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Correlation of AIM2 expression in peripheral blood mononuclear cells from humans with acute and chronic hepatitis B

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ABSTRACT

The AIM2 (absent in melanoma 2) protein promotes host defenses against invading viruses and pathogenic bacteria through corresponding adapter molecules leading to the initiation of innate immune responses. We investigated the expression of AIM2 in peripheral blood mononuclear cells (PBMCs) from patients with acute hepatitis B (AHB) and chronic hepatitis B (CHB) during different clinical phases, and analyzed the correlation between AIM2 and clinical profiles in these groups. This study indicated that there is higher expression of AIM2, IL-1β, and IL-18 in AHB compared with expression in CHB. The expression of AIM2 mRNA was significantly negatively correlated with serum hepatitis B virus (HBV) load, HBeAg, and significantly positively correlated with IL-1β and IL-18 in AHB patients and CHB patients with immune clearance, which suggests that AIM2 expression is correlated with the immune clearance of HBV in the host. We summarized that there is a higher immune status in AHB, and a lower immune response in CHB. This suggests that the down-regulation of AIM2 may be associated with the chronic development of HB.

1. Introduction

Hepatitis B virus is a non-cytopathic human Hepadnavirus that causes acute and chronic hepatitis and hepatocellular carcinoma [1]. The virus contains a circular and partially double-stranded DNA (dsDNA) genome of approximately 3.2 kb that consists of four overlapping open reading frames, the C, S, P, and X regions. The intact HBV particle is referred to as a Dane particle of approximately 42 nm in diameter. HBV is known as the minimal DNA virus that is presently harmful to humans.

The natural history of chronic HBV infection is generally divided into four chronological stages: immune tolerance, immune clearance, inactive-carrier state, and reactivation [2,3]. During the immune tolerance stage, serum HBV DNA levels are high and hepatitis B e antigen (HBeAg) is present, but alanine aminotransferase (ALT) levels remain normal. In the immune clearance stage, most carriers eventually seroconvert from HBeAg in the blood to the appearance of anti-HBe antibodies and subsequent clearance of HBeAg. After HBeAg seroconversion, patients enter the integration or residual stage, which is referred to as the inactive-carrier state, during which serum HBV DNA levels decrease and ALT becomes normal. However, HBV DNA and ALT in some patients increases again or remains high, which is referred to as reactivation or HBeAg-negative chronic hepatitis B.

The outcome of patients with chronic hepatitis B virus (HBV) infection is closely related to their individual immune responses to infection. The patient immune status plays a crucial role in host–virus interactions and greatly influences viral replication and the clinical outcome of HBV infection [4]. Most studies of the immunologic aspects of persistent HBV infection focus on adaptive immunity. A vigorous and sustained polyclonal cytotoxic T lymphocyte (CTL) response to HBV was observed in patients with self-limited acute hepatitis [5]. However, the immune response was significantly reduced or undetectable in patients with persistent HBV infection [5], indicating that adaptive immune response plays an important role in the pathogenesis of HBV infection. Despite these studies, little is known about the role of innate immunity in resistance to HBV infection.

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; BUS, B-mode ultrasonography; HBV, hepatitis B virus; HCV, hepatitis C virus; HBV-M, HBV serum markers; AIM2, absent in melanoma 2; PBMC, peripheral blood mononuclear cells; HB, hepatitis B; CHB, chronic hepatitis B; AHB, acute hepatitis B; dsDNA, double-stranded DNA; CTL, cytotoxic T lymphocyte; FQ-PCR, real-time fluorescence quantitative polymerase chain reaction; TRFIA, time-resolved fluoroimmunoassay; pro-IL1β, pro-interleukin-1β.

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A key innate immune response to infection with microbial or viral pathogens and tissue damage is the rapid activation of multiprotein complexes called inflammasomes [6]. Hornung et al. [7] showed that cytoplasmic DNA triggers the formation of the AIM2 inflammasome by inducing AIM2 oligomerization. The inflammasomes activate caspase-1, a cysteine protease that processes the inactive pro-interleukin-1β (pro-IL1β) and pro-IL18 to their respective active proinflammatory cytokines, IL-1β, and IL-18. AIM2 belongs to a family of HIN200 proteins that include at least four members in humans, IFI16, MNDA, IFIX, and AIM2. HIN200 protein family members are interferon (IFN)-inducible proteins with a 200-amino-acid repeat at the C-terminus, which is known as the HIN domain, and an N-terminal pyrin domain (PYD). Several studies have recently identified AIM2 as a cytosolic sensor that binds dsDNA through its C-terminal HIN domain [7–12]. The AIM2 N-terminal PYD motif can recruit apoptosis-associated speck-like protein containing aCARD (ASC) and thus, caspase-1. Cytosolic dsDNA from both viruses and bacteria can function as a ligand to activate the AIM2 inflammasome [7,13]. These observations have recently been confirmed using AIM2-deficient mice [10,14]. Macrophages from AIM2-deficient mice were defective for caspase-1 activation and the induction of pyroptosis in response to liposome-delivered cytosolic DNA. Furthermore, AIM2 was also required for the activation of caspase-1 in response to infection with Vaccinia virus or Mouse cytomegalovirus (MCMV) in cell culture [10,14].

