Allergic asthma: Environmental factors challenging the immune system
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Very low doses of endotoxin reveal differential cytokine responses between peripheral blood mononuclear cells from allergic asthmatics versus healthy individuals

Marianne A. van de Pol, Tamara Dekker, Barbara S. Smids, Esmeralda J.M. Krop, Jaring S. van der Zee, René Lutter

 Manuscript in preparation
ABSTRACT

Introduction: Multiple epidemiological studies suggest an inverse association between the development of T helper (Th)2-driven allergic inflammation and (early-life) exposure to endotoxin (lipopolysaccharide (LPS)), which is ubiquitous in our living environment. Most in vitro studies, investigating the underlying mechanisms, are performed with relatively high doses of LPS, as compared to daily airborne exposure.

Aim: To investigate the Th1/Th2-cytokine response of peripheral blood mononuclear cells (PBMC) from house dust mite (HDM)-allergic and non-allergic subjects to very low doses of LPS that are comparable to that by airborne indoor exposure.

Methods: PBMC from 29 allergic asthmatics and 30 non-allergic controls, matched by age and gender, were stimulated with very low doses of LPS (10 and 100 pg/ml). The numbers of cells producing Th1- and Th2-cytokines were determined in ELISPOT assays and cytokine production was quantified in 24 h culture-supernatant by luminex assays.

Results: PBMC from allergic subjects produced significantly less IL-10, IL-12p40 and IFNγ to LPS 10 pg/ml, compared to those from non-allergic asthmatics (p < 0.001). Interestingly, allergic subjects also had less IL-10 producing cells/10⁵ cells (mean (se): 101.7 (20.2) vs. 182.9 (23.9), p < 0.001). This differential cytokine production was overcome by exposure to LPS 100 pg/ml, leading to an equally pronounced IL-10, IL-12p40 and IFNγ production by PBMC from both groups. LPS 100 pg/ml also reduced HDM-stimulated production of Th2 cytokines by PBMC from allergic subjects (-69%, p < 0.001 for IL-5, -31%, p = 0.15 for IL-13).

Conclusion: There is an enhanced threshold at very low doses of LPS (10 pg/ml, comparable with indoor LPS exposure) in PBMC from allergic subjects that prevents induction of mediators that can inhibit the production of Th2 cytokines in response to HDM. We postulate that this threshold may contribute to sensitization by allergens.

INTRODUCTION

Allergic asthma is characterized by T helper (Th)2-driven, often eosinophilic inflammation of the airways, with airway hyperresponsiveness (AHR) and recurrent episodes of shortness of breath with variable airflow obstruction¹. The Th2-driven inflammation is characterized by the cytokines interleukin (IL)-4, IL-5 and IL-13 that are responsible respectively for the secretion of IgE, activation and recruitment of eosinophils, and airway remodeling, leading to the pathology characteristic of asthma²,³. Allergic asthmatics have been sensitized to airborne allergens via initiation of a type 2 immune response, which mechanistically is still not fully elucidated⁴. A complex interplay between genetic and environmental factors appears to determine sensitization to allergens.
Epidemiological studies have revealed an inverse association between the development of allergic diseases and (early-life) exposure to endotoxin or lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria, which is ubiquitous in our living environment. In established asthma, however, exposure to LPS is known to aggravate the allergen-induced inflammation. In addition, several studies have demonstrated that the dose of LPS present during allergen sensitization or allergen challenge is critical for the outcome of the immune response. For example, inhalation of low levels of LPS together with allergen induced a Th2-type response with eosinophilic infiltration in the airways. However, inhalation of high levels of LPS led to a Th1 response (characterized by the cytokines IL-12 and interferon (IFNγ)), dampening the Th2-driven inflammation and inducing neutrophilic inflammation. Thus, the effect of LPS exposure on allergic sensitization and asthma appears to depend on the presence or absence of preexisting asthma and the timing and dose of LPS exposure.

Most of the abovementioned studies were performed with concentrations of LPS comparable with LPS levels measured in settled dust samples from floors and mattresses in the homes of allergic and non-allergic subjects, varying from <3000 to >180,000 EU/g dust. These levels are very high compared to the airborne indoor LPS exposure (0.58-1.49 EU/m³) to which the airways of, for example, young children are exposed most of the day. Assuming a daily breathing rate of 3.6 m³/day for a healthy infant aged 0-2 month to 12.1 m³/day for a healthy child aged 5 years, the daily indoor exposure to LPS varies from 2.1 to 18.0 EU/day.

