

A *TLR4* polymorphism is associated with asthma and reduced lipopolysaccharide-induced interleukin-12(p70) responses in Swedish children

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Background: Bacterial signals play an important role in the maturation of the immune system. Polymorphisms in genes coding for receptors to bacterial components can alter the immune responsiveness of the host to microbial agents and may indicate the development of aberrant immune responses that are associated with immune-mediated diseases such as atopic diseases.

Objective: The study's objective was to investigate the relationship between *TLR4* and *CD14* gene polymorphisms, the LPS responsiveness of PBMCs, and the presence of asthma and allergic rhinoconjunctivitis in children.

Methods: The *TLR4* (Asp299Gly) and *CD14*–159 polymorphisms were determined in 115 Swedish children aged 8 and 14 years. LPS-induced IL-12(p70), IL-10, and IFN- γ responses of PBMCs from 69 of the children were analyzed by means of ELISA. The levels of soluble CD14 in serum samples were analyzed by means of ELISA, and the total IgE levels were analyzed by means of UniCAP Total IgE (Pharmacia Diagnostics, Uppsala, Sweden).

Results: Decreased LPS-induced IL-12(p70) and IL-10 responses were associated with the *TLR4* (Asp299Gly) polymorphism and independently with asthma, especially atopic asthma. The *TLR4* (Asp299Gly) polymorphism was associated with a 4-fold higher prevalence of asthma in school-

Abbreviations used

sCD14: Soluble CD14

SPT: Skin prick test

Th: T-helper

TLR: Toll-like receptor

aged children (adjusted odds ratio 4.5, 95% CI 1.1–17.4) but not to allergic rhinoconjunctivitis.

Conclusion: A *TLR4* polymorphism modifies innate immune responses in children and may be an important determinant for the development of asthma. This may influence the outcome of intervention studies that use microbial stimuli as immune modulators. (*J Allergy Clin Immunol* 2004;114:561–7.)

Key words: asthma, *TLR4*, lipopolysaccharide, IL-12, IL-10

Microbial stimulation is important for the postnatal development of the immune system. In animal models, lack of microbial exposure has been associated with a prolonged period of T-helper (Th) 2-like immune responses, including IgE antibody formation and a delayed development of oral tolerance.¹ Colonization with normal commensal bacteria promotes oral tolerance and immune deviation toward Th1-like responses.¹ Th1 cells produce IFN- γ and promote cell-mediated inflammatory reactions including the activation of phagocytosis and cytotoxicity. Moreover, IFN- γ inhibits Th2 differentiation and IgE production, whereas IL-4 is a Th1 antagonist.² The Th2-like responses support humoral immune responses, including the production of IgE inducing IL-4² and eosinophilia promoting IL-5.³

Immune responses in newborns seem to be more Th2 skewed than in adults.^{4,5} Atopic diseases have been associated with a failure to downregulate the dominating neonatal Th2-like immune responses to allergens.^{5,6} A reduced or altered microbial stimulation has been suggested to partially explain the increased prevalence of atopic diseases in industrialized countries during the last decades.⁷

LPS, an endotoxin from gram-negative bacteria, is a potent stimulator of the innate immune system. It

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TABLE I. Demographic data of the 8- and 14-year-old children

	8-year-old children n = 69	14-year-old children n = 46	P value
Boys	n = 40 (58%)	n = 27 (59%)	NS
Atopic heredity	n = 40 (59%)	n = 25 (54%)	NS
Positive SPT result	n = 29 (42%)	n = 18 (39%)	NS
Atopic asthma	n = 15 (22%)	n = 9 (20%)	NS
Non-atopic asthma	n = 4 (6%)	n = 2 (4%)	NS
ARC	n = 18 (26%)	n = 9 (20%)	NS

ARC, Allergic rhinoconjunctivitis; SPT, skin prick test; NS, not significant.

