Genetic Variation in TLR10, an Inhibitory Toll-Like Receptor, Influences Susceptibility to Complicated Skin and Skin Structure Infections

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Background. Toll-like receptors (TLRs) play a central role in the innate immune response to complicated skin and skin structure infections (cSSSIs), with TLR10 being the first family member known to have an inhibitory function. This study assessed the role of TLR10 in recognition of cSSI-related pathogens and whether genetic variation in TLR10 influences susceptibility to cSSSIs.

Methods. Human peripheral blood mononuclear cells (PBMCs) preincubated with anti-TLR10 antibody and HEK-293 cells overexpressing TLRs were exposed to cSSI pathogens, and cytokine secretion was determined by enzyme-linked immunosorbent assay. A total of 318 patients with cSSI and 328 healthy controls were genotyped for 4 nonsynonymous single-nucleotide polymorphisms in TLR10, and functional consequences of the TLR10 SNPs were assessed via in vitro stimulation assays.

Results. PBMC stimulation with cSSI pathogens in the presence of TLR10 neutralizing antibody significantly increased interleukin 6 secretion. Overexpression of TLR10 completely abrogated TLR2-induced interleukin 8 secretion by HEK-293 cells in response to cSSI pathogens. Three polymorphisms in TLR10, I775L, I369L, and N241H, were associated with reduced susceptibility to cSSSIs. The presence of the TLR10 alleles 775L, 369L, or 241H increased interleukin 6 secretion by PBMCs in response to cSSI pathogens.

Conclusions. TLR10 is a modulatory receptor of innate immune responses to cSSI-related pathogens, and genetic variants in TLR10 are associated with protection against cSSSIs.

Keywords. acute bacterial skin and skin structure infections; receptors, pattern recognition; Toll-like receptor 10; polymorphism, single nucleotide; immunity, innate.

Complicated skin and skin structure infections (cSSSIs) were termed by the Food and Drug Administration as infections involving the deeper soft tissue that require significant surgical intervention, such as major abscesses, diabetic foot infections, surgical site and trauma-induced wound infections, and infections in individuals with a significant underlying disease state that complicates the response to treatment. Also included are infections located in an anatomical site in which the chance of involvement of anaerobic or gram-negative pathogens is high [1, 2]. Gram-positive bacteria such as Staphylococcus aureus and ß-hemolytic streptococci are the predominant causative organisms, but the presence of gram-negative bacteria (eg, Escherichia coli) and anaerobes (eg, Bacteroides fragilis), as well as a polymicrobial etiology, is frequent [2–4].

Cells of the innate immune system recognize pathogen-associated molecular patterns on the surface of invading microorganisms through distinct families of pattern-recognition receptors (PRRs). Toll-like receptors (TLRs) are the best-documented family of PRRs,
and 10 members have been characterized in humans [5]. In general, binding of ligands to TLRs results in interaction with downstream adaptor proteins and activation of transcription factors, followed by production of inflammatory cytokines and chemokines and induction of an immune response.

TLRs are essential in the host defense against cSSI pathogens. Cell wall components of gram-positive bacteria such as S. aureus are ligands for TLR2, in combination with TLR1 and TLR6 [6]. Malfunction of TLRs may lead to an inappropriate overactivation or underactivation of the immune response, resulting in the development of cSSSIs. Our group has recently observed that genetic variants in TLR1, TLR2, and TLR6 were associated with an increased susceptibility to cSSSIs. In addition, functional studies of these genetic variants showed an effect on interleukin 6 (IL-6) secretion by peripheral blood mononuclear cells (PBMCs) after exposure to S. aureus [7]. TLR1, TLR2, and TLR6 form a cluster on chromosome 4, together with TLR10.