This has led us to propose that antigen-presenting cells from allergic versus non-allergic individuals respond differently to very low concentrations of LPS. We assessed the dose-dependent effect of LPS including a LPS dose that reflects indoor exposure, on the (allergen-induced) cytokine response of peripheral blood mononuclear cells (PBMC) from well-characterized allergic asthmatics and non-allergic healthy volunteers.

**METHODS**

**Subjects**

In a previously described clinical intervention study, we collected blood at baseline from 29 adult allergic patients with intermittent to mild persistent asthma, according to American Thoracic Society criteria. All patients were sensitized to HDM, as determined by skin prick test and IgE to *D. pteronyssinus* > 0.5 kU/L. Blood from 30 non-allergic healthy volunteers, was obtained at the start of another previously described, longitudinal cohort study of apprentice laboratory animal workers. The non-allergic volunteers were matched by age, gender and the presence of pets at
home, had less than 18 months of occupational contact with animals at the start of the study and developed during the 2-year follow-up no sensitization to the animals with which they were working. All subjects gave written informed consent. Both studies were approved by the AMC Medical Ethics Committee.

Isolation of PBMC from blood samples
Heparin blood was collected at the start of aforementioned studies. PBMC were isolated within 6 hours by standard density gradient techniques and stored in aliquots in liquid nitrogen until analysis.

Cell stimulation assays
We performed ELISpot assays to determine the number of cells producing Th1- and Th2-cytokines and quantified the cytokine production in 24 hours-culture supernatant in a Luminex assay as described in detail earlier\textsuperscript{17}, with minor modifications. Briefly, cryopreserved PBMC were thawed, washed and incubated in culture medium with phytohemaglutinin (PHA, 0.1 μg/ml) as a positive control, LPS (10 pg/ml or 100 pg/ml), HDM (1 μg/ml) or medium alone. The biological activity of LPS 10 pg/ml (Sigma-Aldrich, St.Louis, MO, USA) was 1 EU/ml (determined in Limulus amebocyte lysate (LAL)-assay) and equalled the amount of LPS contamination in our HDM extract (1 EU/μg). We used cytokine-specific capture and detection antibodies for IL-4, IL-13, IL-10, IL-12p40 and IFN\textgamma from Mabtech AB (Nacka Strand, Sweden), according to the manufacturer’s protocol, and incubated the cells at 37°C and 5% CO\textsubscript{2} for 72 hours in 96-wells PVDF-based membrane plates (Millipore, Billerica, MA, USA). After visualization of the spots with streptavidin-ALP and BCIP/NBT substrate (Sigma-Aldrich), the numbers of cytokine-producing cells were determined by ELISpot analysis software from A.EL.VIS GmbH (Hannover, Germany) and were expressed as positive cells per 1x10\textsuperscript{5} stimulated cells.

For quantification of the cytokine production, we stimulated PBMC in round-bottom 96-well plates for 24 hours with the same culture medium and stimuli as used in the ELISpot assays. The amount of several cytokines (IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-13 and IFN\textgamma) was determined in culture supernatant in a Luminex assay with antibodies from Biosource (Camarillo, CA, USA).

Responses from PHA-stimulated cells were treated as positive control and medium-only culture as negative control. All cytokine responses from stimulated cultures were adjusted for background by subtracting the responses from the medium-only culture. Analyses were performed batch-wise to limit inter-assay variation. In addition, we took along control PBMC, which yielded similar results each time.
Flow cytometric analysis
Cryopreserved PBMC were thawed, washed and incubated (10^6/ml) overnight in culture medium with HDM 1 µg/ml, LPS (10 pg/ml or 100 pg/ml) or medium alone. After washing with cold buffer (PBS containing 0.5% (w/v) bovine serum albumin (Sigma) and 2 mM EDTA (Merck), cells were incubated for 5 min with IL-10-specific catch reagent (IL-10 secretion assay (PE), Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) in cold medium, according to manufacturer’s instructions. Subsequently, the cells were resuspended in warm medium and incubated for 45 min in closed tubes at 37°C allowing them to secrete IL-10. After washing with cold buffer, IL-10 detection antibody (PE) and different combinations of the following surface markers were added and incubated for 20 min on ice: CD3 FITC, CD5 APC, CD8 PerCP-Cy5.5, CD25 APC, CD16+56 FITC (all from BD), CD4 APC-AF780, CD14 PE-Cy7 and CD19 APC-AF780 (all from eBioscience). Then, cells were washed with cold buffer and analyzed by FACSCanto (BD Biosciences) multicolor flow cytometry and FlowJo software (Tree Star Inc.).

