Association of Toll-like Receptor 3 Polymorphisms with Chronic Hepatitis B and Hepatitis B-Related Acute-on-Chronic Liver Failure

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Abstract—Hepatitis B virus (HBV) infection is one of the major causes of chronic liver inflammation. Toll-like receptor 3 (TLR3) plays a key role in innate immunity and is responsible for recognizing viral pathogens. It has been reported that the *TLR3* C1234T polymorphism is associated with various diseases. The aim of this study was to investigate whether *TLR3* polymorphisms were correlated with susceptibility to chronic HBV infection. Two polymorphisms in the *TLR3* gene, A952T and C1234T, were tested by direct sequencing in 452 chronic hepatitis B (CHB) patients and 462 healthy controls. Data showed that subjects carrying 1234CT genotype and TT genotype had 1.42-fold and 2.31-fold increased risk of chronic HBV infection compared to those with CC genotype (95 % confidence interval [CI]=1.08–1.86, p=0.012; 95 % CI=1.34–3.96, p=0.002, respectively). Further analysis revealed that the prevalence of 1234CT genotype and T allele was significantly increased in CHB patients with acute-on-chronic liver failure (ACLF) than those without ACLF (odds ratio [OR]=1.55, p=0.030; OR=1.43, p=0.040, respectively). These results indicate that *TLR3* C1234T polymorphism could be a risk factor for the development of chronic HBV infection, especially the CHB-related ACLF.

KEY WORDS: toll-like receptor 3; polymorphism; chronic hepatitis B; acute-on-chronic liver failure.

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major causes of chronic liver inflammation [1]. It is estimated that more than two billion people have been chronically infected by HBV worldwide, and nearly 350 million have become victims of this disease. HBV infection leads to a wide spectrum of outcomes [1]. Although most of the infected individuals can successfully clarify this virus, there are still about 10 % of patients who would develop chronic hepatitis B (CHB) [2]. Among those CHB individuals, some may progress rapidly towards liver failure, a condition referred to as acute-on-chronic liver failure (ACLF) [3]. The outcome of patients with chronic HBV infection is closely related to their immune responses [2].

Toll-like receptors (TLRs) are evolutionary conserved receptors that recognize pathogen-associated molecular patterns and mediate the innate immune responses against invading pathogens [4]. TLRs expressed on immune cells are involved in the uptake and processing of various exogenous and endogenous antigens, such as lipopolysaccharide, viral double-stranded RNA, and unmethylated CpG islands [5]. Upon binding to specific ligands, TLRs promote the maturation of dendritic cells (DCs) and induce production of inflammatory cytokines and activation of adaptive

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immunity [6–8]. As one of the TLRs' family members, TLR3 recognizes poly(I:C), a synthetic double-stranded RNA analog, as well as viral double-stranded RNA [5], which is presumably formed during viral infection. TLR3 binding to cognate ligands modulates downstream cytokine and chemokine production through the activation of the transcription factor NF-κB, which translocates to the nucleus to modulate gene expression [5, 6]. A role for TLR3 in viral infection has been suggested based on the demonstration that TLR3 knock-out mice were unable to mount a full response to cytomegalovirus infection [7], perhaps by contributing to cytotoxic T cell response after the initial infection [8]. TLR3 is localized primarily in intracellular vesicles, although some cell surface expression is observed in human embryonic kidney cells [9]. Recent study has shown that TLR3 signaling may inhibit HBV replication in an animal model [10]. The expression of TLR3 on peripheral DCs in CHB patients is significantly lower than in healthy individuals [11]. These researches indicate that TLR3 is closely correlated with HBV infection.

Previous studies have described a human single-nucleotide polymorphism (SNP) in *TLR3*, C1234T, which causes an amino acid exchange (Leu to Phe) at position 412 [12].

This SNP has been reported to be associated with different diseases such as HIV infection, prostate cancer, non-small cell lung cancer, *etc*. [13–15]. However, there is no paper reporting TRL3 SNPs and HBV infection. In the current study, we have investigated two *TLR3* polymorphisms, A952T and C1234T, and have analyzed their associations with CHB in the Chinese population.

