

The effect of CD14 C159T polymorphism on *in vitro* IgE synthesis and cytokine production by PBMC from children with asthma

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Abstract

Background: Even though the genotype at the promoter region of the CD14 molecule is known to affect the atopic phenotypes, the cellular and molecular basis of this association is largely unknown.

Objective: To investigate the effect of lipopolysaccharide (LPS) on IgE production and cytokine profile by peripheral blood mononuclear cells (PBMC) obtained from asthmatic children with the TT and the CC genotypes at position -159 of the CD14 gene.

Methods: Peripheral blood mononuclear cells from asthmatic children with alternative genotypes at CD14 C159T locus were stimulated with 2 and 200 ng/ml LPS *in vitro*. The IgE, IgG and, IgM response was determined by ELISA and Ig ϵ -germline, IgG, and IgM transcription by real-time PCR. A cluster of cytokines was measured by cytometric bead array.

Results: Asthmatic children with the TT genotype but not those with the CC genotype responded with increased IgE synthesis and germline transcription to LPS stimulation. There were no genotype-related differences in IgG and IgM. TT but not the CC genotype was associated with significantly increased interleukin (IL)-4/IL-12 and IL-4/interferon-gamma (IFN- γ) ratios in the culture supernatant. There were no genotype-related differences in IL-1 β , IL-7, IL-10, IL-13, IL-17A, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, monocyte chemotactic protein, and tumor necrosis factor alpha.

Conclusion: Peripheral blood mononuclear cells from asthmatic children with the TT genotype at position -159 of the CD14 gene make more IgE than those with the CC genotype following LPS stimulation because of increased germline transcription and have an augmented Th2 cytokine profile.

Asthma is a complex genetic disorder that involves interactions between genetic and environmental factors. Within this network of complex interactions, a strong association

Abbreviations

BSA, bovine serum albumin; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; IFN- γ , interferon-gamma; IL, interleukin; LPS, lipopolysaccharide; MCP, monocyte chemotactic protein; MIP-1 α , macrophage inflammatory protein 1 alpha; TNF- α , tumor necrosis factor alpha.

between total IgE levels and asthma has long been recognized (1), and eventually led to the development of a humanized monoclonal anti-IgE antibody for the treatment of severe asthma (2). Because of the significant role of IgE in allergies and asthma, there has been an increasing interest in the environmental and immunological factors such as lipopolysaccharide (LPS) that may regulate IgE expression.

Within the general context of hygiene hypothesis, several studies have shown that endotoxin exposure may protect against atopy (3) and that the effect can already be

programmed *in utero* (4). In addition to the timing and dose of endotoxin exposure (5–8), the genetic variants of the molecules participating in the endotoxin signaling pathway may strongly influence the host response to endotoxin (9–14).

A C–T polymorphism at position –159 in the promoter of CD14 (C159T) (rs2569190 A/G) changes the affinity of Sp family of transcription factors and thus modulates the cellular response to endotoxin (9). Following the pioneering study by Baldini et al. (10) that showed that the C allele is associated with higher IgE and lower soluble CD14 levels, either the T or the C allele was found to be associated with atopic outcomes in various populations (10, 11, 13), whereas some studies have shown no association at all (12, 14, 15). In fact, a more recent analysis found a significant heterogeneity among published studies (12).

To partly account for these observed discrepancies, recent data have suggested that the response to LPS by alternative genotypes can be greatly influenced by its concentration in the environment and that the genotype stratified IgE response is a function of the strength of the environmental exposure (16, 17).

Even though there is accumulating evidence that endotoxin may modulate the IgE response, this information has been obtained basically from population studies and epidemiological observations. The laboratory evidence for this observation has been missing. Specifically, it is unknown whether PBMC from individuals with the TT and CC genotypes at position –159 of the CD14 gene actually synthesize different amounts of IgE upon exposure to LPS and more importantly whether the response to various concentrations of LPS differs between the two genotypes.

During the development of allergic disease, effector Th2 cells produce interleukin (IL)-4, IL-5, IL-9, and IL-13 that play important roles in allergic inflammation (18, 19). The relationship between the cytokines that are operative in IgE synthesis and CD14 –159 genotype has not been extensively studied.

Even though the genetic polymorphism at the CD14 C159T locus apparently affects the IgE response, the information is gathered basically from epidemiological studies, and direct laboratory evidence for this association is lacking. Moreover, the cellular and molecular mechanisms of this relationship are largely unknown. We have previously shown that the CC genotype is associated with higher IgE in a population of Turkish children with asthma (20). Therefore, we recruited children with asthma from the same population with the TT and CC genotypes at position –159 of the CD14 gene and stimulated the cells with increasing doses of LPS and compared IgE, IgM, IgG1, and IgG4 synthesis and Th1, Th2, and Th17 cytokines between the two groups.

