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Elevated pre-treatment IL-18 level is associated with HBeAg seroconversion in HIV–HBV coinfection

Yijia Li[1,2,†], **Jing Xie**[1,3,†], **Huanling Wang**[1], **Yang Han**[1], **Nidan Wang**[1], **Chloe L Thio**[4,*,‡], and **Taisheng Li**[1,3,*,‡]

[1]Department of Infectious Diseases, Peking Union Medical College Hospital, Beijing, China

[2]Present address: University of Pittsburgh Medical Center, Pittsburgh, PA, USA

[3]Clinical Immunology Center, Chinese Academy of Medical Sciences, Beijing, China

[4]Division of Infectious Diseases, Department of Medicine, Johns Hopkins University, Baltimore, MD, USA

Abstract

Background—In hepatitis B virus (HBV) infected patients, hepatitis B e antigen (HBeAg) seroconversion is associated with better outcomes. Interleukin-18 (IL-18) controls hepatitis B replication in a mouse model. However, its role in treatment response in HIV/HBV co-infected patients is unknown.

Methods—We enrolled 35 treatment-naïve, HBeAg positive, HIV/HBV co-infected patients. HBV DNA, HIV RNA, CD4 cell count, HBV surface antigen (HBsAg) quantification (qHBsAg), HBeAg quantification (qHBeAg) and IL-18 levels were measured prior to, at 24 and 48 weeks of HBV-active combination antiretroviral therapy (cART). Multivariate Poisson regression models with robust standard errors were used to determine factors associated with HBeAg seroconversion.

Results—Twenty-one patients received tenofovir (TDF)+lamivudine (3TC) based cART while 14 patients received 3TC-based cART. After 48 weeks of treatment, 10 patients experienced HBeAg seroconversion. Compared with non-seroconverters, seroconverters had higher median HIV RNA (5.22 vs. 4.58 log copies/ml, P=0.030), lower median qHBsAg (3.97 vs. 4.76 log IU/ml, P=0.011), lower median qHBeAg (1.61 vs. 3.01 log PEIU/ml, P=0.004), and marginally higher median IL-18 (2.70 vs. 2.53 log pg/ml, P=0.068) prior to ART. In the multivariate regression, higher baseline IL-18 [adjusted relative risk (aRR) 2.99 per 1 log pg/ml increase, P=0.035], high HIV RNA (aRR 1.84 per 1 log copies/ml, P=0.029) and low qHBeAg (aRR 0.71 per 1 log PEIU/ml, P=0.029) were significantly associated with HBeAg seroconversion.

*Corresponding author: cthio@jhmi.edu.
†Contributed equally to this study
‡Contributed equally to this study

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Authors' contributions: Chloe Thio, Taisheng Li and Huanling Wang designed this study. Yijia Li, Jing Xie and Chloe Thio conducted data analysis. Yijia Li, Jing Xie, Yang Han, and Nidan Wang conducted laboratory assays. Yijia Li, Jing Xie, Chloe Thio, Huanling Wang and Taisheng Li prepared the manuscript.

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Conclusions—In HIV/HBV co-infected patients with HBeAg positivity, higher IL-18 levels, HIV RNA load, as well as low qHBeAg prior to cART were associated with HBeAg seroconversion.

Introduction

Hepatitis B virus (HBV) co-infection is common in people living with human immunodeficiency virus (HIV), and HBV-related liver disease is one of the major causes of morbidity and mortality in the era of combination antiretroviral therapy (cART)[1]. One of the favorable treatment outcomes in those with hepatitis B e antigen (HBeAg)-positive CHB is loss of HBeAg with subsequent antibody development (known as HBeAg seroconversion), since it is associated with lower rate of progression to cirrhosis and hepatic cellular carcinoma (HCC) [2]. However, this event occurs in only around 25%-40% of treated individuals [2, 3]. Understanding factors associated with HBeAg seroconversion in HIV/HBV co-infected patients is useful to improve therapies.

In a HBV-transgenic mouse model, interleukin-18 (IL-18), a cytokine associated with inflammasome activation and interferon-γ (IFN-γ) production inhibited HBV replication [4]. Moreover, IL-18 polymorphisms are associated with immune control of HBV in HBV mono-infected patients [5]. It has been suggested that inflammasome activation, marked by IL-18 and IL-1β secretion, may play a role in the liver damage and T cell activation in HBV infection, but its precise role remain uncharacterized [6]. To this end, we evaluated whether plasma IL-18 levels are associated with HBeAg seroconversion in a cohort of HIV/HBV co-infected patients.