Statistical analysis
SPSS version 15.0.1 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). We used Wilcoxon signed rank test to compare the cytokine responses of different stimuli within subjects. Differences between the allergic and non-allergic group were analyzed with the student’s t-test. When data were not normally distributed, either log transformation was performed (PC_{20} methacholine, total IgE) to get normal distribution or the non-parametric Mann-Whitney U test was used (cytokine responses). Correlations were determined with the Spearman rank test. Two-sided p-values of <0.05 were considered statistically significant.

RESULTS
Subjects
Thirty healthy non-allergic subjects from the apprentice laboratory animal workers cohort were matched with the 29 subjects from the allergic asthmatic group to minimize differences in daily environmental allergen- and LPS exposure. Subject characteristics are described in table 1. As expected, the forced expiratory volume in 1 second (FEV1) and provocative concentration of methacholine causing a decline in FEV1 of 20% from baseline (PC_{20}) of the allergic asthmatic group were significantly lower than those of the healthy group whereas the amount of total immunoglobulin E (IgE) in serum was significantly higher in the allergic asthmatic group.
Table 1. Subject characteristics

<table>
<thead>
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<th></th>
<th>Allergic</th>
<th>Healthy</th>
<th>p-value</th>
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<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>30</td>
<td></td>
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<tr>
<td>gender (male/female)</td>
<td>7 / 22</td>
<td>12 / 18</td>
<td>0.399</td>
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<tr>
<td>age (years) #</td>
<td>26 (18; 51)</td>
<td>24 (18; 40)</td>
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<tr>
<td>FEV1 (% predicted) ‡</td>
<td>94.8 (1.8)</td>
<td>105.4 (2.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC_{20} methacholine (mg/ml) †</td>
<td>1.38 (1.3)</td>
<td>&gt;8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total IgE (kU/L) †</td>
<td>223.2 (1.2)</td>
<td>19.9 (1.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as #median (range), †geometric mean (SE), ‡mean (SE). FEV1, forced expiratory volume in 1 second; PC_{20} methacholine, provocative concentration of methacholine causing a decline in FEV1 of 20% from baseline; IgE, immunoglobulin E.

**Impaired induction of cytokines in PBMC from allergic asthmatics in response to indoor airborne concentrations of LPS**

Stimulation of PBMC from allergic asthmatics and non-allergic controls with indoor airborne concentrations of LPS (10 pg/ml) resulted in a significantly lower level of IL-6, IL-10, IL-12p40 and IFNγ in the supernatant of PBMC from the allergic asthmatics compared to those from the non-allergic subjects (p < 0.001, fig. 1A). A ten-fold higher dose of LPS (100 pg/ml) induced a marked increase of these cytokines in both groups and diminished the difference between the groups (fig. 1B). Interestingly, except for the number of IL-10-producing cells after stimulation with the lowest dose of LPS, no difference between the groups was observed for the numbers of cells producing these cytokines (fig. 1C, D). The production of IL-4 and IL-5 was not influenced by either dose of LPS (data not shown). In contrast, the highest dose of LPS (100 pg/ml) induced a low number of IL-13-producing cells and very low amounts of IL-13. Both were significantly higher in PBMC from allergic asthmatics compared to those from the non-allergic subjects (mean number of IL-13-positive cells/10^5 cells (SE): 6.7 (1.4) vs. 1.3 (0.5), p < 0.001; mean IL-13 production (SE): 1.31 (0.38) pg/ml vs. 0.53 (0.25) pg/ml, p < 0.05). Overall, stimulation with LPS predominantly induced a Th1-like response in PBMC from both groups.