induces IL-12⁸ and IL-10⁹ responses from antigen-presenting cells. IL-12 is an obligatory signal for the differentiation of naive Th cells into Th1 cells.¹⁰ The anti-inflammatory IL-10 has been suggested to be important for the induction of antigen-specific tolerance.¹¹ LPS is ubiquitous in the environment and present indoor as a constituent of house dust. It initiates immune responses through CD14, a multifunctional receptor expressed primarily on the surface of monocytes, macrophages, and neutrophils.¹² A polymorphism in the promoter region of the CD14 gene, a C-to-T transition at bp -159 (*CD14/-159*) from the major transcription start site, has been associated with increased soluble CD14 (sCD14) levels and low levels of total serum IgE in children with positive skin prick test (SPT) results.¹³ In adults, the same polymorphism has been associated with low levels of total serum IgE and a decreased prevalence of positive SPT results and self-reported hay fever and allergic rhinitis.¹⁴ sCD14 is abundant in serum and facilitates the interaction of LPS with CD14 negative cells such as epithelial and dendritic cells.¹⁵ Binding of LPS to CD14 initiates cytoplasmic signal transduction through the Toll-like receptor (TLR) 4.¹⁶ Common, cosegregating missense mutations in *TLR4* (Asp299Gly and Thr399Ile) have been identified in humans and found to be associated with hyporesponsiveness to inhaled LPS.¹⁷ Transfection of the THP-1 cell line demonstrated that the Asp299Gly mutation, but not the Thr399Ile mutation, diminished TLR4-mediated LPS signaling. This clearly shows that gene sequence changes in receptors responsible for LPS signaling can alter the responsiveness of the host to microbial agents.

It is conceivable that gene polymorphisms associated with reduced immune responses to LPS could modify the host immune response to microbial stimuli and thus any putative protection of allergies. In this study we investigated the relationship of TLR4 and CD14 gene polymorphisms with the LPS responsiveness of PBMCs and the presence of asthma and allergic rhinoconjunctivitis in children.

METHODS

Subjects

The association between TLR4 and CD14 gene polymorphisms and the presence of asthma and allergic rhinoconjunctivitis was

analyzed in 115 children, of whom 69 were 8 years old and 46 were 14 years old. All the children participated in larger prospective studies or cross-sectional studies regarding atopic diseases, and they were all examined at the Allergy Centre at Linköping University Hospital. Demographic data of the children are shown in Table I.

Asthma was defined as bronchial obstruction appearing 4 times or more during the last year and verified at least once by a physician. In addition, bronchial hyperreactivity was verified with a positive bronchial provocation test result in the 14-year-old children.¹⁸

The individuals with asthma were further divided into those with atopic and non-atopic asthma according to the presence of at least 1 positive SPT result. Allergic rhinoconjunctivitis was defined as rhinitis and conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection.

SPTs were performed in duplicate on the volar aspects of the forearms with standardized birch, timothy, and cat extracts. In the group of 14-year-old children, SPTs with dog and house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) extracts (Soluprick, ALK, Hørsholm, Denmark) were also included. Histamine hydrochloride (10 mg/mL) was used as a positive control, and albumin diluent was included as a negative control. The test result was regarded as positive if the mean diameter of the wheal was greater than 3 mm. Forty-seven of the children had at least 1 positive SPT result. Thirty of the children had SPT positivity to birch, 27 children had SPT positivity to cat, 13 children had SPT positivity to timothy, and 5 children had SPT positivity to dog allergen. None of the children had SPT positivity to the house dust mite extracts that were tested.

Atopic heredity, that is, children with at least 1 parent with a history of typical atopic symptoms, was observed in 65 of the children. Data regarding atopic heredity were missing for 1 child.

Polymorphism detection

DNA was isolated from whole blood or blood mononuclear cells by using the Qiagen Blood MiniKit (VWR, Stockholm, Sweden) according to the manufacturer's instructions.

Genotyping of the *TLR4* (Asp299Gly) polymorphism was performed by amplification of genomic DNA with PCR by using the forward primer 5'TAGAGGGCCTGTGCAATTTGA3' and reverse primer 5'CTAATTCTAAATGTTGCCATCC3', resulting in a 172-bp fragment. The PCR was performed with approximately 50 ng of template DNA, 200 μM dNTP, 2 mmol/L MgCl₂, 1 μmol/L of each primer, and a final concentration of 20 mmol/L (NH₄)₂SO₄, 0.01% Tween 20, 75 mmol/L Tris HCl (pH = 9.0), and 0.5 U Taq DNA polymerase (Gibco BRL, Life Technologies, Scotland, United Kingdom). Cycling conditions were 94°C for 2 minutes and 35 cycles at 94°C for 30 seconds, annealing at 61°C for 30 seconds, and extension at 72°C for 30 seconds in a thermal cycler (PTC-100, MJ Research, Watertown, Mass).

