

The Toll-like receptor 2 R753Q mutation modifies cytokine production and Toll-like receptor expression in atopic dermatitis

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Background: Impaired host defense mechanisms may crucially modulate the pathogenesis of atopic dermatitis (AD). More than 10% of patients with AD are heterozygous for the Toll-like receptor 2 (TLR-2) R753Q single nucleotide polymorphism (SNP) and exhibit severe eczema.

Objective: To elucidate the functional effect of the TLR-2 mutation and its putative relevance for AD.

Methods: Using the human embryonic kidney 293 transfection system, we characterized the properties of the TLR-2 R753Q SNP. Moreover, TLR-2 expression, IL-8 production, and cytokine secretion were analyzed in monocytes and CD4⁺ T cells of patients with AD with and without the mutant *TLR-2* gene.

Results: Human embryonic kidney 293 transfectants mimicking this heterozygous mutation produced less IL-8 when stimulated with lipoteichoic acid (LTA), heat-inactivated *Staphylococcus aureus* or triacylated lipopeptides requiring the TLR-2/1 heterodimer. Suppressed production of IL-8 was confirmed in monocytes from patients with mutant AD after stimulation with peptidoglycan. Cell surface TLR-2 expression was severely impaired in CD3/CD28 activated CD4⁺ T cells of patients with AD bearing the mutant receptor, which could be restored on LTA stimulation. In contrast, LTA decreased TLR-2 expression among nonatopic individuals and patients with AD with the *TLR-2* wild-type gene. T cells from patients with AD exhibited markedly suppressed IL-2 production after macrophage-activating lipopeptide-2 activation. However, no difference was found between mutant and wild-type patients with AD for IL-5, TNF- α , IFN- γ , and IL-2 production.

Conclusion: Collectively, the outcome of innate and adaptive immune responses in AD is modulated by the TLR-2 R753Q SNP. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergy, innate immunity, Toll-like receptor, skin, inflammation, cytokines

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder of the atopic syndrome with still unknown etiopathogenesis. CD4⁺ T_H cells define the major portion of the cellular infiltrate in lesional skin of AD,¹ and their fundamental effect on the disease has been well documented.² One hallmark of AD is the recurrent skin colonization and subsequent superinfection with *Staphylococcus aureus*, affecting more than 60% of the patients.^{3,4} Many, but not all, staphylococcal strains produce exotoxins with superantigenic properties able to activate T cells in a nonconventional manner^{5,6} and to trigger their recruitment into the dermis.^{7,8}

More recently, the role of innate immunity has gained much attention in the pathogenesis of AD.⁹ Indeed, the decreased expression of antimicrobial peptides such as β defensin-2 and β defensin-3^{10,11} provides evidence for a dysregulation within this compartment of the immune system. Among molecular regulators of host defense, Toll-like receptors (TLRs) are known to recognize distinct conserved structures, the so-called *pathogen-associated molecular patterns*. They are transmembrane proteins with extracellular leucine-rich repeats and an intracellular signaling portion homologous to the IL-1 receptor designated as Toll/IL-1 receptor (TIR) domain.¹² Particularly TLR-2 has emerged as a principle regulator in combating Gram-positive bacteria.¹³

Studies using mice deficient for this receptor revealed a higher susceptibility to *S aureus* growing intradermally on subcutaneous injections,¹⁴ and human beings heterozygous for the TLR-2 R753Q mutation have been reported to be prone to staphylococcal infection.¹⁵ Recently we analyzed the prevalence of several TLR polymorphisms among adult patients with AD and unexpectedly found a considerable percentage of them (23.5%) carrying either the cosegregating D299G/T399I missense mutations within the extracellular portion of the *TLR-4* gene (12%) or the R753Q missense mutation localized in the TLR-2 TIR domain (11.5%).¹⁶ Notably, patients heterozygous for the latter mutation had increased disease severity. Yet it still remains unclear whether this particular polymorphism has any direct functional effect on AD pathogenesis.

