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Hepatitis B virus particles preferably induce Kupffer cells to produce TGF-β1 over pro-inflammatory cytokines

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ABSTRACT

Background: Kupffer cells and related cytokines are thought to play a critical role in liver fibrosis; however, the role played by Kupffer cells in hepatitis B virus-related fibrogenesis is unknown.
Methods: Primary rat Kupffer cells were cultured with different titres of hepatitis B virus particles and the concentrations of transforming growth factor (TGF)-β1, interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α in the culture supernatant were measured every 24 h for 7 days. The mRNA and protein levels of these cytokines in Kupffer cells were also analysed using quantitative real-time polymerase chain reaction and western blotting, respectively.
Results: Kupffer cells maintained normal morphology and function throughout the 7-day exposure to hepatitis B virus. The concentration of TGF-β1 secreted by hepatitis B virus-stimulated Kupffer cells (6 log IU/ml hepatitis B virus) increased 5.38- and 7.75-fold by Days 3 and 7, respectively ($p < 0.01$). Western blotting showed that TGF-β1 expression in Kupffer cells exposed to high titres of hepatitis B virus increased 1.80- and 2.42-fold by Days 3 and 7, respectively ($p < 0.01$). In contrast, Kupffer cell expression and secretion of pro-inflammatory cytokines (IL-6, IL-1 and TNF-α) was unchanged throughout the experiment.
Conclusion: Hepatitis B virus preferentially stimulates Kupffer cells to produce the pro-fibrogenic/anti-inflammatory cytokine TGF-β1 rather than the pro-inflammatory cytokines IL-6, IL-1 and TNF-α. This may partly explain why overt liver fibrosis still presents in cases of chronic hepatitis B virus infection with minimal (or no) necro-inflammation.

1. Introduction

Chronic infection associated with hepatitis B virus (HBV) is a major cause of liver fibrosis and cirrhosis in many Asian countries, including China [1,2]. Although the general theory regarding the pathogenesis of fibrosis suggests that chronic necro-inflammation activates hepatic stellate cells (HSC) to differentiate into myofibroblast-like cells, which produce excess extracellular matrix (ECM), the mechanisms underlying HBV-induced liver fibrosis have not yet been fully elucidated [3–5]. Indeed, some cases of chronic HBV infection with minimal or no evidence of necro-inflammation show overt fibrosis [2,3]. Recent studies in models of toxic or cholestatic liver fibrosis show that Kupffer cells, the largest group of resident liver macrophages, promote fibrogenesis by producing pro-fibrogenic mediators such as transforming growth factor (TGF)-β1 [6–8]. Toll-like receptors expressed by murine Kupffer cells also interact with HBV [9,10]. A recent study concluded that HBV interferes with murine liver macrophage function, suggesting that Kupffer cells recognize and engulf HBV particles [11]. However, it is still not known whether HBV directly stimulates Kupffer cells to produce pro-fibrotic mediators without causing significant levels of necro-inflammation. To address this issue, we stimulated rat Kupffer cells with different titres of purified HBV virions and observed the resulting pro-fibrogenic/anti-inflammatory and pro-inflammatory cytokine profiles. The results showed that high HBV titres stimulated Kupffer cells to produce the pro-fibrogenic/anti-inflammatory mediator, TGF-β1, rather than pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α. Taken together, these results may provide a novel explanation for the relationship between HBV and liver fibrosis.

2. Materials and methods

2.1. Isolation and purification of HBV particles

HBV particles were isolated and purified from the serum of chronic hepatitis B patients with serum HBV DNA levels around 7 log IU/ml as measured by quantitative real-time polymerase chain reaction (RT-PCR). As previously described [12], 2 ml of serum was

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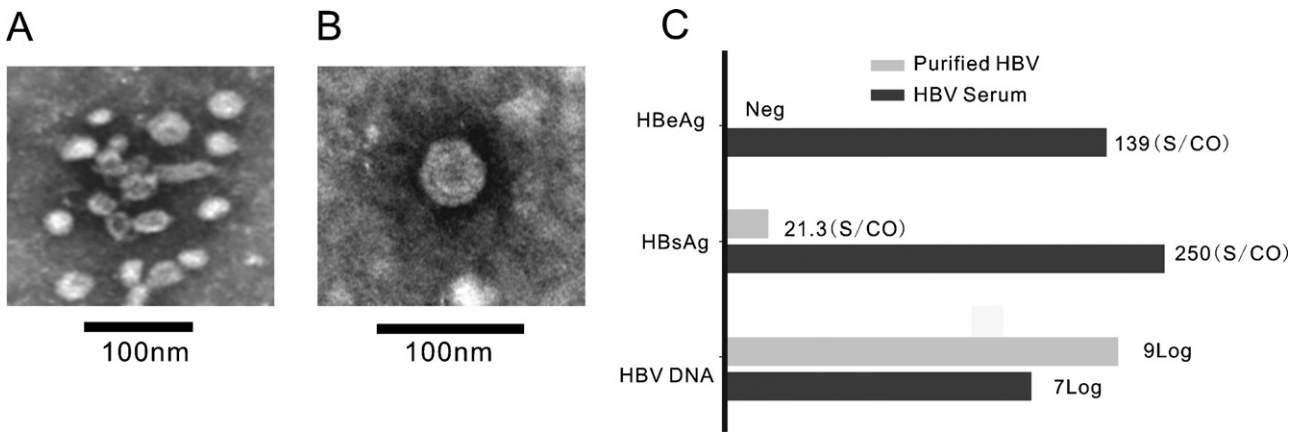

