

Effect of IL-18 on peripheral blood mononuclear cells of chronic hepatitis B and hepatitis B virus DNA released by HepG2.2.15 cell lines

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BACKGROUND: Interleukin-18 (IL-18), a pro-inflammatory cytokine that induces interferon- γ (IFN- γ) production in T cells and natural killer cells, plays a critical role in the T-lymphocyte helper type 1 (Th1) response. This study was designed to explore the effect of IL-18 on peripheral blood mononuclear cells (PBMCs) derived from chronic hepatitis B (CHB) and on hepatitis B virus (HBV) DNA released by HepG2.2.15 cell lines, which were transfected with hepatitis B virus gene in vitro.

METHODS: PBMCs isolated from 25 healthy people and 25 patients with CHB were stimulated with HBcAg and IL-18 of various concentrations for 72 hours. The levels of IFN- γ in the supernatants of cultured PBMCs were determined by ELISA. After the stimulation of IL-18 of various concentrations, PBMCs derived from one patient were co-cultured for 96 hours with HepG2.2.15 cells which had been cultured for 24 hours, and then the supernatants were collected by centrifugation and used for HBV DNA quantitative assay.

RESULTS: When PBMCs were stimulated by HBcAg and IL-18 at various concentrations, the levels of IFN- γ in the supernatants of CHB groups were much higher than those in normal control groups, at 0.2 ng/ml: $t=11.70$, $P<0.01$; at 1.0 ng/ml: $t=16.19$, $P<0.01$; and at 5.0 ng/ml: $t=20.12$, $P<0.01$. In the CHB groups, the levels of IFN- γ in the supernatants of PBMCs stimulated by HBcAg alone were lower than both those stimulated by HBcAg and IL-18 at various concentrations and those stimulated by HBcAg and IL-18 (5.0 ng/ml) together with IL-12 (mild: $t=2.20$, $P<0.05$; moderate: $t=2.97$, $P<0.05$; severe: $t=0.66$, $P>0.05$). The content of HBV DNA in the supernatant of co-cultivation of HepG2.2.15 cells and PBMCs without stimulated materials was higher than that stimu-

lated by HBcAg and IL-18 at various concentrations of HBcAg and IL-18 together with IL-12/IFN- α 1b.

CONCLUSION: IL-18 can induce IFN- γ secretion and probably play a key role in the modulation of both innate and adaptive immunity. It has implications in improving immunoregulatory effect and increasing the ability of immune cells to kill cells infected by virus.

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KEY WORDS: interleukin-18; chronic hepatitis B; peripheral blood mononuclear cells; HepG2.2.15 cells; interferon- γ ; HBV DNA

Introduction

During the course of chronic hepatitis B virus (HBV) infection, HBcAg can preferentially elicit T-lymphocyte helper type 1 (Th1) cells-mediated immune reactions, and HBeAg can preferentially elicit Th2 cells-mediated immune reactions.^[1] In patients with chronic hepatitis B (CHB), weak HBc-specific Th1 responses occur, but they increase during inflammatory exacerbation and subsequent viral clearance. These phenomena demonstrate that HBc-specific Th1 responses have a potential important effect on viral clearance. Thus increasing the HBc-specific Th1 responses will affect the result of HBV infection.

Interferon- γ (IFN- γ) appears to play an important role in T-cell-mediated viral clearance, and there is a markedly increased genital virus load in vaccinated mice treated with anti-IFN- γ antibodies.^[2] And interleukin-18 (IL-18) as a pro-inflammatory cytokine that can induces IFN- γ production in T cells and natural killer (NK) cells^[3] plays a critical role in specific Th1 and innate immune responses.^[4] IL-18 also act as an immune adjuvant to induce the specific humoral immune response.^[5,6] The published research articles suggest that IL-18 might play a role in antiviral effect,^[7-13] but the mechanism of this effect has not been elucidated, and to

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date, the role of this cytokine to HBV and hepatitis C virus (HCV) has been less studied. But it was reported that IL-18 may take part in the host immunity against HBV and is related to the inflammation degree of the liver, and that the transcription and expression of IL-18 in chronic hepatitis B are correlated positively with the serum level of ALT.^[14] The higher levels of IL-18 in the serum of patients with chronic hepatitis C than those of the healthy controls and asymptomatic HCV carriers indicate a significant positive correlation to ALT.^[15] Kimura^[16] found that IL-18 can inhibit HBV replication in the livers of transgenic mice.

