Antiviral Research 140 (2017) 18–24

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

NK cell phenotypic and functional shifts coincide with specific clinical phases in the natural history of chronic HBV infection

Rik A. de Groen [1], Jun Hou [1], Gertine W. van Oord, Zwier M.A. Groothuismink, Marieke van der Heide, Robert J. de Knegt, André Boonstra*

Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form
3 December 2016
Accepted 10 January 2017
Available online 14 January 2017

Keywords:
Hepatitis B
HBV clinical phases
Natural killer cells
NK cells
Cytotoxicity
IFN-γ

ABSTRACT

Background: Chronic HBV infection can be divided into 4 distinct clinical phases: immune tolerant, immune active, inactive carrier, and HBeAg-negative hepatitis. Using a systems biology approach, we recently identified innate immune response components, specifically NK cells as a distinctive factor of specific HBV clinical phases. To expand on this study and identify the underlying immunological mechanisms, we performed a comprehensive profiling of NK cells in chronic HBV infection.
Methods: Peripheral blood from untreated chronic HBV patients was used to analyze phenotypic markers, as well as cytokine production and cytoxicity of NK cells.
Results: The overall composition, phenotype, and cytolytic activity of the NK cells remained constant across all clinical phases, with the exception of a few specific markers (KIRs, NKp46). $CD56^{bright}$ NK cells of chronic HBV patients differed in their ability to produce IFN-γ between the clinical phases pre- and post-HBeAg seroconversion.
Conclusion: This depicts a shift in NK cell characteristics between the immune active, under heavy viral or immune pressure, and inactive carrier phases, that coincides with HBeAg seroconversion. Although these changes in NK cells do not appear to be completely responsible for differences in liver damage characteristic of specific clinical phases, they could provide a step toward understanding immune dysregulation in chronic HBV infection.

1. Introduction

Infection with the hepatitis B virus (HBV) leads to non-cytopathic infections of the hosts' hepatocytes. Control of viral replication and subsequent liver injury are believed to be the consequence of the activity of the host immune response to infection. Contrary to infections with the hepatitis C virus (HCV), chronic HBV infections are characterized by episodes with differentiating serum levels of HBV DNA, alanine transferase (ALT), a marker of liver damage, and HBV envelope antigen (HBeAg). Using these parameters, different clinical phases have been discerned to describe the dynamics of the natural history of chronic HBV infection over a period of many years, and determine the indication for antiviral treatment on the basis of rate of viral replication and ALT elevation. Chronic HBV patients have been categorized into 4 clinical phases: the HBeAg-positive immune tolerant (IT) and immune active (IA) phases, as well as the HBeAg-negative inactive carrier (IC) and hepatitis (ENEG) phases (European Association For The Study Of The Liver, 2012). The nomenclature to describe the natural course of HBV infection has led to confusion on the underlying mechanisms, as the IT phase accurately describes the situation where high levels of HBV DNA are observed without elevated ALT levels in serum, but erroneously suggests that the immune response is more tolerant to the presence of virus than in other phases. This, however, is not the case, since normal HBV-specific T cell responses are observed and no distinctive HBV-specific or global T cell activity could be identified in any of the clinical phases (Bertoletti and Hong, 2014; Park et al., 2016).

Over the last decade the importance of natural killer (NK) cells has been extensively described in chronic viral hepatitis (Rehermann, 2013). Various studies, including our own, have

* Corresponding author. Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, Wytemaweg 80, Room Na-1011, 3015 CE, Rotterdam, The Netherlands.
E-mail address: p.a.boonstra@erasmusmc.nl (A. Boonstra).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.antiviral.2017.01.007

shown that chronic HBV patients and healthy individuals have similar total numbers of $CD56^{+}CD3^{-}$ NK cells in peripheral blood (Tjwa et al., 2011, 2014), whereas the ability of NK cells from patients to produce interferon (IFN)-γ is impaired (Boni et al., 2015; Lunemann et al., 2014; Oliviero et al., 2009; Peppa et al., 2010; Tjwa et al., 2011). However, a myriad of studies have reported conflicting results, observing no difference or even higher levels of IFN-γ production by NK cells in HBV patients compared to healthy individuals (Conroy et al., 2015; Li et al., 2014; Sun et al., 2012; Zhang et al., 2011). Part of the variation in outcome of these studies may be attributed to the large variety in stimuli (e.g. interleukin (IL)-2, IL-12, IL-15, IL-18, IFNα, PMA, and ionomycin) used alone or in combination to trigger IFN-γ production by NK cells. Also, the clinical and virological characteristics of the chronic HBV patients examined are likely to influence the features of the NK cell compartment, as many of the patient cohorts in these studies are an unsegregated mixture of all clinical phases (Lunemann et al., 2014; Oliviero et al., 2009; Peppa et al., 2010; Tjwa et al., 2011), whereas others examined either HBeAg-positive or HBeAg-negative patient groups (Boni et al., 2015; Conroy et al., 2015). This may be of particular relevance, as *in vitro* studies have demonstrated that exposure of NK cells to HBeAg affected IL-18 receptor signaling, and consequently reduced the capacity to produce IFN-γ (Jegaskanda et al., 2014). In addition, HBV infection may alter the activation potential of NK cells by modulating the balance of activating and inhibitory receptors on the cell surface. During viral challenge the balance shifts from inhibition to activation after a critical threshold of activation signals exceeds those of inhibition (Lanier, 2005; Vivier et al., 2011). We previously showed that chronic HBV patients express elevated levels of the inhibitory receptor NKG2A and downregulated expression of activating receptors CD16 and NKp30 (Tjwa et al., 2011), although in general reports of the phenotype of NK cells differ between studies with vast degree of conflicting results (Mondelli et al., 2010).