Genotype determination was performed with denaturing HPLC analysis on a WAVE DNA Fragment Analysis System (Transgenomic Inc, San José, Calif).¹⁹ In brief, PCR products were mixed with known wild-type DNA and denatured at 95°C for 1 minute and 94°C for 3 minutes and then gradually reannealed by decreasing the sample temperature from 96°C to 34°C at a rate of 1.5°C per minute in a thermal cycler (PTC-100, MJ Research). The PCR product (15 μL) was loaded into a temperature equilibrated column (DNASep R Cartridge, Transgenomic Inc, Omaha, Neb) and eluted on a linear acetonitrile gradient (0.1 mol/L triethylammonium acetate/0.1 mol/L triethylammonium acetate with 25% acetonitrile). The temperature for optimal resolution of homoduplex and heteroduplex was predicted at 56.3°C by using WAVEMAKER Software (Transgenomic Inc). The fragments were detected by UV light at 254 nm.

TABLE II. Occurrence of asthma and allergic rhinoconjunctivitis in children with different *TLR4* (Asp299Gly) and *CD14*–159 genotypes

	TLR4			CD14			
	AA	AG	P value	CC	CT	TT	P value
Asthma	24/104 23%	6/11 55%	.03	11/43 26%	13/48 27%	6/24 25%	NS
ARC	23/104 22%	4/11 36%	NS	10/43 23%	11/48 23%	6/24 25%	NS

The χ^2 test was used for categoric variables, and Fisher's exact test was used when the expected frequency was less than 5.
ARC, allergic rhinoconjunctivitis.

Samples displaying different patterns after denaturing HPLC analysis were sequenced by means of a Thermo sequenase ³³P-radiolabeled terminator cycle sequencing kit (USB Corporation, Cleveland, OH). The sequencing reaction products were separated on a 6% denaturing polyacrylamide gel at 70W constant power.

Genotyping of the *CD14*–159 polymorphism was performed similarly to the protocol described by Baldini and colleagues.¹³ Briefly, PCR was performed as described previously with 1 μ mol/L each of forward primer 5'GTGCCAACAGATGAGGTTTAC3' and reverse primer 5'GCCTCTGACAGTTTATGTAATC3'. Cycling conditions were 94°C for 2 minutes and 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 45 seconds, and final extension at 72°C for 10 minutes. PCR-amplified DNA was digested with 10 U *Ava*II and the manufacturer's buffer (1 $\times$) (New England Biolabs Inc, Hitchin Hertfordshire, United Kingdom) at 37°C for 3 hours. Products were separated on 1.5% agarose gels with 0.8 μ g ethidium bromide per milliliter. Reactions with no enzyme added were used as negative controls. *Ava*II digests the PCR product only when the polymorphism (a C-to-T transition) is present. The assay thus yields 1 band of 497 bp for the CC genotype, 3 bands of 144, 353, and 497 bp for CT heterozygotes, and 2 bands of 144 and 353 bp for TT homozygotes. The results of this restriction fragment length polymorphism assay were confirmed by direct sequencing of the –159 promotor region of the *CD14* gene.

Cell culture

PBMCs were available from 69 children (34 of the 8-year-old children and 35 of the 14-year-old children). The PBMCs were isolated from heparinized venous blood with Ficoll Paque density gradient centrifugation (Pharmacia Biotech, Sollentuna, Sweden) and then cryopreserved.

1 $\times$ 10⁶ cells in 1 mL of AIM-V medium (Life Technologies AB, Täby, Sweden) containing 20 μ mol/L mercaptoethanol (Sigma-Aldrich, Stockholm, Sweden) were cultured with 100 ng/mL LPS (*Salmonella typhimurium*, L-6143, Sigma-Aldrich), 2 μ g/mL phytohemagglutinin (PHA) (Sigma-Aldrich), or medium alone in a CO₂ incubator. After 24 and 48 hours (only 48 hours for PHA cultures) of stimulation, the supernatants were collected and stored at –70°C. The cytokine concentrations in the supernatants from nonstimulated cells were subtracted from the cytokine concentrations in the supernatants from cells stimulated with LPS or PHA. Optimal LPS doses and time points for cytokine secretion were determined in pilot experiments including cells from 12 adults. The doses of LPS tested were 0.1, 1, 100, and 10,000 ng/mL, and the time points tested were 12, 24, 48, 36, and 72 hours. The optimum time points were 24 hours for IL-12(p70) and 48 hours for IL-10 and IFN- γ .