First described by Chuang and Ulevitch in 2001, TLR10 was found to be closely related to TLR1, TLR6, and, to a lesser extent, TLR2 (50%, 49%, and 30% amino acid identity, respectively) [8]. TLR10 was predominantly expressed in lymphoid tissues, including spleen, lymph node, thymus, and tonsil specimens [8]. Expression of TLR10 has mainly been observed in immune cells, such as B cells, dendritic cells, monocytes, neutrophils, and eosinophils [9–13], but also in nonimmune cells, such as trophoblasts [14]. TLR10, similar to TLR1 and TLR6, forms heterodimers with TLR2 [9, 15, 16] but differs in its lack of downstream signaling [17]. Recent studies suggest TLR10 is an unusual PRR, with mainly inhibitory functions on TLR2-driven immune responses [17–20], whereas its involvement in the recognition of cSSSI pathogens is unknown.

Genetic variation in TLR10 has previously been associated with inflammatory diseases [21–23] and cancer [24–26], while its role in susceptibility to cSSSIs and consequences for cSSI pathogen-induced immune responses remain to be elucidated.

In this study, we hypothesized that TLR10 influences recognition of cSSI pathogens and that genetic variation in TLR10 may result in an altered immune response to cSSI pathogens, hence influencing susceptibility to infection.

METHODS

Subjects and Blood Samples

A total of 646 white East European individuals were included in our association study, among whom were 318 with cSSI from a randomized, multicenter clinical trial [27] (subtype/diagnosis: major abscess, diabetic foot infection, wound infection, or infected ischemic ulcer) and 328 healthy controls recruited from the community. Detailed information on inclusion criteria in the study cohort are described elsewhere [7]. In addition, 74 white, healthy Dutch individuals were recruited from the community to correlate their genotype with IL-6 secretion upon stimulation of PBMCs. All subjects gave informed consent for genetic analysis. PBMCs were isolated from buffy coats of healthy individuals for experiments regarding antibody blocking of TLR10.

In Vitro PBMC Stimulation Assays

PBMC isolation and stimulation assays were performed as described earlier [28], with minor modifications. PBMCs were isolated by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) from venous blood specimens. Next, PBMCs were washed twice with phosphate-buffered saline (pH 7.4) and resuspended in Roswell Park Memorial Institute 1640 Dutch modified culture medium (supplemented with 2 mM L-glutamine, 1 mM pyruvate, and 50 μg/mL gentamicin; Gibco Invitrogen, Carlsbad, California) for experiments.

PBMCs (5 × 10⁶) were added to 96-well round-bottomed plates (Greiner, Nurnberg, Germany) in the presence of either 1 × 10⁵ colony-forming units/mL of heat-killed S. aureus ATCC 29213, Bacteroides fragilis NCTC 10584 or ATCC 25285, Escherichia coli ATCC 25922, or 10 μg/mL TLR2 ligand Pam3Cys (EMC Microcollections, Tübingen, Germany) in a final volume of 200 μL. After incubation for 24 hours at 37°C and 5% CO₂, supernatants were collected and stored at −20°C. For the TLR10 blocking experiments, PBMCs were preincubated for 1 hour with 10 μg/mL anti-TLR10 antibody (Novus Biologicals, Cambridge, United Kingdom) or IgG1κ isotype control (R&D Systems, Minneapolis, Minnesota) before exposure to cSSI pathogens or Pam3Cys.

Transfection and Stimulation of Human Embryonic Kidney (HEK)–293 Cells

Transfection of HEK-293 cells with human TLR10, TLR2, or both was performed as previously described [18, 20].

