

Induction of IL-6 and IL-8 by House Dust Mite Allergen Der p1 in Cultured Human Nasal Epithelial Cells Is Associated with PAR/PI3K/NFκB Signaling

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Key Words

Allergic rhinitis • Allergen • Signaling pathway • Epithelial cell

Abstract

Objective: The mechanism of action involved in how *Dermaphagoides pteronyssinus* (Der p) 1 initiates the nasal allergic cascade is poorly understood. **Methods:** We detected proinflammatory cytokine production (GM-CSF, TNF-α, IL-1β, IL-6, and IL-8) and associated signal molecules in primarily cultured nasal epithelial cells (NECs) from patients with allergic rhinitis (AR) after Der p1 stimulation, using ELISA, RT-PCR, and Western blot. We also evaluated the importance of protease-activated receptors (PAR)/phosphatidylinositol 3 kinase (PI3K)/NFκB signaling pathways in IL-6 and IL-8 production using glucocorticoids and specific inhibitors, LY294002 and PDTC. **Results:** We observed significantly elevated IL-6 and IL-8 production (both gene and protein) in NECs after Der p1 stimulation, and demonstrated that the expressions of PAR2, pAkt, and pp65 were upregulated after Der p1 stimulation, which were associated with IL-6 and IL-8 production in NECs. **Conclusion:** These findings demonstrate that the

PAR/PI3K/NFκB signaling pathway is involved in the induction of IL-6 and IL-8 in Der-p1-stimulated NECs from AR patients, and may have potential implications for the prevention and treatment of AR and asthma.

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Introduction

Allergic rhinitis (AR) and asthma are immune-dysregulated inflammatory conditions characterized by airway hyperreactivity, glandular hypertrophy, and Th2 cytokine overproduction resulting from aeroallergen sensitization [1]. The house dust mite is the most frequent indoor aeroallergen, and more than 60% of children sensitized to house dust mites have asthma, eczema, and/or rhinitis. Many studies have reported house dust mites as a crucial aeroallergen and an independent risk factor for the development of allergic respiratory diseases [2]. The

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airway epithelium is known to constitute a physical barrier against the delivery of numerous allergens, effectively acting as the first line of host defense. Traditionally, allergens have been considered as innocuous to the airway epithelium. When inhaled into the airway passage, house dust mites make contact with the airway epithelium and initiate inflammatory responses after they are captured and presented by antigen-presenting cells. Recently, some allergens such as the house dust mite have been shown to exert cysteine protease activity that can directly activate the airway epithelium, eliciting an inflammatory response [3], although the molecular mechanisms by which this occurs are not fully understood. In this regard, an investigation of the proinflammatory effects of the house dust mite on airway epithelial cells is essential for understanding the initiation of allergic cascades.

There are many species of house dust mites, the most allergenic of which are *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f). Der p1 is one of the most allergenic major proteins of the house dust mite. It is well known that Der p1 possesses proteolytic activity, which is a central biochemical property that endows molecules with intrinsic allergenicity [3]. Furthermore, airway-derived epithelial cells are capable of increasing the release of proinflammatory cytokines, such as IL-6 and IL-8, in response to the house dust mite and other allergens. In previous studies, Der p1 was shown to directly stimulate airway epithelial cells or to synergize with IL-4 and IL-13 to produce these proinflammatory cytokines [4]. Such activities may not only facilitate access of inhaled allergens to dendritic cells below the epithelial layer, but also modulate the function of immune and structural cells in the airway mucosa.

Proinflammatory effects may be important in the pathogenesis of disorders characterized by airway inflammation. IL-8 is known to: (1) be chemotactic for neutrophils, basophils, and T lymphocytes; (2) stimulate an oxidative burst and release of granule contents; (3) induce 5-lipoxygenase activity in neutrophils, as well as induce histamine release by basophils [5]. For example, IL-6 would be a central regulator in not only inhibiting the Th1 response, but also promoting Th2 and Th17 responses [6]. In addition, IL-6 is thought to play an important role in suppressing T regulatory cell (Treg) activity [7]. In our previous study, we found that Foxp3⁺ Treg insufficiency is responsible for the fundamental immune dysregulation observed in AR patients [8]. Consequently, the release of proinflammatory cytokines induced by Der p1

might be particularly important for the development of allergic sensitization. Therefore, an investigation of the mechanisms underlying the induction of IL-6 and IL-8 in airway epithelial cells might be crucial for understanding the pathogenesis of allergic responses.

IL-6 and IL-8 release was found to be mediated by protease-activated receptors (PAR) located on these epithelial cells [9]. The cellular mechanism of PAR-mediated cytokine release by airway epithelial cells in response to proteolytic allergens remains unclear. To address this issue, we examined the induction of IL-6 and IL-8 in primarily cultured nasal epithelial cells (NECs) obtained from AR patients and normal donors. Based on the report that the phosphatidylinositol 3 kinase (PI3K) and NFκB signaling pathways are usually involved in proinflammatory cytokine production [10], we hypothesized that induction of IL-6 and IL-8 by Der p1 in airway epithelial cells might involve activated PI3K and NFκB signaling pathways. Therefore, the roles of the PI3K and NFκB signaling pathways were assessed using specific inhibitors. The resultant findings will aid in better understanding the pathogenesis of allergic sensitization and facilitate the development of novel therapeutic targets in the management of AR.