Methods

Study participants

This study included patients from previously-established Chinese cohorts, whose inclusion and exclusion criteria have been reported [7]. In brief, these cohorts were established between 2008 and 2014, and the aim of these studies were to evaluate the efficacy of antiretroviral therapy in HIV-infected treatment-naïve adults, including HIV/HBV co-infected patients [7]. Enrollment criteria relevant to this study include: (1) HBV surface antigen (HBsAg) and HBeAg positive at pre-cAR; (2) uninfected with hepatitis C virus (HCV); (3) treatment naïve for HIV and HBV. This study was approved by the Institutional Review Board of Peking Union Medical College Hospital and is consistent with the ethical requirements of the Declaration of Helsinki. Informed consents have been obtained from all participants in this study.

Clinical and laboratory data

HIV RNA, HBV DNA, HBeAg, antibody to HBeAg (anti-HBe), quantitative HBsAg (qHBsAg), quantitative HBeAg (qHBeAg) and IL-18 were measured at each visit [0 (baseline), 24, 48 weeks] using plasma samples stored at -80°C. Measurement of all these parameters except qHBeAg and IL-18 was reported in our previous study. qHBeAg was quantified using Abbott Architect i2000 platform (Abbott Diagnostics, Abbott Park, IL, USA), with the HBeAg reference agent obtained from the Paul Ehrlich Institute (PEI,

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Langen, Germany)[8]. The reference agent with a concentration of 100 PEI Unit (PEIU)/ml was serially diluted to generate a calibration curve. IL-18 levels were determined using the human IL-18 Platinum ELISA kit (Affymetrix eBioscience, Vienna, Austria), following manufacturer's instructions.

Statistical analysis

The primary analysis focused on IL-18 and other factors associated with HBeAg seroconversion after 48 weeks of cART. Secondary analysis determined association of IL-18 with HBV DNA suppression and with qHBsAg and qHBeAg levels after 48 weeks of cART. HBeAg seroconversion (SC) was defined as loss of HBeAg and development of anti-HBe. Categorical variables between participants who experienced HBeAg seroconversion (seroconverters) and those who did not (non-seroconverters) were compared by the Chi square test or Fisher's exact test. Continuous variables were compared by the Kruskal–Wallis test. Relative risks (RR) of variables associated with HBeAg seroconversion and HBV DNA suppression (HBV DNA<20IU/ml) were determined by Poisson regression with a robust error variance [9]. Factors associated with qHBsAg and qHBeAg levels at week 48 were determined by linear regression. IL-18 levels were log transformed due to their right-skewed distribution. Factors with P<0.10 in the univariate model in addition to IL-18 levels, CD4 cell count and HIV RNA were included in the initial multivariate models. Regression with backward stepwise elimination was used for multivariate analyses. Variables with P>0.10 were eliminated in the stepwise elimination. Stata 13 (StataCorp, College Station, TX, USA) was used for all analyses. P values <0.05 were considered statistically significant.

Results

This study included 35 HIV/HBV HBeAg positive co-infected participants who received HBV-active cART where the HBV active drugs were either tenofovir disoproxil fumarate (TDF)+ lamivudine or lamivudine only. The majority were male (82.9%), aged 30-50 years, received TDF+lamivudine-based cART (60%), infected by HBV genotype B or C, and had HBV DNA >20,000 IU/ml (97.1%) prior to cART (Table 1). After 48 weeks of HBV-active cART, 10 [28.6%, 95% confidence interval (CI) 13.7%-52.5%] participants experienced HBeAg seroconversion. Compared to the non-seroconverters, HBeAg seroconverters were more likely to receive TDF+lamivudine (seroconverters vs. non-seroconverters, 90.0% vs. 48.0%, P=0.028), have higher median baseline ALT (49 vs. 36 IU/l, P=0.027), have higher baseline HIV RNA (5.22 vs. 4.58 log copies/ml, P=0.030), have lower median baseline qHBsAg levels (3.97 vs. 4.76 log IU/ml, P=0.011), and have lower median baseline qHBeAg levels (1.61 vs. 3.01 log PEIU/ml, P=0.004). There were no differences between the groups in terms of other demographic parameters including baseline median CD4 cell count (196 vs. 188 cells/μl, P=0.36) and baseline median HBV DNA levels (7.82 vs. 8.04 log IU/ml, P=0.26) (Table 1). There was a trend towards higher baseline IL-18 levels in HBeAg seroconverters (2.70 vs. 2.53 log pg/ml in seroconverters and non-seroconverters, respectively, P=0.068).

