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The Polymorphism P315L of Human Toll-Like Receptor 1 Impairs Innate Immune Sensing of Microbial Cell Wall Components

Katherine O. Omueti,*‡ Daniel J. Mazur,† Katherine S. Thompson,† Elizabeth A. Lyle,† and Richard I. Tapping2†‡

As a pattern recognition receptor, TLR1 mediates innate immune responses to a variety of microbial cell wall components including bacterial lipoproteins. We have previously shown that the central region of the extracellular domain of human TLR1, comprising leucine-rich repeat (LRR) motifs 9–12, is required for the sensing of bacterial lipopeptides. In this study, we have investigated three nonsynonymous single nucleotide polymorphisms (SNPs) located in this region of TLR1 by generating these variants and examining receptor function. We have found that a variant of TLR1 based upon the SNP P315L, located in the loop of LRR motif 11 (LRR11), is greatly impaired in mediating responses to lipopeptides and a variety of other bacterial agonists for this receptor. Despite normal cell surface expression, the P315L variant also fails to bind to GD2.F4, a commonly used anti-TLR1 mAb. Although a number of amino acid substitutions at position 315 impair receptor function, the leucine substitution has the strongest deleterious effect. GD2.F4 inhibits agonist-induced activation of TLR1, supporting a crucial role for the loop of LRR11 in receptor function. These results also suggest that the P315L SNP may predispose certain individuals to infectious diseases for which the sensing of microbial cell components by TLR1 is critical to innate immune defense. The Journal of Immunology, 2007, 178: 6387–6394.

Imnate immunity is the first line of host defense in response to an invading pathogen. TLRs constitute an essential component of the innate immune system that initiates cellular inflammatory responses following infection of the host. These evolutionary conserved receptors act as sensors of viral, bacterial, and fungal structures that, following agonist recognition, mediate responses leading to direct killing of the pathogen and cellular changes that drive the activation of the adaptive immune system (reviewed in Refs. 1–4).

Humans possess 10 TLR family members, subsets of which are expressed by epithelial cells, endothelial cells, and leukocytes. TLRs are type-1 transmembrane receptors composed of an extracellular domain (ECD), a short transmembrane region, and an intracellular signaling domain that shares homology with that of the IL-1 receptor. TLR-mediated cellular activation occurs following the recognition of specific microbial components by the ECD of the receptor. Although TLRs share a number of intracellular signaling pathways, there are qualitative differences in the responses generated by different TLR family members. For example, TLR family members that recognize viral nucleic acids activate the expression of type-1 IFNs. Thus, intracellular signals generated by individual TLRs appear to be tailored to activate responses commensurate with the type of invading pathogen.

Single nucleotide polymorphisms (SNPs) are common allelic variations that exist in the population at a frequency >1%. The association of SNPs found in genes of the human TLR family with immune function is currently an area of intense investigation. Thus far, SNPs in this gene family have been clinically associated with a variety of infections, asthma and a number of chronic inflammatory diseases (reviewed in Ref. 5).

Among members of the TLR family, TLRs 1 and 6 comprise the most highly conserved pair and appear to have arisen more recently during evolution through a gene duplication event. Through cooperation with TLR2, either TLR1 or TLR6 enables cells to respond to a variety of microbial agonists including bacterial lipoproteins and lipopeptides. However, TLR1/2 and TLR2/6 receptor pairs exhibit different specificities toward many microbial agonists including diacylated and triacylated lipopeptides (6–9). Through the reciprocal exchange of extracellular domains between the human TLRs 1 and 6, we previously found that the receptor specificity toward different lipopeptide agonists is determined by the region comprised of leucine-rich repeat (LRR) motifs 9–12 of these receptors (10). In this study, we investigated three nonsynonymous SNPs in this coding region of human TLR1 and found that one, P315L, exhibits highly attenuated activity toward a number of microbial-derived agonists. These findings have potential implications for susceptibility to infectious diseases in certain populations.