Patients and Methods

Patients

Five patients (3 males and 2 females, aged 27 ± 14 years, range: 19–48) with persistent AR sensitized by house dust mite Der p1 were recruited for the study. Diagnosis was based on the case history, patient complaints, nasal/endoscopic inspection, allergen skin prick tests, and specific IgE assays. The patients had positive skin prick tests and specific IgE assays, fitting the standard criteria for AR, as stated in the ARIA guidelines [1]. Three age-matched healthy volunteers (2 males and 1 female) were recruited as normal controls, and the atopic status of the patients and control subjects was evaluated by allergen skin prick tests and specific IgE assays. None of the subjects suffered from asthma and other atopic disorders, and none had taken oral steroids or other medications 4 weeks prior to study recruitment. All subjects were non-smokers and free of upper respiratory tract infection for the 4 weeks preceding the study. This study was approved by the local ethics committee and informed consent was obtained from each subject.

Primary Nasal Epithelial Cell Culture

For primary cell culture, inferior turbinate NECs were collected by nasal scraping under local anesthesia. Collected NECs were cultured in submersion cultures in BEGM medium (Lonza, Walkersville, Md., USA) until passaged. Cells from passage 2 were frozen in liquid nitrogen and stored for use in the passage 2–3 experiments. When 80–90% confluence was reached, the NECs

Table 1. Sequences of primers used for real-time PCR

Gene	Sense (5'-3')	Antisense (5'-3')
PAR1 (NM_001992)	AGTAGGCTATTCCTGAGAGCTGCAT	ATGGCCCTGGCATGTGTCT
PAR2 (NM_005242)	ATTCCTGCCCTGAACATCAC	ATAGGCAGAGGCTGTGAGGA
IL-6 (NM_000600)	CCAGTACCCCCAGGAGAAGATT	TCGAGGATGTACCGAATTTGTTT
IL-8 (NM_000584)	ATTAGCCACCATCTTACCTCACAGT	GTGCTTCCACATGTCCTCACA
β -Actin (NM_001101)	AAGATGACCCAGATCATGTTTGAGACC	AGCCAGGTCCAGACGCAGGAT

were washed with PBS (37°C, pH 7.4), and fresh media without hydrocortisone was added in the presence of Der p1 (0–500 ng/ml) (Indoor Biotechnologies, Charlottesville, Va., USA) or PBS (control). In our study, the concentration of lipopolysaccharides in the cell cultures containing the allergen was less than 0.1 ng/ml, estimated by the Limulus Amebocyte Lysate assay (Cambrex, East Rutherford, N.J., USA). Cells were incubated at 37°C for 0–24 h. In our preliminary study, we found that 500 ng of Der p1 significantly stimulated the cells at 24 h. Thus, cell pellets and supernatants were collected 24 h after stimulation, and stored at –80°C until analyzed by RT-PCR and Western blot analysis.

Cytokine Inhibitor Treatment

Before measurement of IL-6 and IL-8 production in Der-p1-stimulated NECs, dexamethasone (DEX) was used to evaluate the therapeutic effect of glucocorticoids, and specific inhibitors were employed to evaluate the possible signaling pathways underlying IL-6 and IL-8 production. For glucocorticoid administration, various concentrations (0, 10^{-8} , 10^{-7} , 10^{-6} M, respectively) of DEX (Sigma-Aldrich, St. Louis, Mo., USA) or DMSO (Sigma-Aldrich) were added to NECs 6 h before adding Der p1. For specific inhibitor administration, pyrrolidine dithiocarbamate (PDTC) (Sigma-Aldrich), an NF κ B inhibitor, and LY294002 (Sigma-Aldrich, a PI3K-specific inhibitor) were dissolved in DMSO before use. These inhibitors and their corresponding vehicles (<0.1%) were added to the cultures 3 h before Der p1 stimulation. The NECs were then stimulated with Der p1 (500 ng/ml) for 24 h. We observed no cell cytotoxicity of these inhibitors at the doses used in this study based on the trypan blue dye exclusion assay.

Real-Time RT-PCR

Real-time RT-PCR was performed as described in our previous report [8]. Briefly, total RNA was extracted with TRIzol reagent (Invitrogen) following the manufacturer's instructions. The mRNA expression was determined using the ABI Prism 7300 Detection System (Applied Biosystems, Foster City, Calif., USA) and SYBR Premix Taq™ (Takara). The primer sequences are listed in table 1. PRISM samples contained 1× SYBR Green Master Mix, 1.5 μ l of 5- μ M primers, and 25 ng of the synthesized cDNA in a 25- μ l reaction volume. The samples were heated to 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s and annealing extension at 60°C for 60 s. All PCR reactions were performed in duplicate. Melting curve analysis was used to control for amplification specificity. Target gene expression is expressed as the fold increase or decrease relative to the expression of β -actin. The mean value of the replicates for each sample was calcu-

lated and expressed as the cycle threshold (Ct). The amount of gene expression was then calculated as the difference (Δ Ct) between the Ct value obtained for the target gene and the Ct value obtained for β -actin. Fold changes in target gene mRNA expression were determined as $2^{-\Delta\Delta Ct}$.

