SUPPLEMENT ARTICLE

The Importance of Toll-Like Receptor 2 Polymorphisms in Severe Infections

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Toll-like receptor 2 (TLR2) is a member of the TLR family, which plays a central role in the innate immune response to a wide variety of microorganisms. Animal studies have shown that TLR2-knockout mice are more susceptible to septicemia due to *Staphylococcus aureus* and *Listeria monocytogenes,* meningitis due to *Streptococcus pneumoniae,* and infection with *Mycobacterium tuberculosis,* suggesting that functional *TLR2* polymorphisms may impair host response to a certain spectrum of microbial pathogens. In humans, 2 polymorphisms in the exon part of *TLR2,* which attenuate receptor signaling, enhance the risk of acute severe infections, tuberculosis, and leprosy. Because gram-positive bacteria have became the first cause of severe infections, including septic shock, knowledge of the role that alteration or lack of TLR2 function plays in the pathogenesis of infectious diseases could contribute to the design of new therapeutic strategies, including prevention, pharmacological intervention, and vaccine development.

INNATE IMMUNE DEFENSE AND TOLL-LIKE RECEPTORS (TLRs)

Humans are constantly exposed to a wide variety of microorganisms that can cause deadly infection. The ability of a host to sense invasion by pathogenic organisms and to respond appropriately to control infection is paramount to survival. Delayed detection of pathogens contributes to overwhelming infections and exaggerated systemic responses and produces life-threatening tissue damage, organ dysfunction, and death. The role of host genetic factors in determining susceptibility to infections has become more evident; certain persons appear to be predisposed to certain infections, whereas others are protected [1]. A better understanding of the disease process may be achieved by studying the immune response and the genetic makeup of susceptible and resistant persons. For example, human allelic variants of genes involved in the innate immune response have been shown to influence the susceptibility to infection and/or the subsequent rates of progression toward severe sepsis and septic shock [1].

The innate immune system has evolved as the first line of defense against invading pathogens, playing a primary role in acute host defense [2]. It plays a crucial role in destroying pathogens, determining the localization and development of the infectious challenge, and facilitating the adaptive immune response. Innate immunity recognition of invading microorganisms is mediated by a set of soluble and membrane receptors (pathogen recognition receptors) that recognize conserved pathogen-associated molecular patterns shared among each class of infectious agents but absent in higher eukaryotes (nonself).

During the past 5 years, accumulating evidence has shown that pathogen detection can be attributed mainly to germline-encoded pathogen recognition receptors called TLRs. TLRs have been conserved during evolution and are present in creatures as diverse as mammals, insects, and plants. To date, 10 human TLRs (TLR1–TR10) have been cloned from the human genome, and at least 1 ligand has been identified for each

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TLR, except for TLR10 [3]. These receptors have been shown to play an essential role in triggering the innate immune response by recognizing a wide variety of pathogen-associated molecular patterns from bacteria, viruses, protozoa, and fungi. Signals initiated by the interaction of TLRs with their specific ligands orchestrate the inflammatory response, which attempts to clear the offending pathogen, and lead to a specific adaptive immune response. Activation of TLRs stimulates macrophages to produce proinflammatory cytokines and antimicrobial molecules, such as nitric oxide or defensins, and initiates the maturation of dendritic cells, which then migrate to the lymph nodes and overexpress antigen–major histocompatibility complexes and costimulatory molecules (CD80/CD86). Thus, TLR signaling not only represents a key component of the innate immune response to microbial infection but is also essential in linking innate and adaptive immunity.

TLR2 has a special place among the 10 members of the human TLR family. Of all of the mammalian TLRs and, perhaps, of all pathogen recognition receptors, TLR2 is capable of detecting the widest repertoire of pathogen-associated molecular patterns from a large variety of pathogens, including gram-positive or gram-negative bacteria, mycobacteria, fungi, viruses, and parasites. This large repertoire is mainly the result of the ability of TLR2 to recognize ligands as a heterodimer with either TLR1 or TLR6. The fact that TLR2 is crucial for recognition of gram-positive bacteria and mycobacteria is of particular clinical importance. Gram-positive bacteria now represent the most common cause of severe infections associated with organ dysfunction or septic shock in the intensive care unit [4, 5], and tuberculosis remains a leading cause of death worldwide. This review summarizes recent advances in our understanding of the biological importance of TLR2 and describes functional genetic variations of this central component of innate immunity.

