

Systemic Up-Regulation of TLR4 Causes Lipopolysaccharide-Induced Augmentation of Nasal Cytokine Release in Allergic Rhinitis

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Key Words

Allergy · Endotoxin · Inflammation · Innate immunity · Nose · Rhinitis · Toll-like receptors

Abstract

Background: Allergic rhinitis is a systemic disorder, and it is clinically well recognized that it can be aggravated by infection. Activation of the innate immune system constitutes a critical element in the process. Toll-like receptors (TLRs) comprise a part of the innate immune system, and lipopolysaccharide (LPS)-induced activation of TLR4 represents bacterial-induced interactions in various model systems. The present study examines how TLR2 and TLR4 expression is affected by symptomatic allergic rhinitis, and if LPS added upon allergen affects nasal cytokine release. **Methods:** In patients with pollen-induced allergic rhinitis and healthy non-allergic volunteers, nasal lavage (NAL), peripheral blood and bone marrow were sampled before and during the pollen season. TLR2 and TLR4 expression was determined flow cytometrically. Changes in the TLR receptor expression pattern were evaluated by a nasal challenge with allergen followed by LPS, or vice versa. Symptoms along with cells and

cytokines in NAL were analyzed. **Results:** TLR4 expression increased in leukocytes in NAL, peripheral blood and bone marrow during symptomatic allergic rhinitis. A similar increase was seen for TLR2 in neutrophils in blood. Nasal challenge with allergen followed by LPS augmented the release of IL-4, IL-5, IL-10, IL-13, IFN- γ and TNF- α . **Conclusion:** A systemic up-regulation of TLR4 in symptomatic allergic rhinitis may explain why LPS preceded by allergen increases nasal cytokine release.

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Introduction

Like other atopic diseases, allergic rhinitis constitutes a systemic condition where local reactions might only be the most evident sign of a more general reaction involving not only the whole respiratory tract but also other compartments of the body [1]. Upper and lower airways, peripheral blood, bone marrow and mucosa-associated lymphoid tissue are all systemically linked [2, 3]. Allergens are not the sole trigger of the inflammatory reaction in allergic rhinitis. Several studies suggest a synergistic

effect between allergen and various microbes [4, 5]. The microbes trigger an inflammatory response by activating pattern-recognition receptors in the host.

So far, Toll-like receptors (TLRs) are the best characterized pattern-recognition receptors [6]. There are at present 10 TLRs known in humans. TLR2 recognizes bacterial lipoproteins, and TLR4 is activated by lipopolysaccharide (LPS) from Gram-negative bacteria [7]. Both receptors have been shown to affect atopic diseases such as allergic rhinitis and asthma [8–10].

The present study was designed to examine if symptomatic allergic rhinitis could affect TLR2 and TLR4 expression, not only in the nose, the primary target for the allergen, but also in the peripheral blood and bone marrow cells. We also aimed to determine if LPS stimulation in an allergic milieu, provided by an allergen challenge, could influence the cytokine release in the nose.

Materials and Methods

Subjects

Samples of NAL, peripheral blood and bone marrow were obtained during the season from 11 patients (7 men and 4 women) with a history of birch ($n = 5$) and grass pollen-induced rhinitis ($n = 6$). The same sampling was performed outside the season on another set of 10 patients with a similar history (6 men and 4 women) and 9 healthy volunteers without symptoms/signs or a history of allergy (6 men and 3 women). Another 13 patients with seasonal allergic rhinitis were challenged outside the season with LPS, allergen, LPS followed by allergen or allergen followed by LPS (6 grass, 1 birch, and 6 both birch and grass; 4 men and 9 women).

The diagnosis of pollen-induced allergic rhinitis was based on a positive history of seasonal allergic rhinitis for at least 2 years and a positive skin prick test (SPT) to birch and/or timothy pollen. All patients were classified as having moderate/severe symptoms during the birch and/or grass pollen season, and in the previous years they had been treated with anti-histamines and nasal steroids, often in combination with anti-histamine or cromoglycate eye drops. The patients had no symptoms of asthma at the time of visit and did not take any regular asthma medication. Exclusion criteria included a history of perennial symptoms, a history of upper airway infection within 2 weeks before the visit, and treatment with local or systemic corticosteroids within 2 months before the visit. All subjects were evaluated by an ear-, nose- and throat consultant. Individuals with signs or symptoms of chronic rhinosinusitis, hypertrophy of turbinates, severe septum deviation or nasal polyposis were excluded. The study was approved by the regional ethics committee, and informed consent was obtained from all participants.

Study Design

The during-season sampling was performed at the beginning of the pollen season on patients with substantial symptoms of rhinoconjunctivitis (itchy nose and eyes, sneezing, nasal secretion

and nasal blockage) for at least 3 consecutive days. The majority of patients were seen within 5–10 days after the first appearance of symptoms. A local pollen count confirmed the presence of the relevant pollen in the air during this period. Leukocyte expression of TLR2 and TLR4 was investigated using flow cytometry.

Outside-season challenges were performed by spraying 100 μ l of a sterile physiological saline solution with 0.5 μ g/ μ l LPS (*Escherichia coli*, L-2654; Sigma, St. Louis, Mo., USA) in both nostrils, resulting in a total dose of 100 μ g LPS. Twenty-four hours later, the same patients were challenged with 10,000 SQ/U per nostril of either birch ($n = 6$) or grass ($n = 7$) pollen extract. Only allergens with an ultra-low LPS content were used (special delivery from ALK). The same challenge procedure was then repeated 3–6 weeks later, but this time the allergen preceded the LPS. Symptom scores were recorded 5 and 30 min and 6 h after allergen challenge, and 6 h after LPS challenge. NAL was performed before the first challenge and 6 h after each challenge. The total number of cells and the cell distribution in NAL was counted. Cytokine levels were analyzed in samples from 6 of the patients.