MATERIALS AND METHODS

Study Subjects

This study consisted of 452 CHB cases and 462 controls. All subjects were unrelated Han Chinese and recruited from two hospitals, Beijing 302 Hospital and Weihai 404 Hospital between November 2009 and August 2011. The diagnosis of CHB was made based on HBsAg seropositive, anti-HBs seronegative, and continuously abnormal alanine aminotransferase (ALT) and asparate aminotransferase for more than 6 months. The diagnosis of hepatitis B-related ACLF was made based on the criteria of a history of chronic hepatitis B with serum total bilirubin >10 mg/dL, prothrombin

activity <40 %, and recent development of complications. The control population was recruited from people who came for physical examinations in these two hospitals. All the control subjects did not have HBV history. None of them received HBV vaccination by the time of enrollment. People who are relatives or positive for HBsAg were excluded from this study. All the control subjects were matched with patient population in terms of age, sex, and residence area (urban or rural). Written informed consent was obtained from each participant. This study was approved by the institutional review boards of Beijing 302 Hospital and Weihai 404 Hospital.

Polymorphism Detection

Genomic DNA was extracted from peripheral blood lymphocytes using a commercially available kit according to the manufacturer's instructions (Blood Genomic DNA Miniprep Kit, Axygen Biosciences). The PCR fragment carrying A952T SNP was amplified using the following primer pair: 5′-CGCAATTCCAAGATTATTTCCG-3′ and 5′-GGTACCTCACATTGAAAAGCC-3′. Direct sequencing to detect the A952T SNP was conducted using primer 5′-CTTACAGAGAAGCTATGTTTGG-3′. Similarly, the PCR fragment carrying C1234T polymorphism was amplified using the following primer pair: 5′-TGGGATCTCGTCAAAGCCGTTG-3′ and 5′-AAG TATTTCCCTTGCCTCACTCC-3′. Direct sequencing to detect the C1234T SNP was conducted using primer 5′-TTGCTTAGATCCAGAATGG-3′.

Statistical Analysis

The SPSS statistical software package ver.13.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Demographic data between the study groups were compared by the chi-square test and by the Student's *t* test. The polymorphisms were tested for deviation from Hardy–Weinberg equilibrium by comparing the observed and expected genotype frequencies using the chi-square test. For SNP analysis, genotype and allele frequencies were compared between groups using the chi-square test, and odds ratios (OR) and 95 % confidence intervals (CIs) were calculated using unconditional logistic regression. Haplotypes were reconstructed from genotype data and were statistically analyzed by the SHEsis program (http://analysis.bio-x.cn/myAnalysis.php). *p* values <0.05 were considered significant.

RESULTS

Characteristics of the Patients and Controls

Table 1 shows the general characteristics of CHB patients and controls. There was no significant difference in regard to age and sex between cases and controls. All the patients were detected HBsAg and anti-HBc IgG positive, whereas the controls were negative for these two items. The CHB patients could be divided into subgroups, without ACLF (251 cases) and with ACLF (201 cases), based on the clinical diagnosis. We then compared basic clinical parameters between these two subsets. As expected, ALT and total bilirubin were significantly higher in ACLF patients ($p<0.001$) (Table 1).

TLR3 Polymorphisms in CHB and Controls

The genotype and allele frequencies of the *TLR3* A952T and C1234T polymorphisms in CHB cases and controls are summarized in Table 2. The genotype distributions of these two polymorphisms among the controls were in agreement with the Hardy–Weinberg equilibrium ($p>$

0.05). The frequency of 953A allele was relatively low in both CHB cases (2.7 %) and controls (2.3 %). As for the C1234T polymorphism, the prevalence of C and T alleles was 67.3 and 32.7 % in patients and 74.6 and 25.4 % in controls ($p=0.0006$). The 1234CT and TT genotypes showed significantly higher numbers in CHB cases compared to healthy individuals (OR=1.42, 95 % CI 1.08–1.86, $p=0.012$; OR=2.31, 95 % CI 1.34–3.96, $p=0.002$) (Table 2). Also, we analyzed the linkage disequilibrium (LD) and the possible haplotypes constructed by A952T and C1234T polymorphisms. Data showed that there was no significant LD between these two SNPs ($D'<0.008$). Four haplotypes were detected, and prevalence of AT haplotype was significantly increased in CHB cases than in controls ($p=0.0005$) (Table 2). These data suggest that *TLR3* C1234T polymorphism is associated with an increased susceptibility to CHB in the Chinese population.