HEK-293 cells were cultured in Dulbecco’s modified Eagle’s medium, supplemented with 7.5% fetal bovine serum (HyClone, Thermo Scientific, Logan, Utah), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen), at 37°C and 5% CO₂. The plasmid-selecting agents G418 (1 mg/mL; Sigma-Aldrich, St Louis, Missouri) for TLR2, blasticidin (5 μg/mL; Invivogen) for TLR10, and a combination of both for TLR2/10 were added to the culture medium to ensure the presence of these specific TLRs in HEK-293 cells. Correct TLR expression was confirmed by reverse transcription–polymerase chain reaction (PCR) and flow cytometry. When 80% confluency was reached, the HEK-293 cells were passaged and used in stimulation experiments. Nontransfected, TLR10-transfected, TLR2-transfected, and TLR2/10-transfected HEK-293 cells (1 × 10⁶) were added to 96-well flat-bottomed plates (Greiner) in the presence of either 1 × 10⁵ CFU/mL of heat-killed S. aureus ATCC 29213, B. fragilis ATCC 25285, E. coli ATCC 25922, or TLR ligands Pam3Cys (TLR2; 1 μg/mL; EMC Microcollections) and lipopolysaccharide (LPS; TLRI; 10 ng/mL; Sigma-Aldrich) in a final volume of 200 μL. After incubation for 24 hours at 37°C and 5% CO₂, supernatants were collected and stored at −20°C.
Cytokine Detection Assays
IL-6 secretion was selected as the primary readout cytokine after PBMC stimulation because it is crucial in the response against cSSSI pathogens, as blockage of IL-6 by tocilizumab has been recently related to staphylococcal infections [29]. In supernatants from the HEK-293 cells interleukin 8 (IL-8) was determined. IL-6 secretion and IL-8 secretion were determined in supernatants by commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s protocol (Sanquin Reagents, Amsterdam, the Netherlands).

Genotyping of TLR10 Polymorphisms
Genomic DNA from patients with cSSSI was isolated using the PLUS XL manual kit (LGC Genomics, Berlin, Germany). Genomic DNA from healthy controls was isolated using the Gentra Pure Gene Blood kit (Qiagen).

Table 1. Genotyped Nonsynonymous Single-Nucleotide Polymorphisms (SNPs) in TLR10

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Mutation</th>
<th>Nucleotide Change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amino Acid Change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Taqman Assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR10</td>
<td>rs4129009</td>
<td>Missense</td>
<td>A → C</td>
<td>I775L</td>
<td>C__309084_10</td>
</tr>
<tr>
<td>TLR10</td>
<td>rs11096955</td>
<td>Missense</td>
<td>A → C</td>
<td>I369L</td>
<td>C__309086_10</td>
</tr>
<tr>
<td>TLR10</td>
<td>rs11096957</td>
<td>Missense</td>
<td>A → C</td>
<td>N241H</td>
<td>C__309088_10</td>
</tr>
<tr>
<td>TLR10</td>
<td>rs11466653</td>
<td>Missense</td>
<td>T → C</td>
<td>M326T</td>
<td>C__25643406_10</td>
</tr>
</tbody>
</table>

Abbreviation: ID, identification number.
<sup>a</sup> First described nucleotide (and corresponding amino acid) is the ancestral allele and is therefore considered the wild-type allele.

Figure 1. Blocking Toll-like receptor 10 (TLR10) increases interleukin 6 (IL-6) secretion in human peripheral blood mononuclear cells (PBMCs) after exposure to common complicated skin and skin structure (cSSSI) pathogens. PBMCs (5 × 10<sup>6</sup>/mL) were preincubated for 1 hour with 10 µg/mL anti-TLR10 (α-TLR10) antibody or isotype control, followed by incubation for 24 hours with 10 µg/mL Pam<sub>3</sub>Cys (A), 1 × 10<sup>6</sup> colony-forming units/mL of heat-killed cSSSI pathogens Staphylococcus aureus ATCC 29213 (B), Bacteroides fragilis ATCC 25285 (C), or Escherichia coli ATCC 25922 (D). IL-6 secretion was determined in supernatants by an enzyme-linked immunosorbent assay. Data are presented for 9 different buffy-coats performed in 3 separate experiments. *P<.05 and **P<.01, by the Wilcoxon signed rank test.
Four nonsynonymous TLR10 single-nucleotide polymorphisms (SNPs; Table 1), with previously described associations with human diseases and a minor allele frequency of at least 5% among different populations, were selected from the National Center for Biotechnology Information SNP database. TLR10 gene fragments were amplified using commercially available TaqMan SNP genotyping assays according to the manufacturer’s protocol on a 7300 Real-Time PCR system (Applied Biosystems, Foster City, California). Quality control of the assay was warranted by incorporating positive and negative controls and duplicating random samples across different plates. Correct assessment of TLR10 SNPs by Taqman SNP genotyping assays was validated by whole-exome sequencing on a selection of the cohort of healthy, white, Dutch individuals.