Materials and Methods

Methods

**Microbial agonists.** The synthetic bacterial lipoproteins N-palmitoyl-S-[2,3-bis[palmitoyloxy]-propyl]-cysteiny1-[lysyl]3-lysine (Pam₃CSK₄) and R-cysteiny1-(lysyl)₃-lysine (Pam₃Cys)
and N-palmitoyl-S-[2-hydroxy-3-(palmitoyloxy)propyl]-1-(R)-cysteinyl-
(lysolecithin)lysine (PanCysPamSK), were purchased from EMC Microcollections. The microbial agonists Porphyromonas gingivalis LPS, heat-killed Acholeplasma laidlawii, (a mycoplasma) and lipomannan from Mycobacteria smegmatis were purchased from Invitrogen. Other microbial agonists used were zymosan A particles from Invitrogen Life Technologies, Mycobacteria membrane fractions (National Institute of Allergy and Infectious Diseases Contract N01 AI-75320 entitled “Tuberculosis Research Materials and Vaccines Testing”), heat-killed Staphylococcus aureus from Invitrogen Life Technologies, and the Escherichia coli type II heat-labile enterotoxin LT-IIB from Dr. T. Connell (University at Buffalo, State University of New York, Buffalo, NY).

**Antibodies.** The mouse anti-human TLR1 mAb GD2.F4 and the isotype control, a mouse IgG1 mAb, were obtained from eBioscience. A mouse anti-FLAG mAb was obtained from Sigma-Aldrich. A biotin-conjugated control, a mouse IgG1 mAb, were obtained from Jackson ImmunoResearch Laboratories.

**Cell culture**

Human embryonic kidney 293T cells were cultured in DMEM containing 10% (v/v) heat-inactivated FBS (Intergen) and 2 mM L-glutamine. SW620 cells (a human epithelial cell line (American Type Culture Collection no. CCL-227) were cultured in RPMI 1640 containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37°C in a 5% CO2/air environment.

**Cloning**

The S248N, H305L, and P315L variants as well as the TLR1 position 315 mutants were generated as N-terminal FLAG-tagged constructs within pFLAG-CMV (Sigma-Aldrich). Oligos encoding nucleotide changes were designed and used to introduce the amino acid changes as described by either the QuikChange site-directed mutagenesis method (Stratagene) or the technique of overlap extension PCR (Table I) as described by Horten et al. (11). The site-directed mutagenesis of all the variants and mutants was performed in a common variant of human TLR1 represented by National Center for Biotechnology Information (NCBI) accession no. AA109095. Variants and mutants were verified by sequencing.

**Transient transfection**

SW620 colonic epithelial cells were cotransfected with various combinations of TLRs along with a firefly luciferase gene driven by the IL-8 promoter (12) and pRL-null, a Renilla luciferase transfection control (Promega). Transfections were performed with the FuGENE 6 transfection reagent at a 4:1 ratio of FuGENE 6 to DNA (Roche). The total amount of transfected plasmid DNA was equalized by supplementing with an empty vector, pFLAG-CMV.

**Stimulation**

Two days after transfection cells were stimulated with the indicated agonists for 6 h and cell lysates were collected. During Ab blocking experiments the cells were preincubated with either the anti-human TLR1 mAb GD2.F4 or the isotype control, an anti-mouse IgG1 Ab, at a concentration of 10 μg/ml for 30 min before stimulation with an agonist.

**Luciferase assays**

Firefly luciferase and Renilla luciferase enzyme activities were determined using the luciferase assay system (Promega) according to the manufacturer’s instructions. Firefly luciferase activity was normalized to that of Renilla luciferase activities to correct the transfection efficiency. After correcting for transfection efficiency, all values were normalized to those of unstimulated cells transfected with reporters and an empty FLAG-CMV vector.