Cytokine analyses

The levels of IL-12(p70) in cell supernatants were analyzed by means of commercial ELISA kits (R&D, Abingdon, United Kingdom) according to the manufacturer's instructions. The sensitivity limit for quantitative determinations was 0.6 pg/mL.

The levels of IL-10 and IFN- γ in cell supernatants were analyzed by means of commercial ELISA kits according to the manufacturer's instructions (PeliPair reagent set, CLB, Amsterdam, The Netherlands), except that nonspecific protein binding sites were blocked by using low-fat cow's milk (Arla, Stockholm, Sweden). The sensitivity limits for quantitative determinations were 2 and 25 pg/mL, respectively.

Serum analyses

The levels of sCD14 in serum samples diluted 1:100 to 1:500 were analyzed by means of commercial ELISA kits (Quantikine Human sCD14 Immunoassay, R&D) according to the manufacturer's instructions. Total IgE levels were analyzed with UniCAP Total IgE (Pharmacia Diagnostics, Uppsala, Sweden).

Statistics

Because the concentrations of cytokines, IgE antibodies, and sCD14 were not normally distributed, unpaired analyses were performed with the Mann-Whitney *U* test, and correlations were analyzed with the Spearman rank-order correlation coefficient test. Samples with IL-10, IFN- γ , and IL-12(p70) levels below the sensitivity limit were given half the cutoff value for the statistical analysis. The χ^2 test was used for categoric variables, and Fisher's exact test was used when the expected frequency for any cell was less than 5. The ORs for associations between different genotypes and atopic symptoms were calculated in a multivariate logistic regression model adjusting for potential confounders. A probability level of less than 5% was considered to be statistically significant. Calculations were performed with the statistical package StatView 5.0 for PC (SAS Institute Inc, Cary, NC).

Ethical aspects

The Regional Ethics Committee for Human Research at the Linköping University approved the study.

RESULTS

The *TLR4* (Asp299Gly) AA genotype was expressed in 104 of the 115 children (90%), and the remaining 11 children were AG heterozygotes (10%). The *CD14*–159 CC genotype was evident in 43 of 115 (37%) of the children, whereas 48 of 115 (42%) were heterozygotes (CT) and 24 of 115 (21%) had the TT genotype. Heterozygosity of the *TLR4* (Asp299Gly) polymorphism was associated with symptoms of asthma (Table II). In a logistic regression analysis, asthma was more often diagnosed in AG heterozygotes than in individuals carrying the AA (wild-type) genotype (Table III). The association remained after the introduction of potential confounders into the model, that is, atopic heredity and

TABLE III. Crude and adjusted odds ratio for the association between the *TLR4* (Asp299Gly) polymorphism and asthma (both atopic and non-atopic) and atopic asthma at school age (8 and 14 years of age) as analyzed in a multivariate logistic regression model

<i>TLR4</i> (Thr299Gly)	N	Crude OR 95% CI	P value	Adjusted OR 95% CI	P value
Asthma					
AA	24/104 23%	1		1	
AG	6/11 55%	4.0 1.1-14.1	.03	4.5 1.1-17.4	.03
Atopic asthma					
AA	18/98 18%	1		1	
AG	6/11 55%	5.3 1.5-19.4	.01	7.0 1.7-29.1	.007

Atopic heredity and sex were included in the model to adjust for potential confounders.
OR, Odds ratio; CI, confidence interval.

sex. When children with atopic and non-atopic asthma were analyzed separately, an association with the *TLR4* (Asp299Gly) polymorphism was found only among those with atopic asthma (Table III). However, non-atopic asthma was diagnosed in only 6 children. Three of the 6 children with the AG genotype and atopic asthma were sensitized to birch only, 1 child was sensitized to cat only, 1 child was sensitized to both cat and birch, and 1 child was sensitized to cat and dog. Of the 5 children with the AG phenotype who did not have asthma, 1 was sensitized to dog. The *TLR4* (Asp299Gly) polymorphism was not associated with allergic rhinoconjunctivitis (Table II), SPT positivity, or number of positive SPT results (data not shown).