TLR2 LIGANDS AND SIGNALING

The expression profile of TLR2 on human tissues and cells indicates its pivotal role in host defense. High expression of TLR2 in humans is restricted to a small number of cell types mainly involved in acute host defense, with strongest expression detected in hematopoietic system–derived cells (e.g., monocytes, macrophages, granulocytes, and dendritic cells), followed by cell populations from the spleen and lung [6]. For example, on neutrophils, TLR2 is expressed in higher concentrations than is TLR4, which is a receptor for lipopolysaccharide (LPS) and other gram-negative bacterial products [7]. TLR2 is also expressed on the B and T cells that mediate adaptive immunity and on a variety of other cells, including vascular and intestinal endothelial cells, adipocytes, and cardiac myocytes.

Although the expression pattern and the ligand specificity of TLR2 appear to be unique, the structure of TLR2 shares many similarities with other members of the TLR family. TLR2 is encoded by a DNA sequence composed of 2352 bases that specify 784 amino acids [6]. This type I transmembrane receptor is characterized by an extracellular leucine-rich domain (amino acids 1–588), a single transmembrane domain (amino acids 589–609), and a cytoplasmic domain (amino acids 610–784) that resembles the intracellular domain of the IL-1 receptor and contains a conserved region called the toll/IL-1 receptor (TIR) homology domain (amino acids 643–784).

The extracellular domain of *TLR2* plays an important role in defining the ligand specificities of TLR2. A wide variety of bacterial, fungal, protozoal, and viral components are capable of stimulating TLR2. Pathogen-associated molecular patterns and pathogens recognized by TLR2 are listed in table 1.

Human TLR2 plays a key role in response to peptidoglycan (PGN), lipoteichoic acid, and a variety of macromolecules from gram-positive bacteria. As such, TLR2 may be considered as

Table 1. Toll-like receptor 2 (TLR2) ligands.

TLR, origin of ligands	Ligand
TLR2/TLRx	
Gram-positive bacteria	Lipoproteins, peptidoglycan, lipoteichoic acid, fimbriae
Listeria species	Heat-killed bacteria
Group B streptococci	Soluble factors (GBS-F)
Gram-negative bacteria	
Legionella species	LPS
Porphyromonas gingivalis	Atypical LPS
Bordetella species	LPS
Meningococci	Porins
Klebsiella species	Outer membrane protein A
Brucella species	Heat-killed *Brucella abortus*
Mycobacteria	Lipoarabinomannan
Leptospira interrogans	Atypical LPS
Treponema maltophilium	Glycolipids
Trypanosoma cruzi	Glycoinositolphospholipids
Cytomegalovirus	Virions
Anaplasma phagocytophilium	
Measles virus	Hemagglutinin protein
TLR2	
Aspergillus species	
A. fumigatus	Conidia, hyphae
A. niger	Conidia, hyphae
Candida albicans	Conidia, phopsholipomannan
Saccharomyces cerevisiae	Zymosan
TLR1/TLR2	Soluble factors and porins, OspA lipoprotein, 19-kDa lipoprotein, ara-lipoarabinomannan, tri-acyl lipoprotein, PAM
TLR2/TLR6	
Mycoplasma species	MALP-2 (di-acyl lipopeptides)
Staphylococcus species	Phenol-soluble modulin

NOTE. GBS-F, group B streptococci soluble factors; LPS, lipopolysaccharide; MALP-2, macrophage-activating lipopeptide 2; PAM, tri-palmitoyl-S-glyceryl-Cys-Ser-Lys^4.

the most important receptor for gram-positive bacterial products. In vitro, macrophages from TLR2-deficient mice still produce proinflammatory cytokines in response to LPS but are unresponsive to *Staphylococcus aureus* PGN and *Listeria monocytogenes* [8]. Overexpression of TLR2 in Chinese hamster ovary cells conferred responsiveness to *S. aureus, Streptococcus pneumoniae,* and *Bacillus subtilis.* Moreover, human TLR2 has been identified as a receptor for bacterial lipoproteins and lipopeptides that are present in a variety of pathogens, including gram-positive and gram-negative bacteria, mycobacteria, and *Mycoplasma* species. TLR2 recognizes the N-terminal lipid moiety of a variety of bacterial lipoproteins, such as M161 antigen and MALP-2 (macrophage-activating lipoprotein 2) from *Mycoplasma* species [9] and lipoarabinomannan, which is a major cell wall–associated glycolipid derived from *Mycobacterium tuberculosis.* More recently, TLR2 has been involved in the detection of other gram-negative bacterial pathogen–associated molecular patterns. Hence, although TLR4 mediates LPS signaling from gram-negative bacteria (mainly enterobacteria), TLR2 detects "nonclassical" LPS from *Legionella* species, *Bordetella* species, *Leptospira interrogans,* and *Porphyromonas gingivalis* [3]. Similarly, TLR2 detects porins from meningococci and outer membrane protein A from *Klebsiella* species.