Methods

Study population

Children aged 6–18 who were diagnosed with asthma and who were previously genotyped at the CD14 –159 locus were

recruited from the Pediatric Allergy and Asthma Unit of Hacettepe University, School of Medicine, Ankara, Turkey. These children belonged to a cohort of children with asthma who were previously genotyped for another study in our department (20). Of 613 children who were genotyped (20), we selected children with asthma in stable condition, who have had no disease exacerbation, respiratory infections, or received systemic corticosteroids within the last 4 weeks who presented to our department between October 2007 and April 2008. All children had evidence of reversible airway obstruction as defined by at least a 12% improvement in FEV1 following bronchodilator administration.

Spirometric measurements, total IgE, and eosinophil counts were obtained, and skin testing was performed as described previously (20). All study procedures were carried out in accordance with a protocol previously approved by the Ethics Committee of Hacettepe University. All parents provided written informed consent for the study procedures. Even though the parents of these children had initially signed an informed consent for DNA donation, a new Ethics Committee Approval has been obtained, and the parents of all children signed a new informed consent for the current study, and the children provided their assets to allow phlebotomy once more and do tissue culture experiments.

Cell preparation and culture

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by density-gradient centrifugation on Histopaque 1077 (Sigma, St Louis, MO, USA) washed three times in Phosphate buffered saline (PBS) (Sigma) and resuspended in Iscove's Modified Dulbecco's Medium (IMDM + L-Glutamin; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 36 mM sodium bicarbonate, 1% ITS + Premix (containing 12.5 mg/ml recombinant human insulin, 12.5 mg/ml human transferrin, 12.5 µg/ml selenous acid and 10.7 mg/ml linoleic acid; BD Biosciences, Bedford, MA, USA), Penicillin (100 U/ml) (Sigma) and Streptomycin (100 mg/ml) (Sigma), as previously described (21).

In 48-well plates, 1×10^6 PBMC were cultured in complete medium in the absence and presence of LPS (2 and 200 ng/ml), (*Escherichia coli*, 055:B5; Fluka, Buchs, Switzerland) in 500 µl complete medium. Supernatants were collected at 48 h for cytokine determination, RNA was extracted for epsilon germline determination at day 5, and supernatants were collected at day 12 for immunoglobulin determinations (22, 23). All media used in the experiments were free of LPS contamination.

Immunoglobulin measurements

IgE was measured by ELISA. Briefly, ELISA plates were coated overnight at 4°C with anti-IgE (Polyclonal rabbit anti-human IgE antibody, DakoCytomation, Denmark). Plates were blocked with 3% FBS + 3% bovine serum albumin (BSA) in PBS, washed, and standards and samples were added in appropriate dilutions for 2 h. After washing,

HRP-conjugated goat anti-human IgE antibody (Biosource, Camarillo, CA, USA) was added for 2 h. TMB (3,3',5,5'-tetramethyl-benzidine) substrate solution was used to obtain coloration, and the reaction was stopped with 10% H₂SO₄. Optical density was measured at 450 nm. IgG1 and IgG4 and IgM determinations were carried out with the cytometric bead array (Bio-Plex; Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the manufacturer's protocol.

Ig ϵ -germline transcription

Total RNA was extracted from the PBMCs using RNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Approximately 500 ng total RNA was reverse transcribed by ImProm-II Reverse Transcription System (Promega Corporation, Madison, WI, USA) into complementary DNA (cDNA). The PCR primers and probes were designed based on the sequences reported in GenBank with the Primer Express software version 1.2 (Applied Biosystems, Foster City, CA, USA). A reference gene screening has been carried out, and elongation factor (EF)-1 α was used as an endogenous control. Primers used for relative quantification are EF-1 α f 5'-CTGAACCATCCAGGCCAAAT-3', EF-1 α r 5'-GCCGTGTGGCAATCCAAT-3', IgE f 5'-ACATCCACAGGCACCAAA-3', IgE r 5'-TTGCAGCAGCGGGTCAA-3', IgG1 f 5'-CTCTCAGCCAGGACCAGGGA-3', IgG1 r 5'-GGTGGGCATGTGTGAGTTTTG-3', IgG4 f 5'-ACCATGGTCACCGTCTCCTCA-3', IgG4 r 5'-GGGACCATATTTGGACTC-3'. The prepared cDNAs were amplified by using iTaq SYBR Green Supermix with ROX (Bio-Rad, Basel, Switzerland), according to the manufacturer's recommendations in an ABI PRISM 7900 Sequence Detection System (Applied Biosystems) in triplicates. Relative quantification and calculation of the range of confidence was performed by using the comparative $\Delta\Delta$ CT method (24).