Western Blot Analysis

NECs from AR patients were washed 3 times in cold PBS, and total cellular protein was extracted in 100 μ l RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 1% NP-40, 150 mM NaCl, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mM Na_3VO_4 , and 1 mM NaF) at 4°C for 30 min. Cell debris was then removed by centrifugation at 14,000 g for 20 min at 4°C, and the supernatants were collected. The protein concentration was determined by the Bradford method. Samples containing 40 μ g protein were boiled and subjected to SDS-PAGE in 10% Tris-glycine gels and transferred electrophoretically to the polyvinylidene fluoride membrane, which was incubated with 5% fat-free skimmed milk in Tris-buffered solution (TBS) containing 0.05% Tween 20 (1 h, room temperature) and then with mouse anti-human pp65, pAkt (Cell Signaling, Danvers, Mass., USA) and β -actin monoclonal antibody (Santa Cruz) diluted 1:2,000 (overnight, 4°C). The membrane was washed twice with TBS containing 0.05% Tween 20, and then incubated with horseradish peroxidase-linked secondary antibody (goat anti-mouse immunoglobulin G, 1:1,000). After 2 more washes with TBS, the membrane was processed using the ECL chemiluminescence reaction kit (Cell Signaling) according to the manufacturer's instructions and exposed on medical film. The relative band density of the target gene compared to β -actin was quantified with Bio-Rad Quantity One 1-D Analysis Software (Bio-Rad, Hercules, Calif., USA).

ELISA

The cytokine concentrations of (GM-CSF, TNF- α , IL-1 β , IL-6, and IL-8) in the supernatant were determined by ELISA using commercially available solid-phase sandwich ELISA kits (Bioss Co., Beijing, China) and performed according to the manufacturer's instructions. All assays were performed in duplicate. The detection limits were as follows: 15.6 pg/ml for GM-CSF, TNF- α , IL-1 β , and IL-8; 31.2 pg/ml for IL-6.

Statistical Analyses

Data are expressed as means $\pm$ SD. The statistical significance of differences was analyzed using one-way ANOVA and nonparametric Mann-Whitney U test; $p < 0.05$ was considered statistically significant.

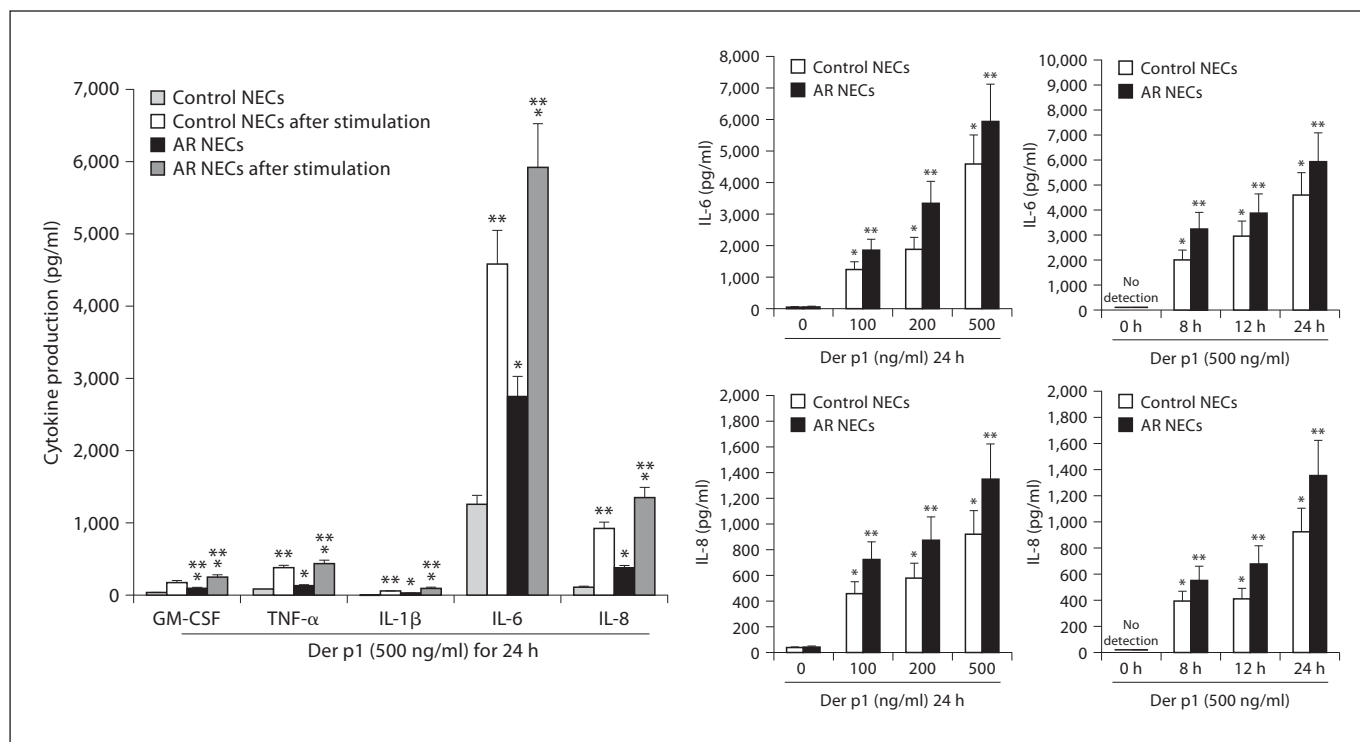


Fig. 1. Effects of the house dust mite allergen Der p1 on IL-6 and IL-8 production in the supernatants of primarily cultured NECs. Cell viability was evaluated using trypan blue dye. Primarily cultured NECs from AR patients and normal controls were treated with Der p1 (500 ng/ml) for 24 h. Of all cytokines, IL-6 and IL-8 were shown to have the most prominent elevation after stimula-

tion. Dose- and time-dependent release of IL-6 and IL-8 after stimulation with aeroallergen Der p1 are presented. Production of GM-CSF, TNF- α , IL-1 β , IL-6, and IL-8 was analyzed by ELISA. Data are expressed as means $\pm$ SD of at least 3 independent experiments. * $p < 0.05$ vs. control NECs; ** $p < 0.05$ vs. unstimulated counterparts.