**Flow cytometry**

293T cells were transfected in 6-well plates with a vector expressing a FLAG-tagged TLR construct or empty vector alone (3.0 μg each). Transfections were performed with the FuGENE 6 transfection reagent at a 3:1 ratio of FuGENE 6 to DNA (Roche). The cell medium was changed 24 h posttransfection, and 48 h posttransfection the cells were removed from the plate with chilled PBS (pH 7.4) and the cell suspension was immediately transferred and split into two 1.5-ml Eppendorf tubes incubated on ice. To determine surface expression, one set of cells was incubated on ice sequentially with a mouse anti-FLAG mAb and a biotin donkey anti-mouse secondary Ab followed by a streptavidin-PE-conjugated tertiary Ab. The other set of cells was incubated on ice sequentially with the mouse anti-human TLR1 Ab GD2.F4 and a biotin donkey anti-mouse secondary Ab.
followed by a streptavidin PE-conjugated tertiary Ab. After labeling, the cells were washed, fixed, and surface fluorescence was quantified using a Coulter Epics XL instrument. Overlays were created using the Summit software program.

Results

Generation and expression of TLR1 variants

Through the examination of chimeric receptors generated by domain exchange between TLR1 and TLR6, previous studies in our laboratory have defined a functional region of human TLR1. This region, comprised of LRR motifs 9–12 of TLR1, is required for the recognition of lipopeptide agonists (10). These findings prompted us to search the NCBI database for naturally occurring polymorphisms in this region. This search revealed three SNPs designated rs4833095, rs3923647, and rs5743613, which are associated with the nonsynonymous amino acid changes S248N, H305L, and P315L, respectively, within this extracellular region of TLR1. Through site-directed mutagenesis these three TLR1 variants were independently generated within a reference TLR1 template that represents a common naturally occurring gene variant found in the human population. To measure the receptor expression levels, all of the constructs were generated as N-terminal FLAG-tagged proteins.

FIGURE 1. Expression profile of TLR1 variants. 293T cells were transfected with 3 μg of either a FLAG-tagged TLR1 vector (solid line), a FLAG-tagged TLR1-SNP vector (solid line), or an empty vector (dashed line) as indicated. After 48 h, cells were removed from the plate and incubated sequentially with either the anti-FLAG mAb (A) or the anti-TLR1 mAb GD2.F4 (B) and the biotin donkey anti-mouse secondary Ab followed by a streptavidin-PE-conjugated tertiary Ab. After labeling, the cells were washed, fixed, and analyzed by flow cytometry for surface expression of TLRs.

FIGURE 2. A TLR1 SNP, P315L, shows attenuated response to synthetic lipopeptide agonists. SW620 cells were cotransfected with various combinations of TLRs as indicated (50 ng/ml TLR2, 200 ng/ml TLR1, and 200 ng/ml TLR1 SNPs), an IL-8 driven luciferase gene (100 ng/ml), and a Renilla transfection control (50 ng/ml). Two days posttransfection cells were stimulated with the indicated agonists at 20 ng/ml for 6 h, after which luciferase activities were measured. After correcting for transfection efficiency, all values were normalized to those of unstimulated cells with a reporter and an empty FLAG-CMV vector. Open bars represent unstimulated cells. The data shown are the mean ± SD of three replicates in a given experiment. Asterisks indicate activity that is significantly lower than that of the wild type receptor (p < 0.05) as determined by Student’s t test.

FIGURE 3. The response mediated by P315L is partially rescued at high agonist concentration. SW620 cells were cotransfected with various combinations of TLRs as indicated (50 ng/ml TLR2, 200 ng/ml TLR1, and 200 ng/ml P315L SNPs), an IL-8 driven luciferase gene (100 ng/ml), and a Renilla transfection control (50 ng/ml). Cells with stimulated with increasing concentrations of the synthetic lipopeptide Pam3CSK4. Luciferase activity was measured as described in the legend of Fig. 2. Each titration experiment was repeated at least twice and one representative result is shown.
H315L variants, it was completely unable to bind the P315L variant (Fig. 1B). These results demonstrate that this amino acid substitution within LRR motif 11 (LRR11) at position 315 destroys the epitope for GD2.F4. To our knowledge, this is the first study to reveal a potential epitope for this mAb.