LPS-induced IL-12(p70) responses were detectable in 24 of 69 children, and the median value and range were 25 (3.4-63) pg/mL in positive samples. The corresponding figures were 24 (3.7-330) pg/mL in 51 of 69 children for IL-10 and 125 (37-2036) pg/mL in 36 of 57 children for IFN- γ . The LPS-induced cytokine levels were similar in the 8- and 14-year-old children (data not shown).

LPS-induced IL-12(p70) and IL-10 secretion was more often detectable among *TLR4* (Asp299Gly) AA genotypes than among AG genotypes—23 of 57 (40%) versus 1 of 11 (9%) ($P = .042$) and 46 of 58 (79%) versus 5 of 11 (45%) ($P = .029$), respectively. Furthermore, the levels of LPS-induced IL-12(p70) production were lower among AG than AA subjects (Fig 1, A), and a similar trend was observed for IL-10 (Fig 1, C). The number of children with detectable levels of IL-10 and IL-12(p70) was similar among *CD14*–159 genotypes. However, the levels of LPS-induced IL-12(p70) were higher among TT subjects than among subjects carrying the C allele (Fig 1, B). In contrast, the levels of IL-10 were similar between subjects with different *CD14*–159 genotypes (Fig 1, D).

The LPS-induced IL-12(p70) secretion was lower in children who had atopic asthma than in the non-asthmatic children (Fig 2, A). None of the 14 children with atopic asthma responded with LPS-induced IL-12(p70) production, compared with 19 of 42 non-asthmatic children ($P = .022$). Furthermore, the LPS-induced IL-10 production tended to be lower in atopic asthmatic than in non-asthmatic schoolchildren (Fig 2, B). Poor LPS-induced IL-12(p70) and IL-10 secretion was not associated with rhinoconjunctivitis (data not shown) or non-atopic asthma

(Fig 2, A and B). The LPS-induced IFN- γ secretion was similar in all of the children (data not shown).

Phytohemagglutinin-induced IL-12(p70) responses were detectable in 32 of 69 children, and the median value and range were 16 (3.4-47) pg/mL in positive samples. The corresponding figures were 73 (5.9-330) pg/mL in 69 of 69 children for IL-10 and 993 (78-4032) pg/mL in 57 of 57 children for IFN- γ . No differences in PHA-induced cytokine responses were observed between different *TLR4* (Asp299Gly) genotypes and *CD14*–159 genotypes or between healthy children and children with atopic and non-atopic asthma.

The *CD14*–159 genotypes were not associated with asthma or allergic rhinoconjunctivitis (Table I), SPT positivity, or number of positive SPT results (data not shown). Eight of the 11 subjects with the *TLR4* (Asp299Gly) AG genotype expressed the *CD14*–159 C allele, and 3 subjects were *CD14*–159 TT homozygotes. Five of the 8 AG-CC/CT genotypes and 1 of the 3 AG-TT genotypes displayed atopic asthma (no statistically significant difference).

No association was found between sCD14 levels in serum and either atopic symptoms or total IgE levels (data not shown). There was no correlation between LPS-induced cytokine secretion and sCD14 or IgE levels (data not shown). The levels of total IgE and sCD14 were similar in subjects with different *TLR4* (Asp299Gly) and *CD14*–159 genotypes. The 14- and 8-year-old children were compared separately because of the higher levels of total IgE in the 14-year-old children. However, the result was similar when all children were analyzed as 1 group (data not shown).