**LPS reduces HDM-induced Th2 cytokine production in allergic asthmatics**

Addition of a low dose of LPS (100 pg/ml) to HDM-stimulated PBMC from allergic asthmatics, reduced the production of Th2 cytokines (-69%, p < 0.001 for IL-5, -31% for IL-13, p = 0.15, IL-4 levels were below detection limit). At the same time, the production of IL-10, IFNγ and IL-12p40 was significantly increased (fig. 2A). A similar Th2-reducing and Th1- and IL-10-increasing effect of LPS was observed for the number of cytokine-
Differential cytokine responses to low dose LPS in allergic asthmatics

Figure 1. Impaired induction of cytokines in allergic asthmatics in response to indoor concentrations of LPS. PBMC from allergic asthmatics (open bars) and non-allergic healthy subjects (grey bars) were stimulated with LPS 10 pg/ml (A, C) and LPS 100 pg/ml (B, D). Levels of IL-6, IL-10, IL-12p40 and IFNγ, measured in 24 h culture supernatant are shown in figures (A) and (B). Figures (C) and (D) represent the number of cells producing IL-10, IL-12 or IFNγ per 100,000 cells, determined in ELISPOT assays. Mean values (SE) are shown and values are corrected for medium control. ** p<0.01, *** p<0.001.

Figure 2. LPS reduces HDM-induced Th2 cytokine production in allergic asthmatics. (A) Cytokine production after 24 hours by PBMC from allergic asthmatics that were stimulated with HDM (1 μg/ml) in the absence (open bars) or presence (black bars) of LPS (100 pg/ml, grey bars). (B) Number of cytokine-producing PBMC from allergic asthmatics that were stimulated with HDM (1 μg/ml, open bars) in the absence (open bars) or presence (black bars) of LPS (100 pg/ml, grey bars) for 72 hours in an ELISPOT assay. Mean values (SE) are shown and values are corrected for medium control. Significant changes from HDM-stimulated values by addition of LPS are marked with ***p<0.001.
producing cells (fig. 2B). Furthermore, PBMC from allergic asthmatics produced less IL-10, IL-12p40 and IFNγ in response to HDM (1 μg/ml, containing 10 pg LPS), compared to those from non-allergic subjects. Addition of LPS (100 pg/ml) to these HDM-stimulated PBMC abrogated the observed difference in cytokine response and led to an equally pronounced IL-10-, IL-12p40- and IFNγ-production by PBMC from both groups (fig. 3). Because LPS-induced IL-10 production by whole blood cultures of allergic asthmatics has been shown to play a role in the reduction of Th2 cytokines by high doses of LPS, we looked more closely to the IL-10 producing cells in our study.

**Figure 3.** Addition of LPS to PBMC from allergic asthmatics leads to cytokine responses comparable with those from non-allergic subjects. Cytokine production by PBMC from allergic asthmatics (open bars) and non-allergic healthy volunteers (grey bars) that were stimulated with HDM (1 μg/ml, contaminated with 10 pg LPS) in the absence or presence of LPS (100 pg/ml). Mean values (SE) are shown and values are corrected for medium control.

**Stronger IL-10 response to indoor airborne levels of LPS in non-allergic subjects**

PBMC from non-allergic subjects, stimulated with low (100 pg/ml) and very low (10 pg/ml) doses of LPS, produced significantly more IL-10 than those from allergic asthmatics (mean (se): 37.06 (9.74) pg/ml vs. 3.43 (1.08) pg/ml, p < 0.001 for LPS 10 pg/ml and 368.0 (56.72) pg/ml vs. 164.5 (22.15) pg/ml, p = 0.006 for LPS 100 pg/ml, fig. 4A). Moreover, stimulation with the lowest dose of LPS induced significantly more IL-10-producing cells in PBMC from the non-allergic subjects compared to those from the allergic subjects (mean (se): 148.6 (20.4) /10⁵ cells vs. 81.7 (17.7) /10⁵ cells, p = 0.002, fig. 4B).

