

CLINICAL STUDIES

Rather than interleukin-27, interleukin-6 expresses positive correlation with liver severity in naïve hepatitis B infection patients

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Abstract

Aims: Effective cytokines can drive the commitment of naïve T cells to regulate immune response after antigen-mediated activation. Aims are to elucidate the clinical role of serum IL-27 and IL-6 in the different stages of naïve hepatitis B virus (HBV)-infected patients. **Methods:** Samples with well-characterized clinical profiles were assessed from 395 HBV-infected patients including chronic hepatitis B (CHB) group in 291 patients, liver cirrhosis (LC) group in 57 patients, hepatocellular carcinoma (HCC) group in 47 patients. Another 139 non-HBV infected individuals were enrolled as control group (CG) including 104 with normal liver function (NF) and 35 with liver dysfunction (LD). **Results:** The HBV-infected group and separated groups presented significantly higher IL-27 and IL-6 expression than the CG or subgroups of CG. In contrast to IL-27, IL-6 showed significant differences with deteriorating liver condition compared with LC or HCC with CHB groups. Furthermore, IL-6, rather than IL-27, showed significant statistical differences in patients with advanced liver disease compared with those of mild or moderate to severe liver disease and in patients with terminal stage HCC compared with those of early to intermediate or advanced stage HCC. The data associated with liver function, including Albumin, Bilirubin, INR, Platelet and AFP levels, were significantly correlated to IL-6 expression, but had weak correlation to IL-27 expression in HBV patients. **Conclusion:** Serum IL-27 can trigger immune response to prevent hepatic injury in different clinical-pathologic stages of HBV-infected patients earlier, but IL-6 may play an extremely important role to determine the liver progression.

Hepatitis B virus (HBV) infection is the major contributive cause of cirrhosis and hepatocellular carcinoma (HCC) in the world (1). Although the standard therapies of HBV infection have been well-established (2, 3), the therapeutic response remains limited and is greatly influenced by the interactions of virus and host cofactors (4). Therefore, the host's immune response may play a crucial role to determine the outcome of HBV infection (5–7).

Interleukin-27 (IL-27), a heterodimeric cytokine belonging to the IL-12 family (8), consists of IL-27p28 and Epstein–Barr virus-induced gene 3 (EBI3) subunits (9, 10). It has been shown that IL-27 acts on hepatocytes

and hepatic stellate cells and that it contributes to the antiviral response in these cells (11, 12). IL-27 is produced by antigen-presenting cells (APC), which protect their local microenvironment from host pathogens, and functions on different cell types expressing the full receptor (R) complex (13–15). Anti-inflammatory function is achieved through inhibiting the development of Th1 (16, 17), Th17 (18, 19), Th2 (20, 21) and regulatory T cells (Treg) (22–24); and anti-tumour activity through indirect mechanisms by either induction of natural killer and cytotoxic T lymphocyte (CTL) response or inhibition of angiogenesis working on induction of CXCL10 and CXCL9 (25, 26). Contrast to IL-27 as protective role, IL-6, a well-recognized inflammatory cytokine, can trigger hepatocyte proliferation and liver

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regeneration. Therefore, high IL-6 levels might reflect more active hepatic necro-inflammation and be associated with the presentation and severity in HCV-infected or HCC studies (27–31).

Previous studies have investigated IL-27 or IL-6 expression associated with the clinical presentation in HBV infected patients (29, 32, 33), but the clinical relationship between IL-27 and IL-6 remains limited and needs to be further clarified in different clinical-pathologic stages of HBV-infected patients. To elucidate the clinical role and relationship of IL-27 and IL-6, we conducted a study on a larger cohort in different clinical-pathologic stages of naïve HBV-infected patients. We envisage that these findings in the current study might offer useful references in viral clearance of HBV-infected patients.

Patients and methods

Patients

A total of 534 patients, including 395 naïve HBV-infected patients and 139 non-HBV infected individuals were enrolled as control group (CG) including 104 individuals with normal liver function (NF) subgroup and 35 individuals with liver dysfunction (LD) subgroup with well-characterized clinical condition were enrolled at China Medical University Hospital, Taichung, Taiwan. The HBV-infected patients ($n = 395$) were categorized into three groups, including 291 patients in the chronic hepatitis B (CHB) group, 57 patients in the cirrhosis (LC) group and 47 patients in the hepatocellular carcinoma (HCC) group, based on clinical biochemical examination, serological diagnoses, pathology and abdomen ultrasonography or computerized tomography results.