Table 1. Clinical characteristics of the study population

Characteristics	CHB patients (N=452) (%)	Controls (N=462) (%)	p value
Age (years)	38.8±11.5	39.1±10.2	>0.05
Sex			>0.05
Male	340 (75.2)	344 (74.5)	
Female	112 (24.8)	118 (25.5)	
HBsAg (+)	452 (100)	0 (0)	
Anti-HBs IgG (+)	0 (0)	462 (100)	
HBeAg (+)	202 (44.7)	0 (0)	
Anti-HBc IgG	452 (100)	0 (0)	
ALT (U/L)	356±371	–	
TBil (mg/dL)	11.3±13.6	–	
Clinical diagnosis			
Without ACLF	251 (55.5)	–	
With ACLF	201 (44.5)	–	
Characteristics (patients)	With ACLF, N=201 (%)	Without ACLF, N=251 (%)	p value
Age (years)	38.2±10.9	39.2±10.5	>0.05
Sex			>0.05
Male	157 (78.1)	183 (72.9)	
Female	44 (21.9)	68 (27.1)	
HBsAg (+)	201 (100)	251 (100)	
Anti-HBs IgG (+)	0 (0)	0 (0)	
HBeAg (+)	60 (29.9)	142 (56.5)	<0.001
Anti-HBc IgG	201 (100)	251 (100)	
ALT (U/L)	511±513	208±285	<0.001
TBil (mg/dL)	22.8±13.9	2.1±3.7	<0.001

CHB chronic hepatitis B, *ACLF* acute-on-chronic liver failure, *ALT* alanine aminotransferase, *TBil* total bilirubin

Table 2. The genotype and allele frequencies of TLR3 SNPs between CHB patients and controls

Polymorphisms	CHB cases (N=452) (%)	Controls (N=462) (%)	OR (95 % CI)	p value
TLR3 A952T				
Genotype				
AA	433 (95.8)	445 (96.3)	Ref.	
AT	14 (3.1)	13 (2.8)	1.11 (0.51–2.38)	0.795
TT	5 (1.1)	4 (0.9)	1.29 (0.34–4.82)	0.710
Allele				
A	880 (97.3)	903 (97.7)	Ref.	
T	24 (2.7)	21 (2.3)	1.17 (0.65–2.12)	0.598
TLR3 C1234T				
Genotype				
CC	198 (43.8)	250 (54.1)	Ref.	
CT	212 (46.9)	189 (40.9)	1.42 (1.08–1.86)	0.012*
TT	42 (9.3)	23 (5.0)	2.31 (1.34–3.96)	0.002*
Allele				
C	608 (67.3)	689 (74.6)	Ref.	
T	296 (32.7)	235 (25.4)	1.43 (1.17–1.75)	0.0006*
Haplotypes (952, 1,234)				
AC	590 (65.3)	673 (72.8)	Ref.	
AT	290 (32.1)	230 (24.9)	1.44 (1.17–1.77)	0.0005*
TC	18 (2.0)	16 (1.7)	1.28 (0.65–2.54)	0.473
TT	6 (0.6)	5 (0.6)	1.37 (0.42–4.51)	0.604

CHB chronic hepatitis B
*$p<0.05$

TLR3 Polymorphisms in CHB Cases with ACLF

Since CHB may progress towards ACLF, which has a rapid disease progression and a high incidence of short- or medium-term mortality of 50–90 %, we therefore investigated whether the *TLR3* polymorphisms were correlated with ACLF. The patient group was divided into two subsets based on with or without ACLF (Table 3). The *TLR3* A952T SNP did not reveal any difference between these two subsets. However, the 1234CT genotype and 1234T allele showed increased prevalence in patients with ACLF than those without ACLF (OR=1.55, 95 % CI 1.04–2.29, $p=0.030$; OR=1.43, 95 % CI 1.17–1.75, $p=0.040$, respectively) (Table 3). These results indicate that *TLR3* C1234T SNP is further correlated with an increased risk of CHB patients with ACLF.