**Statistical Analysis**

$\chi^2$ analysis of deviation from Hardy–Weinberg equilibrium (HWE) were performed for all 4 TLR10 SNPs in healthy controls, using a web-based HWE calculator [30]. The association between susceptibility to cSSSIs and a SNP were investigated by means of univariate logistic regression models with IBM SPSS 18 software (IBM, Armonk, New York). SNPs were evaluated using dominant-model analyses, and $P$ values of <.05 were considered to be statistically significant. Odds ratios including 95% confidence intervals were reported for these tests of association. Pairwise linkage disequilibrium (LD), $D’$ and $r^2$, were calculated using IBM SPSS 18 software.

The statistical analyses and graphical presentation of the cytokine concentrations in the PBMC and HEK-293 cells stimulation assays were performed using GraphPad Prism 5.00 software (GraphPad Software, La Jolla, California).

**RESULTS**

**Blocking TLR10 Increases IL-6 Secretion by Human PBMCs After Exposure to cSSSI Pathogens**

To determine the biological function of TLR10 in the innate immune response to cSSSI pathogens, human PBMCs were preincubated with TLR10 neutralizing antibody or isotype control followed by stimulation with heat-killed S. aureus, B. fragilis, E. coli, or TLR1/2 ligand Pam3Cys (Figure 1). Blocking of TLR10 significantly increased IL-6 secretion after exposure to Pam3Cys ($P = .0078$), S. aureus ($P = .0195$), and B. fragilis ($P = .0039$) and approached significance after E. coli stimulation ($P = .0547$). No differences were observed for PBMCs exposed to TLR4 ligand LPS in the presence or absence of TLR10 neutralizing antibody (Supplementary Figure 1).

**Overexpression of TLR10 Abrogates TLR2-Mediated Cytokine Secretion in HEK-293 Cells After Exposure to cSSSI Pathogens**

To investigate how TLR10 exerts its inhibitory effect on the recognition of cSSSI pathogens, HEK-293 cells were transfected with human TLR2, TLR10 or both TLR2 and TLR10. All combinations were cultured for 24 hours in the presence of the PRR ligands Pam3Cys (TLR1/2) and LPS (TLR4) and the cSSSI pathogens S. aureus, B. fragilis, and E. coli. Thereafter, IL-8 secretion was determined in supernatant by ELISA (Figure 2). Unstimulated cells did not induce significant levels of IL-8. Nontransfected

![Figure 2](image)
Table 2. Distribution of TLR10 Genotypes in 318 Patients With Complicated Skin and Skin Structure Infection (cSSSI) and 328 Healthy Controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Wild-type, %</th>
<th>Heterozygous, %</th>
<th>Homozygous, %</th>
<th>P Valuesa</th>
<th>Odds Ratioa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR10 I775L</td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
<td>.002</td>
<td>0.603 (0.438–0.831)</td>
</tr>
<tr>
<td>Patients with cSSSI</td>
<td>214 (67.3)</td>
<td>89 (28.0)</td>
<td>15 (4.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>180 (55.4)</td>
<td>119 (36.6)</td>
<td>26 (8.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR10 I369L</td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
<td>.019</td>
<td>0.676 (0.487–0.937)</td>
</tr>
<tr>
<td>Patients with cSSSI</td>
<td>123 (38.7)</td>
<td>145 (45.6)</td>
<td>50 (15.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>98 (29.9)</td>
<td>156 (47.6)</td>
<td>74 (22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR10 N241H</td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
<td>.019</td>
<td>0.676 (0.487–0.937)</td>
</tr>
<tr>
<td>Patients with cSSSI</td>
<td>123 (38.7)</td>
<td>145 (45.6)</td>
<td>50 (15.7)</td>
<td></td>
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<tr>
<td>Healthy controls</td>
<td>98 (29.9)</td>
<td>156 (47.6)</td>
<td>74 (22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR10 M326T</td>
<td>TT</td>
<td>TC</td>
<td>CC</td>
<td>.174</td>
<td>0.667 (0.370–1.200)</td>
</tr>
<tr>
<td>Patients with cSSSI</td>
<td>298 (93.7)</td>
<td>20 (6.3)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>298 (90.9)</td>
<td>29 (8.8)</td>
<td>1 (0.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

