Evaluation of Immune Response in Patients with Chronic Hepatitis B Infection. II: Humoral Immunity

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ABSTRACT

The humoral immune response play an essential role during hepatitis B virus (HBV) infection. HBV clearance is associated temporally with production of anti-envelope antibodies. The aim of this study was to evaluate the humoral immune response in patient with chronic hepatitis B virus (CHBV) infection via estimation of serum level of interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10) and immunoglobulins IgG, IgA and IgM. 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 of IL-4, IL-6 and IL-10. Single radial immunodiffusion (sRID) assay was used to estimate serum levels IgG, IgA and IgM.

The results showed insignificant dropping in serum levels of IL-4 and IgG respectively while there insignificant elevation in IL-6, IgM and IgA respectively. IL-10 levels shows significant elevation in CHBV patients group as compared with healthy control group.

**INTRODUCTION**

Hepatitis B virus (HBV) infect more than 400 million people worldwide and is a common cause of liver disease, HBV a member of the hepadnaviridae family, is a small DNA virus (1-4). The chronic HBV and HCV are major causes of morbidity and mortality (5). Patients with chronic hepatitis B (CHBV) are at risk of developing severe liver disease, including cirrhosis and hepatocellular carcinoma (6-8). As HBV is currently viewed as non-cytopathic virus, HBV-associated liver damage is thought to be the consequence of long lasting cytolytic immune response against infected hepatocytes (9,10). HBV replicate in hepatocytes to produce HBsAg particle can be taken up by antigen presenting cells, which degraded the viral proteins to peptides that are able then presented on the cell surface bound to MHC-I or MHC-II molecules (1,11). The degree of inflammatory activity did not correlate with the intensity of HBsAg expression in hepatocytes. However, an inverse relationship between the degree of diffuse membranous HBsAg expression and inflammatory local response in chronic hepatitis B (CHB) of minimal and mild activity was established (12). In CHBV patients, the T-cell response and circulating cytokines profile are associated with viral replication and liver function (13). The clearance of virus was clearly associated with efficient adaptive immune response (14). Activation of the virus-specific cellular immunity followed by the humoral response, which appears at last 10 to 12 weeks after HBV infection. Th2 is able to neutralize HBV by antibodies and inhibit HBV replication through cytokines (15,16). The antibody response in patients with HBV plays a critical role viral clearance through the formation of complexes with viral particles and their removal from the circulation (17-19). Th2 response characterized by IL-4, IL-5 IL-6 and IL-10 (20). However, recent reports on the ability of IL-4 to induce dendritic-cells-specific ICAM-
3 grabbing nonintegrin (DC-SIGN), a myeloid DC-specific lectin, suggest that IL-4 not only suppresses the monocyte and/or microphage lineage but its actively promotes the differentiation of monocytes along the DC lineage (21). Previous reports also suggest that IL-4 alone can activate accessory properties of monocytes and up-regulate MHC-I molecules, co-stimulatory molecules (22,23) and down-regulate CD14 on monocytes (22,24). A number of laboratories have reported that IL-4 inhibits the production of GM-CSF in a variety of cell including human monocytes (25-29). IL-6 is a multifunctional cytokine is critically involved in the acute phase response, T-cell proliferation, B-cell maturation, macrophage maturation and cytotoxic T-cell differentiation. In combination with IL-1, IL-2 and IFN-γ, IL-6 induces expression of IL-2 receptor (IL-2R), thus participating in the activation of resting T-cells (31). Furthermore, IL-6 seems to contribute to MHC-non-restricted cytotoxic activity by inducing natural killer (NK) cell proliferation (32). Interleukin-6 is considered a major source of early Th1/Th2 control during CD4+ T-cells activation at it attributes to the promotion of Th1 polarization. Moreover IL-6 activates the production IL-4 by CD4+ T-cells and their differentiation into Th2 effector cells. Furthermore, it inhibits Th1 differentiation by interference with IFN-γ signaling and the development of Th1 cells (33). Up regulation of Th2 response, associated with cytokines IL-6 and IL-10, may occur to some degree and adversely affected the functioning of Th1 response (34). The severity of liver injury in hepatitis is regulated by balance between aggressive and protective cytokines TNF-α, IFN-γ and IL-4 promote liver inflammation, which is attenuated by IL-6, IL-10 and IL-22 (35). Anti-inflammatory effects of IL-10 have lead to its use in hyper inflammatory status. IL-10 possesses anti-fibrotic activity and may be valuable as therapeutic cytokine for patients with liver cirrhosis (36,37). Successful clearance of the virus as well as the establishment of liver diseases largely driven by complex interaction between the virus and host immune response (38).

**MATERIALS AND METHODS**

Forty five (15-65 years old, mean 39.5 years, 25 males and 20 females) patients with CHBV infection in Kadhimya 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), (eBiosience, England) and ( Biosource, Belgium ) was used to estimate the serum levels of IL-4 and (IL-10,IL-6) respectively.
Single radial immunodiffusion (SRID) assay (Binding site, England) was used to estimate serum levels of IgG , IgM and IgA respectively(39).