Determination of cytokine concentrations

Concentrations of IL-1 β , IL-2, IL-4, IL-5, IL-7, IL-10, IL-12, IL-13, IL-17A, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), monocyte chemotactic protein (MCP), macrophage inflammatory protein 1 alpha (MIP-1 α), interferon-gamma (IFN- γ), and tumor necrosis factor alpha (TNF- α) were determined by cytometric bead array (Bio-Plex; Bio-Rad Laboratories Inc.) according to the manufacturer's protocol.

All measurements were carried out in duplicate with a < 10% variation between the two measurements.

Statistical analyses

Statistical analyses were carried out with SPSS 15 for Windows (SPSS Inc, Chicago, IL, USA) and PRISM 5 for Windows (GARAPHAD Software, Inc., San Diego, CA, USA). All quantitative variables, including age, eosinophil counts, immunoglobulin and cytokine levels, and FEV1, showed non-normal distribution and failed to normalize after various

transformation methods. Therefore, data are given as median and interquartile range, and all statistical comparisons were carried out using Mann-Whitney *U*-test or ANOVA on ranks as appropriate. For all analyses, a *P*-value < 0.05 was considered significant. For pair-wise comparisons of the non-normally distributed data, Mann-Whitney *U*-test with Bonferroni correction was used.

We have performed multiple linear regression analysis to establish the factors that were associated with IgE in culture supernatants after stimulation with LPS 200 ng/ml. Square root transformation was carried out for IgE levels in the culture supernatant. Five of the IgE values were below the detection limit, and we have taken those values as zero. Because logarithmic transformation of zero yields infinity, we preferred square root transformation to log transformation. Using transformed IgE values, we constructed a regression model. With this regression model, we calculated the residuals, which are the difference between observed values and values predicted in the regression. Then, we constructed a box plot as well as a histogram to see whether residual distributions are skewed. Both graphs showed that the distribution of the residuals after the square root transformation was not skewed.

In this regression model, our primary endpoint was the effect of CD14 genotype on IgE levels in the culture supernatant. We considered the following as covariates in the regression model: age, gender, age of onset, skin test positivity, plasma total IgE levels, and eosinophil counts. The model was constructed using backward elimination. To ensure that the assumptions for linear regression analysis are met, we checked for the normality of residuals by Q-Q plots and Shapiro-Wilk test and in addition we constructed a scatter plot of residuals versus predicted values.

Results

Twenty-two asthmatic children with the CC and 23 children with the TT genotype at position -159 of the CD14 gene were included in the study (Table 1). The two groups were similar with respect to demographic and laboratory variables including age, gender, atopy, age of asthma onset, FEV1 (% predicted), total IgE, family history of allergic disease, passive smoke exposure, and pet ownership (*P* > 0.05). Even though the eosinophil counts are slightly different between the two groups, this difference did not reach statistical significance (*P* > 0.05).

LPS induces increased IgE production and ϵ germline transcription by PBMC of asthmatic children with the TT but not the CC genotype

Stimulation of PBMC by 2 and 200 ng/ml of LPS induced different patterns of IgE synthesis in children with alternative genotypes. IgE levels in the supernatant significantly increased in the cultures of PBMCs from asthmatic children with the TT genotype (*P* < 0.0001), but did not change at all in the CC genotype (Fig. 1A,B). At 200 ng/ml of LPS, the amount of IgE synthesized by the PBMC from children with

Table 1 Demographic and laboratory characteristics of the study population*

	CD14 -159 CC	CD14 -159 TT
N	22	23
Age	11.2 (9.2–14.6)	10.5 (9.5–11.6)
Gender		
Boys	11	12
Girls	11	11
Skin test positivity	11	11
Total IgE (kU/l)	110.5 (50.3–309.8)	109.0 (65.0–230.0)
Eosinophil count	292.5 (187.5–399.8)	186.0 (129.0–335.0)
FEV1%	95.5 (88.8–106.3)	100.0 (90.0–108.0)
Age of onset of asthma	6 (4–10)	4 (2.5–7)
Anti-inflammatory treatment	7 (31.8%)	12 (52.2%)
Family history of atopic disease	7	7
Passive smoke exposure	5	5
Pet ownership	2	3

* $P > 0.05$ for all the variables between the two groups.

the TT genotype was significantly higher than that synthesized by the CC genotype ($P = 0.029$) (Fig. 1C). Analyzing the data according to the atopic status demonstrated that LPS induced IgE synthesis only in children with the TT but not CC genotype regardless of their atopic status (Fig. 2A–D).

Multiple linear regression analysis showed that at 200 ng/ml the TT genotype was significantly associated with the IgE levels in the culture supernatant independent of other covariates ($\beta = 0.644$ SE = 0.239, $P = 0.01$). In this model, plasma total IgE had a small but significant effect, as well ($\beta = 0.001$ SE = 0.0002, $P = 0.001$).

