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Expression of Th1 and Th2 Type Cytokines Responding to HBsAg and HBxAg in Chronic Hepatitis B Patients

The cytokine pattern on viral antigen recognition is believed to exert a profound influence on the resolution of viral infections and viral clearance. This study was initiated to investigate whether a cytokine imbalance oriented toward Th2 type response plays a role in chronic hepatitis B. Cytokine profiles of peripheral blood mononuclear cells associated with chronic hepatitis B were analysed by RT-PCR. Upon HBsAg stimulation, expression of IFN-γ, IL-2, IL-4, and IL-10 was detected in 41%, 8%, 41%, and 50% of the patients, respectively. Among these cytokines, the expression of IFN-γ was associated with high levels of serum AST/ALT. However, we could not prove that Th2 type cytokines had a protective effect on hepatocytes. Upon HBxAg stimulation, there was no recognizable association of cytokine patterns with AST/ALT levels. In conclusion, production of a Th1 cytokine, IFN-γ, by HBsAg-reactive cells was associated with hepatocyte damage in chronic hepatitis B, while no counteracting effect of Th2 cytokines produced by those cells was observed.

Key Words : *Hepatitis B virus; Hepatitis B surface antigen; Cytokines; Hepatitis B, chronic*

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INTRODUCTION

Chronic infection with hepatitis B virus (HBV) is the primary source of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (1). Since HBV is a noncytopathic virus, cell-mediated immunity (CMI) appears to be responsible for the elimination of HBV-infected hepatocytes and viral clearance (1). Actually, patients with acute hepatitis B are able to produce vigorous proliferative responses of T cells against various HBV antigens, whereas patients with chronic HBV infection show very weak T cell responses (1-6).

HBV genome contains four open reading frames encoding HBsAg, HBc/eAg, polymerase, and HBxAg. Immune response against each HBV antigen may be different quantitatively and possibly qualitatively. Proliferative responses of T cells against HBc/eAg are readily observed in patients with acute hepatitis B and during the acute exacerbation period of chronic active hepatitis (3-5). In contrast, T cell responses against HBsAg, which may be important in protective immunity against HBV infection, are hardly detectable in both acute and chronic hepatitis (4, 6). Immune responses against polymerase and HBxAg are not well characterized yet (7, 8).

Although the mechanisms of how HBV escapes immunologic surveillance and persists in chronic hepatitis B remain unclear, recent studies on the immunoregulation in microbial infections suggest that balances in cytokine production profiles may play a crucial role in determining the resolution or persistence of infection (9-11). Th1 type cytokines (IFN-γ and IL-2), which are principally involved in CMI, can contribute to protection from viral infections, while predominant Th2 type cytokine productions (IL-4 and IL-10) may inhibit anti-viral CMI (12, 13). Therefore, it can be hypothesized that predominant productions of Th2 type cytokines may be involved in the persistent infection with HBV.

To characterize the cytokine profile associated with chronic hepatitis B, peripheral blood mononuclear cells (PBMCs) obtained from patients with chronic hepatitis B were stimulated with HBsAg or HBxAg and tested for their cytokine expression responses to antigen stimulation by RT-PCR.

MATERIALS AND METHODS

Patients and HBV antigens

Subjects studied were acute and chronic hepatitis B patients attending the gastroenterology services of Ajou University

Medical Center. In chronic hepatitis patients, HBsAg was positive for longer than 6 months. On the same day PBMCs were obtained from patients, serum aspartate transaminase (AST) and alanine transaminase (ALT) were measured.