In addition to bacterial pathogens, TLR2 is involved in the detection of pathogen-associated molecular patterns associated with fungi, protozoa, and viruses. They include zymosan, a component of yeast cell walls, *Candida albicans* cell walls, *Trypanosoma cruzi* glycophosphatidylinositol anchor, cytomegalovirus virions, and the measles virus [10].

How a single TLR can recognize such a variety of chemically diverse ligands is an important question in human biology. The answer for TLR2 probably arises from the potential for TLR2 to form homodimers and heterodimers with TLR1 or TLR6 [11]. Recent functional studies, which used dominant-negative TLRs or TLR-deficient mice or analyzed extracellular and intracellular interactions of different heterodimers, suggested that the composition of the dimers dictates the ligand specificity of TLR2 [12]. TLR1/TLR2 heterodimers recognize bacterial lipopeptides (such as the 19-kDa mycobacterial lipoprotein), the synthetic lipoprotein structure PAM3CYSKA, and other triacylated lipoproteins. Macrophages from both TLR1- and TLR2-knockout mice incubated with *Borrelia burgdorferi* lipoprotein showed impaired production of cytokines and nitric oxide, compared with wild-type controls [13]. On the other hand, expression of dominant-negative TLR2 or dominant-negative TLR6 showed that TLR2 and TLR6 function together to detect gram-positive bacteria, zymosan, and diacylated lipopeptides, such as *Mycoplasma* lipoproteins. TLR2 was shown to interact directly with TLR6, as both are recruited to the phagosome. Although TLR6 knockout mouse macrophages lose responsiveness to MALP-2, single expression of TLR2 still promotes nuclear factor (NF)–κB activation on human cells, and lack of TLR6 only partially impairs M161 antigen–mediated signaling [14]. Hence, TLR2 homodimers are sufficient to signal the presence of this lipoprotein independently of TLR6 in human cells, but not in mouse cells, supporting differences in ligand affinity and specificity among species [15, 16].

Another important question is how TLR2 is activated by microbial cell wall components. Different studies strongly suggest that TLR2, like TLR4 and TLR9 in the TLR family, can directly recognize and bind its ligands (i.e., PGN).

The TLR2 signaling pathway (figure 1) plays an important role in the response to microbial infections. The TLR2-induced signaling cascade is close to that observed for other members of the TIR family. TLR2 stimulation by gram-positive bacteria leads to the recruitment of the adaptor molecules MyD88 and TIRAP to the receptor complex (figure 1). The serine/threonine kinases IL-1 receptor–associated kinase (IRAK)–1 and IRAK-4 are subsequently recruited and activated, allowing interaction with the downstream molecule TRAF6, another adaptor protein. TRAF6 induces activation of TAK1 and MKK6, which, in turn, activate the transcriptional factor NF-κB, the Jun N-terminal kinase (JNK), ERK, and the p38 kinase. The TIR domain–containing adaptors MyD88 and TIRAP are associated with the TIR domain of TLR2 and are essential for PGN-induced NF-κB and MAPK activation, as demonstrated by targeted gene disruption studies. Analysis of MyD88-deficient mice showed that the TLR2 signaling pathway is completely dependent on MyD88 [17]. Hence, in contrast to TLR3 and TLR4, TLR2 has not been shown to use an MyD88-independent pathway. However, MyD88-dependent events through TLR2 were affected by TIRAP deficiency, demonstrating that TIRAP plays an important role in the MyD88-dependent immune response shared by TLR2 and TLR4 [18].