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Abbreviations used

AD: Atopic dermatitis
 FACS: Fluorescence-activated cell sorting
 FITC: Fluorescein isothiocyanate
 HEK: Human embryonic kidney
 NF- κ B: Nuclear factor- κ B
 SNP: Single nucleotide polymorphism
 TLR: Toll-like receptor

METHODS**cDNA expression vectors**

cDNA of human TLR-2, TLR-6, or TLR-9, each coupled to the green fluorescent protein and cloned in pcDNA3 vector (BamHI/XhoI; Invitrogen, Karlsruhe, Germany), were a kind gift from Terje Espevik (University of Science and Technology, Trondheim, Norway). The nuclear factor- κ B (NF- κ B)-dependent luciferase reporter plasmid was provided by Carsten Kirschning (University of Munich, Germany). The TLR-2 R753Q mutant construct was generated with the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, Calif) and verified by automated sequencing. Plasmids were prepared with an endotoxin-free Plasmid Maxi kit (Qiagen, Hilden, Germany).

Transfection, stimulation, and reporter gene assay of human embryonic kidney 293 cells

Human embryonic kidney (HEK) 293 cells were cultured in penicillin/streptomycin-free Dulbecco modified Eagle medium–Glutamax medium (Biochrom, Berlin, Germany) supplemented with 10% heat-inactivated FCS (PAA, Cölbe, Germany). Originally, 2×10^5 cells per 500 μ L were set out for 1 transfection assay performed in a 24-well tissue plate. Using a 1:1 ratio of DNA and the lipofectamin 2000 reagent (Invitrogen, Karlsruhe, Germany), cells were first transiently transfected with 0.25 μ g NF- κ B reporter plasmid along with increasing amounts of TLR expression vectors as indicated in Fig 1. Transfection and stimulation with lipoteichoic acid (30 μ g/mL) lasted 48 hours for each. To mimic the wild-type situation, the homozygous as well as heterozygous TLR-2 R753Q mutation a total of 1.75 μ g DNA was required for 1 transfection assay and in detail was composed of 1 μ g TLR-2 expression plasmid (either wild-type solely: 100% TLR-2 wild-type, the TLR-2 R753Q mutant construct: 100% R753Q, or a 1:1 mixture of wild-type and mutant vector: 50% TLR-2 wild-type + 50% R753Q), 0.5 μ g TLR-6 expression vector (required for recognition of the diacylated lipopeptide MALP-2), and 0.25 μ g NF- κ B reporter plasmid. TLR-1, in addition needed for optimal recognition of the triacylated lipopeptide Pam3Cys, was already endogenously present in HEK293 cells. For some experiments, cells were cotransfected with 0.5 μ g TLR-2 wild-type and 0.5 μ g TLR-9 encoding vectors. Aside from measuring the TLR-2 GFP constructs via fluorescence-activated cell sorting (FACS) analysis, the main approach to ensure similar transfection efficiencies was to make bulk transfections followed by splitting the cells into equal parts. Thus, calculated for 5 transfection assays, 1×10^6 cells were seeded in a 6-well tissue plate and transfected with the grossed up amount of DNA. Twenty-four hours later, cells of each transfection group were split into 5 parts and recultured in a 24-well plate. After a further 24 hours, transfectants were stimulated with LTA (30 μ g/mL; prepared as previously shown¹⁷), Pam3Cys (30 μ g/mL; EMC microcollections, Tübingen, Germany), MALP-2 (0.3 μ g/mL; Alexis, Lausen, Switzerland), or heat-inactivated *S aureus*, strain ATCC29213 (1×10^9 mL). Luciferase activity was assayed by using a Lucite kit (PerkinElmer, Rodgau-Jügesheim, Germany) and a plate-reader luminometer (Isogen Life Science, VA Ijsselstein, The Netherlands).

Preparation of monocytes

PBMCs were isolated by Lymphoprep (Axis-Shield Poc AS, Oslo, Norway) density-gradient centrifugation from informed consented healthy donors, patients with wild-type AD, and patients with AD with the TLR-2 R753Q single nucleotide polymorphism (SNP). AD was determined by the diagnostic

criteria of Hanifin and Rajka. CD14⁺ cells were purified by negative selection according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes were cultured in RPMI medium 1640 supplemented with 10% heat-inactivated FCS (Gibco, Eggenstein, Germany), 2 mmol/L glutamine (Seromed, Berlin, Germany), and 1% penicillin/streptomycin (Seromed, Berlin, Germany). Before initiation of further experiments, cells were analyzed by 2-color flow cytometry (FACS caliber; Becton Dickinson, Heidelberg, Germany) for contaminating CD3⁺ T cells, CD20⁺ B cells, and CD56⁺ natural killer cells. CD14⁺ monocytes had a purity of at least 86%.