Recombinant yeast-derived preparation of HBsAg was kindly provided by the Green Cross Co (Seoul, Korea). HBxAg was prepared by expression of cloned viral DNA in *E. coli* using the glutathione S-transferase (GST) gene fusion system (Pharmacia, Sweden). The expression vector pGEM-4T-1 carrying HBx open reading frame (pGEX-HBx) was kindly given by Dr. W-K Kim, Institute for Medical Science, Ajou University, Suwon, Korea. For the expression of GST-HBx fusion protein, the plasmid was transformed in *E. coli* cells BL21. After cultivating the transformed BL21 in LB medium containing ampicillin until the OD_{600} reached around 0.6, expression was induced by 0.1 mM IPTG (Sigma Chemical Co., St. Louis, MO) for 5-6 hr. Cells were centrifuged at 6,000 *g* for 10 min and washed twice with Tris-HCl buffer solution (pH 7.4, 20 mM Tris-HCl and 1 mM EDTA). The cell pellet was resuspended in the Tris-HCl buffer solution containing 0.5% NP-40, sonicated (Heat system Inc., U.S.A.) for 2 min, cooled on ice, and spun down at 10,000 *g* for 30 min. Soluble cell extract was collected and filtered with a 0.45 μm filter. The GST-HBx fusion protein was purified by Glutathione Sepharose 4B affinity chromatography (Pharmacia, Sweden). Briefly, the cell extract was applied to a Glutathione Sepharose 4B column. The column was washed three times with 10 bed volume of 20 mM Tris-HCl and the fusion protein was eluted with a glutathione elution buffer (pH 8.5, 50 mM Tris-HCl and 10 mM reduced glutathione). The eluate was concentrated using a ultrafiltrator (Amicon Inc., Beverly, MA) and further purified by Sephacryl S-100 gel filtration to use as an antigen.

Culture medium

The culture medium used was RPMI 1640 supplemented with 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 10mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin (all from GIBCOBRL Lab., Grand Island, NY) and 10% human AB serum (Sigma Chemical Co., St. Louis, MO). Recombinant human IL-2 (rhIL-2) was purchased from Sigma Chemical Co.

HBsAg-reactive T cell populations

PBMCs were separated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation. Using 24 well culture plates (Costar, Cambridge, MA), PBMCs (1×10^6/well) in the complete medium were cultured in the presence of HBsAg (5 μg/mL) for 7 days at 37℃ in a humidified 5% CO_2 incubator. Then HBsAg-reactive cells were preferentially expanded for 7 days by adding rhIL-2 (10 U/mL).

For cytokine assays, the HBsAg-reactive cell populations (1×10^6/mL) was restimulated with HBsAg in the presence of irradiated (3,000 Rad; gamma cell 3000 Elan; Nordion, Kanata, Ontario) autologous PBMCs (2×10^6/mL) as feeder/antigen presenting cells. Supernatants of the antigen-stimulated and control cultures were collected after 4 days and human cytokine ELISA kits (Endogen, Woburn, MA) were used for IFN-γ and IL-4.

Cytokine RT-PCR

PBMCs (1×10^6/mL) were cultured in the presence or absence of HBsAg (5 μg/mL). After 12 hr, 24 hr and 72 hr, the cells were collected and subjected to a semi-quantitative cytokine RT-PCR. Antigen-stimulated PBMCs were harvested for total RNA extraction with acid guanidinium thiocyanate-phenol-chloroform and isopropanol precipitation. Vacuum-dried pellets were dissolved in 15 μL DEPC-treated-distilled water and incubated for 65℃ for 10 min. After cooling on ice, the mixture was incubated for 60 min at 37℃ in the presence of 10 μL of 2 x reverse transcriptase buffer (100 mM Tris-HCl, pH 8.3, 150 mM KCl, 6 mM $MgCl_2$, 20 mM DTT), 0.5 μg oligo-dT (18-mer; Amitof Co., Boston, MA), final concentrations of 200 units of reverse transcriptase (Superscript IITM, Gibco BRL, Grand Island, NY), 0.5 mM dNTP, and 25 U of RNasin (Promega, Madison, WI). Tubes were then heated to 95℃ for 10 min, and 130 μL of distilled water was added to the 20 μL reaction mixture. Samples were stored at -20℃ until further use.