Symptom Scores

Subjects were asked to record the severity of three nasal symptoms, i.e. itching/sneezing, secretion and blockage, using a scale from 0 to 3 (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms). A total nasal score was calculated by addition of the three scores. Similarly, a total eye symptom score (based on itch, tears and redness) and a total lung symptom score (based on cough, wheeze and dyspnea) were calculated by addition of the three scores.

Recovery of NAL

NAL was obtained as previously described [11]. After clearing excess mucous by exsufflation, sterile saline solution (8–10 ml; 0.9% NaCl) at room temperature (RT) was aerosolized into each nostril. The nasal fluid was allowed to return passively into a graded test tube, until 7 ml were recovered. The fluid was centrifuged for 10 min at 1,334 g at 4°C. In subjects challenged with LPS and/or allergen, the total number of cells/ml was quantified in a Bürker chamber. The cell pellet was used to prepare slides that were May-Grünwald and Giemsa stained for morphological assessment of the cells. Cell distribution in NAL after challenge with LPS/allergen or the combination was assessed in 12 allergic patients. Flow cytometry of NAL was conducted on samples from 10 allergic patients during the season and 6 allergic patients out of the season, and 8 healthy controls.

Blood Sampling

One sample containing 4 ml of blood was collected in a test tube containing buffered trisodium citrate solution (0.129 mol/l, BD Vacutainer™ 367704; Becton-Dickinson, Plymouth, UK) and analyzed flow-cytometrically. Blood samples were obtained from all of the 11 patients studied during the season, the 10 patients outside the season and the 9 healthy volunteers.

Bone Marrow Aspiration

One sample containing 1–2 ml of bone marrow was aspirated from the posterior iliac crest following local anesthesia with lidocaine hydrochloride (10 mg/ml). The sample was immediately placed in a culture medium containing buffered trisodium citrate solution (0.129 mol/l), RPMI 1640 with 2 mM HEPES and N-ace-

tyl-L-alanyl-L-glutamine (Biochrom AG, Berlin, Germany). Bone marrow aspiration was obtained from 7 patients with symptomatic allergic rhinitis, 9 patients outside the pollen season and from 8 healthy controls.

Flow Cytometry on Leukocytes in Bone Marrow, Peripheral Blood and NAL

Samples of NAL, peripheral blood and bone marrow were used for cell analysis with flow cytometry. NAL and bone marrow samples were filtrated prior to preparation. Both cell surface and intracellular levels of TLR2 and TLR4 were analyzed. All samples were labeled with CD16-Pcy5 (IM2642U, clone 3G8; Immunotech, Marseille, France) and CD45-ECD (IM2710U, clone J.33; Immunotech) for 15 min at RT.

For cell surface staining, cells were labeled with TLR2-PE (mouse monoclonal anti-TLR2, clone TL2.1; ImmunoKontakt, Oxon, UK) and TLR4-FITC (mouse monoclonal anti-TLR4, clone HTA125; ImmunoKontakt) for 15 min RT. Erythrocytes were lysed by mixing with 0.6 ml 0.1% (v/v) formic acid for 3–4 s. The ionic strength was rendered iso-osmotic by addition of 0.28 ml 51 mM Na₂CO₃, 0.20 M Na₂SO₄ and 0.22 M NaCl, and cells were washed and fixed in PBS containing 1% formaldehyde prior to analysis.

Intracellular staining was performed using the IntraPrep™ permeabilization reagent kit (Immunotech) according to the specifications of the manufacturer. Thus, the cells were fixed and permeabilized prior to incubation with TLR2-PE and TLR4-FITC for 15 min at RT. Cells were washed in PBS and resuspended in PBS containing 1% formaldehyde prior to analysis. In a subset of experiments, cells from healthy controls (n = 2) or allergic patients outside the season (n = 3) were also incubated with isotype control antibodies MsIgG2a-PE (Immunotech) for TLR2 and MsIgG2a-FITC (Immunotech) for TLR4.

By gating intact leukocytes on forward scatter (FSC) and side scatter (SSC) properties as well as by their CD16 and CD45 signals, leukocytes were characterized as neutrophil granulocytes, eosinophil granulocytes, basophil granulocytes, monocytes and lymphocytes. Immature granulocytes were gated as high FSC, high SSC and low CD16. Neutrophil granulocytes were the only leukocyte type that could be clearly identified in NAL. The gating strategy and the definitions of the various cell types were carried out in the same manner as in one of our previous publications [12].

A total of 30,000 events were collected in bone marrow and peripheral blood samples, and 3,000 events were collected in NAL. Data were analyzed using Expo32 ADC analysis software (Beckman Coulter). Cell surface staining in NAL proved unreliable. Staining was poor and could not be properly evaluated.

Mediators in NAL

NAL samples obtained from 6 patients before and 6 h after each challenge were analyzed by sandwich-based immunoassays. IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNF-α and IFN-γ were measured using a 10-spot multiplex electrogenerated chemiluminescence immunoassay (Human Th1/Th2 panel; Meso Scale Discovery, Gaithersburg, Md., USA; according to protocol 17061-v2-2006Okt). Spike recovery and freeze-thaw tests were performed for the NAL matrix for the assay. Detection limits for the mediators analyzed were as follows: 0.3 pg/ml for IL-1β, 0.5 pg/ml for IL-2, 1.3 pg/ml for IL-4, 0.3 pg/ml for IL-5, 1.0 pg/ml for IL-8, 1.9 pg/ml for IL-10, 1.1 pg/ml for IL-12p70, 2.8 pg/ml for IL-13, 0.3 pg/ml for TNF-α and 0.8 pg/ml for IFN-γ.