Stimulation of monocytes

Cells were either unstimulated or stimulated for various periods with PGN from *S aureus* (1, 5, or 10 g/mL; Invivogen, Toulouse, France). LPS was not detected in PGN, as determined by the Limulus amoebocyte assay (Haemochrom Diagnostika, Essen, Germany).

Determination of IL-8 and IFN- γ -inducible protein (IP)-10 by ELISA

Concentrations for IL-8 and IP-10 were determined in the culture supernatants by commercially available OptEIA enzyme immunoassay (BD Pharmingen, San Diego, Calif).

T-cell purification and stimulation conditions

PBMCs were obtained by density centrifugation from informed consented healthy donors without TLR-2 mutation ($n = 6$) and patients with AD either carrying the TLR-2 wild-type gene ($n = 4$) or heterozygous for the TLR-2 R753Q SNP ($n = 4$). Cells were used for CD4⁺ T-cell purification by positive selection on the basis of the Dynal technology (Dynal Biotech, Hamburg, Germany) and enriched to more than 99% purity as assessed by staining with phycoerythrin-labeled mAbs to CD4 (BD Biosciences, San Jose, Calif) or CD3 (BD Biosciences) and FACS analysis. Cells (3×10^6 /mL) were cultured in RPMI medium 1640 supplemented with 2 mmol/L glutamine (PAA), 10% heat-inactivated FCS, and 1% penicillin/streptomycin (PAA). After overnight incubation, cells were stimulated with 10 μ g/mL immobilized anti-CD3 antibody (PeliCluster, Freiburg, Germany) and 10 μ g/mL soluble anti-CD28 antibody (BD Pharmingen, Karlsruhe, Germany) for 72 hours to achieve full TLR-2 expression. To investigate the effect of TLR-2 ligands, T cells were stimulated with LTA (30 μ g/mL; 3 μ g/mL) and MALP-2 (0.3 μ g/mL; 30 ng/mL) in conjunction with TCR activation for a further 72 hours.

FACS analysis

Cells were adjusted to 1×10^5 cells per 100 μ L in PBS/5% FCS. T-cell surface markers were measured by successive staining for 30 minutes at 4°C with directly conjugated mAbs: isotype control (fluorescein isothiocyanate [FITC])/isotype control (phycoerythrin [PE]), isotype control (allophycocyanin [APC]), α CD3 (phycoerythrin), α CD4 (APC), α CD4 (FITC), α CD45RO (phycoerythrin), α CD25 (APC), α TLR-2 (FITC). All antibodies were purchased from BD Biosciences except the TLR-2 antibody (clone TL2.1), which was distributed by Alexis and which has been extensively characterized before.¹⁸ Analysis of 10,000 events was performed by using a 4-color FACS caliber flow cytometer (Becton Dickinson) and CELLQuest software version 3.2.1f1, setting the gate on the CD4⁺ population on the basis of forward and side scatter properties.

mRNA isolation and RT-PCR for analysis of TLR-2 and TLR-6 expression

Total RNA from T cells was isolated by using the RNeasy Mini kit (Qiagen, Hilden, Germany) and DNase I digestion (Invitrogen, Karlsruhe, Germany). First-strand cDNA synthesis was performed with Oligo-(dT)₁₈ primers and the Omniscript Reverse Transcription Kit (Qiagen) followed by amplification of the glyceraldehyde 3-phosphate dehydrogenase, TLR-2, and TLR-6 transcripts by using the HotStarTaq PCR kit (Qiagen) and specific primers (sequences and PCR conditions available on request).

