Virology 512 (2017) 48–55

Contents lists available at ScienceDirect

Virology

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

Hepatic immunopathology during occult hepacivirus re-infection

Cordelia Manickam, Amanda J. Martinot, Rhianna A. Jones, Valerie Varner, R. Keith Reeves*

Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA, USA

ARTICLE INFO

Keywords:
GBV-B
Re-infections
Hepatitis C virus
Marmosets
Liver pathology

ABSTRACT

Despite drug advances for Hepatitis C virus (HCV), re-infections remain prevalent in high-risk populations. Unfortunately, the role of preexisting viral immunity and how it modulates re-infection is unclear. GBV-B infection of common marmosets is a useful model to study tissue immune responses in hepacivirus infections, and in this study we re-challenged 4 animals after clearance of primary viremia. Although only low-to-absent viremia was observed following re-challenge, GBV-B viral RNA was detectable in liver, confirming re-infection. Microscopic hepatic lesions indicated severe-to-mild lymphocyte infiltration and fibrosis in 3 out of 4 animals. Further, GBV-B-specific T cells were elevated in animals with moderate-to-severe hepatopathology, and up to 3-fold increases in myeloid dendritic and activated natural killer cells were observed after infection. Our data indicate that occult hepacivirus re-infections occur and that new liver pathology is possible even in the presence of anti-hepacivirus T cells and in the absence of high viremia.

1. Introduction

Hepatitis C infects approximately 180 million infected people worldwide causing chronic hepatitis in 80% of infected persons. While the rapidly expanding field of HCV therapeutics of approved directly acting antivirals (DAA) has a sustained virologic response rate of more than 90% in infected patients, there are still several risks and limitations to DAAs. In addition to the adverse side effects and substantial costs, there is a significant risk of frequent re-infections after successful treatment (Wandeler et al., 2015) which could lead to development of viral resistance and successive disease.

HCV re-infections occur commonly after spontaneous clearance suggesting that natural immunity could be short-lived. Studies in injection drug users have shown that previous spontaneous clearance does not reduce the incidence of new infections (Micallef et al., 2007; Osburn et al., 2010; Page et al., 2009; van de Laar et al., 2009). Peak HCV viral load was generally lower in re-infections than primary infections and clearance was also observed in 50% of participants in a shorter time duration than primary infection (Micallef et al., 2007; Osburn et al., 2010; Page et al., 2009; van de Laar et al., 2009). However, studies in humans can have limitations — most are retrospective studies and are based on heterogeneous individuals of varying age, ethnicity and injecting risk behavior. Therefore, investigations of correlates of protection in spontaneous clearance and re-infections in animal models could provide important information for vaccine development or repeat use of antivirals.

Animal models have played significant roles in identifying and understanding HCV infections in humans. Chimpanzees were the first animal model used to elucidate the natural history of HCV. In re-infected chimpanzees, rapid viral clearance was associated with proliferating $CD4^+$ and $CD8^+$ T cell responses after re-challenge (Bassett et al., 2001; Major et al., 2002; Nascimbeni et al., 2003; Prince et al., 2005; Zubkova et al., 2014). While chimpanzees are now rarely used in biomedical research, new world primates infected with GBV-B are commonly used as surrogate animal models for HCV (Akari et al., 2009; Beames et al., 2001; Bright et al., 2004; Jacob et al., 2004; Manickam et al., 2016). GBV-B belongs to the same family Flaviviridae and genus Hepacivirus and is closely related to HCV (Deinhardt et al., 1967; Karayiannis and McGarvey, 1995; Muerhoff et al., 1995; Tabor et al., 1980). Viremia in primary GBV-B infection clears within 2-to-3 months of infection (Bright et al., 2004; Bukh et al., 1999; Manickam et al., 2016). Several studies have shown viral immunity in primary infection, but there are only few reports of GBV-B re-infection (Bukh et al., 2008; Woollard et al., 2008). Similar to HCV re-infections, GBV-B re-infected animals also clear the virus rapidly and are associated with more robust T cell responses (Bukh et al., 2008; Woollard et al., 2008). However, the immune responses that lead to viral clearance and tissue specific immune responses in re-infection remain incompletely understood. To study the immunological processes involved in GBV-B re-infection and clearance, we re-challenged four marmosets with GBV-B and analyzed immune responses and pathology in blood and tissues within the first 4 weeks of re-infection.