We recently performed a systems biology study of peripheral blood transcriptomes in chronic HBV infection to better identify the mechanisms that govern the distinct clinical phases. Besides enhanced activity of IFN-stimulated genes (ISG) in the IT phase and B cell-function related genes in the IA phase, we also observed that upregulation of cytotoxicity/NK cell activity-related genes clustered in the IA and ENEG phase, i.e. the clinical phases with elevated ALT levels (Vanwolleghem et al., 2015). In the current study, we hypothesized that differential NK cell functionalities contribute to the distinct features observed during the HBV clinical phases, including the fluctuations in liver damage markers and HBV replication. Numerical, phenotypical, and functional analysis of immune parameters, of NK cells obtained from the 4 clinical phases was conducted to obtain an in depth profiling of NK cells throughout the course of natural history of chronic HBV infection.

2. Materials and methods

2.1. Patient selection and characteristics

Prospectively collected peripheral blood mononuclear cell (PBMC) samples from 40 31 untreated chronic HBV patients attending the outpatient hepatology clinic of the Erasmus MC (Rotterdam, The Netherlands) were selected, if there were no concomitant HIV, HCV, or HDV infections or oncological/rheumatological diseases. In addition, patients were excluded if they were pregnant, had significant steatosis on liver ultrasound, other liver pathology on liver biopsy, or had received antiviral treatment within the previous year. Liver fibrosis, as determined by histology or transient elastometry, was restricted to a maximum F2 Metavir score or maximum elasticity of 7.0 kPa. Based on serum HBV DNA, ALT levels, and HBeAg presence at the time of sampling, patients were categorized into 4 clinical HBV phases according to international guidelines (European Association For The Study Of The Liver, 2012). Immune tolerant (IT) patients had detectable serum HBeAg and repetitive normal ALT values (<40 U/L) for at least 1 year. The HBeAg-positive immune active (IA) and HBeAg-negative (ENEG) patients had repetitive or intermittent abnormal serum ALT (>40 U/L) values, and HBV DNA levels >2000 IU/mL. Inactive carrier (IC) patients were HBeAg-negative and had both repetitive normal ALT values (<40 IU/L) and HBV DNA levels below 20,000 IU/mL for at least 1 year. Serum ALT was measured on an automated analyzer, qualitative serum HBsAg and HBeAg levels were measured on an Architect Abbott analyzer, and serum HBV-DNA levels were measured using the COBAS AmpliPrep-COBAS Taq-Man HBVv2test (CAP-CTM; Roche Molecular Systems) (Chen et al., 2012; Feld et al., 2007; Papatheodoridis et al., 2012). Patient characteristics are presented in Table 1. PBMC were isolated from venous blood by ficoll separation (Ficoll-Paque™ plus, Amersham), and stored at −150 °C until used for the various assays. Written informed consent was obtained from all participants. The study protocol was approved by the institutional ethics committee and conducted in accordance with the guidelines of the Declaration of Helsinki.

Table 1
HBV patient characteristics.

	Immune Tolerant (IT)	Immune Active (IA)	Inactive Carrier (IC)	HBeAg- Hepatitis (ENEG)
Sex (M/F)	3/4	4/2	5/3	9/1
Age (Years)	29.7 (24–37)	36.8 (18–49)	38.0 (30–48)	40.9 (29–47)
Ethnicity				
Asian	7	5	5	4
African	0	0	3	3
Other	0	1	0	3
ALT	23.3 (14–39)	131.3 (64–229)	28.9 (8–39)	56.3 (29–73)
HBV DNA (IU/ml)	5.7×10^8 (1.6×10^8–1.1×10^9)	3.8×10^8 (3.4×10^7–1.1×10^9)	1.5×10^3 (2.0×10^1–7.6×10^3)	1.3×10^6 (9.4×10^1–1.3×10^7)
HBsAg (IU/ml)	52930 (150–93730)	36628 (6495–72570)	6152 (1–17970)	5439 (543–13466)
HBeAg	Positive	Positive	Negative	Negative
HBV Genotype				
A	0	1	0	3
B/C	5	5	4	2
D	0	0	0	2
E	0	0	1	0
Unknown	2	0	3	3
Fibrosis				
F0-F1	6	2	8	8
F1-F2	1	4	0	2