Statistics

Statistical analysis was performed using the software Graph-Pad Prism 5 (GraphPad Software, San Diego, Calif., USA). The non-parametric Kruskal-Wallis test (unpaired data) or the Friedman test (paired data) was used in combination with Dunn's multiple comparison test to determine statistical differences. All data are expressed as medians and ranges (minimum to maximum), and n equals the number of subjects in each group. A value of p < 0.05 was considered statistically significant.

Results

TLR2 and TLR4 Expression on Leukocytes in Various Compartments

TLR2 and TLR4 expression on leukocytes derived from NAL, peripheral blood and bone marrow was analyzed with flow cytometry before and during the pollen season as well as in healthy volunteers. Neutrophils were the only leukocyte type that could be clearly identified in NAL. They exhibited an increased intracellular expression of TLR4 during the season compared to that outside the season. No corresponding increase was observed for TLR2 (fig. 1). Neutrophils in the peripheral blood from symptomatic patients displayed an increased intracellular expression of both TLR2 and TLR4 compared to patients outside the season and healthy controls. A similar increase in TLR4 was seen in eosinophils and monocytes (fig. 2). In bone marrow, a seasonal up-regulation of TLR4 was seen in immature granulocytes and neutrophils (fig. 3). No corresponding up-regulation was seen for TLR2 and no up-regulation of either TLR could be seen in eosinophils, monocytes or lymphocytes. Leukocyte surface expression of TLR2 or TLR4 was not affected by the pollen season (data not shown).

Symptom Scores, Cell Distribution and Cytokine Release after Challenge

Allergic subjects examined during the pollen season reported an increase in nasal and eye symptom scores (median value of 5 and 3, respectively) compared to subjects examined outside the season (both median values: 0; p < 0.001, respectively) as well as compared to healthy controls (both median values: 0; p < 0.001, respectively). No differences in lung symptom scores were seen between the three groups (data not shown).

Within the concentration used, LPS caused no local symptoms, neither by itself nor when it was administered 6 h after allergen challenge. The allergen challenge gave transient local symptoms, which had disappeared after 6 h (fig. 4a). The total cell count in NAL was unaffected

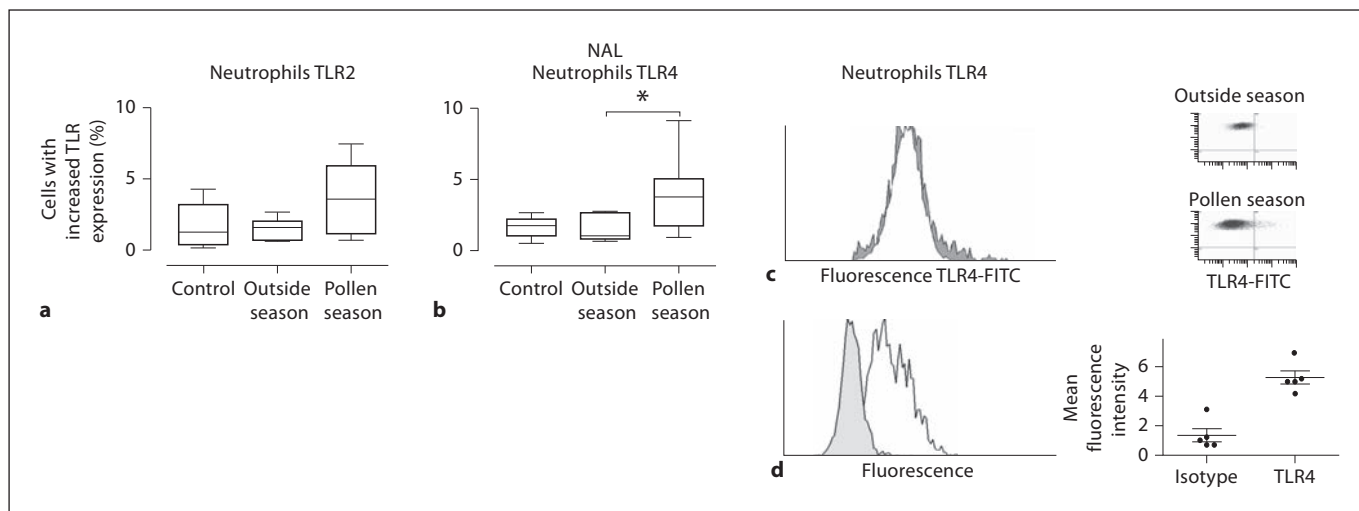


Fig. 1. Expression of intracellular TLR2 (**a**) and TLR4 (**b**) in neutrophils in NAL in control subjects and patients with a history of allergic rhinitis, outside and during the season. **c** TLR4 expression in a subject during the pollen season (dark grey histogram) compared to a subject outside the season (open histogram), and the gating strategy for determining expression of TLR4 is demon-

strated. **d** Isotype staining (light grey histogram) compared to TLR4-stained neutrophils in a healthy control subject (open histogram); the plot displays the corresponding mean fluorescence intensity [medians and ranges (minimum to maximum)]. $n = 5-10$; * $p < 0.05$.