To determine whether the increased IgE concentration in culture supernatants is because of increased transcription, we measured ε -germline transcription using quantitative real-time PCR. There were individuals who responded with elevated IgE transcription in both groups. However, the difference between the groups reached significance only in the TT group (Fig. 3A,B).

Stimulation of PBMC by LPS did not result in any changes in IgG1, IgG4, and IgM concentrations in the culture supernatant in either group but resulted in similar inductions of IgG1 and IgG4 mRNA expression in PBMCs from both the CC and TT individuals (data not shown).

LPS induces higher production of Th2 cytokines by PBMC from asthmatic children with the TT genotype

Among the multiple cytokines that were analyzed, IL-2, IL-4, and IL-13 showed significant increases upon stimulation with 2 and 200 ng/ml LPS in children with both the TT and CC genotypes (Fig. 4A–C). However, as can be seen in Fig. 4, the differences in IL-2 and IL-4 were more striking in the TT genotype. Moreover, IL-4/IL-12 and IL-4/IFN- γ ratios increased significantly only in the TT group ($P = 0.041$ and

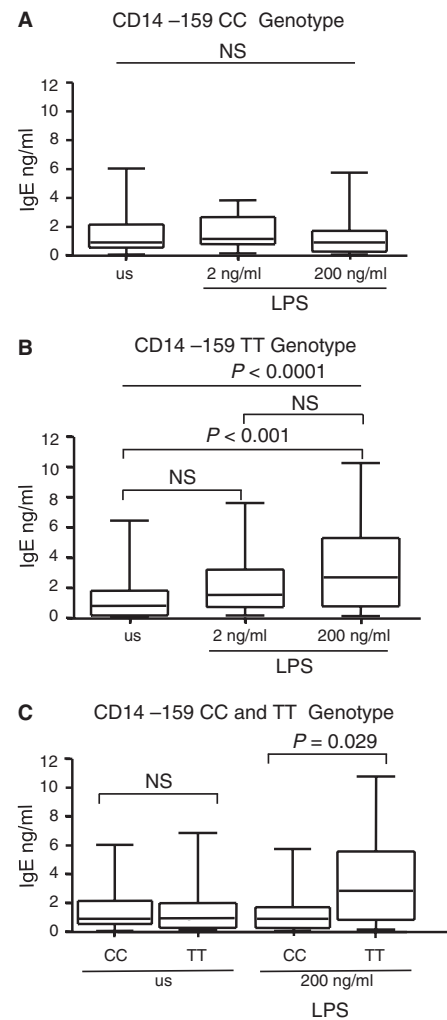


Figure 1 Increased IgE response to lipopolysaccharide (LPS) by cells from asthmatic children with the TT genotype at CD14 -159 position. Peripheral blood mononuclear cells were obtained from asthmatic children with the CC (A) and the TT (B) genotypes and stimulated with indicated doses of LPS for 12 days. Cells from children with the TT genotype synthesized significantly higher amounts of IgE compared to CC genotype upon stimulation with 200 ng/ml of LPS (C). us, unstimulated.

0.05, respectively) (Fig. 5A,B); but not in the CC group ($P > 0.05$ for both). There was a highly significant difference in IL-4/IL-12 and IL-4/IFN- γ ratios between unstimulated samples and 200 ng/ml samples only in the TT group (Fig. 5A,B) ($P = 0.006$ and $P = 0.001$, respectively).

Of the other cytokines that were measured, IL-1 β , IL-7, IL-10, GM-CSF, G-CSF, IFN- γ , and TNF increased similarly in both CC and TT groups; and IL-17, MCP, MIP-1 α did not change in either group (data not shown). IL-5 was present in very small quantities in both groups. Stratification of the data according to the atopic status did not have any significant effect on the results.