DISCUSSION

The *TLR4* (Asp299Gly) polymorphism is associated with a 4-fold higher prevalence of asthma and a 7-fold higher prevalence of atopic asthma in Swedish children. This is in contrast with an earlier study in which no association between genetic variation in the *TLR4* gene and asthma susceptibility was found in a heterogeneous North American and a Canadian cohort.²⁰ These discrepant results are most likely explained by genetic differences between the populations, as well as differences

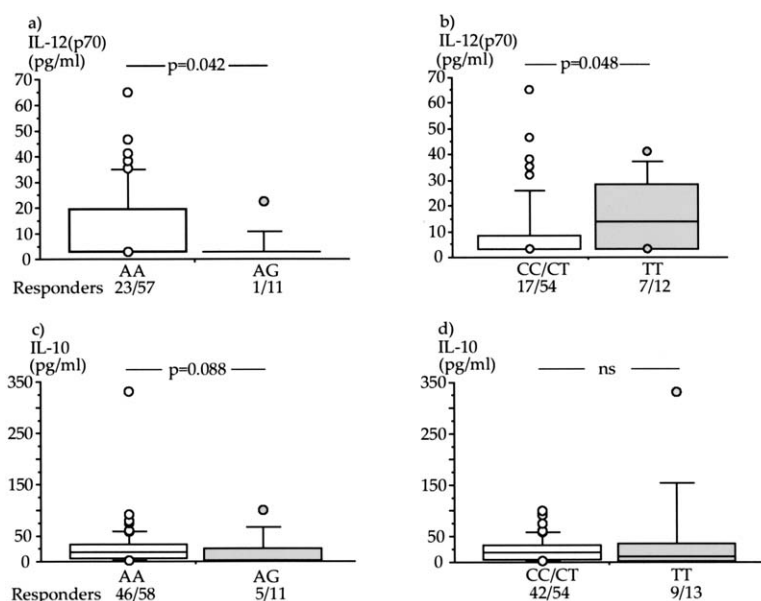


FIG 1. Production of IL-12(p70) (A and B) and IL-10 (C and D) from PBMCs (1×10^6 /mL) after stimulation with LPS (100 ng/mL) for 24 and 48 hours, respectively.

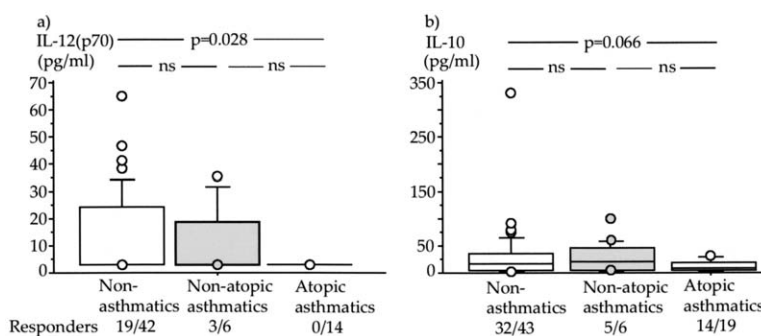


FIG 2. Production of IL-12(p70) (A) and IL-10 (B) from PBMCs (1×10^6 /mL) after stimulation with LPS (100 ng/mL) for 24 and 48 hours, respectively.

in gene–environment interactions in the different studies. The Swedish population is in general homogenous when compared with the American population. The *TLR4* (Asp299Gly) polymorphism was also associated with reduced LPS-induced IL-12(p70) and IL-10 responses by PBMCs from children.

The *TLR4* is expressed on monocyte lineage cells and facilitates transmembrane signaling of LPS activation.¹⁶ The Asp299Gly polymorphism affects the extracellular domain of the *TLR4* receptor and has been linked with hyporesponsiveness to inhaled LPS in humans.¹⁷ Transfection of THP-1 cells with the Asp299Gly allele interrupts LPS-induced nuclear factor- κ B activation and IL-1 α release. In contrast, overexpression of the wild-type allele (Asp299) in airway epithelial cells or alveolar macrophages from individuals with Asp299Gly polymorphism rescued the LPS hyporesponsive phenotype characterized by low IL-1 α and TNF- α responses.¹⁷

Blood cells from children with the *TLR4* (Asp299Gly) polymorphism showed reduced capacity to produce IL-