Based on the above mentioned theories we conducted this research to explore the effect of IL-18 on the peripheral blood mononuclear cells derived from patients with CHB.

Methods

Subjects

Included in this study were 25 patients with CHB (17 men and 8 women, 14 to 57 years old) who had been admitted to the First Clinical Hospital of Harbin Medical University from April to July, 2002. Their diagnoses were based on the diagnostic criteria of the Revised Prophylaxis and Treatment Program for Viral Hepatitis (September, 2000, Xi'an).^[17] All patients were positive for HBsAg and negative for HBsAb, without any history of liver diseases. Among them 15 patients were positive for HBeAg, and 10 patients were positive for HBeAb. Twenty-five healthy subjects (18 men and 7 women, 20 to 35 years old) served as controls, who were negative for HBsAg and HCV antibody, with normal liver enzymes.

Reagents and cells

Recombinant human IL-18 and IL-12 were purchased from Strathmann Biotec Company, Germany. Recombinant HBcAg was purchased from 302 Hospital of PLA, Beijing, China and IFN- α 1b from Kexing Biological Production Company, Shenzhen, China. IFN- γ ELISA kit was bought from Fanbang Chemical Engineering Techniques Co., Ltd., Dalian, China. HepG2.2.15 cell lines were purchased from the Department of Virology of Harbin Medical University, Harbin, China.

Isolation and culture of PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from sterile heparinized venous blood (4 ml/subject) over Ficoll-hypaque density centrifugation in 2 hours, and resuspended in a RPMI 1640 medium. The cells were counted by use of a hemacytometer and examined for viability by trypan blue exclusion (>95%), then they were diluted to 1.0×10^6 cells/ml, then 1 ml of PBMCs was brought to a 24-round-bottom-well plate.

Finally the following stimulated materials were added into the plate: polyhydroxyalkanoate (PHA) (100 μ g/ml); HBcAg (1 μ g/ml); HBcAg (1 μ g/ml)+IL-18 (0.2 ng/ml); HBcAg (1 μ g/ml)+IL-18 (1.0 ng/ml); HBcAg (1 μ g/ml)+IL-18 (5.0 ng/ml); and HBcAg (1 μ g/ml)+IL-18 (5.0 ng/ml)+IL-12 (10 ng/ml). After 72 hours, the supernatants were harvested, centrifuged, collected, and stored at -20 °C immediately until testing.

Culture of HepG2.2.15 cells^[18]

HepG2.2.15 cells were maintained in the RPMI-1640 medium in a 37 °C humidified incubator in an atmosphere of 5% CO₂. The cells were generated every three days, and could be used when HBV DNA was detected stably in the supernatant. One ml of cells was put into a 24-round-bottom-well plate with the density of 2×10^4 cells/ml. After 24-hour adhesion at 37 °C with 5% CO₂ in a humidified incubator, the cells were used in experiments.

Effect of IL-18 alone on HBV DNA released by HepG2.2.15

The following stimulated materials were added into the plate where HepG2.2.15 cells were cultured: unstimulated material; IL-18 (0.2 ng/ml); IL-18 (1.0 ng/ml); IL-18 (5.0 ng/ml); HBcAg (1 μ g/ml)+IL-18 (5.0 ng/ml)+IL-12 (10 ng/ml); and HBcAg (1 μ g/ml)+IL-18 (5.0 ng/ml)+IFN- α (1b 1500 μ /ml). Three parallel test wells were set for each material, and after incubation for 96 hours at 37 °C with 5% CO₂ in an incubator, the supernatants were centrifuged and collected, then stored at -20 °C for immediate detection of HBV DNA.

Effect of HBcAg combined with IL-18 at various concentrations of IL-18 and IL-12 or IL-18 and IFN- α 1b on HBV DNA released by HepG2.2.15 when PBMCs and HepG2.2.15 cells were co-cultured

The medium of HepG2.2.15 cells obtained from method 2 was replaced with 1 ml of RPMI1640 medium per well, then PBMCs derived from a patient with CHB were isolated and brought to the well (1 ml, with a density of 2×10^6 cells/ml. The method of isolation was the same to method 1) filled with HepG2.2.15 cells. Finally the following stimulated materials were added: unstimulated material; HBcAg (1 μ g/ml)+IL-18 (0.2 ng/ml); HBcAg (1 μ g/ml)+IL-18 (1.0 ng/ml); HBcAg (1 μ g/ml)+IL-18 (5.0 ng/ml); and HBcAg (1 μ g/ml)+IL-18 (5.0 ng/ml)+IL-12 (10 ng/ml). Three parallel test wells were set for each material; after 96-hour of incubation at 37 °C with 5% CO₂ in an incubator, the supernatants were centrifuged, collected, and stored at -20 °C for immediate detection of HBV DNA.