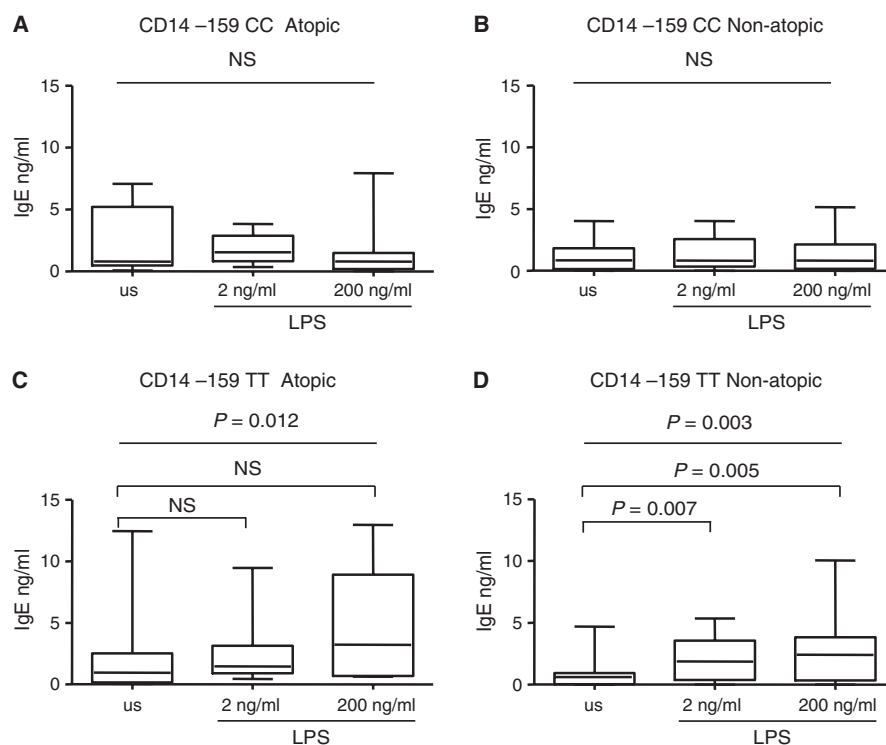


Figure 2 Lipopolysaccharide (LPS) induces IgE production from both atopic and nonatopic children with asthma. Peripheral blood mononuclear cells were obtained from atopic with the TT genotype

and non-atopic asthmatic children with the CC (A and B) and the TT (C and D) genotypes at the CD14 -159 locus and stimulated with indicated doses of LPS for 12 days.

Discussion

Our study shows that PBMC from children with asthma who are homozygous for the T allele at -159 position of the CD14 gene respond with increased *in vitro* IgE synthesis to increasing doses of LPS, whereas those who are homozygous for the C allele do not. This differential cellular IgE response may be associated with a cytokine profile favoring the Th2 response in PBMC with the TT genotype as indicated by increased IL-4/IL-12 and IL-4/IFN- γ in this group.

Even though the association between the CD14 C159T genotype and total IgE levels has been the subject of numerous investigations in many different populations, the cellular and molecular basis of this association is largely unknown. In this study, we provide direct evidence for the first time that under controlled conditions of the *in vitro* environment, PBMCs from individuals with the TT genotype mount an increased IgE synthesis. Importantly, this finding was strongly supported by our linear regression analysis, which showed that a higher IgE in culture supernatant was independently and significantly associated with the TT genotype. The observation that there was a significant increase in ϵ -germline transcription only in the group with the TT genotype suggests that the major mechanism underlying the difference in the protein concentration may be increased transcriptional rate.

We have previously shown that Turkish asthmatic children with the CC genotype have higher levels of circulating IgE in

their plasma (20). Our current study, however, shows that the PBMC from asthmatic children with the TT genotype respond with higher IgE synthesis to increasing doses of LPS. In addition, the difference between the two genotypes becomes significant at 200 ng/ml of LPS concentration. This observation is in line with our previous findings that PBMC from the individuals with the TT genotype secrete higher level of sCD14 into the cultures supernatant (22). Apparently, there is a difference between the *in vivo* plasma IgE-genotype relationship and *in vitro* IgE synthesis-genotype relationship. It seems that the C allele is a risk factor for higher IgE *in vivo*; and the T allele becomes a risk factor for increased IgE synthesis upon stimulation with high doses of LPS *in vitro*. We believe that the explanation to this seemingly contradictory observation may lie in the gene-environment effect that suggests that the genetic influences can be directly and significantly influenced by environmental exposures, which is, in this case, LPS concentration. From a practical point of view, this may be important in preventive strategies as protecting against environmental endotoxin exposure may be helpful in some genotypes but not others. Apparently, the measurement of environmental endotoxin exposure in this cohort would be very helpful in determining the genotype-related changes in total IgE as a function of endotoxin exposure. Unfortunately, however, the level of endotoxin exposure has not been measured in our patients.

