Tumor Necrosis Factor Alpha and Interleukin 6 Productions in Response to Platelet-Activating Factor in Chronic Hepatitis B Virus Infection

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Key Words

Chronic hepatitis B · Platelet-activating factor · Tumor necrosis factor- α · Interleukin-6

between chronic HBV carriers and naturally immune subjects suggest that TNF- α may be a critical mediator of HBV clearance.

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Abstract

Objective: The aim of this study was to determine tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) release in response to platelet-activating factor (PAF) induction in peripheral blood mononuclear cells (PBMCs) from chronic hepatitis B virus (HBV) carriers. **Methods:** Subjects were grouped into three subgroups. The mean age was 37 ± 10 years. Group A ($n = 15$), group B ($n = 10$) and group C ($n = 9$) subjects were HBV serology-negative, had natural immunity after recovery from an acute HBV infection, and were chronic HBV carriers, respectively. **Results:** Compared with group A, PBMCs from naturally immune subjects and chronic HBV carriers produced significantly higher amounts of TNF- α and IL-6 in response to PAF. In chronic HBV carriers, TNF- α ($1,633.3 \pm 793.7$) and IL-6 ($2,533.3 \pm 466.3$) production was statistically lower than TNF- α ($2,630.0 \pm 727.3$) and IL-6 ($3,870.0 \pm 728.4$) obtained from naturally immune subjects to HBV. **Conclusion:** Differences of TNF- α levels

Introduction

Hepatitis B virus (HBV) infection is one of the most common infectious diseases worldwide. Depending on the interaction between the virus and the host response, there is a wide spectrum of clinical courses of viral hepatitis B [1–3]. Impaired clearance of HBV, which occurs in 10% of infected patients, results in chronic infection [4, 5]. Lysis of infected hepatocytes by cytotoxic T lymphocytes is crucial for elimination of the HBV. Like interferons, tumor necrosis factor alpha (TNF- α) is responsible for a number of effects. It is an inhibitor of viral replication [6]. It also induces human leukocyte antigens (HLA) in hepatocytes to activate cytotoxic T lymphocytes. Studies indicate that an altered production of interleukin-6 (IL-6) may contribute to the changes in cellular immune regulation which occur in patients with acute and chronic viral hepatitis [7, 8]. In recent years, the role of biological-

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ly active substances like platelet-activating factor (PAF) in host response to immune destruction in the liver has been investigated [9–12]. PAF is a potent autocoid mediator, with large pharmacologic and biologic properties. It is responsible for cytokine synthesis and release by peripheral blood mononuclear cells (PBMCs), monocytes, and macrophages. The dose-dependent effect of PAF may induce the release of different cytokines at different levels and affect the host immune response. PAF and cytokines potentiate each others' effects in vivo to modulate immune response [13–16].

In this study, TNF- α and IL-6 levels of PBMCs in response to PAF obtained from HBV-naïve subjects, naturally immune subjects to HBV infection and chronic HBV carriers were investigated. The cytokine responses to PAF stimulation were compared with unstimulated results.

Subjects and Methods

Thirty-four individuals (mean age 37 ± 10 years) were divided into three groups according to the HBV serology and the biochemical results. Fifteen healthy individuals (9 male, 6 female) with hepatitis B surface antigen (HBs Ag), antibody to hepatitis B core antigen IgG (anti-HBc IgG) and antibody to HBs Ag (anti-HBs)-negative serology and with normal serum alanine transaminase (ALT) and bilirubin levels served as controls (group A); 10 subjects (5 male, 5 female) with anti-HBc IgG and anti-HBs seroconversions after acute HBV infection and with normal ALT and bilirubin levels served as postexposure subjects (group B), and 9 cases (4 male, 5 female) with HBs Ag anti-HBc IgG seropositivity but anti-HBs seronegativity after a period of 6 months or more after acute HBV infection, with normal ALT and bilirubin levels and without any histological sign of chronic active hepatitis, chronic persistent hepatitis or cirrhosis served as healthy chronic HBV carriers (group C). Histological examination was only performed in chronic HBV carriers. Patients with alcoholic liver disease, drug-induced hepatitis, autoimmune hepatitis (antinuclear antibody-positive patients), α_1 -antitrypsin deficiency, idiopathic hemochromatosis or infection with known viruses including type A, type C, type D, type E hepatitis viruses, cytomegalovirus or Epstein-Barr virus and chronic active hepatitis, chronic persistent hepatitis and cirrhosis due to HBV were excluded from the study. HBV serological tests were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Organon Teknika, Holland). HBs Ag, anti-HBc IgG and anti-HBs were investigated as serological markers. ALT and bilirubin levels were observed as biochemical tests.

PBMC Isolation

PBMCs were separated by a density gradient on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) from freshly drawn heparinized (preservative-free heparin, Immuno A.G., Vienna, Austria) peripheral venous blood, washed thrice in phosphate-buffered saline and resuspended to 5×10^6 PBMCs/ml in RPMI 1640 media (Gibco, Paisley, UK) supplemented with 10% human AB serum (Flow Labs., UK),

Table 1. Clinical details of individuals studied

	n	Age	M/F	ALT, U/l	Bilirubin, mg/dl
Control	15	29–45	9/6	15 ± 7	0.8 ± 0.2
Post-exposure	10	27–47	5/5	24 ± 9	0.7 ± 0.3
Healthy carrier	9	30–43	4/5	28 ± 8	1.0 ± 0.2

M = Male; F = female.

100 IU penicillin/ml, 100 μ g streptomycin/ml and 2 mM *L*-glutamine (Gibco). A hundred milliliters of the cell suspension were pipetted onto cell culture plates (Costar, Cambridge, Mass., USA).