The definitions of the three groups and their severity of HBV-infected patients were as follows: CHB group was defined as HBsAg (+) for longer than 6 months and positive anti-HBc IgG with negative anti-HBc IgM detection. The severity of parenchymal liver disease (PLD) score was classified into mild (PLD score ≤ 6), moderate (PLD score = 7), severe (PLD score = 8) and LC (PLD score > 8) by ultrasonography; or mild (fibrosis ≤ 1), moderate (fibrosis = 2) and severe (fibrosis = 3) accorded to Metavir score for liver biopsy; LC based on the definition of CHB and was further determined by: (i) ultrasonography finding combined with clinical signs with laboratory exams, including ascites, shrunken liver size, splenomegaly, jaundice, oedema, cutaneous arterial 'Spider' angiomas, palma erythema, hyperbilirubinemia, reversed levels of serum aspartate transaminase (AST) and alanine Transaminase (ALT), prolonged serum prothrombin time, decreased serum albumin, increased serum globulins, and the albumin/globulin (A/G) ratio < 1 , or (ii) liver biopsy and histopathology showed Metavir score 4. In addition, the classifications of disease severity were accorded to Child-Pugh

score; HCC based on the definition of CHB further defined by: (i) serum alpha-fetoprotein (AFP) levels greater than 200 mg/L and ultrasonography or computerized tomography showed HCC, or (ii) proved by liver biopsy. The classifications of HCC were accorded to Barcelona Clinic Liver Cancer (BCLC) staging. The presence of liver dysfunction (LD) and symptoms (Symp) was defined as: (i) in CHB group, ALT levels were higher than upper limitation of normal range (40 IU/L) or total bilirubin (Bil) levels were greater than 1.3 mg/dl mean LD; two-fold higher than 40 IU/L or total bilirubin levels were greater than 2.0 mg/dl mean Symp; (ii) in LC group, besides criteria of CHB group, either ascites, or oesophageal/gastric varices with bleeding, or hepatic encephalopathy; and (iii) in HCC group, besides criteria of CHB group, either ascites, or oesophageal/gastric varices with bleeding, or hepatic encephalopathy, or pathologically malignant cells. CG was defined as negative exams for HBsAg and anti-HCV markers with undetectable HBV DNA and HCV RNA viral loads.

In addition, the patients who had (i) co-infection or super-infection with HCV, HDV, or HIV; (ii) previous antiviral agents, i.e. interferon or nucleoside analogues, immunomodulatory or anti-tumour agent; (iii) alcoholic liver disease, autoimmune hepatitis or drug-induced liver disease; (iv) any concomitant illness, i.e. diabetes or hypertension; or (v) acute inflammation within 2 weeks, i.e. gout arthritis, were excluded from the current study.

Serological markers and liver biochemical assays methodology

Serum HBV markers, including HBsAg, anti-HBs, HBeAg and anti-HBe, were assessed by a commercial enzyme immunoassay (AxSYM; Abbott, North Chicago, IL, USA), and anti-HCV antibody was assessed by a commercial enzyme immunoassay (Abbott HCV EIA 2.0; Abbott Laboratories). Serum albumin, total bilirubin, ALT, creatinine, coagulation tests and AFP were determined using an autoanalyzer (TBA-30FR; Toshiba, Tokyo, Japan).

Estimation of serum IL-6 and IL-27

After informed consent, venous blood samples were obtained from a peripheral vein of all enrolled cases and then immediately centrifuged. The serum was stored at -80 degrees and IL-27 and IL-6 concentrations checked within 2 weeks by specific ELISA using commercially available kits (eBioscience). Results were expressed in picograms per millilitre (pg/ml) by reference to a standard curve obtained with recombinant IL-27 and IL-6.

Statistical analysis

The baseline data were expressed as the mean \pm standard deviation (Table) and mean \pm standard error devi-

ation (Figures). Each group of experiments was repeated twice to confirm the data. Correlations between continuous variables were assessed by Student *t*-test and Pearson correlation. All statistical tests were two-tailed. A *P*-value <0.05 was defined as statistically significant.

Results

The baseline characteristics of 395 naive HBV-infected patients and 139 nonHBV-infected individuals are shown in Table 1. Among the separated groups, patients in the HCC group were older than those in LC group, CHB group and CG (60.11 ± 12.39 vs. 52.91 ± 10.61 vs. 43.84 ± 12.12 vs. 43.87 ± 13.39 years respectively), which was compatible with the distribution of natural course of HBV infection.