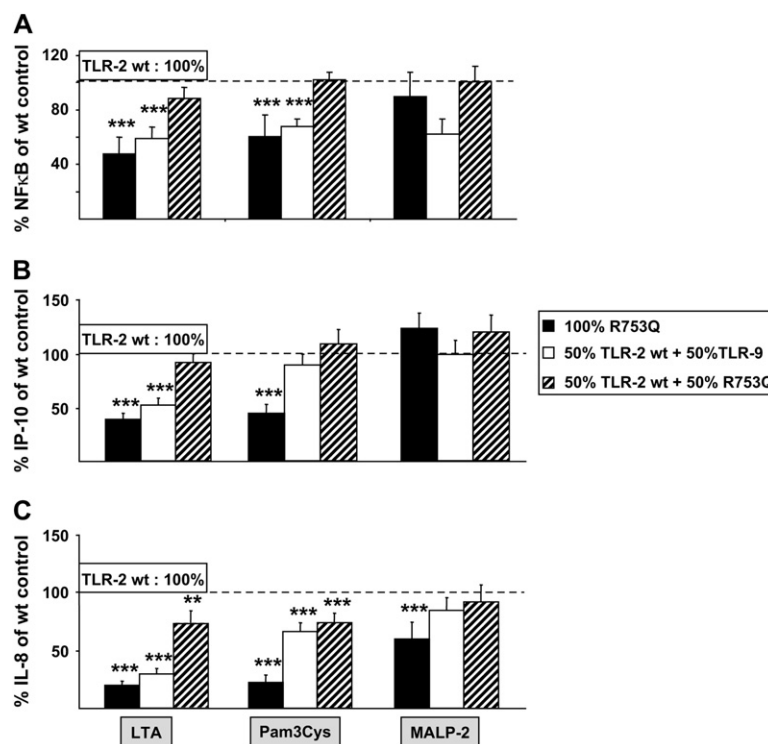


FIG 1. The TLR-2 R753Q SNP in HEK293 cells. NF- κ B activity (A), IP-10 (B), and IL-8 secretion (C) of homozygous (100% R753Q) or heterozygous (50% TLR-2 wt + 50% R753Q) TLR-2 R753Q transfectants are displayed relative to the TLR-2 wild-type mediated response (100% TLR-2 wt), which was normalized as 100%. Shown are means $\pm$ SDs from $n = 8$ separate experiments. ** $P < .01$ and *** $P < .001$.

Determination of T_H1/T_H2 cytokines in cell culture supernatants via the Cytometric Bead Array system

Supernatants of 72-hour-stimulated CD4⁺ T cells in the absence or presence of TLR-2 ligands were applied for the Cytometric Bead Array Kit (BD Pharmingen, San Diego, Calif) that simultaneously and quantitatively measures multiple cytokines in a single sample. Beads coated with capture antibodies specific for various analytes were mixed with phycoerythrin-labeled detection antibodies in the presence of standards or test samples to form sandwich complexes. Sample data were obtained by using the multicolor FACSscan flow cytometer (Becton Dickinson), and results were calculated by using BD CBA Software version 1.4.

Statistical analysis

Data are shown as the means $\pm$ SDs. Significant difference was evaluated by the unpaired Student t test with 2-tailed distributions (* $P < .05$; ** $P < .01$; *** $P < .001$).

RESULTS

Reduced IL-8 production in HEK293 cells mimicking the heterozygous TLR-2 R753Q mutation

Initially, the degree of altered TLR signaling mediated by the TLR-2 R753Q mutation was analyzed using an *in vitro* transfection system. HEK293 cells were transiently transfected at various doses with either the wild-type or the mutant construct followed by stimulation with LTA and subsequent determination of NF- κ B activity by reporter gene assay. There was a clear dose-response relationship between the TLR-2 expression and an increased NF- κ B response. This effect was a result of neither variations within transfection efficiency nor differing expression levels

between mutant and wild-type receptor as revealed by comparable mean fluorescence intensities of the GFP-tagged constructs (data not shown).

Expression of the heterozygous TLR-2 missense mutation was simulated by transfecting HEK293 cells at a 1:1 ratio with human TLR-2 wild-type and TLR-2 R753Q mutant constructs. Expression of either wild-type or mutant transfection vector alone resembled the wild-type and mutant homozygous situations respectively. All transfectants were stimulated with ligands requiring the TLR-2/TLR-1 (Pam3Cys) or the TLR-2/TLR-6 heterodimer (MALP-2) to elucidate the effect of the mutation on a nonexclusively TLR-2-mediated pathway. Stimulation of the heterozygous mutant receptor with LTA and Pam3Cys resulted in significantly reduced expression levels of IL-8. No effect was observed on NF- κ B and IP-10 expression levels. The highest degree in reduction of NF- κ B, IP-10, and IL-8 signals was observed with the homozygous mutant TLR-2 receptor (Fig 1, A-C). These effects were observed again for stimulation with LTA and Pam3Cys, but not after MALP-2 (with the exception of IL-8 production, which was also reduced by using the homozygous mutant receptor; Fig 1, C).