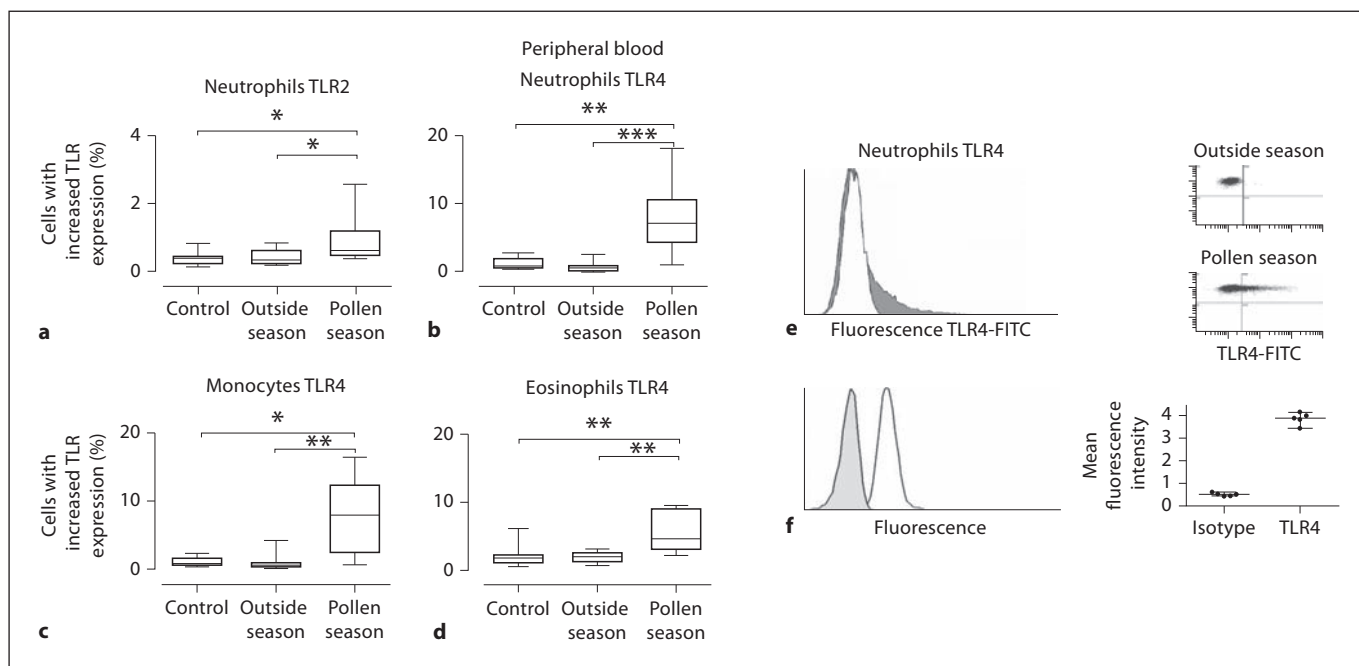


Fig. 2. Expression of intracellular TLR2 and TLR4 in leukocytes from the peripheral blood in control subjects and patients with a history of allergic rhinitis, outside and during the season. **a, b** Staining of neutrophils for TLR2 (**a**) and TLR4 (**b**). **c, d** Staining of monocytes (**c**) and eosinophils (**d**) for TLR4. **e** TLR4 expression in a subject during the pollen season (dark grey histogram) compared to a subject outside the season (open histogram), and

the gating strategy for determining expression of TLR4 is demonstrated. **f** Isotype staining (light grey histogram) compared to TLR4-stained neutrophils in a healthy control subject (open histogram) as well as the corresponding mean fluorescence intensity [medians and ranges (minimum to maximum)]. $n = 5-11$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