* Data are based on a dominant-model analysis.

Figure 3. Correlation of the TLR10 I775L genotype with interleukin 6 (IL-6) secretion by human peripheral blood mononuclear cells (PBMCs) after exposure for 24 hours to common complicated skin and skin structure infection (cSSSI) pathogens. PBMCs were exposed to 10 μg/mL Pam3Cys (A) or 1 × 10^6 colony-forming units/mL of the heat-killed cSSSI pathogens Staphylococcus aureus ATCC 29213 (B), Bacteroides fragilis NCTC 10584 (C), or Escherichia coli ATCC 25922 (D). Boxes represent the median values and interquartile ranges; whiskers represent minimum and maximum values. IL-6 secretion was determined in supernatants by an enzyme-linked immunosorbent assay, and results were stratified for the TLR10 I775L genotype. Dark gray represents individuals with the AA genotype (n = 47), light gray represents individuals with the AC genotype (n = 22), and white represents individuals with the CC genotype (n = 1). *P < .05, **P < .01, and ***P < .001, by the Mann–Whitney U test.
HEK-293 cells or transfected with only TLR10 were unable to induce significant IL-8 secretion in response to any of the stimuli (Figure 2). HEK-293 cells transfected with TLR2 alone did not respond to LPS but demonstrated a strong induction of IL-8 secretion in the presence of Pam3Cys, as well as the cSSSI pathogens S. aureus, B. fragilis, and E. coli (Figure 2). In contrast, the presence of TLR2 in combination with TLR10 resulted in a significant reduction of IL-8 secretion after exposure to Pam3Cys (P = .0022) and cSSSI pathogens S. aureus (P = .0050), B. fragilis (P = .0022), and E. coli (P = .0022) in HEK-293 cells (Figure 2).

Genetic Variation in TLR10 Is Associated With Reduced Susceptibility to cSSSIs

Data from 318 patients with cSSI and 328 healthy controls were included to assess the role of TLR10 polymorphisms I775L, I369L, N241H, and M326T in susceptibility to cSSSIs (Table 2). All TLR10 polymorphisms were in HWE for the healthy controls. LD analyses revealed that I369L and N241H were in complete linkage (P < .0001, r² = 1.00, D' = 1.00), whereas moderate LD was observed for I775L with I369L and N241H (P < .0001, r² = 0.64, D' = 0.82), and weak LD was observed for M326T with I775L (P < .0001, r² = 0.01, D' = 0.47) and M326T with I369L and N241H (P < .0001, r² = 0.25, D' = 0.52).

Univariate logistic regression analysis indicated that 3 TLR10 polymorphisms were associated with susceptibility to cSSSIs (Table 2): I775L (P = .002) and I369L and N241H (P = .019). For all 3 TLR10 polymorphisms, patients with cSSI were significantly less often heterozygous and homozygous for the allelic variant, compared with the healthy controls. The TLR10 polymorphism M326T was not associated with susceptibility to cSSSIs (P = .167).