Although cell signaling associated with TLR2 or TLR4 has been reported to be similar, some studies have demonstrated marked differences, including the importance for TRIF in TLR4 signaling (and not in the TLR2 cascade) and the existence of an NF-κB transactivation pathway for TLR2 [19]. Hence, signaling via TLR2 involves specific tyrosine phosphorylation and a cascade that includes the small GTPase Rac1, PI3-K, and Akt before activation of NF-κB and induction of inflammatory responses. Blockade of PI3-K does not inhibit NF-κB nuclear translocation but prevents phosphorylation of the p65 subunit of NF-κB that is necessary for maximal transcriptional activity of this central transcription factor of the inflammatory response. Recently, it has also been shown that inhibition of PI3-K stopped activation of p38 and ERK 1/2 after PGN stimulated TLR2 in neutrophils [20]. Hence, Akt has a central role in TLR2-induced production of cytokines and chemokines [20]. Because TLR1, TLR2, and TLR6, but not TLR3, TRL4, or TLR5, contain a consensus-binding motif (YXXM) for the PI3-K p85

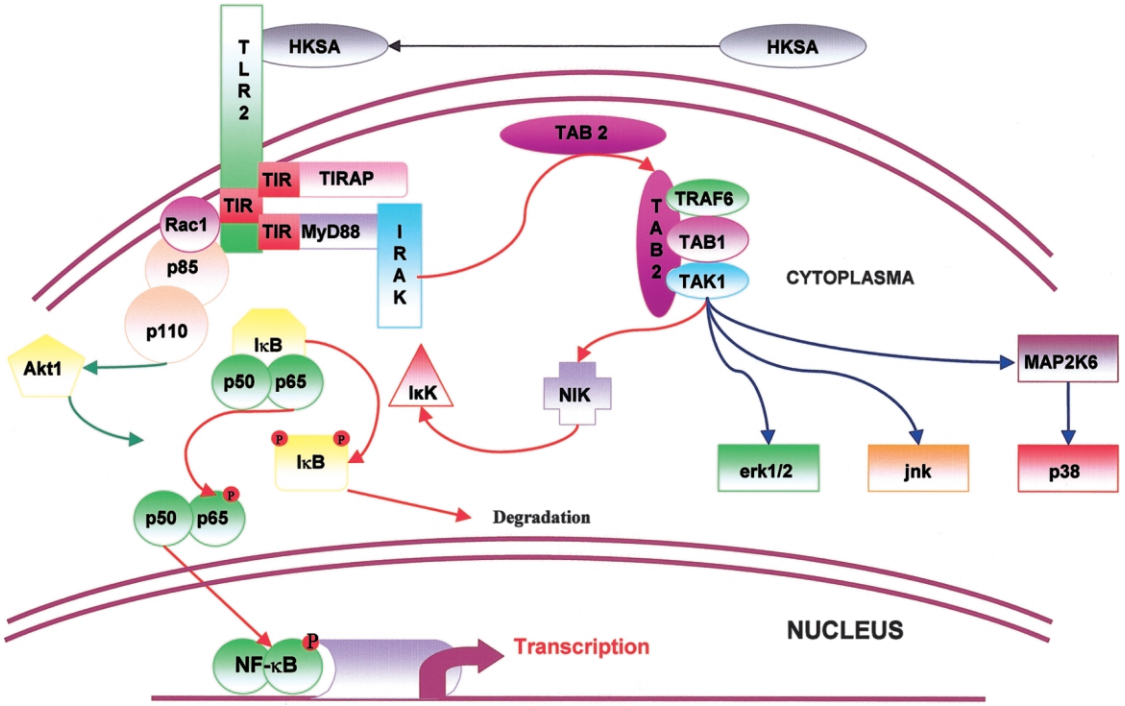

Figure 1. Toll-like receptor 2 (TLR2) signaling pathways. Green arrows indicate signaling pathway that is specific to TLR2 and leads to nuclear factor (NF)-κB transactivation. HKSA, heat-killed *Staphylococcus aureus;* IRAK, IL-1 receptor–associated kinase; MAP2K, mitogen-activated protein 2 kinase; NIK, NF-κB–inducing kinase; TIR, toll/IL-1 receptor; TIRAP, TIR domain–containing adapter protein; TRAF, TNF receptor–associated factor.

subunit [21], it may be predicted that PI3-K regulates TLR2 signaling directly or through interaction with TLR1 or TLR6 activation.