Sequences of cytokine-specific primer pairs, designed to be complementary to the sequences in different exons, and probes are as follows: IL-2 senseprimer: 5'-CCTCA ACTCCTGCCACAATG-3' IL-2 anti-sense primer: 5'-TTGCTGATTAAGTCCCTGG G-3' (340 bps), IL-2 probe: 5'-TACATGCC CAAGAAGGCCACAGAACTGA AA-3', IFN-γ sense primer: 5'-TCGTTTTGGGTTCTCTTGGC-3', IFN-γ anti-sense primer: 5'-GCAGGCAGGACAAC-CATTAC-3' (477 bps), IFN-γ probe: 5'-CTGACTA ATTATTCGGTAACTGACTTGAAT-3', IL-4 sense primer: 5'-GCTTCCCCCTCT GTTCTTCC-3', IL-4 anti-sense primer: 5'-TCTGGTTGGCTTCCTTCACA-3' (371 bps), IL-4 probe: 5'-TTTGCTGCCTCCAAGAACACAACT-GAGAAG-3', IL-10 sense primer: 5'-CTGAGAAC-CAAGACCCAGACATCAAGG-3', IL-10 anti-sense primer: 5'-CAATAAGGTTTCTCAAGGGGCTGG-3' (351 bps), IL-10 probe: 5'-AATGCCTTTAATAAGCTCCAAGA-GAAAGGC-3', GAPDH sense primer: 5'-GCAGGGGG GAGCCAAAAGGG-3' GAPDH anti-sense primer: 5'-TGCCAGCCCCAGCGT CAAAG-3' (567 bps), GAPDH probe: 5'-GTGGAAGGACTCATGACCACAGTC CAT-GCC-3'. Twenty-five μL of each cDNA were amplified in 0.2 mL MicroAmp reaction tubes (Perkin-Elmer, Branchburg, NJ) in the presence of 200 μM final concentration of

primers, 200 μM dNTP, 2.5 U of *Taq* polymerase (Gibco BRL, Grand Island, NY), and PCR buffer containing 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl, pH8.4 in a final volume of 50 μL. PCR was performed in a thermal cycler (Perkin Elmer-Cetus, Emeryville, CA) for 35 cycles: 45 sec denaturation at 94℃, 45 sec annealing at 52℃, and 45 sec extension at 72℃. After amplification, 10 μL of the PCR product was analyzed by electrophoresis in 1.2% agarose gel, and the DNA fragments were transferred onto a nitrocellulose paper. The authenticity of the amplified products was confirmed by Southern blotting and hybridization with ^{32}P-labeled specific oligonucleotide probes. Size of the amplified target sequences was validated by running in parallel ^{32}P-labeled molecular size marker. A relative amount of each cytokine mRNA to GAPDH mRNA was quantitated using a phosphor imaging analyzer (BAS2500, Fuji, Japan).

RESULTS

Production of IFN-γ by HBsAg-reactive T cell populations

To investigate whether there are any differences in cytokine profiles of HBsAg-reactive T cells between acute and chronic hepatitis, HBsAg-reactive T cell populations were prepared from PBMCs of hepatitis B patients and analysed for their production of IFN-γ and IL-4 by ELISA. Upon stimulation with HBsAg, IFN-γ was produced in 80% of the acute hepatitis patients. Production of IFN-γ was also observed in 25% of the chronic hepatitis patients (Table 1). In contrast, production of IL-4 was not detected in any patient (Table 1). Since some chronic hepatitis patients showed IFN-γ production, we decided to examine cytokine profiles associated with chronic hepatitis in more detail using RT-PCR.

Cytokine expression profiles of PBMCs upon stimulation with HBsAg in chronic hepatitis B patients

For further investigation on cytokine patterns in the immune response against HBsAg during chronic HBV infection, PBMCs were obtained from 12 patients showing various serum levels of AST/ALT, and analysed for their production of IL-10, IL-4, IL-2 and INF-γ by RT-PCR. Expression of each cytokine was assessed at the time of 12 hr, 24 hr, and 72 hr incubation. Age of the patients was 34±11 years old and all patients were male except one female patient.