Detection of IFN- γ and HBV DNA in the culture supernatants

The concentrations of IFN- γ in the culture supernatants were determined using monoclonal antibody (mAb) in a specific enzyme-linked immunosorbent assay (ELISA); HBV DNA was amplified by PCR.

Statistical analysis

All data were presented as mean \pm standard errors of the means (SEM), statistical significance calculated using Student's *t* test between the two means was analyzed by the Kruskal-Wallis test and Wilcoxon's rank-sum test in comparison of several means. A *P* value less than 0.05 was considered statistically significant.

Results

IFN- γ levels in the culture supernatants secreted by PBMCs between the control and CHB groups stimulated by different antigens

The IFN- γ levels of PBMCs to PHA alone were higher in the control group than in the CHB group (442.70 ± 110.54 pg/ml vs 363.58 ± 79.79 pg/ml, $t=2.91$, $P<0.01$); the IFN- γ levels of PBMCs to HBcAg alone were lower in the control group than in the CHB group (361.05 ± 67.66 pg/ml vs 536.02 ± 67.08 pg/ml, $t=9.18$, $P<0.01$); the IFN- γ levels of PBMCs stimulated by HBcAg and IL-18 (0.2 ng/ml) were higher in the CHB group than in the control group (574.07 ± 70.95 pg/ml vs 373.33 ± 47.78 pg/ml, $t=11.70$, $P<0.01$); the IFN- γ levels of PBMCs stimulated by HBcAg and IL-18 (1.0 ng/ml) were higher in the CHB group than in the control group (795.59 ± 111.38 pg/ml vs 379.10 ± 64.45 pg/ml, $t=16.19$, $P<0.01$); the IFN- γ levels of PBMCs stimulated by HBcAg and IL-18 (5.0 ng/ml) were higher in the CHB group than in the control group (1148.31 ± 183.68 pg/ml vs 382.71 ± 49.75 pg/ml, $t=20.12$, $P<0.01$); the IFN- γ levels of PBMCs stimulated by HBcAg and IL-18 (5.0 ng/ml) together with IL-12 were higher in the CHB group than in the control group (1313.20 ± 187.76 pg/ml vs 390.75 ± 23.94 pg/ml, $t=23.94$, $P<0.01$).

IFN- γ levels secreted by PBMCs to different antigen in patients with CHB of various degrees

In patients with CHB of various degrees, the IFN- γ levels of PBMCs to HBcAg and IL-18 (5.0 ng/ml) were compared with those to HBcAg and IL-18 (5.0 ng/ml) together with IL-12. In mild CHB group the former was lower than the latter (1027.55 ± 182.44 pg/ml vs 1205.64 ± 179.59 pg/ml, $t=2.20$, $P<0.05$); in moderate and severe CHB groups, the former was lower than the latter, e.g. moderate: 1488.69 ± 180.43 pg/ml vs 1800.98 ± 183.81 pg/ml, $t=2.97$, $P<0.05$; and severe: 1055.58 ± 126.60 pg/ml vs 1107.75 ± 199.40

pg/ml, $t=0.66$, $P>0.05$.

IFN- γ levels secreted by PBMCs derived from patients with HBeAg and those with HBeAb

The IFN- γ levels of PBMCs to HBcAg in the group of HBeAg positive were lower than those of HBeAb positive (471.02 ± 67.45 pg/ml vs 633.52 ± 110.87 pg/ml, $t=4.57$, $P<0.01$); the IFN- γ levels of PBMCs to HBcAg and IL-18 (0.2 ng/ml) in the group of HBeAg positive were lower than those of HBeAb positive (489.82 ± 74.45 pg/ml vs 700.44 ± 118.30 pg/ml, $t=5.48$, $P<0.01$); the IFN- γ levels of PBMCs to HBcAg and IL-18 (1.0 ng/ml) in the group of HBeAg positive were lower than those of HBeAb positive (707.82 ± 87.52 pg/ml vs 927.24 ± 132.01 pg/ml, $t=5.01$, $P<0.01$).