Results

Der-p1 Stimulation Increases IL-6 and IL-8 Protein in the Supernatants of Primarily Cultured NECs

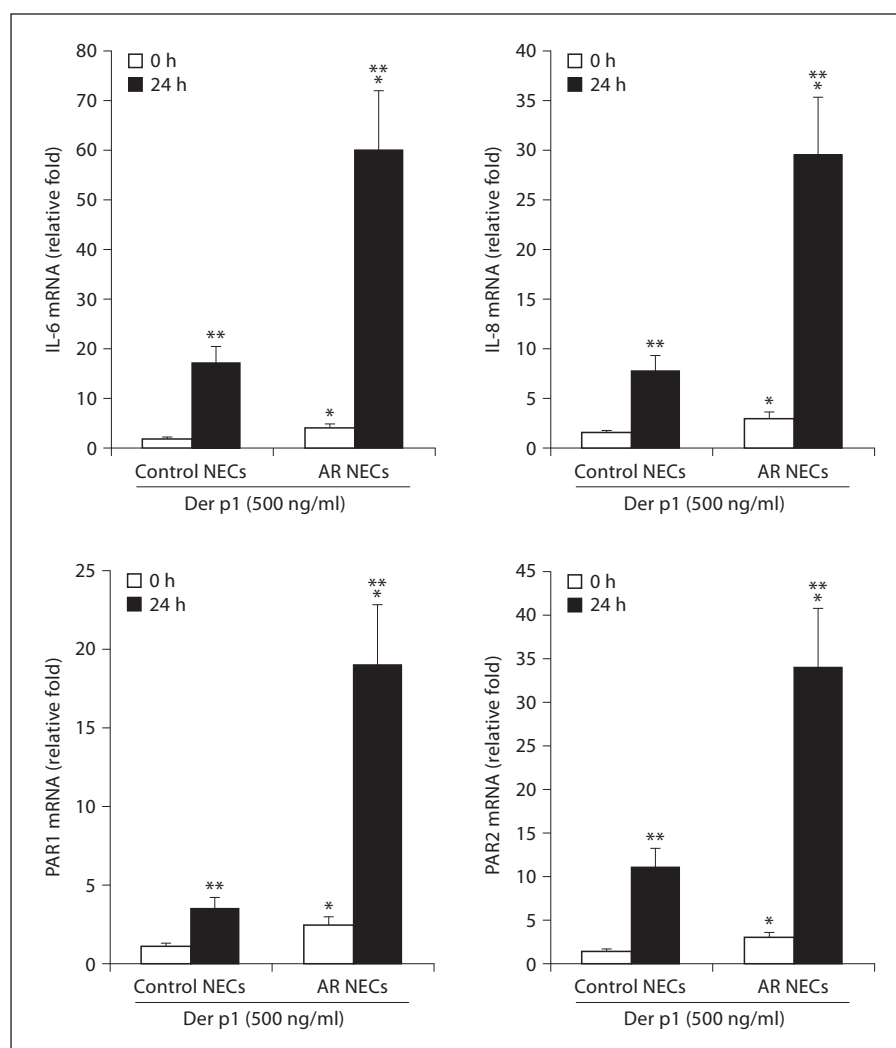
Based on the literature showing that Der-p1 possesses proteolytic activity and is capable of inducing airway-derived epithelial cells to release proinflammatory cytokines, we examined the levels of proinflammatory cytokines (GM-CSF, TNF- α , IL-1 β , IL-6, and IL-8) in the supernatants of primarily cultured NECs from AR patients and normal controls stimulated with Der p1. After stimulation for 24 h, we found that Der-p1 (500 ng/ml) significantly increased the release of proinflammatory cytokines. As illustrated in figure 1, the production of proinflammatory cytokines – including GM-CSF, TNF- α , IL-1 β , IL-6, and IL-8 – were significantly elevated ($p < 0.05$), with IL-6 and IL-8 demonstrating the most prominent elevation. Increased production of IL-6 and IL-8 was detected after stimulation in NECs obtained from

AR patients compared to those obtained from normal controls. Therefore, our findings indicated that Der-p1 exerts significant effects on proinflammatory cytokine production in allergic nasal epithelium as well as normal epithelium. To further understand the effects of Der-p1 on the levels of IL-6 and IL-8 in cultured airway epithelial cells, cytokine production was measured in response to different doses of Der-p1 stimulation (0–500 ng/ml) at different time points (0–24 h). Dose- and time-dependent release of IL-6 and IL-8 was observed in response to Der p1 stimulation. As illustrated in figures 2 and 3, the levels of IL-6 and IL-8 showed the most prominent elevation after stimulation with Der p1 (500 ng/ml) for 24 h.

Der p1 Stimulation Elevates the mRNA Expression of IL-6, IL-8, PAR1, and PAR2 in Primarily Cultured NECs

We accordingly examined the effect of Der p1 on IL-6 and IL-8 mRNA expression in the primarily cultured

Fig. 2. Effects of aeroallergen Der p1 on IL-6, IL-8, PAR1, and PAR2 mRNA expression in primarily cultured NECs. Primarily cultured NECs from AR patients and normal controls were stimulated with Der p1 (500 ng/ml) for 24 h. Der p1 significantly increased the mRNA expression of IL-6 and IL-8 in epithelial cells (17.5- and 8.1-fold in AR NECs, 10.1- and 5.2-fold in control NECs, respectively), as well as PAR1 and PAR2 in epithelial cells (3.2- and 11.3-fold in AR NECs, 2.5- and 7.8-fold in control NECs, respectively). Data are expressed as means $\pm$ SD of at least 3 independent experiments. * $p < 0.05$ vs. control NECs; ** $p < 0.05$ vs. unstimulated counterpart.