Stratification Analysis of TLR3 Polymorphisms in CHB Patients with ACLF

In order to understand whether *TLR3* polymorphisms were correlated with certain characteristics in patients, we performed stratification analysis in CHB patients with ACLF (Table 4). *TLR3* SNPs were compared in ACLF cases based on different age (≥ 40 or <40), sex (male or female), and HBeAg status (positive or negative). No significant difference was observed between patients with different parameters ($p>0.05$) (Table 4). These data

Table 3. Comparison of TLR3 SNPs between CHB-related ACLF and CHB without ACLF

Polymorphisms	With ACLF (N=201) (%)	Without ACLF (N=251) (%)	OR (95 % CI)	p value
TLR3 A952T				
Genotype				
AA	190 (94.5)	243 (96.8)	Ref.	
AT	8 (4.0)	6 (2.4)	1.71 (0.58–5.00)	0.326
TT	3 (1.5)	2 (0.8)	1.92 (0.32–11.60)	0.470
Allele				
A	388 (96.5)	492 (98.0)	Ref.	
T	14 (3.5)	10 (2.0)	1.78 (0.78–4.04)	0.166
TLR3 C1234T				
Genotype				
C	76 (37.8)	122 (48.6)	Ref.	
CT	104 (51.7)	108 (43.0)	1.55 (1.04–2.29)	0.030*
TT	21 (10.5)	21 (8.4)	1.61 (0.82–3.14)	0.164
Allele				
C	256 (63.7)	352 (70.1)	Ref.	
T	146 (36.3)	150 (29.9)	1.43 (1.17–1.75)	0.040*

ACLF acute-on-chronic liver failure

*$p<0.05$

Table 4. Stratification analysis of TLR3 SNPs in ACLF patients

Polymorphisms	Age ≥40 (N=92) (%)	Age <40 (N=109) (%)	OR (95 % CI)	p value
TLR3 A952T				
Genotype				
AA	86 (93.5)	104 (95.4)	Ref.	
AT	4 (4.3)	4 (3.7)	1.21 (0.29–4.98)	0.792
TT	2 (2.2)	1 (0.9)	2.42 (0.22–27.14)	0.460
Allele				
A	176 (95.7)	212 (97.2)	Ref.	
T	8 (4.3)	6 (2.8)	1.61 (0.55–4.72)	0.385
TLR3 C1234T				
Genotype				
CC	35 (38.0)	41 (37.6)	Ref.	
CT	48 (52.2)	56 (51.4)	1.00 (0.55–1.82)	0.989
TT	9 (9.8)	12 (11.0)	0.88 (0.33–2.33)	0.795
Allele				
C	118 (64.1)	138 (66.3)	Ref.	
T	66 (35.9)	80 (36.7)	0.96 (0.64–1.45)	0.864
Polymorphisms	Male (N=157) (%)	Female (N=44) (%)	OR (95 % CI)	p value
TLR3 A952T				
Genotype				
AA	150 (95.5)	40 (90.9)	Ref.	
AT	5 (3.2)	3 (6.8)	0.44 (0.10–1.94)	0.269
TT	2 (1.3)	1 (2.3)	0.53 (0.05–6.04)	0.606
Allele				
A	305 (97.1)	83 (94.3)	Ref.	
T	9 (2.9)	5 (5.7)	0.49 (0.16–1.50)	0.203
TLR3 C1234T				
Genotype				
CC	58 (37.8)	18 (48.6)	Ref.	
CT	81 (51.7)	23 (43.0)	1.09 (0.54–2.21)	0.804
TT	18 (10.5)	3 (8.4)	1.86 (0.49–7.06)	0.355
Allele				
C	197 (63.7)	59 (67.0)	Ref.	
T	117 (36.3)	29 (33.0)	1.21 (0.73–1.99)	0.458
Polymorphisms	HBeAg (+) (N=60) (%)	HBeAg (−) (N=141) (%)	OR (95 % CI)	p value
TLR3 A952T				
Genotype				
AA	57 (95.0)	133 (94.3)	Ref.	
AT	2 (3.3)	6 (4.3)	0.78 (0.15–3.97)	0.762
TT	1 (1.7)	2 (1.4)	1.17 (0.10–13.13)	0.901
Allele				
A	116 (96.7)	272 (96.5)	Ref.	
T	4 (3.3)	10 (3.5)	0.94 (0.29–3.05)	0.915
TLR3 C1234T				
Genotype				
CC	22 (36.7)	54 (38.3)	Ref.	
CT	30 (50.0)	74 (52.5)	0.99 (0.52–1.91)	0.988
TT	8 (13.3)	13 (9.2)	1.51 (0.55–4.15)	0.422
Allele				
C	74 (63.7)	182 (64.5)	Ref.	
T	46 (36.3)	100 (35.5)	1.13 (0.73–1.76)	0.584

demonstrate that A952T and C1234T SNPs may not be associated with age, sex, or HBeAg in CHB patients with ACLF.