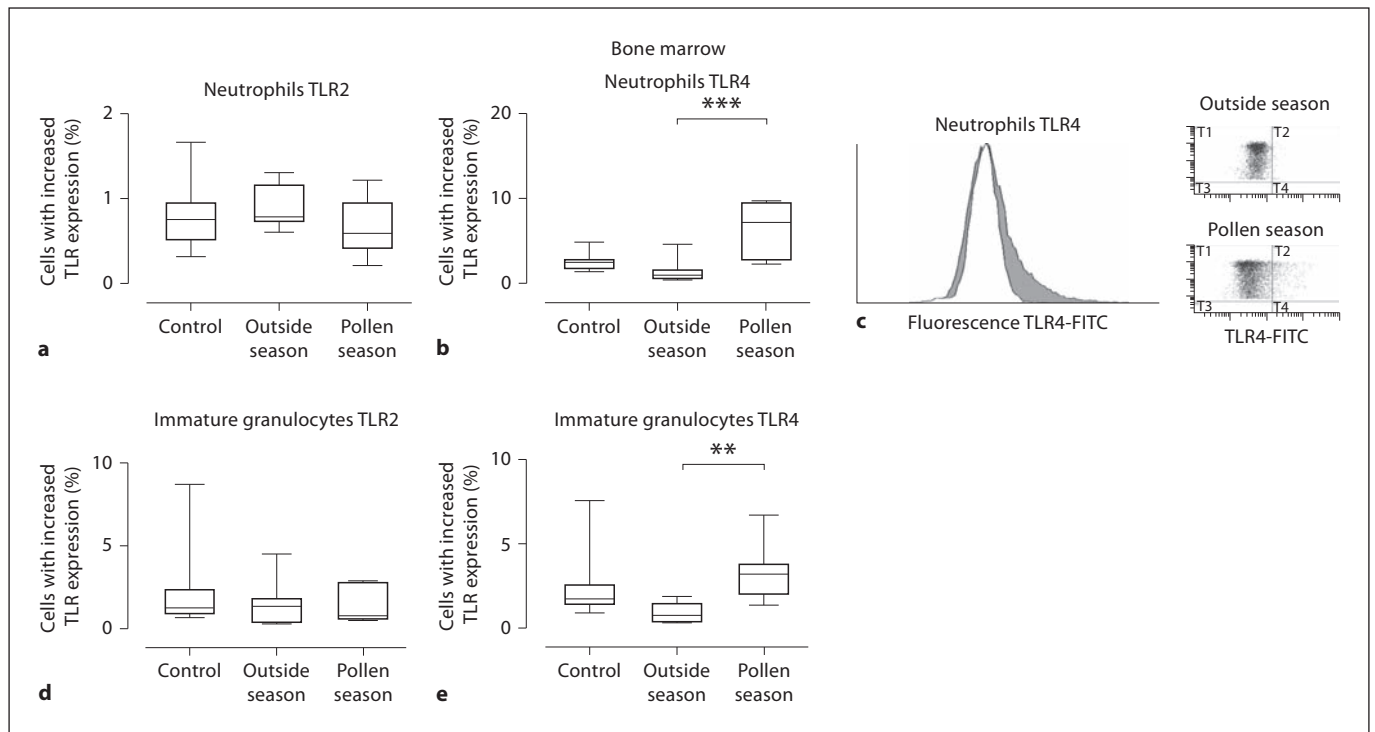


Fig. 3. Expression of intracellular TLR2 and TLR4 in leukocytes from the bone marrow in control subjects and patients with a history of allergic rhinitis, outside and during the season. The top row displays staining of neutrophils for TLR2 (a) and TLR4 (b). c TLR4 expression in a subject during the pollen season (grey his-

togram) compared to a subject outside the season (white histogram), and the gating strategy for determining expression of TLR4 is demonstrated. d, e Staining of TLR2 (d) and TLR4 (e) in immature granulocytes [medians and ranges (minimum to maximum)]. n = 7–11; ** p < 0.01, *** p < 0.001.

by allergen challenge and LPS per se (fig. 4b), but LPS increased the percentage of neutrophils (fig. 4c). Allergen or LPS alone did not affect the levels of any of the measured cytokines. Challenge with LPS following allergen caused an increase in the levels of the Th2 cytokines IL-4, IL-5, IL-10 and IL-13 compared to untreated controls. Furthermore, an increase in the levels of the Th1 cytokine IFN- γ and TNF- α was also observed after LPS following allergen (fig. 5). IL-1 β , IL-2, IL-8 and IL-12p70 were unaffected by all treatment conditions (data not shown).

Discussion

The present study demonstrates an up-regulation of TLR4 on leukocytes derived from NAL, blood and bone marrow during on-going pollen-induced allergic rhinitis. Further, challenge with LPS in a milieu of allergic inflammation causes release of cytokines from the nose which is not seen in response to either allergen or LPS alone, nor when LPS precedes allergen.

We observed no change in the surface expression of the examined TLRs. TLR2 and TLR4 are often categorized as cell surface receptors, but they are also found intracellularly [13–15]. There is a continuous circulation of TLR4 from the Golgi apparatus to the cell surface [16], demonstrating that these receptors are not restricted to the plasma membrane. An increased intracellular expression may therefore reflect an increased production and mobilization of these receptors to the cell surface. Furthermore, since the subjects with symptomatic allergic rhinitis were sampled at the beginning of the pollen season, it is possible that the observed up-regulation would be more pronounced later in the pollen season. Hence, it is possible that the increased expression of intracellular TLRs might be followed by an increased expression of cell surface TLRs.

We have previously observed an up-regulation of TLR4 in epithelial cells upon allergen exposure [10]. Combined with the present findings of increased TLR4 expression in leukocytes in the nose, blood and bone marrow as a consequence of the pollen season, this sug-

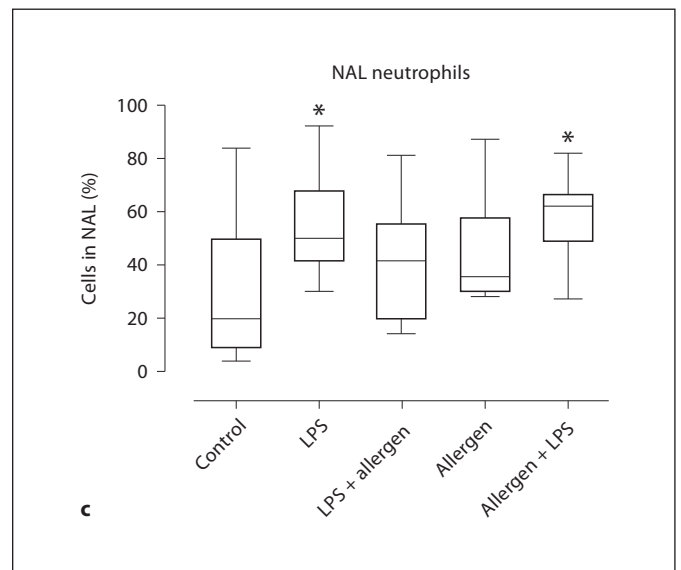
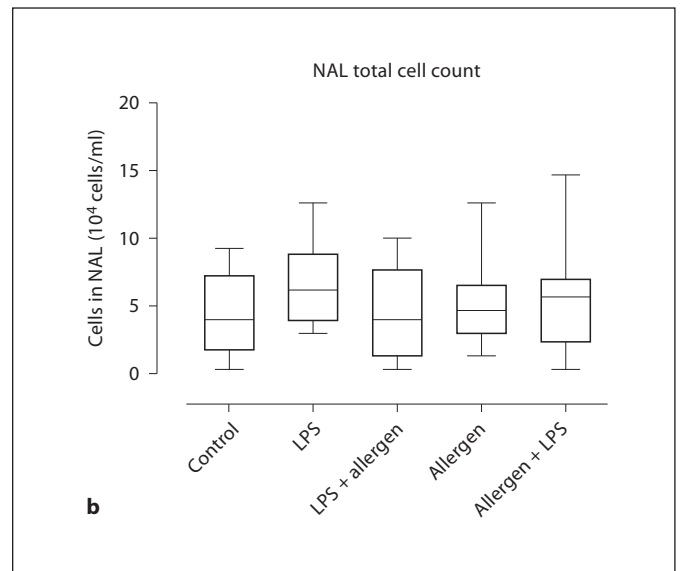
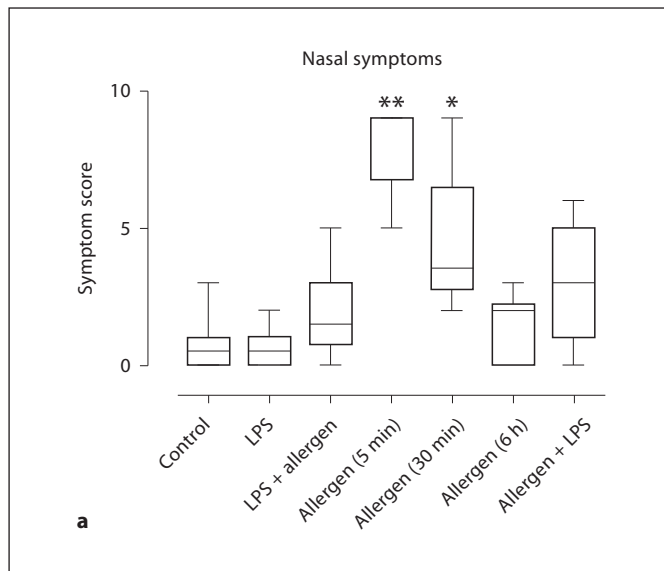