NECs. Primarily cultured NECs from AR patients and normal controls were treated with Der p1 (500 ng/ml) for 24 h. As illustrated in figure 4, Der p1 significantly increased the mRNA expression of IL-6 and IL-8 in epithelial cells (17.5-fold and 8.1-fold in AR NECs, 10.1-fold and 5.2-fold in control NECs, respectively). Furthermore, the mRNA expression of PAR1 and PAR2 was evaluated in primarily cultured NECs, based on reports that PAR is responsible for triggering the Der p1 signaling pathway. In addition, as illustrated in figure 4, we found that Der p1 significantly increased the mRNA expression of PAR1 and PAR2 in epithelial cells (3.2-fold and 11.3-fold in AR NECs, 2.5-fold and 7.8-fold in control NECs, respectively), and increased mRNA levels of IL-6 and IL-8 were detected after stimulation in NECs obtained from AR than in those obtained from normal

controls. These findings confirmed that the PAR-mediated pathway plays an essential role in the house dust mite allergen Der-p1-triggered biological effects, including induction of the proinflammatory cytokines IL-6 and IL-8.

DEX Inhibits IL-6 and IL-8 Production in Primarily Cultured NECs after Der p1 Stimulation

Since glucocorticoids represent the mainstay of treatment for allergic airway diseases, we evaluated the effects of DEX on IL-6 and IL-8 production in Der-p1-stimulated NECs. Primarily cultured NECs from AR patients and normal controls were treated with various concentrations of DEX (0, 10^{-8} , 10^{-7} , 10^{-6} M, respectively). As illustrated in figure 4, DEX significantly decreased the levels of IL-6 and IL-8 in epithelial cells in a dose-dependent manner.

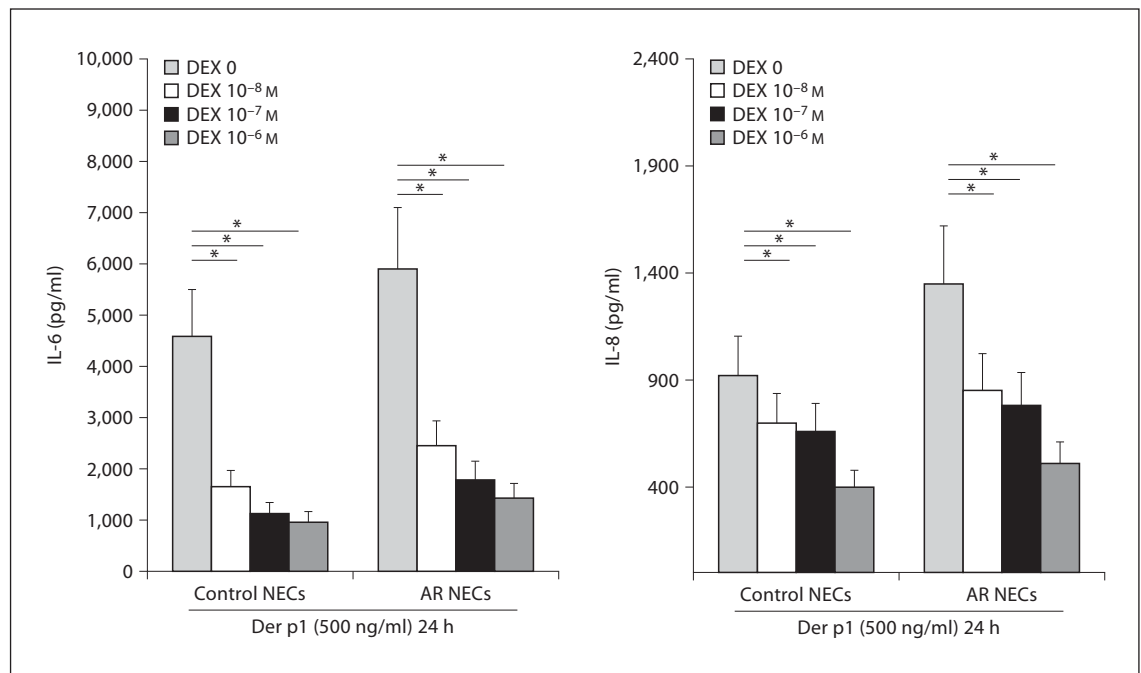


Fig. 3. Effects of DEX on IL-6 and IL-8 production in Der-p1-stimulated NECs. Primarily cultured NECs from AR patients and normal controls were treated with DEX (0, 10⁻⁸, 10⁻⁷, 10⁻⁶ M, respectively) for 6 h and then stimulated with 500 ng/ml Der p1 for 24 h. DEX significantly and dose-dependently decreased the levels of IL-6 and IL-8 in allergic epithelial cells. Data are expressed as means $\pm$ SD of at least 3 independent experiments. * $p < 0.05$ vs. cells stimulated with 500 ng/ml Der p1 for 24 h without DEX treatment.

These findings indicate that DEX interfered with Der-p1-triggered biological effects, including induction of the proinflammatory cytokines IL-6 and IL-8. Based on the report that DEX is able to interfere with NF κ B and PI3K signaling pathways in epithelial cells, it is possible that induction of IL-6 and IL-8 by Der p1 in airway epithelial cells might be associated with activation of NF κ B and PI3K signaling pathways.