Since baseline qHBsAg and qHBeAg were significantly correlated (Spearman's rho=0.61, P<0.001), separate multivariate regression models were constructed with each of these

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variables to evaluate baseline factors associated with HBeAg seroconversion. In the multivariate model including qHBeAg, IL-18 [adjusted relative risk (aRR) 2.99 per 1 log pg/ml increase, P=0.035], HIV RNA (aRR 1.84 per 1 log copies/ml increase, P=0.029) and qHBeAg (aRR 0.71 per 1 log PEIU/ml increase, P=0.029) remained significantly associated with HBeAg seroconversion (Table 2). In the model including qHBsAg, IL-18 (aRR 3.71 per 1 log pg/ml increase, P=0.025), HIV RNA (aRR 1.79 per 1 log copies/ml increase, P=0.016) and qHBsAg (aRR 0.26 per 1 log IU/ml increase, P<0.001) were significantly associated with HBeAg seroconversion, while CD4 cell count was marginally associated (aRR 1.63 per 100 cells/μl, P=0.050) (Table 2).

After 48 weeks of cART, HBV DNA suppression occurred in 34.3% (12/35) of individuals, and was greater in the seroconverters than in the non-seroconverters (80.0% vs. 16.0%, P=0.001). In a multivariate model with qHBeAg, baseline IL-18 (aRR 1.37 per 1 log pg/ml, P=0.29) was not independently associated with HBV DNA suppression whereas TDF +lamivudine use (aRR 9.76, P=0.016) and lower baseline HBV DNA were (aRR 4.10 for HBV DNA<8 log IU/ml, P=0.005). Examining the secondary outcome of qHBeAg level after 48 weeks of cART, TDF+ lamivudine-based cART (Coefficient -0.88, P=0.019) and higher baseline ALT (Coefficient -1.08 for ALT>40 IU/l, P=0.010) were associated, while higher baseline IL-18 was only marginally associated (Coefficient -0.70 per 1 log pg/ml, P=0.094). None of these factors were significantly associated with qHBsAg level after 48 weeks of treatment (data not shown). Only one subject experienced HBsAg clearance after 48 weeks of ART, and none had HBsAg seroconversion; thus this outcome could not be examined.

Discussion

This is the first study to demonstrate that higher pre-treatment IL-18 levels are independently associated with HBeAg seroconversion in HIV/HBV co-infected participants receiving HBV-active cART. In particular, every 1 log increase in IL-18 increases the likelihood of HBeAg seroconversion nearly three-fold.

IL-18, as a pro-inflammatory cytokine upstream to IFN-γ release, is cleaved into its active form with activation of the inflammasome [10]. In HBV mono-infected patients receiving IFN-α treatment, IL-18 levels peaked before HBeAg seroconversion [11], suggesting its role in HBeAg seroconversion. In HIV/HBV co-infected patients initiating HAART, IL-18 levels correlated with ALT at the time of hepatic flare suggesting IL-18 as a mediator of the immune response [12]. Taken together with our data, greater inflammasome activation during anti-HBV therapy may be beneficial to achieving a more favorable immune response to treatment.

Further support for a role for IL-18 in HBV comes from a transgenic mouse model, when IL-18, synergized with IL-12, suppressed HBV replication through induction of IFN-γ [4]. IL-18 polymorphisms are associated with HBV spontaneous recovery in HBV mono-infected patients [5]. We did not find that IL-18 was strongly associated with HBV DNA suppression, but this is likely because the potency of TDF+lamivudine in inhibiting HBV replication was stronger than the effects of IL-18. Interestingly, HBeAg expression

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suppresses IL-18 signaling; thus, the association of lower levels of qHBeAg with HBeAg seroconversion may also be related to IL-18 [13].

Interestingly, elevated HIV RNA in our patients was independently associated with HBeAg seroconversion. One potential reason is that HIV induces inflammasome activation [14], leading to a greater pro-inflammatory state and favoring HBeAg seroconversion. Alternative explanations could be that elevated HIV RNA leads to greater activation of toll-like receptors or higher levels of other interferon-stimulated genes that may improve the anti-HBV immune response [15].

The main limitations of this study include the small sample size and relatively short follow-up duration. The small sample size precluded us from including HBV genotype in a multivariate analysis. All the patients in this study were of Chinese origin, so whether the findings can be generalized to other ethnicities needs further study. Furthermore, we did not have HBV mono-infection group to further demonstrate the role of IL-18 in HBV mono-infection. Another limitation is that hepatitis delta virus serology was not assessed in this cohort.

This is the first study to demonstrate that higher pre-treatment IL-18 is associated with HBeAg seroconversion in patients with HIV/HBV co-infection after 48 weeks of HBV cART, which suggests a role for the inflammasome in a favorable response to treatment. Further studies are warranted to determine if IL-18 is associated with HBeAg seroconversion in HBV monoinfection and mechanisms by which IL-18 leads to a favorable anti-HBV immune response to treatment.