The results of the studies that have investigated the association between CD14 -159 genotype and atopic outcomes can

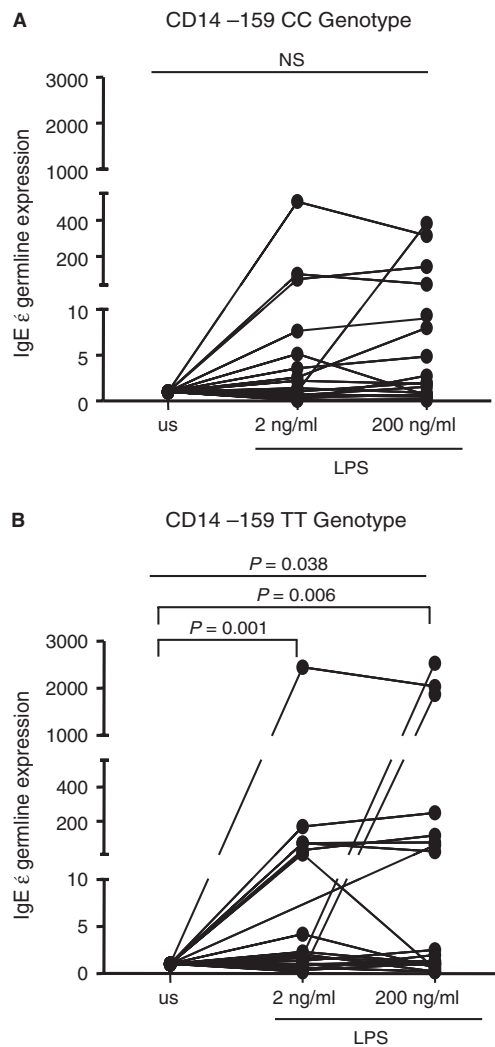


Figure 3 Increased IgE class switch in response to lipopolysaccharide (LPS) in CD14 -159 TT genotype. Total RNA was extracted on day 5 and relative ϵ germline expression quantified with real-time PCR. For each individual, measurements after LPS stimulations were expressed as fold increase relative to the measurement from unstimulated cells.

be evaluated in three distinct categories. Kedda et al. and Ober et al. have shown that the presence of the T allele is associated with atopy (9–13), whereas others have shown that it is the C allele that is associated with atopic phenotypes such as sensitization to molds (25), and more positive allergen-specific IgE tests (26) or total IgE (20). In addition, there are other studies that have shown no association between this specific genotype and atopic phenotypes (14, 15). Within this complexity, when the results of the asthma and endotoxin study (6) were stratified according to the endotoxin exposure (27), it was shown that the C allele was associated with a higher risk of allergic sensitization at low levels of endotoxin exposure and lower risk at high levels of exposure. This observation was later substantiated by two independent

studies (16, 17). Our data provide strong *in vitro* evidence to support this observation, as only the cells from individuals with the TT genotype have responded with increasing IgE synthesis to high doses of LPS. These findings suggest that the combination of environmental factors and genetic determinants may have different stimulatory effects on the same population depending on the CD14 genotype (28). It is not exactly possible to make a one to one comparison between the environmental exposure studies, which measures the relative amount of LPS per unit area or per gram of dust with the pure LPS stimulation used under the highly controlled environment in the test tube. In addition, like all other *in vitro* studies and unlike environmental exposures, our study cannot provide any data regarding long-term continuous exposure.

The results of our study show that LPS induced IgE synthesis only in children with the TT but not CC genotype regardless of their atopic status. However, as shown in Fig. 2, there appears to be more IgE variability in the atopic children, indicating that the CD14 -159 polymorphism may be less important in altering IgE levels as a result of LPS exposure in atopic children. Alternatively, the number of samples may be too small to say anything conclusive about this.

Various studies searching the association of the CD14 genotype on the atopic status in asthmatics and nonasthmatics have shown that the effects of CD14 genotype on atopic phenotypes can be influenced by many factors. For instance in their pioneering study, Baldini et al. (10) showed that the effects of CD14 genotype on total IgE levels can only be observed in skin test-positive individuals but not in skin test-negative individuals. Similarly, O'Donnell et al. (29) have shown that the influence of CD14 -159C on the atopic phenotype may be age specific, exerting an effect during mid childhood, which is no longer apparent by early adulthood. Therefore, these observations including ours imply that the genotypic effects on CD14 can depend on other co-existing entities such as asthma, atopy, or the age.

In this study, to provide some explanation for the cellular basis of our observation, we measured a variety of cytokines in culture supernatants. LPS increased IL-2 and IL-4 production by PBMC in the culture supernatants from both genotypes. However, cells from individuals with the TT genotype had a stronger response. In addition, the increase in the Th-2/Th-1 cytokine ratios such as IL-4/IFN- γ and IL-4/IL-12 was significant only in the TT group. These results have strong implications regarding the differential regulation of IgE in alternative genotypes. IL-2 was identified as a T-cell growth factor that is produced by T cells following activation by mitogens or antigens (30, 31) and was shown to stimulate the growth and differentiation of many cell types including B cells and monocytes/macrophages (32, 33). On the other hand, IL-4 is the most important factor leading to IgE switch in B cells, whereas IL-12 and IFN- γ are the major Th-1 cytokines and counteract the actions of IL-4 on IgE synthesis both at the transcriptional and at the receptor level (34). Th1 cells also efficiently contribute to the effector