Fig. 4. Nasal symptoms (**a**), along with the total cell count (**b**), and the percentage of neutrophils (**c**) in NAL after challenge with LPS (6 h after challenge), allergen (6 h after challenge) or the combination of these (30 and 6 h after challenge) [medians and ranges (minimum to maximum)]. $n = 10-13$; * $p < 0.05$, ** $p < 0.01$.

gests a systemic role for TLR4 in allergic rhinitis. Previous studies have found an increased release of granulocytes from the bone marrow in response to allergen. Those studies have suggested that this is due to granulocyte maturation and proliferation caused by cytokine release from T cells attracted to the bone marrow during the early-phase response [3, 17]. It may be speculated that the alteration in TLR4 expression seen in the bone marrow granulocytes is mediated by similar mechanisms and that the responsible cytokine is one which is also present locally.

Furthermore, up-regulation of TLR4 in response to various inflammatory mediators has been described in other cell systems. Platelet-activating factor and granulocyte macrophage colony-stimulating factor have both been shown to induce an increase in TLR4 [18, 19] and are found in allergic inflammation [20, 21]. Similarly, histamine can cause an up-regulation of TLR4 and confer greater responsiveness to LPS [22].

Allergen or LPS alone caused no persisting symptoms and no significant cytokine release. Trends were seen but significances were not reached. This might partly be related to the applied doses of allergen and LPS, but also to