**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**

**Interleukin-10:** Serum levels of IL-10 showed significant elevation in CHB patients group on ( 23.67 ± 4.68 pg/ml ) as compared with healthy control group ( 4.29±1.08 pg/ml ). Fig.1

**Interleukin-6:** Serum levels of IL-6 showed insignificant elevation in CHBV patients ( 20.57± 7.70 pg/ml ) as compared with healthy control group ( 17.67 ± 4.88 pg/ml ).Fig.1

**Interleukin-4:** Serum levels of IL-4 showed insignificant dropping in CHB patients group (26.36 ± 3.43 pg/ml ) as compared with healthy control group ( 27.00 ± 354.36 pg/ml ). Fig.1

**Immunoglobulin G:** Serum levels of IgG showed insignificant dropping in CHB patients group (9188.33±1311.64 mg/dl) as compared with healthy control ( 9574.44 ± 1035.47 mg/dl ). Fig.2
Immunoglobulin M: Serum levels of showed insignificant elevation in CHB patients (95.67±16.52 mg/dl) as compared with healthy control group (77.42 ± 15.76 mg/dl). Fig.3

Immunoglobulin A: Serum levels of IgA showed insignificant elevation in CHB patients group (39.50 ± 3.62 mg/dl) as compared with healthy control group (33.44 ± 4.93 mg/dl). Fig.3

Conventional definition of Th1 and Th2 cells depend strictly on the ability to secrete IFN-γ and IL-4 respectively (40,41). Th2 response associated with cytokines IL-6 and IL-10 may occur to some degree and adversely affected the functioning of Th1 response (34). IL-10 is an immunoregulatory cytokine and its on of the key cytokines in the Th2 response. The decrease in serum IFN-γ levels coincides with increase in the levels of IL-10 which inhibit IFN-γ synthesis (42,43). Interleukin-10 levels were significantly increase while significant decrease in IL-4 levels were observed in patients with HBV infection (44). Both of TGF-β and IL-10 negatively regulate...
production of Th$_1$ and Th$_2$ cytokines. Their over expression may impair the immune mechanisms directed against pathogen and tumor antigen (45-47). The persistence of high IL-10 levels in the convalescent phase is important in the secretion of surface antibodies against HBV and development of immunity. Anti-HB$_s$Ag block the adherence of viral particles to non-infected cells and remove from the circulation the free antigenic particles, protecting the individual against reinfection (48). The current study showed significant elevation in serum levels of IL-10.

The significant elevation of IL-10 may contribute to its production by CD4$^+$CD25$^+$ regulatory cells (Tregs). Tregs are immunosuppressive T-cells that play an essential role in controlling immune responses and autoimmunity (49). Recent finding suggest that Tregs also play a role in regulating immune responses to HBV infection (50). High levels of Tregs have been detected in CHB and are thought to be responsible for the chronicity of HBV infection, probably by inhibiting HBV- specific T-cell response (8).

It was shown that HBV was recognized by Kupffer cells (KC), although the virus does not replicate in these cells, and within hours post infection, this recognition lead to the activation of NF$_\kappa$B and subsequently to release IL-6 and other pro-inflammatory cytokines (43, 51). The current study shown insignificant elevation of serum levels of IL-6 in CHBV patients, our results agree with other papers which revealed the elevation in serum levels of IL-6 (51). Interleukin-6 elevation may refer to Th$_2$ activation, in another words slightly dominant of Th$_2$ response.

Type 2 cytokines such as IL-4 and IL-5 may also involved in the clearance of circulating virus by promoting the production the neutralizing antibodies against the HBV surface and core antigens (51). Current study agree with Mansour et al (53), which refer to IL-4 dropping in HBV patients. The elevation of IL-10 leads to suppression of IL-4 production in HBV patients (44), while the elevation of serum levels of IL-4 and IL-6 found in patients with autoimmune CHBV infection (54).

The elevation of IL-4 in CHBV patients with liver damage may revealed the dominant of Th$_2$ response (20,55). Severity of liver injury in hepatitis
patients is TNF, IFN-γ and IL-4 promote liver inflammation, which is attenuated by IL-6, IL-10 and IL-22 (35). Th2 cytokines inhibit growth of extracellular parasites and suppress phagocytosis. They augment B-cells proliferation derive antibody production switch IgG to IgE class of antibodies (41). Current study showed slightly elevation of serum levels of IgM while showed slightly dropping in each of IgG and IgA. Serum levels of immunoglobulins were ranged between normal to slightly elevation in HBV patients (56). If the host is able to clear the infection eventually the HBsAg will become undetectable and will be followed by IgG antibodies to the HBsAg and core antigen (57). HBV clearance is associated temporally with production of anti-envelope antibodies (58), through the formation of antivirus immunoglobulins-viral particles complex and there removal from the circulation (17-19).

**REFERENCE**


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