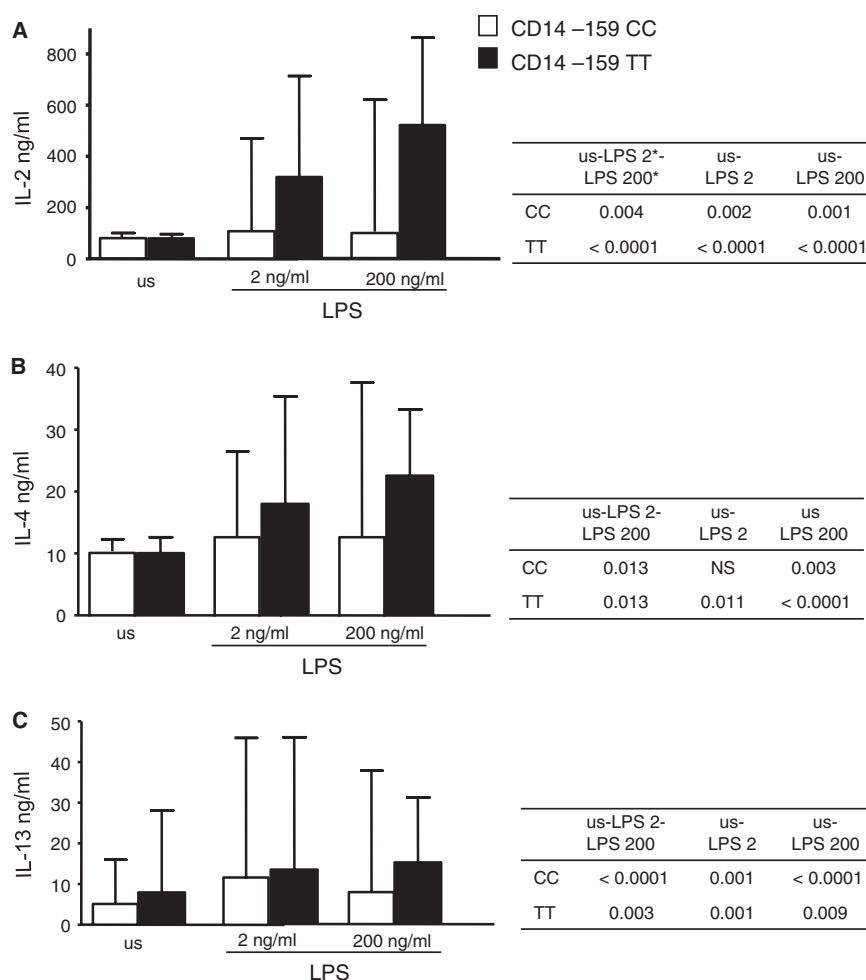


Figure 4 Cytokine concentrations in the peripheral blood mononuclear cells culture supernatants with alternative CD14 genotypes. Culture supernatants were collected after 48 h of stimulation with indicated doses of endotoxin. (*) denotes the concentration of

lipopolysaccharide in the cell culture medium. The comparisons are between the different lipopolysaccharide concentrations within the same genotype.

phase in allergic diseases (35) or dampen allergic inflammation depending on specific disease model and stage of the inflammation (36). They play a role in apoptosis of the epithelium in asthma and atopic dermatitis (35), and predominant Th2 profile in atopic diseases might be a result of the increased tendency to activation-induced cell death of high IFN- γ -producing Th1 cells (37). Taken together, our data suggest that the PBMC from children with asthma carrying the TT genotype may have an increased T-cell response (IL-2) followed by an exaggerated Th-2/Th-1 (IL-4/IFN- γ and IL-4/IL-12), which eventually lead to higher IgE synthesis. However, the molecular mechanisms underlying the genotype-related differential cytokine expression remain to be discovered. It was previously shown that the transcriptional activity of the different alleles at the CD14 C/T locus may depend on the relative expression of various Sp family transcription factors (9). In addition, LPS stimulation activates a variety of intracellular molecular cascades culminating in the activation of a variety of transcription factors such as IRF3,

IRF7, AP1 and NF- κ B (38). It is possible that this particular genotype at the CD14 gene may result in a preferential activation of one transcription factor over the other and thus result in altered ratios of Th1/Th2 cytokine leading to altered IgE synthesis. Even though our data may suggest that IL-17 responses in children with asthma are not regulated by the CD14 promoter -159 genotypes, this statement should be approached with caution because of the low power of the study because of its small sample size. Alternatively, this lack of difference might be because of the presence of TGF-B in the serum, which may have modulated the IL-10 and IL-17 responses.