Incubation Procedure

TNF- α and IL-6 production by PBMCs was induced by the addition of 1 iM synthetic PAF (PAF C16, Sigma Co., St. Louis, Mo., USA). Cells were incubated for 72 h at 37°C in a humidified atmosphere containing 5% CO₂ (Sanyo, Japan). After the incubation period, supernatant was removed and kept at –70°C until assayed. TNF- α and IL-6 were analyzed by ELISA (Genzyme Co., USA) method from supernatant. Assays were performed as the kit manufacturers recommended. Results were expressed as picograms/ 5×10^6 cell.

Statistics

Data are expressed as mean $\pm$ standard deviation. Kruskal-Wallis test was performed to compare TNF- α and IL-6 levels between the groups. Since the results were significant between the groups, Bonferroni-adjusted Mann-Whitney U test was used to compare two groups at a time. To reduce the type 1 error risk, the degree of importance was divided by the number of comparisons and alpha level was found as 0.0167.

Results

Details of the subjects studied are summarized in table 1. Stimulation of PBMCs by PAF in chronic HBV carriers and in control cases revealed higher amounts of TNF- α and IL-6 than those by unstimulated PBMCs. The levels of TNF- α in response to PAF stimulation in naturally immune subjects to HBV and in cases with chronic HBV carriers were $2,630.0 \pm 727.3$ and $1,633.3 \pm 793.7$, respectively, compared to 53.7 ± 13.2 in the control group. Similar results were observed for the serum IL-6 level in response to PAF. The stimulated levels of these cytokines in naturally immune subjects and chronic HBV carriers were higher than those in control cases. Low amounts of TNF- α and IL-6 were secreted by unstimulated PBMCs into the culture supernatant and no differences were observed between the postexposure and healthy carrier groups. As shown in table 2, statistical dif-

Table 2. TNF- α and IL-6 levels in PBMC culture unstimulated and stimulated with PAF C16

	Spontaneous response (unstimulated)		Stimulated with PAF C16	
	IL-6	TNF- α	IL-6	TNF- α
Group A	15.1 $\pm$ 6.7	14.7 $\pm$ 4.5	64.8 $\pm$ 11.3	53.7 $\pm$ 13.2
Group B	410.0 $\pm$ 74.9	352.0 $\pm$ 64.9	3,780.0 $\pm$ 728.4	2,630.0 $\pm$ 727.3
Group C	353.3 $\pm$ 65.7	276.6 $\pm$ 44.1	2,533.3 $\pm$ 466.3	1,633.3 $\pm$ 793.7
p = 0.02.				

ferences were also observed between naturally immune subjects and chronic HBV carriers in response to PAF, the levels of TNF- α and IL-6 being lower in chronic HBV carriers ($p \leq 0.0167$).

Discussion

Differences in the immunological host response are thought to be the major factors that determine the course of hepatitis B infection. Cytokines have an important role in the pathophysiology of acute and chronic viral hepatitis. In the present study, PBMCs from control individuals, subjects with natural immunity after recovery from an acute HBV infection and chronic HBV carriers produced considerable amounts of TNF- α and IL-6 without stimulation. Possibly, PBMCs were activated in in vitro culture medium and stimulated by a very low level of lipopolysaccharide contamination in human serum. Increased TNF- α and IL-6 production were seen after PAF stimulation in naturally immune subjects and chronic HBV carriers. This finding indicates that production of both cytokines reflects a response to antigenic stimulation.

PAF is synthesized and secreted from PBMCs, monocytes, and macrophages. Specific PAF receptor is present on the cell membranes of both lymphocytes and monocytes. After binding this receptor, PAF initiates intracellular secondary mRNA synthesis and conducts the signal received via nuclear factor-kappa B to the nucleus and leads to the synthesis of cytokines. The type and amount of cytokines produced may vary according to the dosage and time of exposure to PAF [13, 16].

TNF- α and IL-6 have an important role in immunoregulation and inflammation. It has been shown that TNF- α like interferon- α activates HLA in hepatocytes, which induces cytotoxic T lymphocytes. Lysis of infected hepatocytes by cytotoxic T lymphocytes is crucial for elimination of the virus. It has been shown that TNF- α synthesis by stimulated PBMCs of patients with chronic hepatitis is

increased [17]. In an experimental study, it is shown that a combination of interferon therapy with TNF and IL-2 may improve HBV clearance [18]. An altered production of IL-6 may contribute to the changes in cellular immune regulation that occur in patients with acute and chronic viral hepatitis. Although in patients with acute hepatitis B and C, elevated IL-6 serum levels were observed [19, 20], in another study impaired in vitro production of IL-6 by lipopolysaccharide-stimulated PBMCs from patients with chronic hepatitis B was detected [21]. In this study it is shown that the serum levels of IL-6 in naturally immune subjects to HBV after acute HBV infection and in chronic HBV carriers have been augmented. Elevated and persistent TNF- α and IL-6 levels in PBMCs from naturally immune subjects to HBV after acute infection may be due to residual HBV replication and remnant liver inflammation after recovery from acute HBV infection [22].

Conclusion

Our results indicate that PAF can stimulate PBMCs, leading to the release of TNF- α and IL-6. Compared with the control group, PBMCs from naturally immune subjects and chronic HBV carriers produced higher amounts of both TNF- α and IL-6 in response to PAF. In naturally immune subjects TNF- α and IL-6 productions were higher than those in chronic HBV carriers. The differences in TNF- α levels between chronic HBV carriers and naturally immune subjects suggest that TNF- α may be a critical mediator of HBV clearance ($p \leq 0.0167$).

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