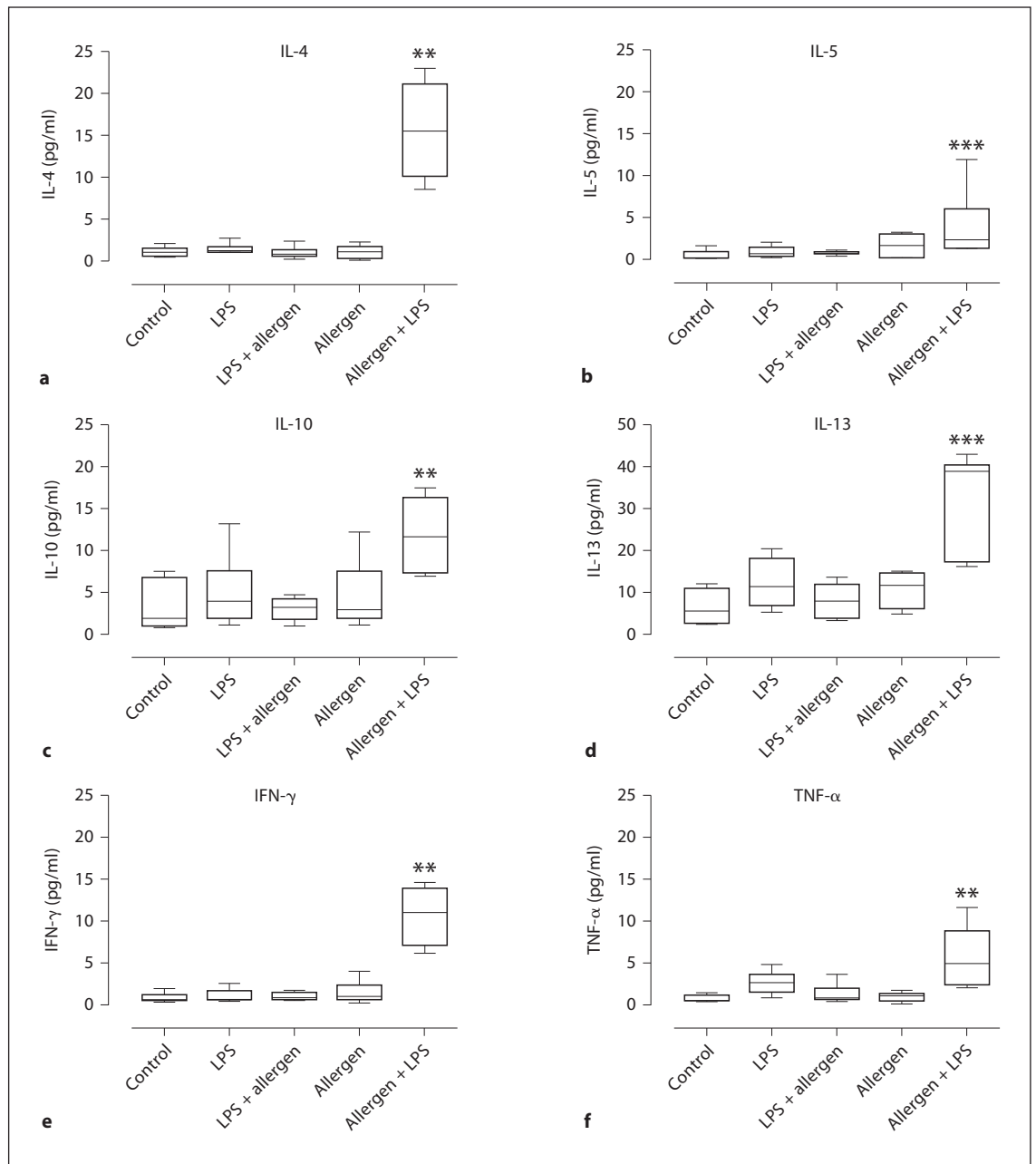


Fig. 5. The levels of IL-4 (a), IL-5 (b), IL-10 (c), IL-13 (d), IFN- γ (e), TNF- α (f) secreted after treatment with LPS (6 h after the challenge), allergen (6 h after the challenge), LPS followed by allergen (30 h after the LPS challenge and 6 h after the allergen challenge) or allergen followed by LPS (30 h after the allergen challenge and 6 h after the LPS challenge) [medians and ranges (minimum to maximum)]. $n = 6$; ** $p < 0.01$, *** $p < 0.001$. vs. control.

the low number of patients examined. Along with several groups, we have shown that challenge with allergen, but not LPS, can cause local nasal symptoms [23] and that both allergen and LPS have the ability to release an array of cytokines in the nose [23–29].

In contrast to the present lack of response seen after allergen and LPS alone, the combination of allergen followed by LPS caused a marked increase in Th2 cytokines in NAL such as IL-4, IL-5, IL-10 and IL-13. This increase was accompanied by a rise in IFN- γ , which is considered

a typical Th1 cytokine, and a rise in the levels of TNF- α . No such increases were seen when allergen was preceded by LPS. Since there was no increase in the total number of cells, we do not believe that the increased levels of nasal cytokines are a result of a quantitative effect on the cell content in NAL, but rather the result of a qualitative change manifested by an increase in TLR4, making the cells more responsive to LPS. It is therefore tempting to suggest that the cytokine release observed upon LPS stimulation following allergen challenge may be the result of an allergen-induced up-regulation of TLR4. Such a suggestion is supported by earlier studies demonstrating that an allergen challenge can augment the LPS-induced cellular response in NAL and that allergen in combination with LPS has the ability to amplify nasal symptoms [30, 31].

The presence of both Th1 and Th2 cytokines is not surprising. While it is known that LPS induces Th1 responses [32, 33], other findings show that LPS-induced TLR4 signalling is also important for the induction of Th2 responses to inhaled allergen [34] and suggested that the induction of a Th1- or a Th2-type inflammation by LPS is dose dependent. Furthermore, a response with

both Th1 and Th2 cytokines after the same LPS dose, dependent on time, has been described [35].

To summarize, the present study demonstrates a systemic up-regulation of TLR4 in symptomatic allergic rhinitis, which might lead to an increased response to LPS when TLR4 is activated in an allergic setting.

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References

- 1 Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al: Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and Allergen). *Allergy* 2008;63(suppl 86):8–160.
- 2 Braunstahl GJ: The unified immune system: respiratory tract-nasobronchial interaction mechanisms in allergic airway disease. *J Allergy Clin Immunol* 2005;115:142–148.
- 3 Togias A: Systemic effects of local allergic disease. *J Allergy Clin Immunol* 2004;113:S8–S14.
- 4 Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN: House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;15:410–416.
- 5 Michel O: Role of house-dust endotoxin exposure in aetiology of allergy and asthma. *Mediators Inflamm* 2001;10:301–304.
- 6 Janeway CA Jr, Medzhitov R: Innate immune recognition. *Annu Rev Immunol* 2002;20:197–216.
- 7 Sabroe I, Read RC, Whyte MK, Dockrell DH, Vogel SN, Dower SK: Toll-like receptors in health and disease: complex questions remain. *J Immunol* 2003;171:1630–1635.
- 8 Gangloff SC, Guenounou M: Toll-like receptors and immune response in allergic disease. *Clin Rev Allergy Immunol* 2004;26:115–125.
- 9 Sabroe I, Parker LC, Wilson AG, Whyte MK, Dower SK: Toll-like receptors: their role in allergy and non-allergic inflammatory disease. *Clin Exp Allergy* 2002;32:984–989.
- 10 Fransson M, Adner M, Erjefalt J, Jansson L, Uddman R, Cardell LO: Up-regulation of Toll-like receptors 2, 3 and 4 in allergic rhinitis. *Respir Res* 2005;6:100.
- 11 Fransson M, Benson M, Wennergren G, Cardell LO: A role for neutrophils in intermittent allergic rhinitis. *Acta Otolaryngol* 2004;124:616–620.
- 12 Fransson M, Benson M, Erjefalt JS, Jansson L, Uddman R, Björnsson S, et al: Expression of Toll-like receptor 9 in nose, peripheral blood and bone marrow during symptomatic allergic rhinitis. *Respir Res* 2007;8:17.
- 13 Hassan F, Islam S, Tumurkhuu G, Naiki Y, Koide N, Mori I, et al: Intracellular expression of Toll-like receptor 4 in neuroblastoma cells and their unresponsiveness to lipopolysaccharide. *BMC Cancer* 2006;6:281.
- 14 Uronen-Hansson H, Allen J, Osman M, Squires G, Klein N, Callard RE: Toll-like receptor 2 (TLR2) and TLR4 are present inside human dendritic cells, associated with microtubules and the Golgi apparatus but are not detectable on the cell surface: integrity of microtubules is required for interleukin-12 production in response to internalized bacteria. *Immunology* 2004;111:173–178.
- 15 Satoh M, Shimoda Y, Maesawa C, Akatsu T, Ishikawa Y, Minami Y, et al: Activated Toll-like receptor 4 in monocytes is associated with heart failure after acute myocardial infarction. *Int J Cardiol* 2006;109:226–234.
- 16 Latz E, Visintin A, Lien E, Fitzgerald KA, Monks BG, Kurt-Jones EA, et al: Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the Toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. *J Biol Chem* 2002;277:47834–47843.
- 17 Johansson AK, Sergejeva S, Sjöstrand M, Lee JJ, Lotvall J: Allergen-induced traffic of bone marrow eosinophils, neutrophils and lymphocytes to airways. *Eur J Immunol* 2004;34:3135–3145.