* Corresponding author.
E-mail address: rreeves@bidmc.harvard.edu (R.K. Reeves).

http://dx.doi.org/10.1016/j.virol.2017.08.037
Received 28 April 2017; Received in revised form 28 August 2017; Accepted 31 August 2017

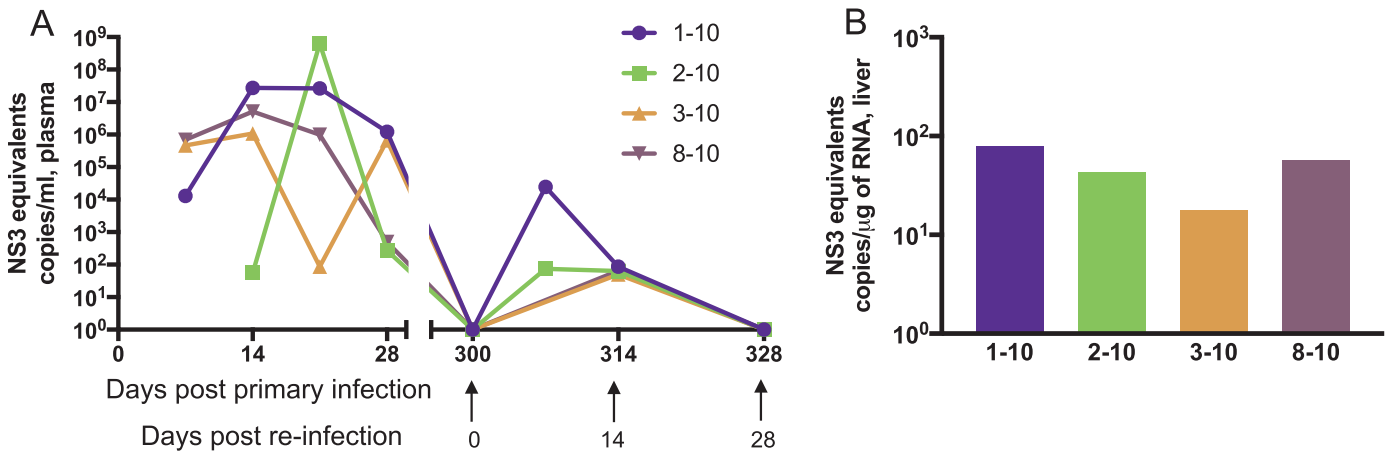

Fig. 1. Viremia in primary infection and re-infection with GBV-B. (A) Post-primary viral clearance animals were re-infected with GBV-B at 300 days after primary infection. Viral loads were quantified in plasma samples at serial time points following GBV-B challenge. (B) Viral loads per µg of total RNA isolated from liver at day 28-post re-infection. Standard equivalents were obtained using GBV-B NS3 plasmid as previously described (Manickam et al., 2016).

2. Results

2.1. Rapid control of viremia following re-challenge

Following primary infection, all animals rapidly developed viremia with variable clearance (Fig. 1) (Manickam et al., 2016). Prior to re-challenge, all animals were confirmed to be aviremic on day 300 relative to primary infection (heretofore day 0 of re-infection) (Fig. 1A). Since the goal of this study was to identify viral immunity in GBV-B re-infection, we describe the immunological and pathological data only relative to re-infection. After re-infection, only transient and low level viremias were observed suggesting a more rapid control of virus replication compared to primary infection. Furthermore, only one animal, 01-10, had plasma virus loads above 100 copies/ml. Similarly, low levels of tissue virus were observed in the liver of all animals at day 28 (Fig. 1B).