Table 1. Cytokine production by HBsAg-reactive T cell populations obtained from PBMCs of viral hepatitis B patients

Groups of patients	No. of positive T cell population (%)*	
	IFN-γ	IL-4
Acute hepatitis	4/5 (80%)	0/5 (0%)
Chronic hepatitis	2/8 (25%)	0/8 (0%)

*Production of cytokines was analysed by ELISA after stimulation with HBsAg.

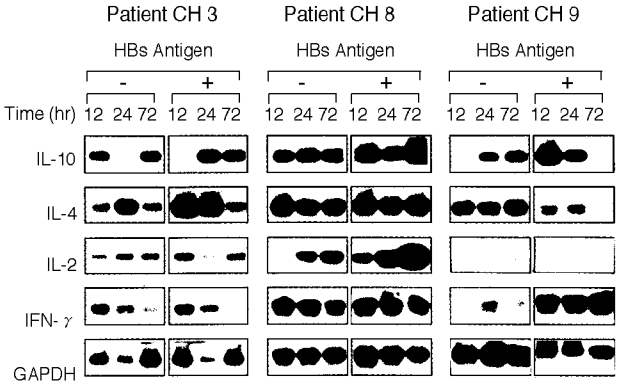

Fig. 1. Expression of cytokine mRNAs in PBMCs of chronic hepatitis B patients responding to HBsAg stimulation. PBMCs (1 × 10^6/mL) were cultured in the presence or absence of HBsAg (5 μg/mL). After 12 hr, 24 hr, and 72 hr, the cells were collected and subjected to semi-quantitative RT-PCR. Representative autoradiogram results are shown.

Table 2. Cytokine expression prefiles of PBMCs stimulated with HBsAg in chronic hepatitis B patients

Patients	AST/ALT (IU/mL)	IL-10	IL-4	IL-2	IFN-γ
CH 1	32/44	-	-	-	-
CH 2	74/143	+	+	-*	+
CH 3	97/152	+	+	-	-
CH 4	156/845	+	-	-	-
CH 5	199/220	-	+	-	-
CH 6	440/1090	-	-	-	-
CH 7	470/1140	-	-	-	-
CH 8	533/402	+	-	+	-
CH 9	550/860	+	-	-*	+
CH10	590/1490	+	+	-*	+
CH11	941/1150	-	-	-	+
CH12	1462/1500	+	+	-	+

Cytokine expression levels of PBMCs stimulated with HBsAg were compared with levels of unstimulated PBMCs. -: no difference, +: increased, -*: not detectable

Various patterns of the cytokine expression were observed depending on the individuals (Fig. 1). For example, patient CH3 showed an increased expression of Th2 type cytokine (IL-4), while patient CH9 showed increased expressions of both Th1 type cytokine (IFN-γ) and Th2 type cytokine (IL-10) (Fig. 1). Patient CH8 was the only patient who showed an increased expression of IL-2 (Fig. 1, Table 2). Fig. 2 shows example of results analysed by a phosphor imaging analyzer.