- 18 Soliman A, Michelsen KS, Karahashi H, Lu J, Meng FJ, Qu X, et al: Platelet-activating factor induces TLR4 expression in intestinal epithelial cells: implication for the pathogenesis of necrotizing enterocolitis. *PLoS One* 2010;5:e15044.
- 19 O'Mahony DS, Pham U, Iyer R, Hawn TR, Liles WC: Differential constitutive and cytokine-modulated expression of human Toll-like receptors in primary neutrophils, monocytes, and macrophages. *Int J Med Sci* 2008; 5:1–8.
- 20 Gajewska BU, Wiley RE, Jordana M: GM-CSF and dendritic cells in allergic airway inflammation: basic mechanisms and prospects for therapeutic intervention. *Curr Drug Targets Inflamm Allergy* 2003;2:279–292.
- 21 Alfaro V: Role of histamine and platelet-activating factor in allergic rhinitis. *J Physiol Biochem* 2004;60:101–111.
- 22 Gutierrez-Venegas G, Cruz-Arrieta S, Villeda-Navarro M, Mendez-Mejia JA: Histamine promotes the expression of receptors TLR2 and TLR4 and amplifies sensitivity to lipopolysaccharide and lipoteichoic acid treatment in human gingival fibroblasts. *Cell Biol Int* 2011;35:1009–1017.
- 23 Fransson M, Adner M, Uddman R, Cardell LO: Lipopolysaccharide-induced down-regulation of uteroglobin in the human nose. *Acta Otolaryngol* 2007;127:285–291.
- 24 Linden M, Greiff L, Andersson M, Svensson C, Akerlund A, Bende M, et al: Nasal cytokines in common cold and allergic rhinitis. *Clin Exp Allergy* 1995;25:166–172.
- 25 Erin EM, Zacharasiewicz AS, Nicholson GC, Tan AJ, Higgins LA, Williams TJ, et al: Topical corticosteroid inhibits interleukin-4, -5 and -13 in nasal secretions following allergen challenge. *Clin Exp Allergy* 2005;35:1608–1614.
- 26 Danuser B, Rebsamen H, Weber C, Krueger H: Lipopolysaccharide-induced nasal cytokine response: a dose-response evaluation. *Eur Arch Otorhinolaryngol* 2000;257:527–532.
- 27 Peden DB, Tucker K, Murphy P, Newlin-Clapp L, Boehlecke B, Hazucha M, et al: Eosinophil influx to the nasal airway after local, low-level LPS challenge in humans. *J Allergy Clin Immunol* 1999;104:388–394.
- 28 Sigsgaard T, Bonefeld-Jorgensen EC, Kjaergaard SK, Mamas S, Pedersen OF: Cytokine release from the nasal mucosa and whole blood after experimental exposures to organic dusts. *Eur Respir J* 2000;16:140–145.
- 29 Ekman AK, Fransson M, Rydberg C, Adner M, Cardell LO: Nasal challenge with LPS stimulates the release of macrophage inflammatory protein 1 α . *Int Arch Allergy Immunol* 2009;149:154–160.
- 30 Eldridge MW, Peden DB: Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma. *J Allergy Clin Immunol* 2000;105:475–481.
- 31 Braga CR, Rizzo MC, Naspitck CK, Sole D: Nasal provocation test (NPT) with isolated and associated Dermatophagoides pteronyssinus (Dp) and endotoxin lipopolysaccharide (LPS) in children with allergic rhinitis (AR) and nonallergic controls. *J Investig Allergol Clin Immunol* 2004;14:142–148.
- 32 Le J, Lin JX, Henriksen-DeStefano D, Vilcek J: Bacterial lipopolysaccharide-induced interferon-gamma production: roles of interleukin 1 and interleukin 2. *J Immunol* 1986; 136:4525–4530.
- 33 D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste M, et al: Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J Exp Med* 1992;176:1387–1398.
- 34 Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K: Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;196:1645–1651.
- 35 Mukherjee S, Chen LY, Papadimos TJ, Huang S, Zuraw BL, Pan ZK: Lipopolysaccharide-driven Th2 cytokine production in macrophages is regulated by both MyD88 and TRAM. *J Biol Chem* 2009;284:29391–29398.