TLR2-DEFICIENT MICE

The characteristics of mice harboring a null allele for TLR2 provide strong in vivo evidence that TLR2 is involved in innate immune responses to gram-positive bacteria, mycobacteria, spirochetes, and fungi. In gene disruption experiments, TLR2-deficient mice were unable to respond to PGN and had significantly decreased survival after intravenous challenge with *S. aureus,* compared with wild-type controls. Impaired outcome was associated with higher bacterial counts in the blood and kidneys of TLR2-knockout mice [17]. Similarly, TLR2-knockout mice were shown to be more prone to morbidity and mortality due to pneumococcal meningitis. Significantly decreased bacterial clearance from the CNS was also reported in this model [22]. The same authors have demonstrated that meningitis caused by *Listeria monocytogenes* induced greater mortality in TLR2-deficient mice than in wild-type mice. Similar results have been reported with *S. pneumoniae* in TLR2-knockout mice.

As previously mentioned, monocytes/macrophages detect mycobacterial cell wall components, such as *Mycobacterium* lipoproteins and lipoarabinomannan, through TLR2. Studies of aerosol challenge with *M. tuberculosis* involving TLR2-, TLR4-, and CD14-deficient and control mice have shown that only TLR2-knockout mice exhibit decreased resistance to tuberculosis after high-dose exposure [23]. TLR2-deficient mice have reduced bacterial clearance after mycobacterial aerosol challenge, fail to mount an effective granulomatous response, and develop chronic lethal pneumonia, despite an exacerbated pulmonary immune response (Th1 response with high alveolar levels of TNF, IL-12 p40, and IFN-γ) [24].

Innate host defense against the spirochete *B. burgdorferi,* the causative agent of Lyme disease, has been studied using TLR1- and TLR2-deficient mice [13, 25]. Two weeks after infection, a significantly higher number of spirochetes were found at all sites (skin, ankle joints, and heart) in both TLR1- and TLR2-deficient mice than in controls (increased ankle swelling due to Lyme arthritis and 10-fold more bacteria in the heart) [13].

Similar results have recently been reported for murine defense against *C. albicans.* Despite conserved capacity for phagocytosis and identical production of reactive oxygen species, TLR2-deficient mice infected intraperitoneally or intravenously with *C. albicans* showed dramatically impaired survival, compared with control mice [26]. This susceptibility to fungal infection seems to be related to an impaired production of cytokines and chemokines. These results confirm the pivotal role of TLR2 in TNF production in response to zymosan [27].

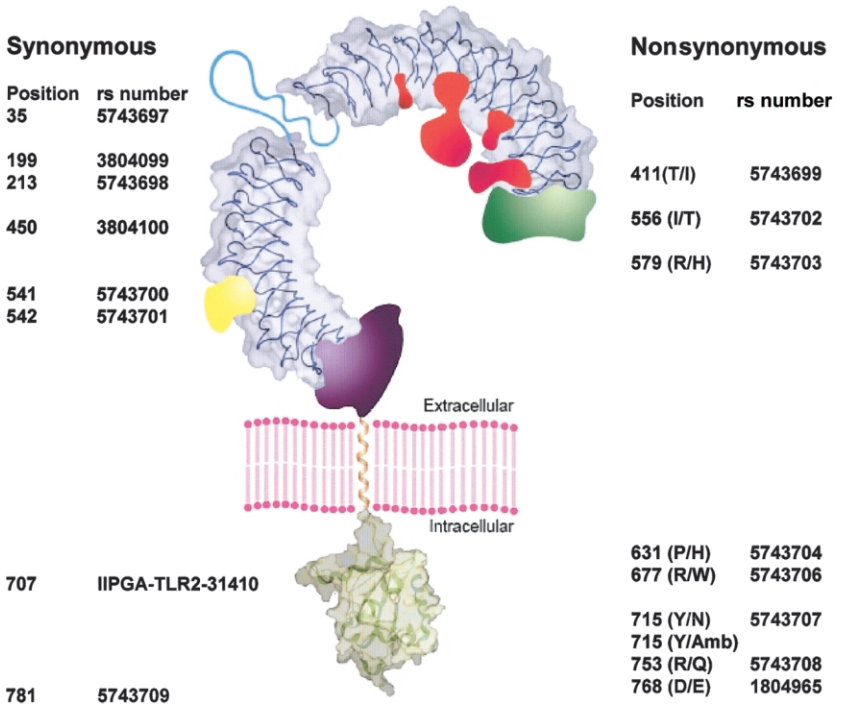

Figure 2. Coding variants of toll-like receptor 2 (TLR2). Position: amino acid number in *TLR2* sequence, followed by the amino acid change. rs, Reference source. Adapted with permission from Bell et al. [28].