As Table 2 shows, about 75% of the patients showed

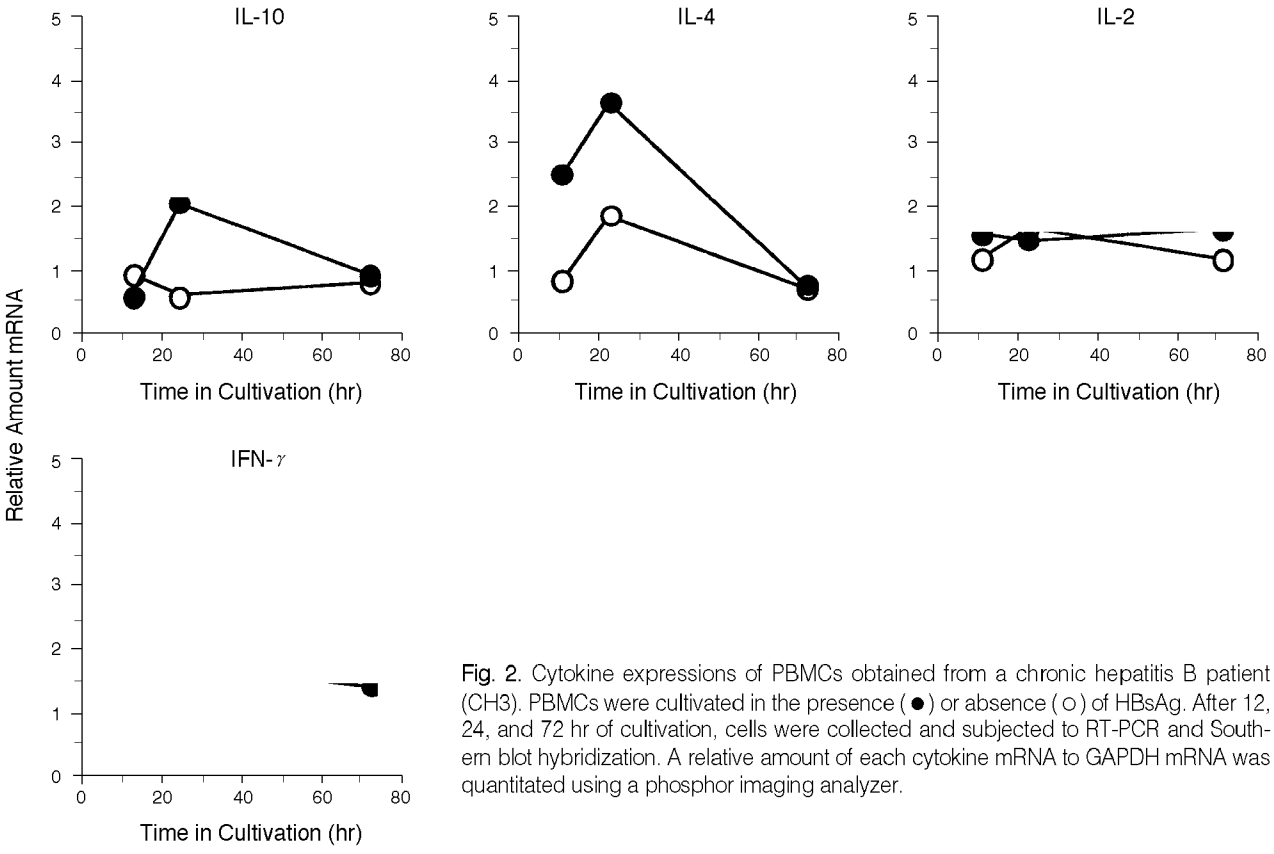

Fig. 2. Cytokine expressions of PBMCs obtained from a chronic hepatitis B patient (CH3). PBMCs were cultivated in the presence (●) or absence (○) of HBsAg. After 12, 24, and 72 hr of cultivation, cells were collected and subjected to RT-PCR and Southern blot hybridization. A relative amount of each cytokine mRNA to GAPDH mRNA was quantitated using a phosphor imaging analyzer.

cytokine production responses upon HBsAg stimulation. Depending on cytokine patterns, the patients could be divided into three groups: production of Th2 type alone was observed in 25% of the patients, both Th2 and Th1 type was 42%, and Th1 type alone was 8%. While each cytokine expression of IL-10, IL-4, and INF-γ was observed in approximately 50% of the patients, IL-2 expression was observed only in one patient (8%). This result was consistent with poor proliferative responses of T cells to HBsAg stimulation (data not shown).

