Evaluation of Immune Response in Patients with Chronic Hepatitis B Infection. I: Innate Immunity

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The innate immune system has role not only in protecting the host during the initial period of virus infections but also shaping the nature of adaptive immune response. The aim of this study was to evaluate the innate immune response in patients with chronic hepatitis B virus (CHBV) infection via estimation of serum levels of granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-1alpha (IL-1α), interleukin-8 (IL-8) and

ABSTRACT

The innate immune system has role not only in protecting the host during the initial period of virus infections but also shaping the nature of adaptive immune response. The aim of this study was to evaluate the innate immune response in patients with chronic hepatitis B virus (CHBV) infection via estimation of serum levels of granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-1alpha (IL-1α), interleukin-8 (IL-8) and
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complement components C3 and C4. Venus blood samples were collected from 45 patients with CHBV infection. Other 20 blood samples were collected from healthy individuals as control group. Enzyme linked immune sorbent assay (ELISA) was used to estimate the serum levels GM-CSF, IL-1α and IL-8. Single radial immuno-diffusion (sRID) assay was used to estimate serum levels of C3 and C4. The results showed significant dropping in serum levels of GM-CSF and insignificant dropping in C3 and C4, respectively. While there was insignificant elevation in serum levels of IL-1α and IL-8, respectively in CHBV patients group as compared with healthy control group.

INTRODUCTION

Chronic infection with hepatitis B virus is a member of hepadnaviridae, a heterotropic non-cytopathic DNA virus is a major cause of liver disease worldwide [1,2]. More than 400 million people are persistently infected and at risk of developing chronic liver inflammation resulting in liver cirrhosis and hepatocellular carcinoma. 1 million death each year at directly attributable to be HBV – related liver disease [3-5]. Innate immune cell (mainly monocytes, neutrophils, and dendritic cells) and molecules play a central role in promptly controlling infections in the early phases and providing environment required for priming efficient adaptive immune response [6-11]. Both innate and adaptive arms of immune system are generally involved in responding to viral infection with innate responses being important for control of viral replication and dissemination very early after infection [12-14]. Due to the large number of immune cells present, the liver may be considered an immunological organ, with particular innate features, and therefore thought to be play an active role in the first line host defense against pathogens [15,16]. After sensing the presence of virus professional innate cells kupffer cells (KC), dendritic cells (DCs),natural killer (NK) and natural killer T cells (NKT) produce cytokines and chemokines that have antiviral properties (e.g., IFN-α, IFN-β, TNF-α, …) or that are meant to attract and stimulate adaptive immune cells (e.g., IL-2, IL-6, IL-10 …) [2,10,17].
In general, infected cells can detect the presence of viral components of PAMPs (pathogen-associated molecular patterns) via cellular sensor or PRRs (patterns recognition receptors), such as Toll-like receptors (TLRs), RIG-like helicases (RLHs), or Nod-like receptors (NLRs) [18,19], and produce antiviral type1 interferons (IFNs) ,IFNα and IFNβ as well as other pro-inflammatory cytokines (e.g., IL-1β, IL-6 …) [20-23].

TLRs recognize microbes either at the cell surface or on lysosome/endosome membranes, while pathogens that invade the cytosol are detected by cytoplasmic PRRs such as RLHs or NLRs [18,19]. Various TLRs were expressed in parenchymal and non parenchymal cells of the liver [24]. Hepatocyte expressed mRNA for all TLRs [25,26]. Whereas KCs express TLR4 and TLR2 [27,28]. In the case of lymphocytes T and NK cells express TLR 1,2,4,5 and 9, whereas B cells express high levels of TLR 1,6,7,9 and 10 [29].

Dendritic cells can be myeloid (mDC) or lymphoid (plasmacytoid, or pDC) origin, and represent an important component of innate immunity in the liver. Both recognize and present antigen to T cells but are distinct in their TLR expression and cytokine production profile [29-31]. Plasmacytoid DCs express TLR 7 and 9 and produce large amounts of IFN-α, whereas, mDCs express TLR 2, 3, 4 as well as 9 and produce pro-inflammatory cytokines and IFN-β but not IFN-α [29,32].

While virtually all liver cells types express RLRs [33]. The downstream effect of any IFN-α produce may be attenuated in antigen-activated cells [34,35] or modified by increase in other cytokines such as IL-1 [36] and IL-8 [37,38]. The level of IL-8 typically increase with the increase in HBV DNA, in keeping with reported ability of HBV to transactivate the IL-8 gene [39]. NK cells have been shown to express the high affinity IL-8 receptor CXCR1 and migrate in response to IL-8 [40].

