin-2 (Figs 1–3). The other novel finding of our study is that blocking the functioning receptor of IL-10 (IL-10Rα) restored the inhibitory effect of LPS only on IL-5 production (Fig. 4). These data suggest that the effect of LPS against the manifestation of allergic asthma is achieved by reducing eosinophilic inflammation through the up-regulation of IL-10 production. In support of this finding, IL-10 has been shown to exhibit anti-allergic activity in sensitized mice by preventing IL-5 release and antigen-induced $CD4^+$ T lymphocyte and eosinophil accumulation [22].

Systemic administration of endotoxin to healthy subjects produced a selective induction of Th1, as confirmed by increased IL-2 production *versus* decreased IFN-gamma production and Th2 chemokine ligands such as CCR4 receptor [34]. These *in vivo* data were not in agreement with ours in terms of different patterns of IFN-gamma production. It would be interesting to evaluate whether IL-10 production is elevated in a human endotoxin model, but this has not yet been attempted. In contrast to systemic administration, inhalation of endotoxin induced different patterns of reaction in the airways. Inhalation of endotoxin has been recognized as an important factor in the aetiology of occupational lung diseases, including non-allergic asthma [35]**.** Eosinophilic inflammation is generally considered to be the main feature of allergic asthmatic airways and is presumed to be crucial in the pathogenesis of allergic asthma [36]. Endotoxin in house dust is associated with exacerbations of pre-existing asthma in children and adults [16,37], and induces neutrophilic airways inflammation via IL-8 secretion [38]. The switching of eosinophilic inflammation into neutrophilic inflammation in the acute exacerbation of allergic asthma is contributed mainly by up-regulation of neutrophilic chemokines such as IL-8. In addition, down-regulation of IL-5 and eotaxin may be one mechanism to reduce the eosinophilic inflammation in the LPS-induced neutrophilic airway inflammation of asthmatics, as shown in experimental models [39], although this has not been revealed in the airways of asthmatics. IL-10 may exert an inhibitory effect on eotaxin-2 production via another pathway such as IL-10Rβ [40], or an unknown pathway. Intriguingly, IFN-γ treatment enhanced the production of IL-5 and eotaxin-2 by antigen-stimulated PBCs, while IL-12 had no effect (Fig. 4a). This observation is in line with previous findings that the suppression of airway eosinophilia and AHR by LPS [41] or killed mycobacteria [42] is not attributable to a Th1 shift.

In summary, two important conclusions can be drawn from our results: first, specific antigen-stimulated whole-blood cultures from asthmatics and normal controls with or without specific IgE to D.p. produce unique patterns of IL-5 and eotaxin-2. Secondly, LPS inhibits antigen-induced production of IL-5 and eotaxin-2 via IL-10 secretion. The inhibitory effect of endotoxin may be associated with its ability to attenuate eosinophilic inflammation or eosinophil-mediated immune responses.

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