TLR10 Polymorphisms Modulate Immune Response to cSSSI Pathogens

To study the functional consequences of TLR10 polymorphisms, PBMCs were obtained from an additional cohort of 74 healthy volunteers. PBMCs were exposed to the cSSI pathogens S. aureus, B. fragilis, and E. coli and the PRR ligands Pam3Cys (TLR1/2) and LPS (TLR4). After 24 hours, IL-6 secretion was measured by ELISA and stratified by TLR10 genotype.
Unstimulated PBMCs did not induce detectable IL-6 secretion (data not shown). No differences were observed between genotypes for all TLR10 polymorphisms after PBMC exposure to LPS (Supplementary Figure 2). Heterozygosity for TLR10 polymorphism I775L (AC; n = 22) significantly increased IL-6 secretion, compared with the wild-type genotype (AA; n = 47), after PBMC exposure to Pam3Cys, S. aureus, and B. fragilis, and a similar trend was observed for E. coli stimulation. Homozygosity for TLR10 polymorphism I775L (CC; n = 1) induced even higher IL-6 secretion, compared with the wild-type and heterozygous genotype after exposure to Pam3Cys, S. aureus, B. fragilis, and E. coli, but only 1 volunteer carried this genotype (Figure 3).

The TLR10 polymorphisms I369L and N241H were also in complete linkage for the cohort of 74 healthy volunteers, and therefore results were combined in Figure 4. Homozygosity (CC/CC; n = 9) and heterozygosity (AC/AC; n = 38) for TLR10 polymorphism I369L/N241H significantly increased IL-6 secretion, compared with the wild-type genotype (AA/AA; n = 26) after exposure to Pam3Cys and B. fragilis. A similar increasing trend was observed after S. aureus and E. coli stimulation (Figure 4).

No differences in IL-6 secretion were detected between heterozygous and wild-type genotypes for TLR10 polymorphism M326T after PBMC exposure to Pam3Cys, S. aureus, B. fragilis, and E. coli. In addition, no homozygotes for TLR10 polymorphism M326T were present among the 74 individuals (Figure 5). Secretion of interleukin 1β and IL-8 by PBMCs showed similar patterns as that of IL-6 for all TLR10 polymorphisms (Supplementary Figures 3 and 4).

**DISCUSSION**

The present study demonstrates that TLR10 is an inhibitory receptor of TLR2-driven immune activation by the common cSSSI pathogens S. aureus, E. coli, and B. fragilis. Genetic variants I775L, I369L, and N241H in TLR10 were significantly associated with a reduced susceptibility to cSSSIs, and the presence of the TLR10 alleles 775L, 369L, or 241H increased cytokine secretion in response to cSSSI pathogens.
Since its discovery, much effort has been put in delineating the ligand and function of TLR10. Coimmunoprecipitation and molecular modeling studies have repeatedly suggested TLR10’s ability to form homodimers and heterodimers with TLR2, but also combinations with TLR1 and TLR6 have been described [9, 15–17]. Downstream, TLR10 was found to associate with MyD88 [9], but it failed to activate typical TLR signaling pathways via nuclear factor-kB [17]. Molecular modeling of the TLR10/2 complex suggested that Pam3Cys might be the ligand [16]. To study the involvement of TLR10 in the recognition of cSSI pathogens, we first neutralized the function of TLR10 on human PBMCs following exposure to cSSI pathogens or Pam3Cys and observed increased IL-6 secretion. Second, we overexpressed TLR2, TLR10, or both in HEK-293 cells, to model the responses of primary immune cells. TLR10 abrogated the TLR2-mediated cytokine secretion after exposure to cSSI pathogens. Similar effects for TLR10 were observed after exposure to the TLR2 ligand Pam3Cys, the TLR6/2 ligand FSL-1, and the pathogens Borrelia burgdorferi and Yersinia pestis [18, 20]. These results were further underscored by in vivo experiments using human TLR10 transgenic (TLR10tg) mice, as mice do not have a functional TLR10 [17]. TLR10tg mice injected intraperitoneally with Pam3Cys produced lower cytokine levels than their wild-type counterparts, confirming the suppressive effect of TLR10 [20].