Heterozygous transfectants secrete inadequate IL-8 in response to heat-inactivated *S aureus*

The TLR-2 R753Q mutation has been previously linked to increased staphylococcal infection.¹⁵ In an approach to elucidate whether signaling through the mutant receptor leads to an altered response on *S aureus* stimulation, homozygous and heterozygous TLR-2 transfectants as well as HEK293 cells expressing the wild-type receptor alone were incubated with heat-inactivated

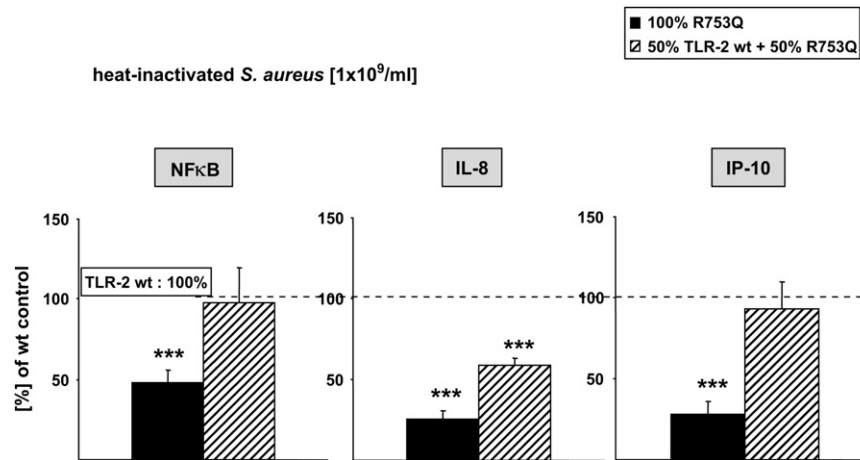


FIG 2. Effect of heat-inactivated *S aureus* on NF-κB response, IL-8, and IP-10 secretion in HEK293 transfectants. Cells were transfected as described and stimulated with 1×10^9 /mL heat-inactivated *S aureus* for 48 hours. Results are expressed as percentage of the wild-type mediated response of $n = 8$ independent experiments. *** $P < .001$.

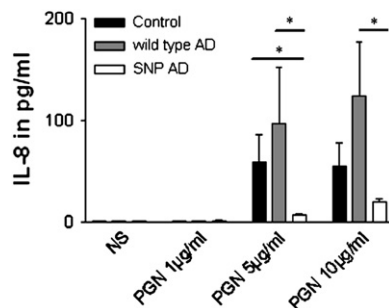


FIG 3. Effect of TLR-2 stimulation with PGN on IL-8 secretion in monocytes from patients with AD with ($n = 6$) and without ($n = 7$) TLR-2 mutation and healthy controls ($n = 6$). Monocytes 1×10^5 were stimulated for 48 hours with PGN (1 μ g/mL, 5 μ g/mL, and 10 μ g/mL). Cell culture supernatants were analyzed for concentrations of IL-8 by ELISA. The mean value $\pm$ SEM is shown. * $P < .05$.

S aureus followed by measurements of NF-κB activity and IL-8 and IP-10 production. Compared with the wild-type situation, homozygous transfectants were markedly affected regarding IL-8 production, and even heterozygous transfectants had about a 40% reduced capacity to produce this chemokine (Fig 2). Notably, the NF-κB and IP-10 levels were again unaltered in the heterozygous state.

In atopic dermatitis, the TLR-2 R753Q SNP is associated with a lower IL-8 production by monocytes stimulated with the TLR-2/nucleotide-binding digomerization domain ligand PGN

The HEK293 expression system revealed the most prominent effect on IL-8 production. To assess whether this finding is of relevance for patients with AD, monocytes were prepared from various patient groups. PGN induced a significant lower IL-8 secretion in monocytes from patients with AD heterozygous for the TLR-2 R753Q SNP compared with patients with AD bearing the TLR-2 wild-type gene (Fig 3). PGN-induced IL-8 mRNA levels were lower in monocytes from all tested patients with AD (patients

with AD with and without the TLR-2 R753Q mutation) compared with healthy controls, but did not significantly differ between patients with TLR-2 wild-type and mutant AD (data not shown).

Human CD4⁺ T cells express TLR-2 and TLR-6 mRNA

Because one of the consequences of *S aureus* colonization in patients with AD is the massive influx of T cells into lesional skin, we investigated whether T cells express TLR-2 under certain conditions. For this purpose, mRNA was isolated from resting or activated CD4⁺ T cells of nonatopic individuals. Via PCR, TLR-2 as well as TLR-6 transcripts were clearly detectable throughout all cell preparations, and there were no major differences in mRNA levels between stimulated and nonstimulated CD4⁺ T cells (Fig 4, A).