Fig. 1. Purified hepatitis B virus (HBV) particles. (A) HBV particles were identified by electron microscopy (×97,000). Many of the virus particles measured approximately 42 nm in diameter. (B) A single HBV particle. (C) Characteristics of HBV before and after purification. The HBV DNA titre increased, whereas that of Hepatitis B surface antigen (HBsAg) dramatically decreased. Hepatitis B e antigen was not detectable after purification.

carefully layered onto a 10%/20%/30%/40%/50% (w/w) sucrose gradient in TNE buffer (20 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 1 mM EDTA) in a 12 ml ultracentrifuge tube, and ultracentrifuged at 200,000 × *g* for 16 h at 4 °C in a Beckman Sw41Ti rotor (Beckman 100xp, Brea, CA, USA). The fraction with a density of 24–30 g mL^{-1} was collected, diluted five-fold in TNE buffer and re-centrifuged for 2 h at 200,000 × *g*. The supernatant was discarded and the HBV virions precipitated using 200 μl Dulbecco's modified eagle's medium (DMEM) containing 10% FBS. The solution was then diluted 10–1000-fold and tested for HBsAg, HBeAg and HBV DNA, and observed under an electron microscope (Fig. 1).

2.2. Isolation and purification of Kupffer cells

Male Wistar rats (~300 g) were anaesthetized by intraperitoneal injection of 10 mg/ml sodium pentobarbital in saline (0.05 mg g^{-1} body weight). The portal vein was cannulated using a 20-gauge needle connected to a perfusion fluid container. The perfusion fluid

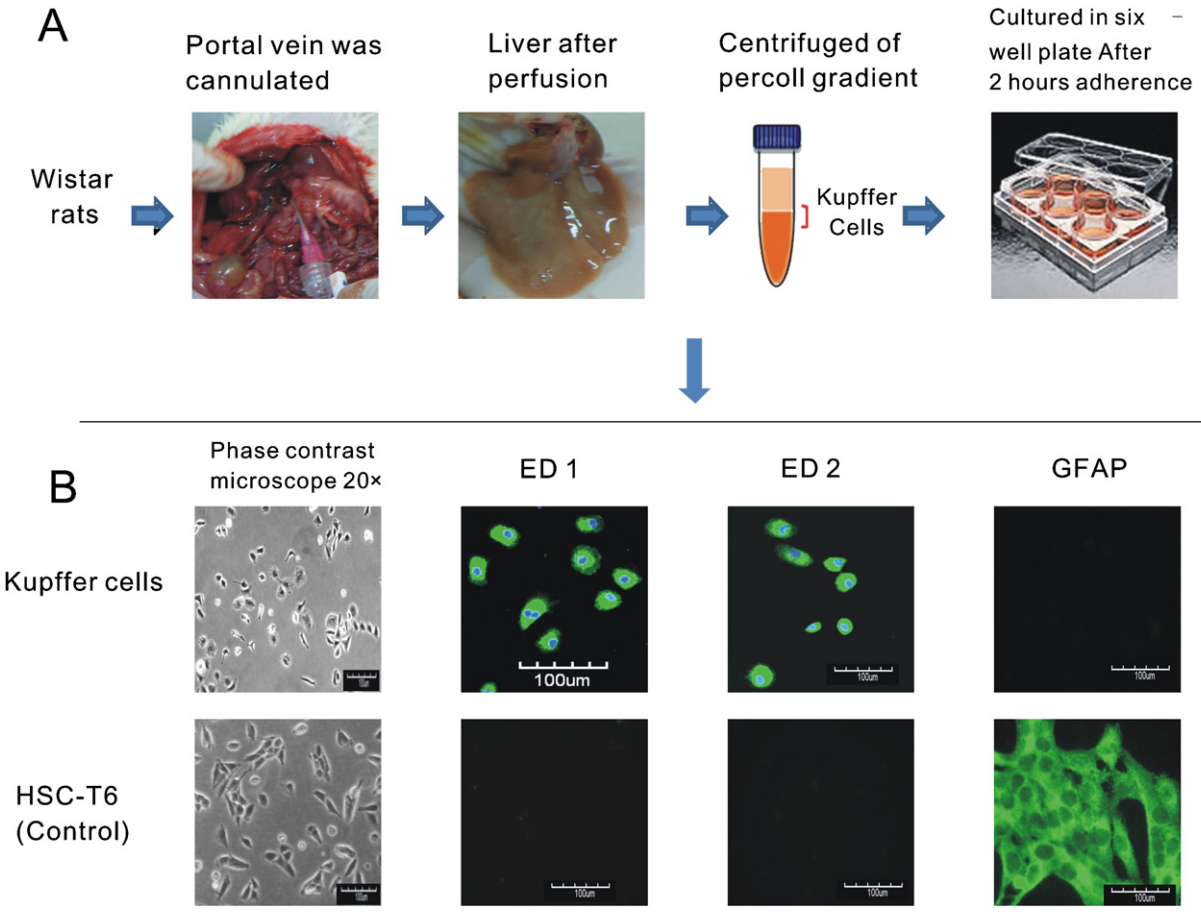

Fig. 2. (A) Kupffer cell isolation. (B) Immunofluorescence staining of Kupffer cells and hepatic stellate cells (HSC). Kupffer cells expressed ectodermal dysplasia antigen 1 (ED1) and ectodermal dysplasia antigen 2 (ED2) (not expressed by HSC), whereas HSC expressed glial fibrillary acidic protein (GFAP) (not expressed by Kupffer cells).