Der-p1-Stimulated Induction of IL-6 and IL-8 in Primarily Cultured NECs Is Associated with Activation of NF κ B and PI3K Signaling Pathways

To further understand the molecular mechanisms underlying the induction of IL-6 and IL-8, we evaluated the NF κ B and PI3K signaling pathway activation in primarily cultured NECs. The expression of pp65 and pAkt in primarily cultured NECs obtained from AR patients was assessed after stimulation with Der p1 by indirect immunofluorescence staining to evaluate the localization of pp65 and pAkt expression in primarily cultured NECs obtained from AR patients. As illustrated in figure 4, pp65 and pAkt immunostaining was weak in the

untreated NECs, but was increased significantly in the cytoplasm and somewhat in the nucleus of NECs after Der p1 stimulation. These results provide evidence that the NF κ B and PI3K signaling pathways are activated in primarily cultured NECs in response to Der p1 stimulation. To further test this, we used the specific inhibitors PDTC and LY294002 to prevent Der-p1-induced NF κ B and PI3K signaling activation, respectively, in AR NECs, and evaluated the levels of pp65 and pAkt in epithelial cells by Western blotting. As illustrated in figure 4, PDTC and LY294002 significantly decreased the levels of pp65 and pAkt in the epithelial cells in a dose-dependent manner. Furthermore, the levels of IL-6 and IL-8 were examined in primarily cultured NECs after treatment with PDTC and LY294002, respectively. As illustrated in figure 5, both PDTC and LY294002 significantly decreased the levels of IL-6 and IL-8 in Der-p1-stimulated epithelial cells in a dose-dependent manner. These findings demonstrate that the NF κ B and PI3K signaling pathways are crucially involved in the induction of IL-6 and IL-8 in Der-p1-stimulated NECs obtained from AR patients.

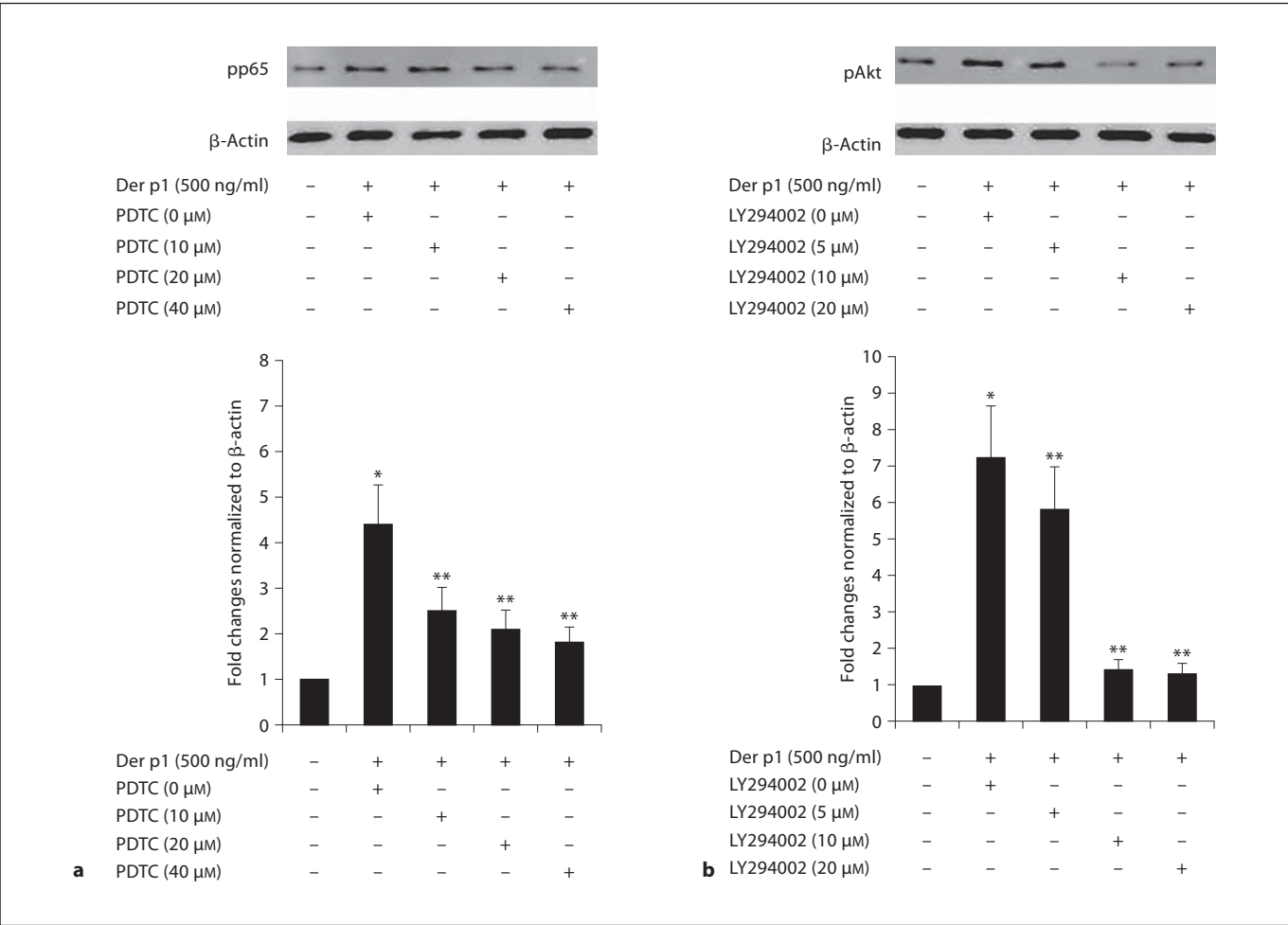


Fig. 4. The specific inhibitors PDTC and LY294002 inhibited Der p1-induced NFκB and PI3K signaling activation in AR NECs, respectively. Primarily cultured NECs from AR patients were treated with PDTC (0, 10, 20, 40 μM) and LY294002 (0, 5, 10, 20 μM) 3 h before Der p1 stimulation. PDTC and LY294002 significantly