Given the low viral loads in liver detectable by standard assays, a novel in-situ hybridization approach, RNAscope, was used to detect single RNA molecules per cell in liver sections. In animal 01-10 clusters of punctate cells positive for GBV-B viral RNA were observed in the liver at day 28 following re-infection (Fig. 2A). Similarly, viral RNA was detectable in 02-10 but not in 03-10 and 08-10 (data not shown). These data clearly indicate a take of infection and active replication in the liver following re-challenge concomitant with detectable viremia in plasma. Positive punctate virus RNA staining was also found in greater numbers in liver sections from marmosets acutely infected following primary GBV-B challenge as a positive control (Fig. 2B), but not in livers of naïve animals as negative controls (Fig. 2C).

2.2. Liver pathology in re-infection

Our previous study of acute and chronic GBV-B infection suggested immune activation induces significant acute liver pathology in marmosets (Manickam et al., 2016). Strikingly, in this re-infection study, pathological manifestations were observed in the livers of re-infected animals despite only very low levels of virus replication. Hematoxylin & Eosin (H & E) stained liver sections showed multifocal moderate to severe predominantly periportal lymphocytic infiltration with random hepatocellular degeneration in all animals, with the most significant hepatitis in 01-10 and 08-10 (Table 1, Fig. 3A) and mild lesions in 02-10 and 03-10 (data not shown). Additionally, moderate fibrotic changes characterized by deposition of trichome positive sinusoidal connective tissue were observed in 01-10 and at mild levels in 08-10 (Fig. 3B). The animals were scored in comparison to normal animals as reported previously (Manickam et al., 2016).

2.3. Elevated GBV-B-specific T cell responses in re-infected animals

Virus-specific responses were evaluated in mononuclear cells from tissues and PBMC by ELISpot assay. Robust responses to all viral antigens (core, NS3, NS3/4a and NS5b) were observed in the liver in all four animals (Fig. 4A). IFN-γ responses against GBV-B core peptides were significantly elevated at day 28 compared to day 0 ($p = 0.037$), but NS3 peptides were the most dominant antigens eliciting significantly elevated responses at day 28 in comparison to day 0 and day 14 ($p = 0.032$). However, PBMC responses were varied with increasing responses against all peptides in animal 01-10 at day 14 and day 28 while low IFN-γ responses in general were observed in the other three animals (Fig. 4B). The overall percentage of IFN-γ secreting cells was lower in PBMC than in liver suggesting higher virus specific responses at the site of virus replication. In other tissues such as spleen, mesenteric lymph nodes (MLN) and axillary lymph nodes (AxLN), elevated responses were observed only in animal 01-10 which had the highest systemic and liver viral loads (Fig. 4C).

IFN-γ secreting $CD8^+$ T cells are known to be major effector cells that can suppress HCV replication (Frese et al., 2002; Major et al., 2004; Sung et al., 2014; Thimme et al., 2002). In order to fractionate the IFN-γ responses observed in ELISpot, we stimulated mononuclear cells with NS3 and NS3/4a pooled peptides for 6 h and analyzed for IFN-γ secreting $CD4^+$ and $CD8^+$ T cells by intracellular cytokine staining (ICS). Interestingly, antigen-stimulated $CD4^+$ T cells showed higher IFN-γ secretion than $CD8^+$ T cells in liver ($p = 0.06$), spleen and PBMC (Fig. 5). The highest percentage of IFN-γ^+ $CD4^+$ T cells was observed in animal 01-10 compared to near undetectable levels in 02-10. Both the ELISpot and the ICS results indicate that animal 01-10 with detectable viremia, showed the highest virus specific IFN-γ responses in re-infection among the four animals.

2.4. Innate immune cell mobilization in circulation

Innate immune cells can also play major roles in modulating Hepacivirus infections (Barth et al., 2011; Gonzalez et al., 2010; Kanto