TLR2 GENE ORGANIZATION AND POLYMORPHISMS IN HUMANS

The fact that TLR2-deficient mice are highly susceptible to infections suggests that polymorphisms that affect TLR2 expression or structure may impair host response to a given spectrum of microbial pathogens. The Human Genome Project has yielded an ever-growing list of allelic variation in the genes involved in innate immunity, including *TLR2,* which may account for individual differences in the response to invading pathogens. Although functional information about the biological consequences of allelic variants of the human *TLR2* gene is still very limited, 89 single-nucleotide polymorphisms (SNPs) have been reported for the *TLR2* gene (for more information, see http://www.innateimmunity.net and http://www.ncbi.nlm.nih.gov). Of these, 26 SNPs concern the 5′-untranslated region, 17 are in the 3′-untranslated region, 29 SNPs are located in the intronic part of the gene, and 17 SNPs modify bases of the third exon of *TLR2* (figure 2).

The human *TLR2* gene is located on chromosome 4q32 and is composed of 3 exons. The first and the second exons are noncoding, and the complete open-reading frame is located on exon III [29] (BAC clone GenBank accession number AC013303 and cDNA GenBank accession number AF051152). Alternative splicing of exon II has been detected in human blood monocytes, generating up to 5 different mRNA species, with the relative abundance of TLR2 splicing forms varying among individuals. Although alternative splicing does not change the open-reading frame and all mRNA isoforms encode identical proteins, differences in the 5′-untranslated region might influence mRNA stability or secondary structure and, therefore, affect the extent of TLR2 production. Thorough analysis of human genomic sequences revealed that a noncoding copy of exon III of the *TLR2* gene is located ~4 kb upstream of the transcribed *TLR2* gene. The presence of this pseudogene should be taken into account for genomic analysis.

Noncoding Variants of the 5′-Untranslated Region

The minimal promoter for human *TLR2* has been extensively characterized. Its sequence contains binding sites for several transcription factors, including Ets and Sp1 family members [29]. None of the 26 reported 5′-untranslated region SNPs are located within this sequence. In the 5′-untranslated region of *TLR2,* a highly polymorphic (GT)n dinucleotide repeat has been reported 100 bp upstream of the *TLR2* translational start site in intron 2, with numbers of GT repeats varying from 12 to 28 [30]. The overall distribution of alleles (number of repeats) is significantly different between African Americans and white Americans and between African Americans and Koreans but is not different between Koreans and white Americans. Functionally, *TLR2* 5′-untranslated regions with shorter (12) or longer (28) GT repeats have higher promoter activity than do medium-sized (20) GT repeats after stimulation with IFN. Be-

cause the basal level of TLR2 expression and its inducible regulation may both influence responses to microbial infection, it is possible that this microsatellite polymorphism plays a role in susceptibility to infectious disease. Recently, an insertion-deletion polymorphism in the 5′-untranslated region of *TLR2* has been reported in a Japanese population [31]. Gene reporter study indicates that the deletion allele has a reduced transcriptional activity, compared with that of the insertion allele. The importance of the deletion allele or of the nucleotide repeats in determination of the susceptibility to infectious diseases is still unknown.

Coding Variants

SNPs are DNA sequence variations that occur when a single nucleotide in the sequence genome is altered. Many SNPs have no effect on cell function, either because they do not result in amino acid change (synonymous SNP) (e.g., CGC, CGT, CGA, and CGG code for arginine) or because the amino acid change does not affect the protein structure or characteristics. Literature on functional polymorphisms in the coding sequence of the *TLR2* gene is limited. Among the 17 SNPs described until now, only 9 of them are nonsynonymous.