MATERIALS AND METHODS

Forty five (15-65 years old, mean 39.5 years, 25 males and 20 females) patients with CHBV infection in Kadhamiya teaching hospital, during period from October 2008 to march 2010 were used for this study. the cause of chronic liver disease was determined using standard diagnostic criteria.
chronic hepatitis B was diagnosed by positive serological tests for serum hepatitis B surface antigen (HBsAg) for at least 6 months. Twenty (18-55 years old, mean 33.4 years, 10 males and 10 females) healthy individuals were used as control group.
Five ml of venous blood were collected from each individual in plain tube. Serum were separated, put in eppendorf tubes and stored at (deep freezing -20°C).

**Immunological assays:**

Enzyme-linked immune-sorbent assay (ELISA), Immunotech, France) was used to estimate serum levels of GM-CSF, IL-1α, and IL-8.
Single radial immunodiffusion (SRID) assay (Binding site, England) was used to estimate serum levels of C4 and C3 respectively (41).

**STATISTICAL ANALYSIS**

Statistical analysis has been performed using (SPSS, version 11.0) for windows. Continuous variables were expressed as mean ± standard error (SE). data were analysed using independent sample student’s t test. Significance was assigned for p values (<0.05) with 95% confident interval.

**RESULTS AND DISCUSSION**

**GM-CSF:** There was insignificant dropping of serum levels of GM-CSF in patients with chronic hepatitis B infection (13.00±2.74 pg/ml) as compared with healthy control group (35.5±2.57 pg/ml). Fig.-1

**Interleukin-1 alpha:** Serum levels of IL-1α showed insignificant elevation in CHBV patients (14.04±2.51 pg/ml) as compared with healthy control group (11.34±1.83 pg/ml).Fig.2

**Interleukin-8:** Serum levels of IL-8 showed insignificant elevation in CHB patients group (872.86±173.35 pg/ml) as compared with healthy control group (694.44±354.36 pg/ml). Fig.2
C3 complement: Serum levels of C3 showed insignificant dropping in CHB patients group (1260.5±205.48 mg/dl) as compared with healthy control (1774.78±221.33 mg/dl). Fig.3

C4 complement: Serum levels of C4 showed insignificant dropping in CHB patients (19.91±5.1 mg/dl) as compared with healthy control group (30.91±3.63 mg/dl). Fig.4.

Macrophage activation represent one of the first events of innate resistance against intracellular infection. In response to pathogens, macrophages and other inflammatory cells. Secret cytokines IFNγ, IL1,IL6,IL8 and TNFα. Some of these cytokines lead to activate against pathogens, activate effector cells involved in the cellular interaction that occur during inflammation and are part of acute and chronic stages of viral hepatitis [42,43].
The significant dropping of serum level of GM-CSF refer to suppression of its production by macrophages . GM-CSF paly an important role in activation , proliferation and differentiation of granulocytes monocytes and macrophages [44-49].
A number of laboratories have reported that IL4 inhibit the production of GM-CSF in a variety of cells including human monocytes [50-54] by down-regulation of mRNA precursor [55]. Th1/Th2 cytokines producing T cells were significantly lower in chronic HBV patients as compared to normal individuals [5]. The Th2 cytokines inhibit growth of extracellular parasites and suppress phagocytosis [56].

That’s mean suppression of GM-CSF production which regulate phagocytosis function by phagocytic cells particular macrophage.

IL-1α is a main key of many of cellular responses, by regulation of GM-CSF production by macrophages [57,58].

The elevation of serum levels of IL-1α agree with Missate (59) which showed an increase of IL-1 in serum of CHB patients. HBV causes an inflammatory illness characterized by mononuclear and polymorphonuclear cellular infiltrate with evidence hepatic macrophage activation [60]. These inflammatory cells produce such cytokines as TNF-α, IFN-γ, IL-1α and IL-6, [60] which mediate the inflammatory process and which contribute to the successful clearance of virus [61]. IL-1 induce expression of IL2R, thus participating in the activation of resting T-cells [62].
So, the elevation of IL-1α in CHB patient may help in support of immune response.

Mahe [39] had showed that increase levels of IL8 associated with the increase in HBV DNA. The elevated levels of serum IL-8, IFN-α and NK cell tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to patients with HBV infection with active liver inflammation a apposed to HBV carriers or control [63]. CHB patients with liver inflammation were accompanied by increase in NK cell activation and surface TRAIL expression [3]. TRAIL pathway revealed that IL8 is capable of up-regulation a death-inducing receptor TRAIL.

The complement system plays an important role in immunological and inflammatory response. Complement deficiency may increase patients susceptibility to invasive infection. One of the causes of reduced production of complement components may be hepatic function disturbances in patients with chronic viral hepatitis [63]. Our results agree with (Bussone and Mouthon)[64] and Sjoholm [65], which (revealed dropping in serum levels of C3 and C4 in patients with hepatitis B infection. Hepatic function disturbances in course of chronic viral hepatitis B and C may lead to deficiency of complement components (hypocomplementaemia) and further to the risk of invasive bacterial infection [63].

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