12(p70) and IL-10 in response to LPS, indicating aberrant function of the innate immune system. IL-12(p70) is a key signal for the differentiation and maturation of Th1 cells and a major inducer of IFN- γ production.²¹ IgE and other Th2-like responses are associated with the atopic phenotype and regulated by inhibitory signals from Th1 cells.²¹ Therefore, not only exposure but also appropriate immune responses to microbial structures like LPS may be a prerequisite to regulate fetal Th1 and Th2 imbalance and to protect against development of atopic disease. The reduced LPS-induced IL-12(p70) and IL-10 responses in children with atopic asthma are possibly explained by poor maturation of Th1-type responsiveness, combined with impaired downregulation of Th2 responses and IgE production. Decreased IL-10 production in response to LPS has been reported in atopic asthma.²² This cytokine is important for the induction of antigen-specific tolerance,¹¹ inhibits IgE production,²³ and has been suggested to counter-regulate Th2-like immunity.²⁴ In children with asthma, the *TLR4* polymorphism occurred only in children

with SPT positivity. However, SPT positivity as such and atopic diseases other than asthma were not associated with *TLR4* polymorphism. Thus, our results indicate that the significance of *TLR4* polymorphism may be associated with the susceptibility of atopic children to develop asthma but not to the development of atopic reactivity and IgE production as such. This challenges the mechanistic explanations based on the Th1 and Th2 polarization and indicates that additional pathways, related to the function of *TLR4*, are involved in the development of atopic asthma.

Human airway epithelium from individuals with Asp299Gly mutation showed low *TLR4* expression,¹⁷ which may imply the importance of local effects of the polymorphism in the airways. The *TLR4* Asp299Gly allele is associated with a higher incidence of gram-negative infections,²⁵ which may contribute to the development of asthma in atopic children. Because non-atopic asthma was infrequent in our study, a possible association between the *TLR4* (Asp299Gly) polymorphism and non-atopic asthma remains open.

We found low IL-12(p70) production from PBMCs in response to LPS in children with atopic asthma, even in those without the *TLR4* polymorphism. This indicates that additional factors regulate cytokine responses to LPS and may affect the development of atopic asthma. Genes other than *TLR4* have been reported to be involved in the response to inhaled LPS in mouse models.²⁶ Exposure to environmental LPS has been linked to an increased risk for asthma and other atopic diseases, although this issue is controversial.²⁷ High levels of house dust LPS are associated with less atopy during childhood.²⁸⁻³⁰ In support of the role of LPS exposure as a modifier of immune deviation in early life, inhalation of endotoxin at the time of primary allergen exposure prevents IgE antibody formation, even in the presence of a Th2-promoting adjuvant.³¹ Our results indicate that not only the LPS exposure but also the genetic factors modifying LPS responsiveness may influence susceptibility to atopic asthma. The complex interaction of LPS exposure and host responsiveness to LPS, which is modified by both genetic and environmental factors may partially explain the discrepant findings about *TLR4* polymorphisms and the risk of atopic diseases.²⁰ In particular, the gene-environment interaction in Swedish children may be different from that in North American and Canadian subjects.

We could not show any association between the *CD14*/–159 polymorphism and the development of atopic symptoms in children. This is in accordance with a previous study in which no association was found between the *CD14*/–159 polymorphism and the doctor-diagnosed asthma.¹⁴ In that study, CC homozygotes had a higher frequency of self-reported hay fever and allergic rhinitis compared with CT and TT genotypes. However, the association disappeared after the introduction of skin test positivity into the analysis model.

The *CD14*/–159 T allele has been suggested to increase sCD14 expression in children and to be associated with

lower total serum IgE levels in children¹³ and adults¹⁴ with SPT positivity. No such associations were observed in our study. In the present study, a tendency to increased LPS-induced IL-12(p70) production was observed in children with the TT genotype when compared with children with CC or CT genotypes. This fits with the hypothesis that the T allele is associated with a deviation toward a Th1-phenotype. It has been suggested that the absence of CD14 activation reduces, rather than abolishes, LPS-induced cell activation through *TLR4*.³² Thus, it is possible that the clinical effect of CD14 mutations is modest, whereas the *TLR4* polymorphism may be of more functional importance for intracellular signaling and activation of transcriptional factors.

In conclusion, we found that gene polymorphisms may alter the responsiveness of the host to microbial agents. A polymorphism in the *TLR4* gene (Asp299Gly), which affects transmembrane signaling of LPS, was associated with decreased LPS-stimulated IL-12(p70) and IL-10 secretion and development of asthma. Thus, genetic variants, which modify immune responses to microbes, may be important determinants for development of atopic diseases and may modify the outcome of intervention studies using microbial stimuli as immune modulators.

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