Relationship between expression of INF-γ in response to HBsAg and hepatocyte injury

Expression of each cytokine was analysed for its correlation with hepatocyte damage which is reflected by serum AST/ALT levels. Whereas IFN-γ expression appeared to correlate positively with serum AST/ALT levels, IL-4 expression did not appear to have any correlations (Fig. 3). Association of IFN-γ expression with severe liver damage is consistent with the finding that IFN-γ production was observed in a majority of the acute hepatitis B patients (Table 1), in which active destruction of HBV-infected hepatocytes occurred.

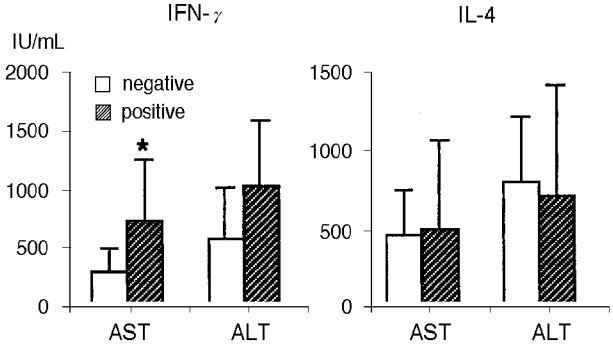

Fig. 3. Relationship between cytokine production and serum AST/ALT levels. PBMCs from chronic hepatitis B patients were stimulated with HBsAg and determined for their IFN-γ and/or IL-4 expression response by RT-PCR. Serum AST/ALT levels of patients who showed increased expressions of IFN-γ or IL-4 (▨) were compared to those of patients who did not (□).

Cytokine production response to HBxAg stimulation

HBxAg was prepared as a GST-HBx fusion protein. Fig. 4 shows purified GST-HBx protein analysed by western blot. Since a large portion of protein purified by the glutathione-sepharose 4B affinity chromatography had been

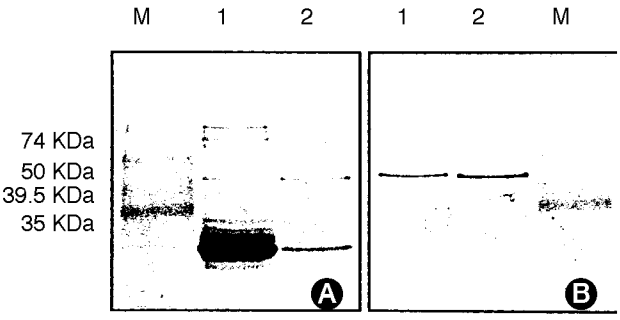

Fig. 4. Western blot analysis of purified GST-HBx proteins. Panel A shows the result of western blot using anti-GST antibody, and panel B shows the result of western blot using rabbit anti-HBx serum. Lane 1; GST-HBx fusion protein purified by Glutathione Sepharose 4B affinity chromatography, Lane 2; GST-HBx fusion protein further purified by Sephacryl S-100 gel filtration to use as an antigen.

Table 3. Cytokine expression prefiles of PBMCs stimulated with HBxAg in chronic hepatitis B patients

Patients	AST/ALT (IU/mL)	IL-10	IL-4	IL-2	IFN-γ
CH 1	32/44	-	-	+	-
CH 2	74/143	-	-	-*	-
CH 3	97/152	-	+	+	+
CH 4	156/845	-	+	-	-
CH 5	199/220	-	-	-	-
CH 6	440/1090	-	-	+	-
CH 7	470/1140	-	-	-	-
CH 8	533/402	-	-	-	-
CH 9	550/860	-	-	-*	-
CH10	590/1490	+	-	-*	+
CH11	941/1150	-	-	-	-
CH12	1462/1500	-	-	-	-

* Cytokine expression levels of PBMCs stimulated with GST-HBx antigen were compared to levels of PBMCs stimulated with GST.
-: no difference, +: increased, -*: not detectable

degraded (Fig. 3, lane 1), the protein was further purified by sephacryl S-100 gel filtration (Fig. 3, lane 2).