The P315L variant exhibits highly attenuated responses to a variety of microbial agonists

We next tested the ability of the variants to respond to TLR1 agonists. Because TLR1 signals as a heterodimer with TLR2 and requires the latter receptor to mediate responses to microbial agonists (7, 14–17), we transfected the TLR1 variants along with TLR2 in SW620 cells. This human epithelial cell line lacks endogenous expression of these receptors and is ideal for the reconstitution of receptor activity as measured by induction of an IL-8-driven luciferase gene (10). We found that all variants exhibit attenuated responses to the synthetic lipopeptides Pam3CKS4 and PamCysPamSK4 (Fig. 2). Interestingly, the P315L variant was the most impaired and displayed a loss of 60–80% of the original lipopeptide-mediated response.

In an effort to understand the nature of the severely attenuated response of the P315L variant, we examined the ability of this variant to induce the IL-8 luciferase reporter at varying agonist concentrations. Although the activity of the reference TLR1 saturated at low lipopeptide concentrations, the activity of the P315L variant did not and was suboptimal even at high lipopeptide concentrations (Fig. 3). This suggests that the ability of the P315L variant to engage the receptor signaling complex is greatly attenuated and cannot be overcome by using high concentrations of a lipopeptide agonist.

To ascertain the generality of our results, we tested additional microbial-derived agonists of TLR1 in our cell activation assay including LPS derived from Porphyromonas gingivalis, mycobacterial membrane fractions, and yeast zymosan particles. We observed that, in comparison with the reference TLR1, cells reconstituted with S248N and H305L were slightly attenuated in their ability to respond to all of the tested agonists, whereas the activity of the P315L variant of TLR1 was severely attenuated (Fig. 4). Thus, the deficiency of the P315L variant in mediating cell activation appears to be a general one that affects cellular responses to a variety of microbial-derived agonists of TLR1.

A variety of mutations at position 315 attenuate TLR1-mediated responses to microbial agonists

To investigate the role of proline at position 315 of TLR1, we introduced various amino acid substitutions within LRR motif 11 (LRR11) at position 315 destroys the epitope for GD2.F4. To our knowledge, this is the first study to reveal a potential epitope for this mAb.

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A variety of mutations at position 315 attenuate TLR1-mediated responses to microbial agonists

To investigate the role of proline at position 315 of TLR1, we introduced various amino acid residues at this site with altered size, polarity, and charge using site-directed mutagenesis. The staining of transfected cells with the anti-FLAG mAb revealed that all of the mutants are equally expressed with the exception of P315G, which exhibits significantly lower expression levels (Fig. 5A). These results suggest that the complete rotational freedom afforded by the glycine residue may contribute to receptor misfolding. Fig. 5B shows that three of the four mutants, alanine, serine and glycine, are able to bind the anti-TLR1 mAb GD2.F4 while the arginine mutant does not. The observation that the GD2.F4 mAb is unable to bind the arginine mutant, similar to the leucine variant, suggests that the introduction of a large amino acid side chain destroys the epitope for GD2.F4.
mosan, or 6
transfected SW620 cells with either the isotype control or the
TLR1 activity in our cellular assay. To this end, we preincubated
This prompted us to examine the ability of GD2.F4 to inhibit
compassing this region of LRR1 is important for TLR1 function.
mAb GD2.F4. Together, these results suggest that the epitope en-
variant, unlike the reference TLR1, does not bind the anti-TLR1
mAb GD2.F4 (10 μg/ml) or an isotype control mAb (10 μg/ml) for 30 min. The
two days posttransfection cells were blocked with either the anti-human TLR1 mAb
GD2.F4 before stimulation with lipopeptide agonists. Fig. 8
demonstrates that the GD2.F4 mAb mediated a 50% reduction in the
activity of the reference TLR1 toward lipopeptides. As expected,
GD2.F4 also inhibited the activity of the P315G, P315A, and the
P315S mutants but had no effect on the activity of the P315R and
P315L mutants. These results confirm the previous flow cytometry
results indicating that the epitope for GD2.F4 is destroyed in these
We next assessed the activity of the TLR1 mutants by recon-
stitution in SW620 epithelial cells followed by stimulation with
various TLR1 agonists. Fig. 6A shows that all four mutants,
P315G, P315A, P315S, and P315R, display attenuated responses to
lipopeptide agonists in comparison to the reference receptor.
Fig. 6B shows that all the TLR1 mutants exhibit attenuated activity
toward a variety of microbial-derived agonists. Interestingly,
every agonist tested the P315L variant was the most severely at-
tenuated and, compared with the reference TLR1, typically exhib-
ted only ~20% of the reference activity.