DISCUSSION

In this case–control study, we investigated the role of the *TLR3* A952T and C1234T SNPs in susceptibility to CHB in the Chinese population and identified that C1234T was a risk factor for this disease. Further analysis revealed that the *TLR3* C1234T polymorphism was associated with increased risk of ACLF in CHB patients. To our knowledge, this is the first study showing the correlation between *TLR3* SNPs and CHB, especially CHB-related ACLF. Our results indicate that *TLR3* C1234T polymorphism may act as a potential maker for the prognosis CHB.

TLR3 is able to recognize dsRNA, which may be the virus genome or replication intermediate, and plays a key role in host antiviral defenses [5]. Several studies have demonstrated the pivotal position of TLR3 in host responses against a number of viruses (*e.g.*, HIV, West Nile virus, and respiratory syncytial virus) [16–18]. Also, TLR3 has been reported to participate in HCV-induced cellular activation and cytokine production [19]. As for HBV infection, recent research has indicated that TLR3 ligand inhibits HBV replication in the liver of HBV transgenic mice [20]. The expression of TLR3 and INF-beta in DCs is significantly lower in ACLF than in healthy individuals [11]. The protein level of TLR3 in PBMC is dramatically decreased in CHB and ACLF patients [21]. Our results have revealed that a functional *TLR3* SNP is associated with increased risk of CHB and ACLF. All these studies indicate that TLR3 may play critical roles against HBV infection and may influence the progression of this disease.

SNPs could potentially be associated with a better or worse clinical outcome for patients with infectious disease. To date, there have been more than 136 SNPs described within the human *TLR3* gene; however, only four were predicted to cause changes in the TLR3 protein molecule [22]. Two of these four *TLR3* SNPs (A952T [rs5743316] and C1234T [rs3775291]) have been shown to affect TLR3 function [22]. The *TLR3* A952T and C1234T SNPs are considered nonsynonymous because the nucleotide changes result in amino acid substitutions from asparagine to isoleucine (at position 284) and leucine to phenylalanine (at position 412), respectively [22]. *In vitro* experiments have demonstrated a complete functional impairment in TLR3 signaling in the presence of A952T SNP (N284I),

whereas a markedly diminished function is observed in the presence of the C1234T SNP (L412F) [22]. Hence, these *in vitro* findings would support their potential role in the clinical setting. The C1234T polymorphism has been identified in different populations and the T allele is about 25 % in the Chinese population. Studies have demonstrated that the C1234T SNP is associated with different diseases such as HIV infection, prostate cancer, non-small cell lung cancer, *etc.* [13–15]. Our data showed that 1234CT and TT genotypes were correlated with increased susceptibility to CHB, whereas the 1234CT genotype revealed association with higher risk of ACLF (Tables 2 and 3). The A952T is a relatively rare SNP. The 952T allele is only about 2 % in the Chinese population. We did not find any significant association between this polymorphism and CHB or CALF (Tables 2 and 3). However, due to the low frequency of 952T, it is important to conduct similar analysis in a larger study population.

In summary, our study demonstrated for the first time that the *TLR3* C1234T SNP was associated with elevated susceptibility to CHB, especially hepatitis B-related ACLF in the Chinese population. These results may provide support for using TLR3 as a therapeutic target and a prognostic marker in the future.

Conflict of Interest. No competing financial interests exist.

REFERENCES

1. Tan, Y.J. 2011. Hepatitis B virus infection and the risk of hepatocellular carcinoma. *World Journal of Gastroenterology* 17: 4853–4857.
2. Gish, R., J.D. Jia, S. Locarnini, and F. Zoulim. 2012. Selection of chronic hepatitis B therapy with high barrier to resistance. *The Lancet Infectious Diseases* 12: 341–353.
3. Olson, J.C., and P.S. Kamath. 2012. Acute-on-chronic liver failure: What are the implications? *Current Gastroenterology Reports* 14: 63–66.
4. Lee, C.C., A.M. Avalos, and H.L. Ploegh. 2012. Accessory molecules for Toll-like receptors and their function. *Nature Reviews Immunology* 12: 168–179.
5. Horscroft, N.J., D.C. Pryde, and H. Bright. 2012. Antiviral applications of Toll-like receptor agonists. *The Journal of Antimicrobial Chemotherapy* 67: 789–7801.