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Table 1

Baseline characteristics

	Overall (n=35)	HBeAg seroconverters (n=10)	HBeAg non-seroconverters (n=25)	p*
Male sex [n (%)]	29 (82.9)	9 (90.0)	20 (80.0)	0.65[a]
Age [Median (IQR)]	34 (28, 48)	39 (32, 53)	33 (28, 46)	0.14
Route of transmission [n (%)]				0.12[a]
MSM	8 (22.9)	0 (0.0)	8 (32.0)	
Heterosexual	20 (57.1)	7 (70.0)	13 (52.0)	
Others/unknown	7 (20.0)	3 (30.0)	4 (16.0)	
TDF+3TC use[b] [n (%)]	21 (60.0)	9 (90.0)	12 (48.0)	**0.028**[a]
ALT, IU/l [Median (IQR)]	37 (30, 49)	49 (33, 103)	36 (24, 46)	**0.027**
ALT>40 IU/l [n (%)]	15 (42.9)	7 (70.0)	8 (32.0)	0.062[a]
CD4+ T-cell count, cells/μL [Median (IQR)]	190 (121, 278)	196 (136, 357)	188 (96, 276)	0.36
HIV RNA, log copies/ml [Median (IQR)]	4.69 (4.39, 5.32)	5.22 (4.52, 5.87)	4.58 (4.24, 4.87)	**0.030**
HBV DNA, log IU/ml [Median (IQR)]	8.04 (7.14, 8.04)	7.82 (6.97, 8.04)	8.04 (7.33, 8.04)	0.26
HBV DNA≥8 log IU/ml [n (%)]	20 (57.1)	4 (40.0)	16 (64.0)	0.27
qHBsAg, log IU/ml [Median (IQR)]	4.64 (3.94, 4.89)	3.97 (3.78, 4.05)	4.76 (4.44, 4.90)	**0.011**
qHBeAg, log PEIU/ml [Median (IQR)]	2.98 (1.66, 3.09)	1.61 (0.19, 2.73)	3.01 (2.80, 3.13)	**0.004**
HBV genotype [n, (%)]				0.88[a]
B	13 (37.1)	10 (40.0)	3 (30.0)	
C	17 (48.6)	11 (44.0)	6 (60.0)	
Unknown	5 (14.3)	4 (16.0)	1 (10.0)	
IL-18 levels, log pg/ml [Median (IQR)]	2.55 (2.24, 2.79)	2.70 (2.45, 3.05)	2.53 (2.18, 2.75)	0.068

IQR, interquartile range; MSM, men who have sex with men; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; ALT, alanine transaminase; IU, international unit; qHBsAg, quantification of hepatitis B surface antigen; qHBeAg, quantification of hepatitis B e antigen; PEIU, Paul Ehrlich Institute unit; IL-18, interleukin-18.

*comparison is between seroconverters and non-seroconverters.

[a]Fisher's exact test.

[b]the remaining patients received 3TC-based treatment.

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Table 2

Baseline factors associated with HBeAg seroconversion.

	Univariate			Multivariate Model with qHBeAg			Multivariate Model with qHBsAg		
	RR	**95% CI**	**P values**	**RR**	**95% CI**	**P values**	**RR**	**95% CI**	**P values**
Sex									
Male	1								
Female	0.54	0.08-3.58	0.52						
Age (per 10 years)	1.35	0.90-2.01	0.14						
HBV-active cART									
3TC -based	1								
TDF+3TC -based	6.00	0.83-43.49	0.076						
CD4 cell count (per 100 cells/μl)	1.30	0.85-2.00	0.22				1.63	1.00-2.67	0.050
HIV RNA (per 1 log copies/ml)	2.27	1.29-3.98	0.004	1.84	1.07-3.17	0.029	1.79	1.12-2.87	0.016
HBV DNA									
≥8 log IU/ml	1								
<8 log IU/ml	2.00	0.67-5.94	0.21						
qHBeAg (per 1 log PEIU/ml)	0.53	0.39-0.74	<0.001	0.71	0.52-0.97	0.029			
qHBsAg (per 1 log IU/ml)	0.27	0.12-0.61	0.002				0.26	0.13-0.51	<0.001
HBV genotype									
B	1								
C	1.53	0.46-5.08	0.49						
Unknown	0.87	0.11-6.69	0.89						
IL-18 (per 1 log pg/ml)	3.16	1.23-8.11	0.017	2.99	1.08-8.29	0.035	3.71	1.18-11.65	0.025
ALT>40 IU/l	3.11	0.94-10.25	0.062	2.46	0.96-6.28	0.060			

RR, relative risk; IQR, interquartile range; MSM, men who have sex with men; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; ALT, alanine transaminase; IU, international unit; qHBsAg, quantification of hepatitis B surface antigen; qHBeAg, quantification of hepatitis B e antigen; PEIU, Paul Ehrlich Institute unit; IL-18, interleukin-18.

Factors with P value<0.10 in the univariate model in addition to IL-18, CD4 cell count and HIV RNA were included in the initial multivariate models. Regression with backward stepwise elimination was built for this analysis. Variables with P>0.10 were eliminated in the stepwise elimination.