Previous reports have shown that CD14 enhances the stimulation
of immune cells mediated by a variety of TLR2 agonists in-
cluding mycobacterial lipomannans (17, 18). Similarly, we have
observed that CD14 greatly enhances the ability of TLR reconsti-
tuted SW620 epithelial cells to respond to the B pentamers of E.
coli type II heat-labile enterotoxins LT IIaB, heat-killed S. aureus,
and heat killed A. laidlawii (Fig. 7). To investigate the activity of
the TLR1 position 315 mutants toward CD14-dependent agonists,
we stimulated SW620 epithelial cells that were reconstituted with
these receptors. As previously observed, in comparison to the ref-
erence TLR1 the P315L variant exhibits the greatest deficiency
with loss of ~80% of the original activity (Fig. 7). These results
demonstrate that the CD14 coreceptor is unable to rescue the ac-
tivity of the P315L variant of TLR1.

The GD2.F4 mAb, which binds LRR1, inhibits TLR1 activity

We have demonstrated that the P315L variant exhibits highly at-
tenuated responses to a variety of microbial agonists and that this
variant, unlike the reference TLR1, does not bind the anti-TLR1
mAb GD2.F4. Together, these results suggest that the epitope en-
compassing this region of LRR1 is important for TLR1 function.
This prompted us to examine the ability of GD2.F4 to inhibit
TLR1 activity in our cellular assay. To this end, we preincubated
transfected SW620 cells with either the isotype control or the

![FIGURE 6. P315L displays a more attenuated response than other position 315 mutants. SW620 cells were cotransfected with TLR2 (50 ng/ml), either TLR1 (200 ng/ml) or position 315 mutants (200 ng/ml) as indicated, an IL-8 driven luciferase gene (100 ng/ml), and a Renilla transfection control (50 ng/ml). Cells were stimulated with 20 ng/ml Pam3CSK4 or PamCysPamSK4 (A) or 20 ng/ml P. gingivalis LPS, 10^7 particles/ml zymosan, or 6 μg/ml Mycobacteria membrane fraction (Myco.Mem.Frac.) as indicated (B). Luciferase activity was measured as described previously. The data shown are the mean ± SD of three replicates in a given experiment. Asterisks indicate activity that is significantly lower than that of the wild-type receptor (p < 0.05) as determined by Student's t test.](http://www.jimmunol.org/content/181/6/6389/F6)

![FIGURE 7. P315L displays a more attenuated response to CD14-dependent agonists than other position 315 mutants. SW620 cells were cotransfected with TLR2 (50 ng/ml), CD14 (100 ng/ml), either TLR1 (200 ng/ml) or position 315 mutants (200 ng/ml) as indicated, an IL-8 driven luciferase gene (100 ng/ml), and a Renilla transfection control (50 ng/ml). Cells were stimulated with 2 μg/ml LT-IIaB from E. coli or 10 ng/ml lipomannan from M. smegmatis (A) 10^7 particles/ml heat-killed Staphylococcus aureus (HKSA) or 2 μg/ml heat-killed A. laidlawii (HKAL) (B). Luciferase activity was measured as described previously. The data shown are the mean ± SD of three replicates in a given experiment. Asterisks indicate activity that is significantly lower than that of the wild-type receptor (p < 0.05) as determined by Student’s t test.](http://www.jimmunol.org/content/181/6/6389/F7)