6. Hornung, V., S. Rothenfusser, S. Britsch, A. Krug, B. Jahrsdorfer, T. Giese, *et al*. 2002. Quantitative expression of Toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *Journal of Immunology* 168: 4531–4537.

7. Schwarz, K., T. Storni, V. Manolova, A. Didierlaurent, J.C. Sirard, P. Rothlisberger, *et al*. 2003. Role of Toll-like receptors in costimulating cytotoxic T cell responses. *European Journal of Immunology* 33: 1465–1470.

8. Kaisho, T., and S. Akira. 2003. Regulation of dendritic cell function through Toll-like receptors. *Current Molecular Medicine* 3: 373–385.

9. Bsibsi, M., R. Ravid, D. Gveric, and J.M. van Noort. 2002. Broad expression of Toll-like receptors in the human central nervous system. *Journal of Neuropathology and Experimental Neurology* 61: 1013–1021.

10. Zhou, Y., X. Wang, M. Liu, Q. Hu, L. Song, L. Ye, D. Zhou, and W. Ho. 2010. A critical function of toll-like receptor-3 in the induction of anti-human immunodeficiency virus activities in macrophages. *Immunology* 131: 40–49.

11. Li, N., Q. Li, Z. Qian, Y. Zhang, M. Chen, and G. Shi. 2009. Impaired TLR3/IFN-beta signaling in monocyte-derived dendritic cells from patients with acute-on-chronic hepatitis B liver failure: Relevance to the severity of liver damage. *Biochemical and Biophysical Research Communications* 390: 630–635.

12. Ueta, M., C. Sotozono, T. Inatomi, K. Kojima, K. Tashiro, *et al*. 2007. Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens–Johnson syndrome. *The British Journal of Ophthalmology* 91: 962–965.

13. Sironi, M., M. Biasin, R. Cagliani, D. Forni, M. De Luca, I. Saulle, *et al*. 2012. A common polymorphism in TLR3 confers natural resistance to HIV-1 infection. *Journal of Immunology* 188: 818–823.

14. Mandal, R.K., G.P. George, and R.D. Mittal. 2012. Association of Toll-like receptor (TLR) 2, 3 and 9 genes polymorphism with prostate cancer risk in North Indian population. *Molecular Biology Reports* 39: 7263–7269.

15. Dai, J., Z. Hu, J. Dong, L. Xu, S. Pan, Y. Jiang, *et al*. 2012. Host immune gene polymorphisms were associated with the prognosis of non-small-cell lung cancer in Chinese. *International Journal of Cancer* 130: 671–676.

16. Breckpot, K., D. Escors, F. Arce, L. Lopes, K. Karwacz, *et al*. 2010. HIV-1 lentiviral vector immunogenicity is mediated by Toll-like receptor 3 (TLR3) and TLR7. *Journal of Virology* 84: 5627–5636.

17. Daffis, S., M.A. Samuel, M.S. Suthar, M. Gale Jr., and M.S. Diamond. 2008. Toll-like receptor 3 has a protective role against West Nile virus infection. *Journal of Virology* 82: 10349–10358.

18. Klein Klouwenberg, P., L. Tan, W. Werkman, G.M. van Bleek, and F. Coenjaerts. 2009. The role of Toll-like receptors in regulating the immune response against respiratory syncytial virus. *Critical Reviews in Immunology* 29: 531–550.

19. Li, K., N.L. Li, D. Wei, S.R. Pfeffer, M. Fan, and L.M. Pfeffer. 2012. Activation of chemokine and inflammatory cytokine response in hepatitis C virus-infected hepatocytes depends on Toll-like receptor 3 sensing of hepatitis C virus double-stranded RNA intermediates. *Hepatology* 55: 666–6675.

20. Wu, J., M. Lu, Z. Meng, M. Trippler, R. Broering, A. Szczeponek, *et al*. 2007. Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. *Hepatology* 46: 1769–1778.

21. Wang, K., H. Liu, Y. He, T. Chen, Y. Yang, *et al*. 2010. Correlation of TLR1-10 expression in peripheral blood mononuclear cells with chronic hepatitis B and chronic hepatitis B-related liver failure. *Human Immunology* 71: 950–956.

22. Ranjith-Kumar, C.T., W. Miller, J. Sun, J. Xiong, J. Santos, I. Yarbrough, *et al*. 2007. Effects of single nucleotide polymorphisms on Toll-like receptor 3 activity and expression in cultured cells. *The Journal of Biological Chemistry* 282: 17696–17705.