Expression of TLR-2 on activated but not resting CD4⁺ T cells

In contrast with the mRNA data, TLR-2 surface protein could not be stained in resting T cells of nonatopic subjects but was evidently present after activation with anti-CD3 and anti-CD28 antibodies as shown by flow cytometry (Fig 4, B). Similarly, activated CD25⁺CD4⁺ T cells of patients with AD with the TLR-2 wild-type gene expressed this receptor to a lower degree, whereas cell surface TLR-2 expression in patients heterozygous for the TLR-2 R753Q mutation was severely impaired (Fig 5). In turn, 75% of the CD25⁺TLR-2⁺ T-cell population belonged to the memory phenotype as revealed by expression of CD45RO (Fig 4, C).

Opposite effects of LTA stimulation on TLR-2 expression in patients with AD with the wild-type or mutant TLR-2 gene

We next analyzed whether coactivation of CD3/CD28 stimulated T cells with TLR-2 ligands would alter the expression level of the receptor. Notably, T cells from nonatopic subjects as well as from patients with AD carrying the TLR-2 wild-type gene showed an almost 25% decline in TLR-2 expression on activation with

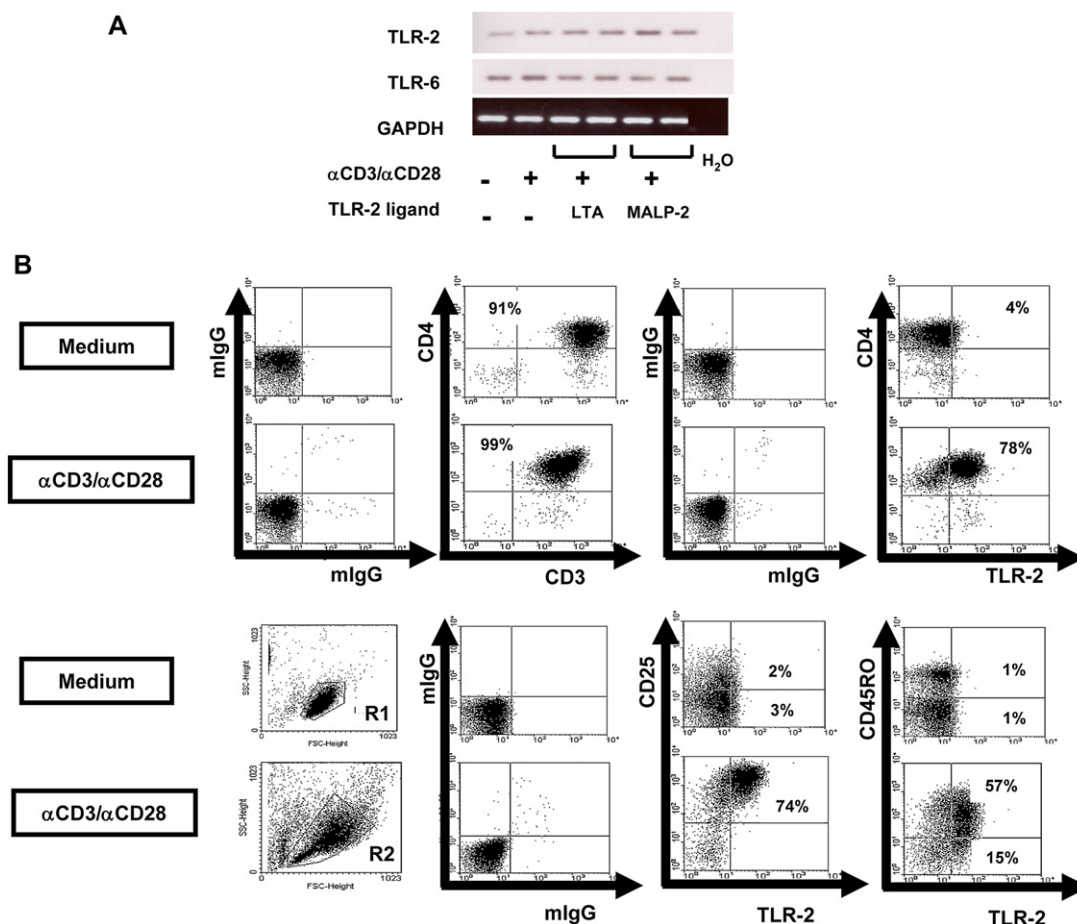


FIG 4. TLR-2 expression in peripheral blood CD4⁺ T cells. **A**, cDNA of CD4⁺ T cells directly on purification or on TCR activation with (+) or without (-) LTA and MALP-2 at previously mentioned doses was used for PCR. **B**, Cell-surface TLR-2 was assayed in CD3/CD28 activated and nonactivated T cells via FACS. Expression profile from 1 representative nonatopic subject is shown. *mlgG*, Mouse IgG.

LTA. On the contrary, stimulation with this ligand increased expression (as much as 20%) in patients with AD heterozygous for the TLR-2 R753Q mutation (Fig 5), whereas MALP-2 did not influence the expression level in all study groups.

Effect of TLR-2 ligation on T-cell cytokine production