Similar to the relationship between CD14 -159 genotype and IgE, the data on cytokines are also conflicting. For example, Böttcher et al. have shown that the levels of LPS-induced IL-12(p70) were higher in Swedish subjects with TT genotype than subjects carrying the C allele (39). Reijmerink et al., (40) on the other hand, have failed to show an association between CD14 -159 genotype and IL-12 and IL-10

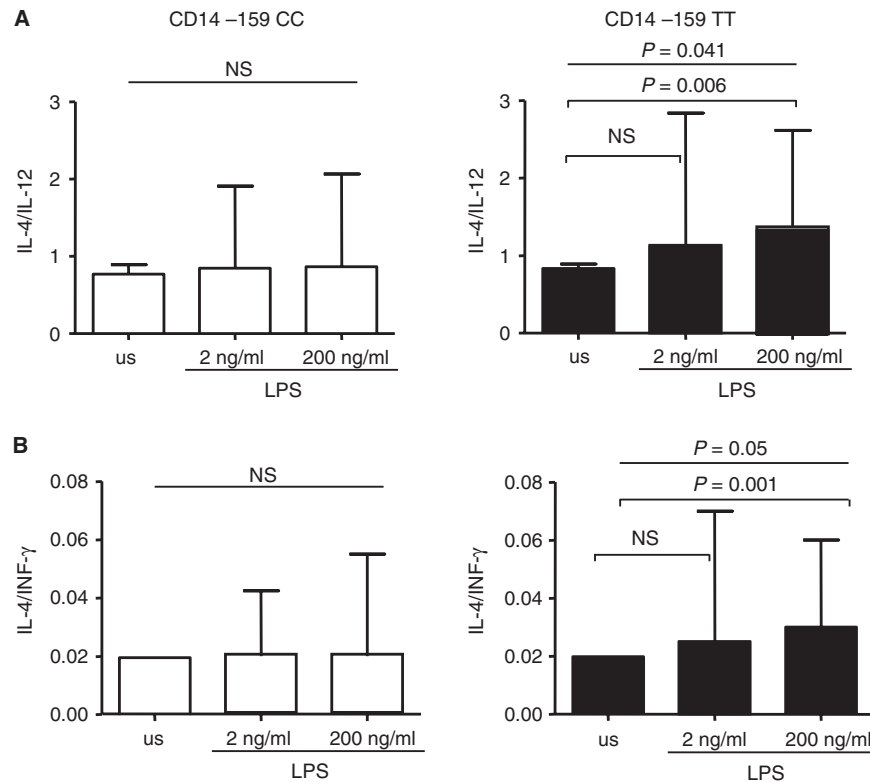


Figure 5 IL-4/IL-12 (A) and IL-4/IFN- γ (B) ratios in peripheral blood mononuclear cells culture supernatants following lipopolysaccharide (LPS) stimulation from children with asthma homozygous

at position -159 of the CD14 gene. The comparisons are between the different LPS concentration within the same genotype.

cytokines in a limited number of Dutch subjects with asthma. The reasons for the observed discrepancies remain to be determined.

Investigation into other immunoglobulin isotypes showed that there were no genotype-related differences in IgG1 and IgG4 protein concentrations suggesting that the genotype-specific effect that we observed is, in fact, specific for IgE. The increase in the transcription of IgG1 and IgG4 mRNA without any accompanying increases in the concentration of these proteins may be because of a couple of reasons. First of all, our experiments were designed specifically to investigate the IgE regulation; and therefore, the experimental time points were ideal for IgE measurement in the culture supernatant and for measurement of ϵ -germline transcription but not for other immunoglobulin isotypes. Secondly, secreted IgG may have been captured by the Fc receptors that are abundantly present on the surface of many cells that are present in the PBMC mixture. Alternatively, the high basal rate of these immunoglobulins resulting from spontaneous secretion may have prevented a difference from becoming apparent.

As can be seen in the figures related to the measurement of IgE protein and ϵ -germline transcription, there is still variance among individuals of the same genotype. Obviously, the genetic variants of other molecules participating in the

endotoxin response pathway or the haplotypes conferred by other polymorphisms on the CD14 gene might also significantly influence the results of our analysis. Alternatively, blocking experiments using monoclonal antibodies against CD14 could be helpful in determining the specificity of the CD14 genotype-related IgE response. These can be the subjects of another study. It should also be noted our study is basically aimed at delineating the molecular mechanisms underlying the endotoxin response and has a small sample size. Therefore, these findings should be interpreted with caution and need to be replicated in a larger and independent sample.

In conclusion, our study provides strong *in vitro* evidence that the cellular response to LPS is a function of both LPS concentration and the genotype at the CD14 C159T locus, which may significantly affect the cytokine production and important atopic phenotypes such as IgE.

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