![FIGURE 8. GD2.F4 mAb inhibits the activity of the reference TLR1. SW620 cells were cotransfected with 50 ng/ml TLR2, either 200 ng/ml TLR1 or 200 ng/ml TLR1 mutants as indicated, an IL-8 driven luciferase gene (100 ng/ml), and a Renilla transfection control (50 ng/ml). Two days posttransfection cells were blocked with either the anti-human TLR1 mAb GD2.F4 (10 μg/ml) or an isotype control mAb (10 μg/ml) for 30 min. The cells were subsequently stimulated with an agonist for 6 h after which the cell lysate was collected and luciferase activity measured as described previously. The data shown are the mean ± SD of three replicates in a given experiment. Asterisks indicate activity that is significantly lower in the presence of GD2.F4 in comparison to the corresponding isotype control mAb (p < 0.05) as determined by Student’s t test.](http://www.jimmunol.org/content/181/6/6389/F8)
action between these receptors on the concave side of the solenoid would be sterically improbable. Indeed, a recent model based on the mutagenesis work of TLR3 bound to dsRNA proposes an interaction of this agonist with the convex side of receptor dimers (25). Thus, TLRs may interact with cognate agonists in a different manner from that of other LRR proteins such as the RNase inhibitor protein, the Nogo receptor, and the glycoprotein 1b receptor that bind their cognate ligands on the concave side of the molecule (26–28).

In this study we show that P315L, a SNP that is located in the outer loop of the LRR11 of TLR1, is associated with a greatly attenuated receptor function. This finding further supports the theory that the outer loops, which make up the convex side of the TLR1 ECD, provide the interface for interaction with a ligand, the TLR2 coreceptor, and/or with other components of the TLR1 activation complex.

Prolines have one fixed dihedral angle and segments containing proline generally have increased rigidity and can provide a focus for protein folding and assembly (29, 30). This residue is often present in turns and is not averse to being exposed to water. Another residue commonly found in turns is glycine, which was therefore a logical choice for substitution in our mutational studies. Although the leucine variant was by far the most greatly attenuated, all of the mutations, including the glycine mutant, exhibited a significantly reduced response to all of the TLR1 agonists examined. This finding suggests that the fixed dihedral angle that is unique to proline plays a role in assuring the correct local conformation of the LRR11 loop in TLR1. These results also show that a large hydrophobic residue, such as leucine, greatly disrupts receptor function. The orientation of this leucine toward the hydrophobic core of the solenoid would be energetically favorable and, by altering the conformation of the outer loop of LRR11, could affect the ability of TLR1 to interact with the agonist and/or other coreceptors, such as TLR2, of the activation complex. Our results show that this inefficient interaction cannot be overcome with high concentrations of a lipopeptide agonist (Fig. 3).

An interesting observation from our results is that the P315L variant is attenuated in its ability to mediate responses to agonists of varying chemical nature. One possibility is that rather than binding this agonist, this region of TLR1 is involved in interactions with TLR2 or other components of the signaling complex. It is also formally possible that, upon binding a cognate agonist, an as yet unidentified accessory molecule(s), similar to MD-2 in the TLR4 signaling complex, undergoes a conformational change that is sensed by the TLR1 signaling complex, leading to receptor activation (31). If this is the case, then a SNP of TLR1 could provide an interaction point at which a proline provides the best local conformation of LRR11 for protein-protein interaction. Clearly,

### Table II. Alignment of known TLR1 orthologs around human TLR1-LRR11

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Sequence</th>
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<tr>
<td>Mus musculus</td>
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</tr>
</tbody>
</table>

*The primary sequences of known TLR1 orthologs were aligned by using the ClustalW algorithm. Residues correspond with the following NCBI accession numbers:* NP_001039969.1 (M. mulatta), NP_001039969.1 (R. taurus), NP_001026945.1 (S. scrofa), and NP_109607.1 (M. musculus). LRR-11 alignment is based on human TLR alignment as defined by Bell et al (20).

