Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population

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Toll-like receptors (TLRs) play a pivotal role in an innate immunity system, which controls inflammation responses and further instructs development of adaptive immunity. We enrolled 250 Han Chinese in Taiwan screening for the single nucleotide polymorphisms (SNPs) in TLRs associated with viral infection, including TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9. The 6 SNPs not hitherto identified in Chinese populations, including TLR3 1377 C>T, TLR3 −7 C>A, TLR7 Gln11Leu, TLR7 IVS1+1817 G>T, TLR8 Met1Val, and TLR8 −129 G>C, had minor allele frequencies of 38%, 23%, 22.3%, 3%, 16.0%, and 16.0%, respectively. The frequencies of 2 common SNPs, TLR9, −1486 T>C and 2848 G>A, were 28% and 44%, respectively. As compared with other ethnic populations, Chinese displayed an opposite allele frequency of TLR8 Met1Val and TLR8 −129 G>C to Caucasians and African Americans. In addition, TLR2 Arg677Try, TLR2 Arg753Gln, TLR4 Asp299Gly, and TLR4 Thr399Ile that were apparent in approximately 10% of Caucasians were not detected in Chinese. In conclusion, obvious ethnic differences in TLR polymorphisms may in part reflect the ethnic diversity of host viral susceptibility. (Translational Research 2007;150:311–318)

Abbreviations: DC = dendritic cell; PAMP = pathogen-associated molecular pattern; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; SNP = single nucleotide polymorphism; ssRNA = single-stranded RNA; TLR = Toll-like receptor

The response to infection of microbial pathogens is variable between individuals of different ethnicities.[1–5] Evidence supports the hypothesis that the genetic makeup of the host plays an important role in the susceptibility to, and the development of, infections.[6,7] The highly specific adaptive immune system requires days to weeks to refine humoral and cell-mediated immune recognition systems to eliminate invading pathogens. In contrast, innate immune systems target structurally conserved pathogen-associated molecular patterns (PAMPs), thereby allowing immediate and sufficient responses to limit or eradicate invading microbes. Toll-like receptors (TLRs) are primary transmembrane proteins of immune cells that play a critical role in innate and adaptive immunity. Currently, 13 TLRs (TLR1–TLR13) have been identified in mammalian species, including 11 in humans.[8,9] TLRs have been assigned to play a central role in the detection of PAMPs and to mediate cellular responses particularly in mice with genetic defects affecting the TLR system.[10,11]

Viruses are highly infectious pathogens that depend on host cellular machinery for survival and replication.

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The innate immune responses of TLRs are involved in the recognition of various viral proteins and nucleic acids.[12] TLR2 and TLR4 are expressed on the cell surface and are thus capable of recognizing viral proteins on the virion. Respiratory syncytial virus and Coxsackievirus B4 induce immune responses through TLR4.[13,14] Measles virus and human cytomegalovirus activate inflammatory responses via TLR2.[15–17] However, TLRs 3, 7, 8, and 9 are the major pattern recognition receptors that recognize distinct types of virally derived nucleic acids that enter the endosome through endocytosis.[18,19] TLR3 is activated by the dsRNA analog poly-rI:rC as well as by host-cell–derived mRNA and dsRNA purified from reovirus.[20,21] Murine TLR7 and human TLR8 recognize analogs single-stranded RNA (ssRNA), which suggests that the ssRNA virus may initiate immunity via TLR7 and TLR8.[22,23] RNA derived from HIV activates NF-κB in HEK 293 cells expressing human TLR8.[24] In addition, wild-type mice but not mice lacking TLR7 respond to G/U-rich ssRNA from HIV-1, vesicular stomatitis virus, and influenza virus by activating cytokine expression, dendritic cell (DC) maturation, and B-cell proliferation.[22–24] Viruses with a DNA genome, such as herpesviruses, may potentially activate TLR9, which is activated in response to CpG DNA, to induce cytokine expression.[25] Altogether, virus infections are sensed by cells through both extracellular recognition of viral proteins and intracellular alarm systems detecting cytoplasmic nucleic acid structures in endosomes. The specificities in signal transduction pathways activated by different TLRs may at least partly explain why different viruses evoke overlapping, yet slightly, different cellular responses to infection. Ample evidence exists that TLRs have an important role in viral PAMPs stimulation and host defense against vial infection.[26]

Because of the role of TLRs at the interface between host and environment and furthermore as key molecules for both innate and adaptive immunity, genetic variations within these genes could have a major impact on host defense or inflammatory disease pathogenesis.[27–29] It has been hypothesized that loss of function of particular TLRs causes the host to become more susceptible to infections. A growing body of evidence concerning host responses to infectious disease supports the suggestion that people with specific single nucleotide polymorphisms (SNPs) of genes encoding TLRs play an important role.[30] TLR genotypic profiling studies have begun, but as yet the number of identified functional TLR polymorphisms is limited; thus far, the data are disparate and even contradictory. Most of these studies have been deliberately ethnically matched to avoid genetic diversity. Gene variants of the potential antiviral TLRs (TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9) certainly have relevance in terms of susceptibility to epidemical viral infection diseases, but only a few polymorphisms have been described in these TLRs in the Chinese population. The aim of this study is to test host ethnicity difference in SNPs of TLRs associated with viral infection. It could be useful for future genomic studies of viral infection in the Chinese population.

Table I. Characteristics of subjects enrolled in this study

Parameter	Male n = 133	Female n = 117	Normal range
Age, years (SD)	55.11 (12.55)	51.87 (12.93)	
Current smoker	42%	0%	
Alcohol (>5 drinks/week)	0	0	
Glucose, mg/dL (SD)	105.6 (32.29)	99.95 (18.7)	75–115
AST, IU/L (SD)	53.25 (19.53)	33.73 (6.65)	0–42
ALT, IU/L (SD)	32.5 (27.7)	26.54 (11.04)	0–42
WBC, N /mL (SD)	6665 (1943)	6376 (1789)	4000–10,000
CRP, mg/L	<5	<5	<5

Abbreviation: SD, standard deviation.

MATERIALS AND METHODS

Subjects. Blood samples were collected from 250 subjects (133 men of mean age 55.11 ± 12.55 years, and 117 women of mean age 51.93 ± 12.93 years) attending health examination in National Cheng Kung University Hospital, Tainan, Taiwan. A complete medical history and physical examination was carried out. Alcohol consumption, smoking habits, and physical activity were also queried. Subjects who had a hereditary genetic disorder or severe immune disease were excluded from the database. The basic characteristics of these subjects are summarized in Table I. All patients provided written informed consent, and the study was reviewed and approved by the National Cheng Kung University Hospital ethics committee.

TLRs genotyping. Genomic DNA was extracted from the buffy coat using the Blood & Tissue Genomic DNA Extraction Miniprep kit (Viogene; Hsichih, Taiwan) following manufacturer's instructions. Most TLR genotypes, except that of TLR2 SNPs, were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods (Tables II and III). TLR gene fragments were PCR-amplified using primers designed according to genomic reference sequences (accession number AC106865 for *TLR2*, AC104070 for *TLR3*, NC000009 for *TLR4*, AC005859 for *TLR7*, *TLR8*, and *TLR9*) and OLIGO6 software (Molecular Biology Insights, Cascade, Colo). The sequences of designed primer pairs are listed in Tables II and III. Amplification conditions were initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 45 s, 55°C (slightly vary with different primer sets) for 45 s, and 72°C for 30 s, with final extension for 7 min at 72°C. PCR products were digested by