decreased the levels of pp65 (**a**) and pAkt (**b**) in epithelial cells in a dose-dependent manner. Data expressed as means $\pm$ SD of at least 3 independent experiments. * $p < 0.05$ vs. cells unexposed to Der p1; ** $p < 0.05$ vs. cells exposed to 500 ng/ml Der p1 and specific inhibitor at 0 μM.

Discussion

NECs form the first line of defense against exposure of the airway and lungs to inflammatory stimuli and antigens. They have long been known to be a rich source of proinflammatory cytokines, such as TNF-α, GM-CSF, IL-6, and IL-8, which might play a central role in airway host defense and allergic asthma and rhinitis [11]. Increasing evidence shows that airway epithelial cells are able to release various cytokines in response to allergen challenge, indicating that epithelial cells mediate innate immune responses and regulate adaptive immune responses, in addition to functioning as a physical barrier.

However, little is known about the proinflammatory properties of NECs during allergic responses and the associated signal pathway. In the present study, we examined the levels of proinflammatory cytokines (GM-CSF, TNF-α, IL-1β, IL-6, and IL-8) in the supernatants of primarily cultured NECs from AR patients and normal controls stimulated with proteolytic Der p1 protein. We found the production of proinflammatory cytokines GM-CSF, TNF-α, IL-1β, IL-6, and IL-8 was significantly elevated in response to Der p1 stimulation, especially for IL-6 and IL-8. Interestingly, increased mRNA and protein levels of IL-6 and IL-8 were detected after stimulation in NECs obtained from AR compared to those ob-

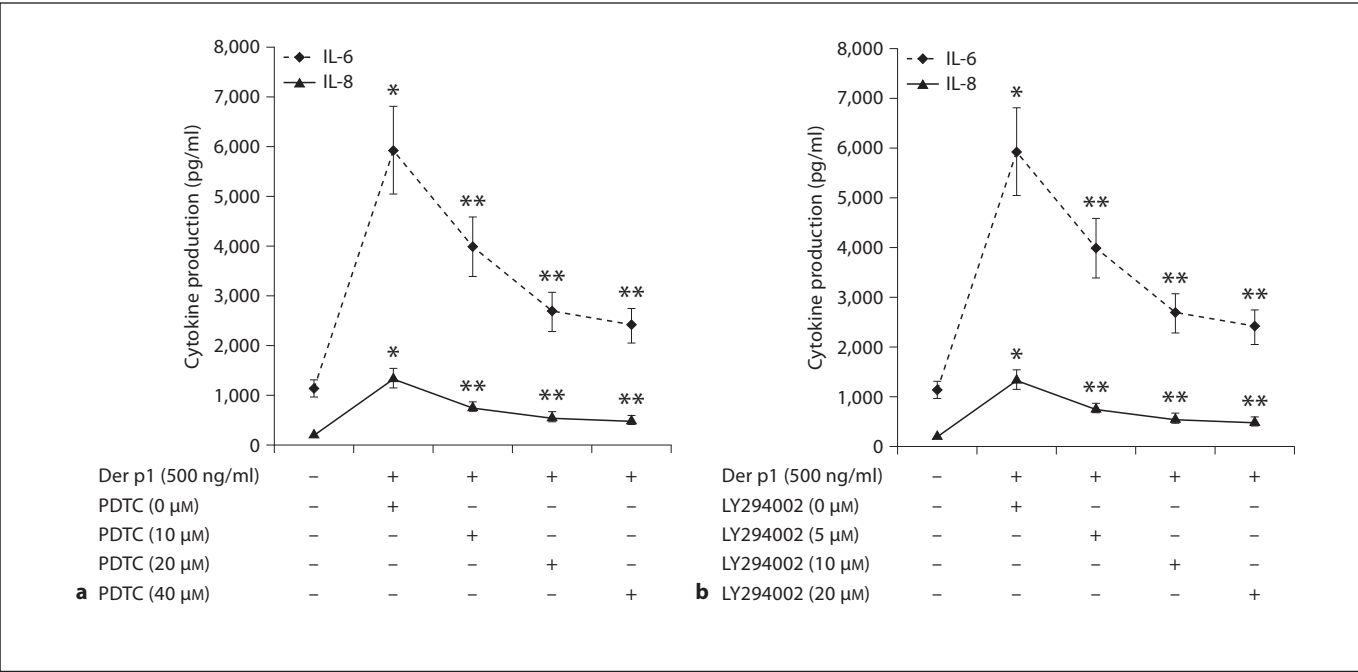


Fig. 5. The specific inhibitors PDTC and LY294002 attenuated Der-p1-induced IL-6 and IL-8 production in AR NECs. Primarily cultured NECs from AR patients and normal controls were treated with PDTC (0, 10, 20, 40 μM) and LY294002 (0, 5, 10, 20 μM) 3 h before Der p1 stimulation. PDTC (**a**) and LY294002 (**b**)

significantly decreased the levels of IL-6 and IL-8 in epithelial cells in a dose-dependent manner. Data expressed as means ± SD of at least 3 independent experiments. * $p < 0.05$ vs. cells unexposed to Der p1; ** $p < 0.05$ vs. cells exposed to 500 ng/ml Der p1 and specific inhibitor at 0 μM.

tained from normal controls, somewhat differing from a report describing chronic rhinosinusitis [12]. Therefore, our results provide the clues that the proinflammatory cytokines IL-6 and IL-8 might be involved in the initiation of allergic nasal responses.