The apparent difference between both groups in the HDM-stimulated cultures that can be observed in figure 4, was found to be strongly related to the difference in spontaneous production of IL-10 shown in the medium-only cultures, and was, in contrast to the LPS-induced IL-10 production, non-significant when adjusted for background. Furthermore, plotting the amount of LPS-induced IL-10 against the number of IL-10-producing cells indicated that non-allergic subjects not only had more IL-10 producing cells, but also produced relatively more IL-10 per cell compared with allergic asthmatics, especially
Differential cytokine responses to low dose LPS in allergic asthmatics

**Figure 4.** Stronger IL-10 response to very low-dose LPS in non-allergic subjects.  
(A) Production of IL-10 in pg/ml by PBMC of allergic (circles) and non-allergic (triangles) subjects after 24 h incubation with medium alone, HDM (1µg/ml), LPS (10 pg/ml or 100 pg/ml) and a combination of HDM and LPS (100 pg/ml). (B) Number of IL-10-producing PBMC (IL-10-positive spots/10⁵ cells in ELISPOT assay) of allergic (circles) and non-allergic (triangles) subjects after 72 h incubation with medium alone, HDM (1µg/ml), LPS (10 pg/ml or 100 pg/ml) and a combination of HDM and LPS (100 pg/ml). Although the data from the stimulated cultures in this figure are not adjusted for medium-control, the observed significant differences between both groups are calculated with values that are corrected for background and are marked with ** p<0.01, *** p<0.001.  
(C, D): Correlation of the number of IL-10-producing PBMC with the amount of IL-10 produced by PBMC after stimulation with (C) LPS (10 pg/ml) and (D) LPS (100 pg/ml) in allergic (circles, dashed lines) and non-allergic (triangles, solid lines) subjects.

when exposed to indoor airborne levels of LPS (10 pg/ml: Rs = 0.611, p < 0.001 for non-allergics vs Rs = 0.392, p = 0.035 for allergic asthmatics, fig.4C). Thus, PBMC from non-allergic subjects seem to have more IL-10-producing cells that, in addition, produce more IL-10 than those from allergic asthmatics in response to indoor airborne LPS concentrations.

No differences between allergic and non-allergic subjects in IL-10 secreting T- or B-cells  
To investigate whether the phenotypes of these IL-10 producing cells were different for both groups, we incubated PBMC from a subgroup of 6 allergic asthmatics and 6 healthy
non-allergic subjects overnight with medium alone, LPS or HDM. The next day, IL-10-positive cells were detected with an IL-10-secretion assay and phenotyped with surface markers for T cells (cytotoxic: CD3$^+$/CD8$^+$, helper: CD3$^+$/CD4$^+$ or T regulatory (Tr)1: CD3$^+$/CD4$^+$/CD25$^{++}$), B cells (classical: CD3$^-$/CD19$^+$ or B regulatory (Br)1: CD3$^-$/CD19$^+$/CD5$^+$), monocytes (CD3$^-$/CD14$^+$) and NK (CD3$^-$/CD16+56$^+$) cells. No differences between allergic and non-allergic subjects were found with regard to any of these IL-10-secreting phenotypic lymphocytes (data not shown), neither in unstimulated PBMC nor after stimulation with LPS or HDM. Differences in monocytes could not be quantified in this assay, because LPS-stimulated monocytes tended to adhere to the culture plate and were not detectable by flow cytometry.

**DISCUSSION**

In the present study, we show that a very low dose of LPS (10 pg/ml equivalent to 1 EU/ml, determined in LAL-assay), comparable with indoor airborne LPS exposure, can induce cytokines, such as IL-10, IL-12 and IFN$\gamma$, in PBMC from non-allergic subjects, but not in PBMC from allergic asthmatics. After stimulation with a 10-times higher dose of LPS the production of these cytokines that can attenuate the production of Th2 mediators, increased in the allergic asthmatic group to levels similar to those of the non-allergic group. Furthermore, after stimulation with HDM (1 $\mu$g/ml, containing 10 pg/ml LPS) the production of IL-10, IL-12 and IFN$\gamma$ by PBMC from allergic asthmatics was less pronounced compared to that by PBMC from non-allergic subjects. Adding LPS (100 pg/ml) to HDM-stimulated PBMC from allergic asthmatics significantly up regulated the production of IL-10, IL-12 and IFN$\gamma$, again leading to cytokine levels similar to those of non-allergic subjects. Moreover, as has been shown before with high doses of LPS$^{20}$, addition of this relatively low amount of LPS to HDM-stimulated cells significantly reduced the allergen-induced production of Th2 cytokines in PBMC from allergic asthmatics. The same shift to a non-allergic profile was seen in the number of cells producing these cytokines when LPS was added to HDM-stimulated PBMC from allergic asthmatics.