The IFN- γ levels of PBMCs to HBcAg and IL-18 (5.0 ng/ml) in the group of HBeAg positive were lower than those of HBeAb positive (996.79 ± 122.61 pg/ml vs 1375.58 ± 187.28 pg/ml, $t=6.13$, $P<0.01$); the IFN- γ levels of PBMCs to HBcAg and IL-18 (5.0 ng/ml) together with IL-12 in the group of HBeAg positive were lower than those of HBeAb positive (1109.12 ± 170.02 pg/ml vs 1619.32 ± 213.75 pg/ml, $t=6.40$, $P<0.01$).

Effect of IL-18 alone on HBV DNA released by HepG2.2.15 cells

There were no significant differences among the test groups of the quantity of HBV DNA in supernatants.

Effect of HBcAg combined IL-18 of various concentrations of IL-18 and IL-12 or IL-18 and IFN- α 1b on HBV DNA released by HepG2.2.15 cells when PBMCs and HepG2.2.15 cells were co-cultured

After the Kruskal-Wallis test, statistical difference was seen in the experimental group ($H=19.92$, $P<0.01$). Subsequently Wilcoxon's rank-sum test showed that there was difference in the experimental group and control group.

The quantity of HBV DNA in the group stimulated by HBcAg and IL-18 of various concentrations was lower than that in the group unstimulated (0.2 ng/ml IL-18: $q=2.78$, $P<0.01$; 1.0 ng/ml IL-18: $q=3.79$, $P<0.05$; 5.0 ng/ml IL-18: $q=4.32$, $P<0.05$; IL-12 + 5.0 ng/ml IL-18: $q=7.75$, $P<0.01$). To eliminate the interference to HBV DNA in the supernatants, we detected the HBV DNA in the wash water the last time during the isolation of PBMCs and the supernatants of PBMCs cultured alone by qualitative analysis.

Discussion

In this study stimulated by HBcAg and IL-18 at various concentrations, PBMCs from the patient group secreted

significantly more IFN- γ than those did from the control group and the group stimulated by HBcAg alone. The result was consistent with that reported by Tsuji-Takayama and others.^[4,19,20] Dual signaling consisting of IL-18-induced NF-kappa B activation and TCR/CD₃ mediated nuclear factor of activated T cells (NFAT) activation is considered crucial to IFN- γ production by IL-18 in murine Th1 cells. IL-18 receptor (IL-18R)^[21] and T cell receptor (TCR) of HBcAg are all expressed on Th1 cells of patients with CHB. Stimulated by IL-18 and HBcAg in this study, PBMCs including Th1 cells produced a considerable amount of IFN- γ by dual signaling; but in the control group, only a small amount of IFN- γ was produced, because PBMCs had never been stimulated by HBcAg of high level and the dual signaling was limited. This finding was similar to that in the group stimulated by HBcAg alone. Moreover, we found that the level of IFN- γ was increasing while adding IL-18 with increased concentrations and that IL-18 synergized with IL-12 and IFN- α to induce the secretion of IFN- γ .^[22-25] The activity of IL-18 is initiated via an IL-18 receptor complex, which is made up of a IL-1R-related protein (IL-1RrP/IL-18R α)^[26] and accessory protein (AcPL/IL-18R β).^[27,28] In human NK and T cells, IFN- α and IL-12 can strongly up-regulate mRNA expression of the IL-18R components—IL-1RrP and AcPL.^[29] When IL-18 is combined with IL-1RrP, the signal is transferred into cells by AcPL, thus inducing NF-kappa B activation.^[30,31] Being similar to HBcAg, when HBcAg is combined with TCR, the signal is transferred into cells by CD₃, then mediated NFAT activation. Finally a considerable amount of IFN- γ are produced, and innate and specific Th1 immune responses appear. This conclusion has been proved in our research. In our study the quantity of HBV DNA in the group of patients with CHB stimulated by HBcAg and IL-18 at various concentrations was lower than that in the group unstimulated, and the quantity of HBV DNA in the group stimulated by HBcAg and IL-18 (5.0 ng/ml) together with IL-12/IFN- α 1b was lower than that in the group stimulated by HBcAg and IL-18 (5.0 ng/ml); all the differences were of statistical significance. These results indicate that IL-18 can augment immune responses of PBMCs. With complicated biological activities, IL-18 is able to induce IFN- γ secretion and play a key role in the modulation of both innate and adaptive immunity. Hence it is implicated in improving immunoregulatory effect and increasing the ability of immune cells to kill virus-infected cells.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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