Polymorphisms of the extracellular domain of TLR2. The ectodomain of *TLR2* consists of a series of 19 leucine-rich repeats flanked at the carboxy-terminal end by a cysteine-rich cluster. The extracellular domain of *TLR2* is crucial for 2 reasons: first, it defines the specificities of TLR2 for its various ligands, and second, it is necessary for dimerization of TLR2 with TLR1 or TLR6. Using deletion mutants of *TLR2,* it has been shown that the extracellular region Ser40-Ile64, which is located in the region of the first leucine-rich repeat (amino acids 53–76), is critical for the recognition of *S. aureus* PGN [32] and for the recognition of mycoplasmal diacylated lipoproteins [33]. Moreover, point mutants of *TLR2* have revealed that 3 leucines (L107, 112, and 115), which are located in the third leucine-rich repeat (amino acids 101–124), are necessary for PGN-induced and diacylated lipopeptide–induced NF-κB activation. Because each of these motifs is crucial for NF-κB activation, these results suggest that these pathogen-associated molecular patterns are recognized by >1 leucine-rich repeat motif of *TLR2.* Analysis of SNPs of the *TLR2* extracellular region reveals that none of the 3 nonsynonymous SNPs is situated in these important areas.

***Nonsynonymous polymorphisms of the cytoplasmic domain of* TLR2.** Six nonsynonymous SNPs of the *TLR2* gene change amino acids of the cytosolic part of this receptor, including 5 SNPs within the TIR domain (amino acids 643–784) that are critical to TLR signaling and dimerization (figure 2) [2, 34, 35]. Only 2 of them have been linked to reduced NF-κB activation and to increased risk of infection, confirming animal models that suggest that defective TLR2 signaling is a causative factor for increased susceptibility to bacterial disease.

The first nonsynonymous *TLR2* SNP consists in a C→T substitution at nucleotide 2029 from the start codon of *TLR2* predicted to replace arginine (Arg; R) with tryptophan (Trp; W) at position 677. This amino acid variation is located in close proximity to the dominant-negative Pro681His mutation of the *TLR2* gene that abolishes the binding of MyD88 with TLR2 [36]. This polymorphism, which is present in Asian and African populations, seems to be absent among white populations (authors' unpublished data). In vitro, the *TLR2* Arg677Trp polymorphism inhibits both *Mycobacterium leprae*–mediated and *M. tuberculosis*–mediated NF-κB activation and production [37, 38]. Moreover, patients carrying the *TLR2* 677W allele had lower basal and *Mycobacterium*-stimulated serum IL-12 levels, providing a potential mechanistic explanation for clinical association between this *TLR2* variant and mycobacterial disease. Because IL-12 is necessary for the activation of the IFN-γ pathway and the induction of the Th1 responses paramount to cell-mediated immunity to intramacrophagic pathogens, it is not surprising that this missense variant has been associated with lepromatous leprosy in a Korean population [39] and with susceptibility to tuberculosis in a Tunisian population [40]. The Korean study consisted of sequence analysis of the intracellular domain of *TLR2* in a group of 86 patients infected with *M. leprae.* The R677W substitution was present in 22% of the patients with lepromatous leprosy, who had large bacillary loads, but was absent in patients with tuberculosis, who had smaller bacillary loads, and in the control subjects. The Tunisian case-control study included a small number of patients with well-defined tuberculosis. The frequency of patients carrying the T allele (677Trp) was significantly higher in patients with tuberculosis than in the control group (94% vs. 31%; $P<.0001$). The inhibitory mechanism of the Arg677Trp polymorphism is not clear, but, because the mycobacterial 19-kDa lipoprotein activates TLR2 through the heterodimer TLR2/TLR1, this *TLR2* variant might affect the association of these 2 TLRs. Despite the importance of fungi such as *C. albicans* in causing human diseases, no information is available on the association of *TLR2* variants and susceptibility to or severity of candidiasis. Only one study searched an association between chronic mucocutaneous candidiasis and several polymorphisms, including the *TLR2* R677Trp, but no conclusion could be drawn because this polymorphism was not present in a small Dutch population [41].

The second functional *TLR2* variant consists of a G→A substitution at nucleotide 2257 from the start codon of *TLR2* predicted to replace arginine with glutamine (Gln; Q) at position 753. This polymorphism within a group of highly conserved amino acids at the C-terminal end of *TLR2* was identified in 3% of healthy white blood donor control subjects [42]. Transfection of the 753Q *TLR2* variant revealed decreased cellular

activation in response to lipoproteins, and this polymorphism was identified in patients with gram-positive septic shock [42]. In a recent report, Ogus et al. [43] found that the frequency of the *TLR2* 753Q allele was 4.7% in a Turkish population (1.7% homozygous carriers and 6% heterozygous patients), suggesting ethnic differences in the allelic frequency of *TLR2* 753Q. In that report, the R753Q polymorphism was associated with an increased risk of developing tuberculosis (6- and 1.6-fold for carriers of the AA and GA genotypes, respectively).