The production of cytokines after stimulation with the TLR-2 ligands LTA and MALP-2 was analyzed in CD3/CD28 activated T cells from nonatopic healthy individuals and patients with AD with and without the heterozygous TLR-2 R753Q mutation. A significant difference between atopics and nonatopics could only be seen for MALP-2-induced IL-2 secretion. Here, MALP-2 was unable to trigger any significant IL-2 production in patients with AD compared with the marked stimulatory effect in healthy controls. The production of IFN- γ , IL-5, and TNF- α was not different between the study groups (data not shown).

DISCUSSION

Atopic dermatitis is a chronic pruritic skin disease particularly complicated by the colonization with *S aureus*. Enhanced susceptibility toward these bacteria may result from an insufficient host defense. Indeed, patients with AD lack endogenous antimicrobial

peptides and just marginally express IL-8 and nitric oxide.^{19,20} Keratinocytes exposed to *S aureus* have been shown to secrete both mediators through TLR-2.²¹ Nevertheless, the effect of this receptor in AD and the mechanisms by which staphylococcal components other than superantigens can worsen eczema are still poorly understood.

Activated memory CD4⁺ T cells define the cellular infiltrate of the skin in AD, implying that staphylococci may directly interfere with T cells and aggravate eczema. In this regard, we could most recently show that *S aureus* α toxin directly triggers a strong T_H1 response in CD4⁺ T cells of patients with AD through a yet unknown mechanism.²² In this study, we assume direct TLR-2-mediated activation of T cells by staphylococcal components that have penetrated the disrupted epithelium. Cell surface TLR-2 expression by human CD4⁺ T cells was initially described by Komai-Koma et al,²³ also demonstrating the strong T_H1-like stimulatory capacity (IFN- γ , TNF- α , and IL-2) of Pam3Cys, a typical TLR-2 agonist, on TCR activation.

Addressing the aspect of TLR-2 expression in peripheral blood T cells, TLR-2 mRNA was detectable in both resting and CD3/CD28 activated T cells. Yet TLR-2 protein could only be seen in activated T cells, indicating the necessity of a TCR signal for receptor trafficking and the higher sensitivity of myeloid cells to TLR agonists. Importantly, the extent of cell surface TLR-2 expression declined in the following order: healthy, nonmutant