PBMCs obtained from the chronic hepatitis B patients were stimulated with HBxAg and examined for IL-10, IL-4, IL-2, and IFN-γ expression by RT-PCR. Expressions of cytokines in the presence of GST-HBx protein were compared with those in the presence of GST protein.

Approximately 40% of the patients showed an increased expression of at least one kind of cytokine upon HBxAg stimulation, and the expression of each cytokine was observed in 8-16% (Table 3). Different from that IFN-γ expression upon HBsAg stimulation correlated with relatively high levels of AST/ALT, there was no correlation of any cytokine with AST/ALT levels upon HBxAg stimulation.

DISCUSSION

Immune responses are not very successful in eliminating HBV from the body in chronic hepatitis B. Since HBV is known to have no cytopathic effect on the infected hepatocytes, cell-mediated immunity is thought to play an important role in the pathogenesis of hepatocellular damage and HBV clearance (1). Even though immune evading mechanisms used by HBV are largely unknown, defects in T cell responses have been suspected as a major factor involved in the pathogenesis of chronic hepatitis B (1-6). Different from acute self-limited hepatitis in which protective immunity develops after elimination of HBV, immune responses fail to remove HBV-infected hepatocytes in chronic hepatitis B. Even more, barely detectable proliferative response of T cells to HBsAg has been consistently reported in patients with hepatitis B, whereas humoral and cellular immunity to HBsAg are easily induced in vaccined individuals (4, 6). Although the exact mechanisms of T cell tolerance to a HBsAg are unknown, patterns of cytokine production responding to HBsAg can provide a clue to understanding the pathogenic mechanisms of chronic hepatitis B. To test the hypothesis that immune responses favoring Th2 type cytokine production may lead to chronic infections with HBV, in the present study, we examined cytokine profiles associated with hepatitis B.

At first, we attempted to clone HBsAg-specific T cells from PBMCs of hepatitis B patients to analyze cytokine profiles. Unfortunately, due to the weak proliferative response of T cells obtained from the hepatitis B patients to HBsAg, we could not clone HBsAg-specific T cells (data not shown). Instead, HBsAg-reactive T cell populations were prepared using rhIL-2. When these T cell populations were tested for IFN-γ production upon HBsAg stimulation, 25% of the patients with chronic hepatitis B as well as 80% of the patients with acute hepatitis B showed IFN-γ production analysed by ELISA (Table 1). As expected, none of the HBsAg-reactive T cell populations showed significant in vitro proliferative responses to HBsAg stimulation (data not shown). This may be explained by hardly detectable production of IL-2 by these T cell populations (data not shown). This result indicates that HBsAg-reactive T cells have defects in the ability to produce IL-2 upon antigenic stimulation, but they are still able to produce other Th1 type cytokine, i.e. IFN-γ, even in chronic hepatitis B. In contrast, production of IL-4 was not detected by ELISA.

Th1 type pattern of secreted cytokines can be considered as an appropriate response of the immune system to inhibit viral replication and HBV eradication. Mechanisms by which IFN-γ favors the elimination of HBV may include enhancement of CTL activation, direct anti-viral activity, in-creased expression of MHC class I molecules on infected cells, and activation of macrophages (25-28). Anti-HBV effect of

HBV was shown by a transgenic mouse model showing that adoptive transfer of cytotoxic T lymphocytes producing IFN-γ could inhibit HBV replication without cell lysis (16).

Recently, an association of a predominant Th1 cytokine profile of HBcAg-specific T cells with acute self-limited hepatitis B was reported (17). Although T cell proliferative responses to HBsAg are not as remarkable as those to HBcAg, roles of HBsAg-reactive T cells have been implicated in viral clearance by several investigators (19-22). For example, Chisari and his colleagues reported CTL responses to epitopes of HBsAg in most patients with acute hepatitis, while there were no such responses in patients with chronic hepatitis (22). In addition, the results of our previous study showed a correlation of IFN-γ production by HBsAg-reactive T cells with acute self-limited hepatitis B (18).