IL-6 is a pleiotropic cytokine that is involved in pro-inflammatory, anti-inflammatory, and proliferative responses in the lung, where different cell types – including alveolar macrophages, epithelial cells, endothelial cells, lymphocytes, and dendritic cells – secrete IL-6. The essential role of IL-6 in allergic responses is thought to involve modulation of Tregs and Th17 cells on the basis of recent findings [6, 13, 14]. IL-6 has been shown to render pathogen-specific T cells refractory to the suppressive activity of CD4+CD25+ Tregs and induce Th17 cell differentiation in vitro, and control lung CD4+CD25+ Treg development and function in vivo, although the mechanisms underlying IL-6 bioactivity in inhibiting peripheral tolerance are not fully understood [14]. On the other hand, IL-8, a potent mediator of inflammation, is the prototypical member of the CXC chemokine family. Expression of IL-8 is elevated in the bronchial lavage and

sputum of patients with asthma. IL-8 is also a powerful activator, chemoattractant, and survival agent for polymorphonuclear leukocytes, T cells, natural killer cells and B cells [15, 16]. Therefore, by inducing IL-6 and IL-8 production, Der p1 might contribute to the progression of the allergic cascade by linking the structural cells and inflammatory cells. Thus, blocking the associated signal pathway and inhibiting cytokine production may be beneficial in controlling allergic asthma and rhinitis sensitized by aeroallergens.

To address this issue, we evaluated the effect of DEX on the expression of IL-6 and IL-8 in NECs stimulated with Der-p1. Glucocorticoid is considered to be the mainstay treatment for allergic asthma and rhinitis, and DEX is a synthetic glucocorticoid that elicits a myriad of pharmacological effects on allergic inflammation [17]. Using different concentrations of DEX, we demonstrated that DEX significantly decreases the levels of IL-6 and IL-8 in epithelial cells in a dose-dependent manner. These findings suggest that DEX interferes with house dust mite allergen Der-p1-triggered biological effects, including induction of the proinflammatory cytokines IL-6 and IL-8.

Although the mechanisms underlying these findings were not well defined, the available literature suggests that the release of proinflammatory cytokines such as IL-6 and IL-8 was mediated by PAR activation in these cells [18]. To further support this, we assessed the mRNA expression of PAR1 and PAR2 in primarily cultured NECs. We found the mRNA levels of PAR1 and PAR2 in AR NECs were significantly higher than that in the control, and Der p1 stimulation significantly increased PAR1 and PAR2 mRNA in AR and control NECs. Together with other reports, our findings therefore suggested that the PAR-mediated pathway plays a possible role in Der-p1-triggered biological effects, including induction of the proinflammatory cytokines IL-6 and IL-8.

However, the downstream signal of PAR pathway underlying allergen-induced IL-6 and IL-8 production in NECs is still poorly understood. A variety of stimuli were found to enhance IL-6 production via transcriptional regulation of the IL-6 gene by NF κ B, AP1, and C/EBP β regulatory elements [19]. Additionally, PI3K and NF κ B signaling pathways were also reported to be involved in IL-6 and IL-8 cytokine production [19, 20]. Together with the report that DEX is able to interfere with the PI3K and NF κ B signaling pathways in epithelial cells, the possibility is raised that induction of IL-6 and IL-8 by Der p1 in NECs might be associated with activation of PI3K and NF κ B signaling pathway as well. To evaluate the importance of activated NF κ B and PI3K signaling pathways in induction of IL-6 and IL-8 in nasal epithelium, we examined the inhibition of NF κ B and PI3K signaling pathways in primarily cultured NECs using the specific inhibitors PDTC and LY294002. We found Der-p1-stimulated PI3K activity, indicated by pAkt production, was significantly attenuated by LY294002. This inhibition of PI3K activity

was associated with a significant concentration-dependent reduction in IL-6 and IL-8 production. For PDTC, we showed that Der-p1-stimulated NF κ B activity, indicated by pp65 production, was significantly attenuated – similar to the observations for LY294002 – and IL-6 and IL-8 production was significantly inhibited by PDTC in a concentration-dependent manner. Overall, these findings demonstrate that PAR/PI3K/NF κ B signaling pathways are involved in the induction of IL-6 and IL-8 in Der-p1-stimulated NECs obtained from AR patients, and further study is still needed to clarify the importance of these cytokines in the maintenance of the allergic nasal condition.

Conclusion

In summary, the present study provides evidence that proteolytic Der p1 possesses proinflammatory properties by inducing IL-6 and IL-8 in primarily cultured NECs and linking structural cells with inflammatory cells. This proinflammatory property may be associated with PAR/PI3K/NF κ B signaling activation. Therefore, modulation of PAR/PI3K/NF κ B signaling activation in the nasal epithelium may represent a beneficial way of regulating nasal inflammation and preventing the development of AR.

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