Interestingly, PBMC from non-allergic subjects not only produced significantly more IL-10, but also had more IL-10-producing cells in response to indoor airborne levels of LPS compared to PBMC from allergic asthmatics. The production of IL-10 by PBMC is facilitated by monocytes, T cells and B cells. IL-10 has been shown to limit immune responses to allergens, inhibit the overproduction of inflammatory cytokines and induce tolerance$^{21-24}$. According to Min et al.$^{20}$, the induction of IL-10 in particular, but not of IL-12 or IFN$\gamma$, was pivotal for the Th2-cytokine inhibiting effect of high doses of LPS. With this in mind, we wondered if the non-allergic subjects had more or different IL-10-secreting cells that were possibly deficient in allergic asthmatics and/or if they just had a lower threshold to LPS.
To determine if the difference in IL-10-producing cells in our study was related to regulatory Tr1 and Br1 cells, recently described by Akdis and Hussaarts, we stimulated the PBMC from both groups with the very low dose of LPS and analyzed the IL-10-secreting cells with flow cytometry. In contrast to previously mentioned studies, we observed no differences in the number of IL-10-secreting Tr1 or Br1 cells between the PBMC from allergic asthmatics and non-allergic subjects. Also IL-10-producing monocytes have been shown to play an important role in the reduction of Th2-cytokines and induction of tolerance during, for example, allergen-specific immunotherapy (SIT). Unfortunately, a high percentage of the monocytes in our study adhered to the culture plate after stimulation with LPS, which made it impossible to analyze and quantify these cells accurately with flow cytometry. Still, the fact that besides IL-10 also the production of IL-6 and IL-12p40, which are mainly produced by monocytes, significantly increased after stimulation with LPS might indicate that the LPS-induced ‘Th2-reducing’ effect is mainly the result of IL-10-secreting monocytes. Further research is needed to confirm the role of IL-10 and monocytes in this process.

With regard to the reduced responsiveness of allergic asthmatics to LPS, conflicting studies have been published concerning Toll-like receptor (TLR)-4, the major signaling receptor for LPS. In the periphery, TLR-4 is mainly expressed by monocytes and forms a complex with CD14, LPS binding protein (LBP), myeloid differentiation protein (MD)-2 and intracellular components (reviewed in 33 and 34). Polymorphisms of CD14 and TLR-4, have been suggested to be associated with allergy and asthma. However, other studies have not found this association. In our study, the similar cytokine response to LPS 100 pg/ml in PBMC from both groups indicates that it is not likely that there is a marked difference between allergic asthmatics and non-allergic healthy subjects in the overall expression of TLR-4 and CD14 by PBMC. The fact that the cells of the allergic asthmatics failed to respond to the low indoor levels of LPS in contrast to the cells of the non-allergic subjects suggests a different threshold in the signaling cascade. Upon binding to the TLR-4 complex, LPS can activate several signal transduction pathways including the MyD88-dependent pathways leading to downstream activation of nuclear factor kappa B (NFκB) and mitogen-activated protein kinase (MAPK) p38, which is followed by an increased production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukins; the MyD88-independent pathway via TRIF (Toll-interleukin-receptor (TIR)-domain-containing adaptor protein inducing IFN), leads to activation of IFN-regulatory factor 3 (IRF3), which increases the production of IFNβ. Differences in the presence or absence of pivotal proteins in these pathways might explain the higher threshold to LPS in allergic asthmatics. It remains to be determined which proteins are responsible for the higher threshold and whether this difference in threshold also applies to other cells such
as airway epithelial cells, dendritic cells and alveolar macrophages, which make up the first line of defense against inhaled antigens.

The results of this study are in line with the protective role of LPS during the development of Th2-responses to allergens. Our results suggest that allergic asthmatics need a higher dose of LPS to shift from a Th2 profile to a Th1 profile and that the indoor airborne LPS levels in homes of industrialized countries are currently too low to overcome this threshold. The higher LPS-induced IL-10 levels and number of IL-10-producing cells in non-allergic subjects suggest a “regulatory” role for IL-10 in this process. Possible (epi)-genetic differences in the induction of dampening- or activating-proteins of the TLR-4 signaling pathways that might be responsible for this difference in threshold, might also explain the exaggerated response to LPS in established asthma.

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