Intriguingly, some studies have indicated that AIM2 is unable to recognize certain DNA viruses or that some viruses have evolved the ability to inhibit AIM2 signaling that may involve blocking AIM2-mediated recognition of viral genomic DNA [10]. Peripheral blood mononuclear cells (PBMCs) are a very important part of the peripheral immune system and have a vital function in invading exogenous microorganisms [15–17].

Table 1
Diagnostic criteria used for determining the phases of chronic HBV infection.

Phase	HBVDNA (**log10** copies/mL)	HBeAg (DRU/mL)	ALT (U/L)	B-mode ultrasonography
Immune tolerance	>5	>0.5	<40	Normal or abnormal
Immune clearance	>3	>0.03	>40	Normal or abnormal
Inactive-carrier state	<3	<0.03	<40	Normal or abnormal
Reactivation	>3	<0.03	>40/<40	Normal or abnormal

Our laboratory has investigated whether AIM2 is involved in hepatitis B (HB), and whether HBV and HBV DNA are also recognized by AIM2 in CHB and AHB. To our knowledge, the current study was the first clinical study in which patients with both AHB and CHB were used to investigate AIM2. The possible association between the AIM2 expression and various clinical parameters was also analyzed to investigate the dynamic role of the innate immune responses in HB.

2. Subjects and methods

2.1. Study subjects

From August 2010 to October 2011, 80 patients with acute hepatitis B ($n = 20$) and chronic hepatitis B ($n = 60$) with a disease history of >12 months, were randomly selected from outpatients at the Department of Infectious Diseases of the Affiliated Hospital of Yan'an University, China. CHB patients ($n = 60$) were divided into three groups in different clinical phases, namely, immune tolerance ($n = 20$), immune clearance ($n = 20$), inactive-carrier state ($n = 20$). In addition, 20 healthy individuals were recruited as normal controls (NC). The characteristics of patients and NC are listed in Table 2. Signed informed consent was provided by every patient, and the study protocol was approved by Human Subject Research Committee, Medical College of Xi'an Jiaotong University and Human Subject Research Committee, The Affiliated Hospital of Yan'an University.

The diagnosis of patients with CHB conformed to the guidelines of prevention and treatment for CHB. All patients were negative for antibodies to Hepatitis C virus (HCV), Hepatitis D virus (HDV) or Human immunodeficiency virus type 1 (HIV-1), Cytomegalovirus (CMV), and Epstein–Barr virus (EBV) [2,18]. Clinical samples were collected from those who had not received antiviral treatment or immunotherapy in the previous six months and all patients had not received any surgical treatment and did not display any other disease or other state of stress that may lead to up-regulation of AIM2 [19]. Blood samples from healthy donors were obtained from the Health Examination Center in the Affiliated Hospital of Yan'an University and were negative for HIV, HBV, HCV, CMV, and EBV.

2.2. Isolation of PBMCs

Venous blood (5 mL) was obtained from each subject and anticoagulated with heparin. Peripheral blood mononuclear cells (PBMCs) were isolated via density gradient centrifugation using Ficoll-Paque plus (GE Healthcare, Piscataway, NJ), according to the manufacturer's instructions. PBMCs were resuspended in RBC lysis buffer (123 mM NH_4Cl, 8 mM $KHCO_3$, and 25 μM

Table 2
Characteristics of patients with chronic hepatitis B (CHB) patients, acute hepatitis B (AHB), and normal controls (NC).

Parameter	NC	AHB	CHB		
			Immune tolerance	Immune clearance	Inactive-carrier state
Patients (n)	20	20	20	20	20
Age[a] (y)	33.2 (6.3)	37.6 (3.6)	23.7 (4.2)	31.6 (5.7)	45.6 (8.2)
Gender (M/F)	12/8	13/7	9/11	8/12	11/9
WBC[a] (10^9/L)	5.39 (2.7)	6.91 (2.5)	5.16 (2.3)	5.83 (3.2)	5.56 (2.9)
ALT[a] (IU/L)	<40	879 (609.3)	<40	188 (104.2)	<40
HBV load[a] (**log10** copies/mL)	NT	6.92 (0.94)	6.84 (0.61)	6.16 (1.04)	<3
HBeAg[a] (DRU/mL)	NT	1.16 (0.42)	1.39 (0.14)	1.25 (0.31)	<0.03
IL-1β[a] (pg/mL)	57.5 (8.02)	3195 (909.2)[c]	57.95 (10.02)	386.4 (104.1)[b]	56.89 (10.36)
IL-18[a] (pg/mL)	56.7 (6.5)	453 (66)[c]	63.5 (4.97)	230.35 (24.2) [b]	58.75 (7.11)

HBV, Hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; NT, not detected.
[a] For age, white blood cell (WBC) count, ALT, HBeAg IL-1β, AIM2 and viral load the mean (SD) for each group is shown.
[b] $P < 0.001$ *vs.* NC, Immune tolerance and Inactive- carrier state.
[c] $P < 0.001$ *vs.* NC and CHB.