Meanwhile, Barnaba et al. cloned CD4+ HBV envelope antigen-reactive T cells from chronic active hepatitis patients, and found that these T cells showed signs of cytotoxicity only when they produced IFN-γ (20). In agreement with this finding, we observed that the production of IFN-γ by PBMCs upon HBsAg stimulation was associated with higher levels of hepatocyte damage (Fig. 3). The importance of Th1 type immune responses in the development of cell-mediated immunity is also supported by a recent longitudinal study showing that serum levels of IL-12, a cytokine which is essential for the differentiation of T cells towards Th1 type, was associated with the clearance of HBV and anti-HBe seroconversion (23).

On the other hand, a cytokine balance favoring Th2 type cytokine production such as IL-4 and IL-10 has been associated with progressive virus infections. For example, the shift from Th1 to Th0 or Th2 profiles was reported in acquired immune deficiency syndrome (14, 15). Because clinically overt acute hepatitis B is generally self-limited, it is not easy to examine cytokine profiles of the initial immune response to HBV in the chronic hepatitis B patients. Therefore, the present study was performed on chronic hepatitis patients, showing variable degrees of liver damages as evidenced by increased serum AST/ALT levels.

Expression of both Th2 type cytokine(s) and Th1 type cytokine(s) was observed in approximately 50% of chronic hepatitis B patients in our study. This result is in agreement with a recent report showing that T cell populations in the liver infiltrate secreted both Th1 (IFN-γ) and Th2 cytokines (IL-4 and IL-5) (24). As the study was performed on HBV non-specific T cells in the liver of chronic hepatitis B patients, cytokine profiles of HBV antigen-specific T cells were not demonstrated. At the moment, available information about the cytokine production patterns of HBsAg-specific T cells in chronic hepatitis B is limited, because few patients and clones have been studied (20). Results of the present study indicate the existence of a Th2 type response to HBsAg in chronic hepatitis B patients and it appeared to be combined with Th1 response in the patients with more severe liver damage (Table 2).

Our results indicated that the production of Th1 cytokines could contribute to antiviral protective immunity in both acute and chronic hepatitis B. The effect of Th1 response might be insufficient for complete removal of HBV in chronic hepatitis B. There is a possibility that concomitantly produced Th2 type cytokines might counteract the antiviral effect of Th1 cytokines. However, we could not observe the protective effect of Th2 type cytokine on the hepatocytes. This may be possibly due to relatively weak T cell responses to HBsAg compared to those of HBcAg (4, 5). Unfortunately cytokine profiles of HBcAg-reactive T cells in chronic hepatitis B are not clear yet.

To investigate cytokine expression patterns on HBxAg stimulation, PMBCs from patients with chronic hepatitis B were stimulated with GST-HBx fusion protein. Although HBx protein has been reported to be immunogenic on a T cell level in acute hepatitis B (8), in our hands proliferative responses of T cells to HBxAg were hardly detectable in chronic hepatitis B (data not shown). However, by RT-PCR approximately 40% of patients showed cytokine expression response upon HBxAg stimulation analysed. There was no significant relationship between cytokine profiles and hepatocyte damage. It is likely that Th1 responses to HBxAg seems to be weaker than those to HBsAg.

In conclusion, we could not show that a predominant production of Th2 type cytokines upon HBsAg or HBxAg antigen stimulation may cause persistent infection of HBV. However, lacking data from other investigators on cytokine profiles of HBV-specific T cells, the roles of Th2 type responses in viral persistency should be further studied. As our data consistently indicated, the production of IFN-γ by HBsAg-reactive cells was associated with HBV clearance. Recently it was reported that IL-12 could induce proliferation and IFN-γ production of HBsAg-stimulated PBMCs in vitro (29). If we can find a way to induce a strong Th1 type immune response to HBsAg, successful resolution of HBV in chronic hepatitis B can be possible.

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