PERSPECTIVES AND CONCLUSIONS

The reasons that only some persons exposed to a pathogen develop uncontrolled disease and others eradicate or limit the disease remain unknown. Only 5%–15% of the population infected with *M. tuberculosis* will develop clinical tuberculosis during their lifetimes. Individual differences in clinical manifestations and outcome of infectious diseases in intensive care medicine often cannot be explained by known phenotypic risk factors alone, such as cirrhosis or chronic heart failure. Unequivocal evidence shows that genetic factors may be important determinants of increased susceptibility to development of infectious disease.

The sequence of the human genome is now considered to be essentially completely known. There are probably >4 million SNPs in the human genome (200,000 of which occur in coding sequences), underlying the extent of genetic variability and its potential effects on susceptibility to disease and individual responses to treatment. Recent data suggest associations between specific genotypes and the risk of severe sepsis and adverse clinical outcomes. These include pathogen detection (i.e., TLR2, TLR4, and CD14), inflammatory responses (i.e., TNF-α and IL-10), or coagulation (PAI-1 and factor V) polymorphisms, with infectious diseases such as pneumonia, meningitis, and septic shock, as well as mortality among critically injured patients.

Polymorphisms of *TLR2* might help us to understand normal and abnormal innate immune responses to a wide repertoire of pathogens. To date, associations between *TLR2* polymorphisms and leprosy, tuberculosis, and staphylococcal infection have been reported in small association studies. Both genetic and biochemical data on TLR2 will support the importance of this receptor in the innate immune defense. Future studies will identify some of the missing pieces of the puzzle regarding specificities of *TLR2* genetic polymorphisms that are just beginning to be solved. It appears necessary to screen simultaneously other candidate genes, such as those encoding receptors that interact with TLR2 (TLR1, TLR6, and CD14) and proteins of the TLR2 signaling pathway, to fully characterize the genetics of gram-positive and mycobacterial infection. For example, the presence of CD14 enhances the efficiency of recognition of many specific ligands by TLR2. The −159C→T CD14 promoter polymorphism, which increases CD14 expression, has been associated with susceptibility to *Chlamydia pneumoniae* infection and septic shock and, therefore, represents an excellent genetic risk factor to be tested [44, 45]. Similarly, the 79 and 53 variants of TLR1 and TLR6 (for more information, see http://www.innateimmunity.net) could change the efficacy of pathogen detection by TLR2. Sufficient statistical power when studying genes with relative small effects requires analysis of DNA from a large population: 300–500 case patients versus 300–500 control subjects is thought to confer enough power in association studies [46]. Such sepsis-association studies are in progress in different countries.

Elucidating the functional role of genetic factors of TLR2 is expected to enhance our understanding of the pathogenesis of gram-positive septic shock, to enrich our therapeutic arsenal with novel agents such as TLR2 antibodies or soluble TLR2, and to decrease morbidity and mortality through improved risk assessment and early administration of prophylactic therapies. For example, soluble forms of TLR2 have been shown to be present in human plasma and breast milk and to modulate cell activation [47]. Because depletion of soluble TLR2 leads to an increased inflammatory response to bacterial lipopeptide, infusion of soluble TLR2 in patients carrying functional *TLR2* polymorphisms, $TLR2_{677W}$ or $TLR2_{753Q}$, might be beneficial for the prevention or treatment of severe gram-positive infections or tuberculosis. In contrast, antagonistic TLR2 antibodies that prevent TLR2-driven septic shock syndromes might be avoided in *TLR2* SNP carriers [48]. Finally, to prevent the development of severe sepsis in such high-risk patients, vaccine strategies could be proposed to foster acquisition of a potent humoral response to pathogens that are normally detected by TLR2. Recently, it has been proven that TLR2-deficient mice, which have a profoundly impaired production of Th1 cytokines, compared with control mice, are equally capable of mounting a specific response to *C. albicans* and developing vaccine-induced resistance [26].

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