In contrast to the inhibitory function reported here, two recent studies have suggested that TLR10 acts as a stimulatory receptor in the innate immune recognition of Listeria monocytogenes and influenza virus [31, 32]. Possible reasons for the dissimilarity might lay in different engagement of the TLR10 receptor by these pathogens, or differences in the experimental setup. Both studies mainly base these conclusions on experiments involving small interfering RNA (siRNA), using epithelial and macrophage cell lines, which hampers a direct comparison with our findings. Inhibition of TLR10 expression in primary human cells by using a siRNA approach has previously been shown to have varying efficiency, but this approach also revealed upregulation of IL-6 secretion by TLR10-silenced macrophages, confirming our results [20].

All 4 studied TLR10 polymorphisms have previously been associated with other diseases. TLR10 polymorphisms I775L, I369L, and N241H all influenced susceptibility to prostate cancer [24, 25] and Crohn’s disease [23]. In addition, TLR10 polymorphism I775L was associated with susceptibility to asthma [21, 22]. TLR10 polymorphism I369L was also associated with susceptibility to Ménière’s disease [33] and sarcoidosis [34], whereas TLR10 polymorphism M326T was only found to be associated with small tumor size of papillary thyroid carcinoma [26]. We found TLR10 polymorphisms I775L, I369L, and N241H but not M326T to be significantly associated with a susceptibility to cSSSI. For all 3 associated TLR10 polymorphisms, healthy controls were more often heterozygous and homozygous for the allelic variant (775L, 369L, or 241H), compared with patients with cSSI, indicating a reduced risk of cSSSI. Similar findings were reported in most other TLR10 association studies, and the type of disease possibly explains findings in which this was not the case.

Genetic variation in TLR10 may modulate the balance between the proinflammatory and antiinflammatory responses and as such explain the influence on susceptibility to cSSSIs. The presence of the TLR10 alleles 775L, I369L, or 241H increased IL-6 secretion after exposure to the cSSI pathogens S. aureus, E. coli, and B. fragilis. In line with our results, recent publications indicate that the same genetic variants increased proinflammatory cytokine secretion in response to the TLR1/2 ligand Pam3Cys and the pathogens B. burgdorferi (Lyme disease) and Y. pestis (plague) [18–20].

In conclusion, the present study demonstrates that TLR10 has a suppressive function on TLR2-driven immune activation by cSSI-related pathogens. Furthermore, genetic variation in TLR10 influences the susceptibility to cSSSI and results in an impaired TLR10 function and enhanced immune response to cSSI pathogens. Further studies are warranted to decipher the exact mechanism by which TLR10 induces its inhibitory effect and to elucidate the potential of these TLR10 polymorphisms in risk assessment, individual diagnosis, and treatment for patients with cSSI.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank all patients, investigators, and study centers, for participating in the RELIEF clinical trial; Johanna Beekman (Bayer Healthcare, Berlin, Germany), for collection, preparation, and storage of patient blood samples for genotypic analysis; and Liesbeth Bruckers (CenStat, Hasselt University, Hasselt, Belgium) and Rob ter Horst (Department of Internal Medicine, Radboud University Medical Center, Nijmegen, the Netherlands), for assistance with statistical analysis.

Financial support. This study is part of the ‘Limburg Clinical Research Program (LCRP) UHasselt-ZOL-Jessa’, supported by the foundation Limburg Sterk Merk, province of Limburg, Flemish government, Hasselt University, Ziekenhuis Oost-Limburg and Jessa Hospital. This work was supported by an unrestricted grant from Bayer Healthcare (to I. C. G.). M. G. N. and M. O. were supported by ERC Consolidator Grant-310372 (to M. G. N.). P. R. was an employee of Bayer Healthcare.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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