<table>
<thead>
<tr>
<th>Study</th>
<th>Ethnic Group (Country)</th>
<th>Sample Size</th>
<th>PP (%)</th>
<th>P/L (%)</th>
<th>Estimated Minor Allele Frequency (%)</th>
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<td>6.9</td>
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<td>97.6</td>
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*SNP frequencies for the P315L variant were compiled from the NCBI SNP database (www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=5743613).
additional biochemical data are needed to resolve this mechanistic issue.

Since GD2.F4 was first generated it has become a widely used mAb for detecting cell surface expression of human TLR1 (13). We have found that the alteration of a proline at position 315 to either a leucine or arginine abrogates the ability of the GD2.F4 mAb to bind to TLR1, suggesting that the loop of LRR11 constitutes part of the epitope for this mAb. The addition of this mAb before stimulation inhibits ~50% of TLR1 activity in our assay (Fig. 8). This level of inhibition is comparable to that found in at least two other studies where this mAb was used (32, 33). The fact that the epitope for GD2.F4 maps to the outer loop of LRR11 confirms the importance of this region in receptor function.

A phylogenetic analysis of the TLRs identified in all vertebrate species to date shows that all family members, including the related subfamily comprising TLRs 1, 6 and 10, have evolved under strong purifying selection (34). Thus, rare rather than common TLR variants are expected to be associated with an increased susceptibility to infectious disease. This notion is supported by a study in which an excess of rare SNPs in TLR4 has been observed among patients with meningococcemia compared with healthy controls (35). The proline at position 315 of TLR1, as well as the surrounding amino acids, is highly conserved in a variety of other mammals, consistent with the idea of strong purifying selection (see Table II). Additionally, the P315L polymorphism is relatively rare in human populations with alleles thus far observed predominantly in individuals of African descent and even more rarely in individuals of European heritage (Table III). Although no individuals homozygous for the P315L SNP have been observed, heterozygous individuals may exhibit susceptibility to disease due to the random silencing of one allele or to a gene dosage effect.

Similar to the P315L of TLR1, two well documented polymorphisms in TLR4 are predicted to be located in the outer loop regions of the LRRs of the receptor. These polymorphisms, D299G and T399I are two coexisting polymorphisms located in the loops of LRR motifs 10 and 15 of the receptor, respectively, and are responsible for hyporesponsiveness to inhaled LPS (36). These TLR4 polymorphisms are associated with an increased incidence of respiratory syncytial virus infection and Gram-negative bacterial septic shock (37, 38). Conversely, there have also been reports that D299G and T399I are associated with decreased susceptibility to atherosclerosis, diabetic neuropathy, and the rejection of allografts (reviewed in Ref. 5). Transfection studies show that these TLR4 variants do not exhibit a general defect in the transport of the receptor to the cell surface (39). Similarly, we have shown that the hyporesponsiveness to TLR1 agonists exhibited by the P315L variant is not due to lack of cell surface expression (Fig. 1). The precise mechanism underlying the reduced activity of these outer loop mutations will require additional mutational, biochemical, and structural analyses.

Because TLR2 is an essential TLR1 signaling partner, clinical association studies of SNPs in the TLR2 gene may provide insight into the putative role of the TLR1-P315L SNP in disease susceptibility. In this regard, P631H, R677W, and R753Q are three non-synonymous SNPs in the signaling domain of the TLR2 gene that have individually been associated with resistance to meningitis or susceptibility to leprosy or tuberculosis (reviewed in Ref. 5). As expected, each of these variants exhibit diminished activity in vitro. Similar to P315L, these three TLR2 SNPs display variable allele frequencies among different races. Clinically, the heterozygosity of each TLR2 variant was sufficient to confer disease susceptibility, indicating that individuals heterozygous for P315L could exhibit increased susceptibility to these infectious diseases. TLR1 mediates responses to a wide variety of microbial and fungal agonists, and a full determination of the effect of the P315L allele on susceptibility or resistance to a given disease will require large-scale genetic association studies between patient and healthy populations.

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Disclosures

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References