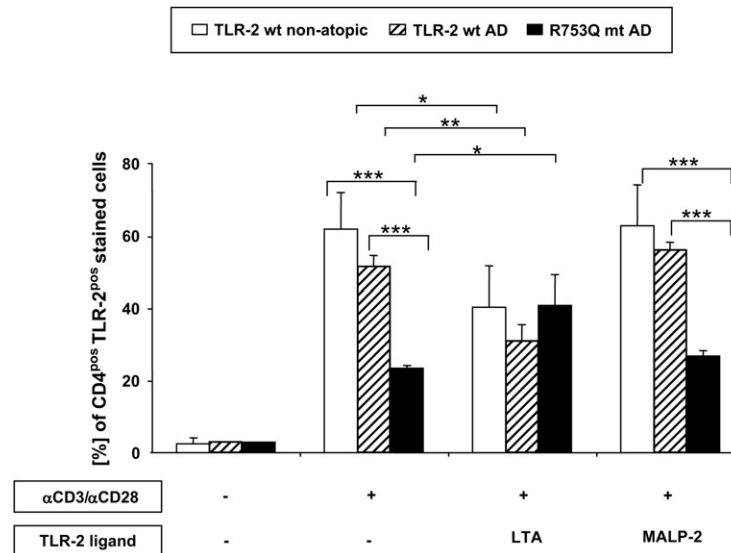


FIG 5. Surface TLR-2 expression in CD4⁺ T cells after TLR-2 stimulation. TCR activation of CD4⁺ T cells from healthy donors (n = 6) and patients with AD with (n = 4) and without (n = 4) TLR-2 mutation was performed with or without LTA (30 μg/mL) or MALP-2 (300 ng/mL). Data represent mean percentage of TLR-2 and CD4 positive T cells ± SD. **P* < .05; ***P* < .01; ****P* < .001.

subjects > patients with AD with the *TLR-2* wild-type gene > patients with AD heterozygous for the *TLR-2* R753Q mutation. Thus, reduced TLR-2 levels in AD could possibly be linked to the higher risk and prevalence of bacterial colonization and superinfection. Because TLR-2 expression was most severely impaired in patients carrying the R753Q missense mutation, one may hypothesize that arginine in position 753 might support TLR-2 delivery to the cell surface. In this context, Nakao et al²⁴ could most recently show that C30S and C36S mutations within TLR-2 indeed affected membrane trafficking. Further investigation is needed to clarify this aspect.

Coactivation of T cells with LTA lowered TLR-2 expression in healthy subjects as well as in patients with AD with the wild-type receptor, implying that LTA might function as a negative regulator and thus limits receptor expression through yet undefined mechanism. Surprisingly, LTA induced the opposite effect in the originally low TLR-2-expressing T cells of patients with AD with the mutant receptor. Although TLR-2 is currently known to be upregulated by its cognate pathogen-associated molecular patterns in monocytes and dendritic cells,^{25,26} we also describe here LTA-mediated downregulation of this receptor in selective cases.

So far, little effort was made to examine the functional consequences of the heterozygous TLR-2 R753Q mutation. The current knowledge relies on data performed in a human whole blood model showing impaired TNF-α and IFN-γ release after exposure to *Borrelia* lysate (mainly composed of triacylated lipopeptides) and unaltered IL-8 production on LTA stimulation.^{27,28} The innate immune system plays a critical role in initial recognition of microbial pathogens followed by initiation of adequate immune responses. In this regard, it was an important finding that the heterozygous TLR-2 R753Q SNP in HEK293 transfectants revealed lower IL-8 levels when exposed to LTA and Pam3Cys.

The current opinion holds that TLR-mediated recognition of pathogens results in dendritic cell activation and subsequent initiation of T-cell responses. Our data on enhanced responsiveness of activated memory CD4⁺ T cells to APC-independent TLR activation stratify the emerging concept of direct T-cell

modulating capacity of TLR ligands as published most recently for the γδ T cells and CD4⁺CD25⁺ regulatory T cells.^{29,30} The impaired responsiveness to produce IL-8 after TLR-2 ligation was confirmed in patients with AD heterozygous for the TLR-2 R753Q mutation. This finding also validates the HEK293 transfection system for studying TLR signaling as a result of such impaired innate defense responses. Indeed, Zhang et al³¹ could detect high amounts of LTA on the skin from impetiginized AD lesions, pointing toward its relevance in AD pathogenesis. Keratinocytes (analogous to HEK293 cells) expressing the heterozygous TLR-2 R753Q SNP may secrete less IL-8 on LTA exposure, resulting in reduced neutrophil chemotaxis and insufficient bacterial clearance. The massive uncleared bacterial load may further augment the mixed cytokine pattern of T_H1 as well as T_H2 cells that has been well described by many investigators over the years^{32,33} and which is characteristic of the chronic inflammatory phase of AD lesions.

In conclusion, the TLR-2 R753Q SNP is prevalent in a subset of patients with AD characterized by very high total, allergen-specific, and superantigen-specific IgE titers and a higher mean SCORing Atopic Dermatitis score than patients with AD bearing the wild-type TLR-2.¹⁶ Here, we have delineated functional alteration after signaling through this mutant receptor with potential consequences in innate and adaptive immune responses. Our data further strengthen that patients with AD have a marked dysregulation after signaling through TLRs on the level of innate-adaptive immune cooperation.

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Clinical implications: The heterozygous TLR-2 R753Q mutation is accompanied by an impaired production of critical mediators known to promote the course of AD.

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