Compared with the control group, IL-27 and IL-6 expressions showed predominance in the HBV-infected and separately HBV-infected groups

There were significantly strong IL-27 and IL-6 expressions in the HBV-infected group compared with CG or NF and DF subgroups of CG (163.36 ± 20.29 pg/ml vs. 31.12 ± 7.02 pg/ml or 24.04 ± 5.23 pg/ml or 52.14 ± 23.01 pg/ml in IL-27, *P* < 0.001; 5.62 ± 0.69 pg/ml vs. 1.16 ± 0.18 pg/ml or 0.98 ± 0.16 pg/ml or 1.69 ± 0.54 pg/ml in IL-6, *P* < 0.001 respectively) (Fig. 1a,b). Among the separated groups of HBV-infected patients, IL-27 expression was significantly higher in patients with CHB, LC, or HCC group than those with CG (159.83 ± 20.67 pg/ml vs. 31.12 ± 7.02 pg/ml, *P* < 0.001; 197.96 ± 86.49 pg/ml vs. 31.12 ± 7.02 pg/ml, *P* = 0.003; 143.27 ± 43.11 pg/ml vs. 31.12 ± 7.02 pg/ml, *P* = 0.013 respectively) or NF subgroup of CG (159.83 ± 20.67 pg/ml vs. 24.04 ± 5.23 pg/ml, *P* < 0.001; 197.96 ± 86.49 pg/ml vs. 24.04 ± 5.23 pg/ml, *P* = 0.002; 143.27 ± 43.11 pg/ml vs. 24.04 ± 5.23 pg/ml, *P* = 0.009 respectively) (Fig. 2a). IL-6 also showed higher expression in patients with CHB, LC, or HCC groups than those of CG (2.67 ± 0.35 pg/ml vs. 1.16 ± 0.18 pg/ml, *P* < 0.001; 13.71 ± 3.56 pg/ml vs. 1.16 ± 0.18 pg/ml, *P* < 0.001 respectively) or NF subgroup of CG (2.67 ± 0.35 pg/ml vs. 0.98 ± 0.16 pg/ml, *P* < 0.001; 13.71 ± 3.56 pg/ml vs. 0.98 ± 0.16 pg/ml, *P* < 0.001 respectively) (Fig. 2b).

Contrast to IL-27, IL-6 showed significant expression in patients with advanced liver disease (PLD > 8) or terminal-stage HCC

There were no significantly statistical differences in IL-27 expression between patients with mild (PLD = 5–6, *n* = 275 cases), moderate to severe (PLD = 7–8, *n* = 16 cases), and advanced (PLD > 8, *n* = 57 cases) liver disease (165.36 pg/ml ± 21.79 pg/ml vs. 64.69 pg/ml

Table 1. Disposition and baseline characteristics of non-HBV-infected (control group) and HBV-infected group (*N* = 534)

Demographics	HBV-infected group (<i>n</i> = 395)			
	Control group (<i>n</i> = 139)	CHE (<i>n</i> = 291)	Cirrhosis (<i>n</i> = 57)	HCC (<i>n</i> = 47)
Age (years) (range)	43.87 ± 13.39 (17–73)	43.84 ± 12.12 (19–82)	52.91 ± 10.61 (32–74)	60.11 ± 12.39 (29–86)
Gender (Male) (%)	79 (56.8%)	184 (63.2%)	47 (82.5%)	38 (80.9%)
Liver score (1/2/3/4)	–	275/10/6/–	0/0/57	6/1/5/35
Child-Pugh score	–	–	27/13/17	–
Biochemical values				
Albumin (g/dl)	4.34 ± 0.35 (3.6–4.9)	4.33 ± 0.44 (2.5–5.1)	3.41 ± 0.7 (2.0–4.6)	3.29 ± 0.7 (2.2–4.6)
Bilirubin (mg/dl)	0.91 ± 0.54 (0.25–5.29)	0.97 ± 0.67 (0.21–6.7)	6.12 ± 10.12 (0.52–38.82)	3.12 ± 3.7 (0.4–20.07)
Creatinine (mg/dl)	0.82 ± 0.22 (0.46–1.51)	0.82 ± 0.18 (0.48–1.28)	1.28 ± 1.25 (0.51–7.75)	0.87 ± 0.31 (0.42–1.82)
ALT (IU/L)	42.67 ± 98.09 (10–1084)	58.84 ± 165.26 (9–2040)	167.98 ± 576.4 (13–4200)	56.87 ± 37.79 (16–201)
INR	0.98 ± 0.08 (0.86–1.12)	1.05 ± 0.13 (0.86–1.7)	1.50 ± 0.76 (0.89–3.93)	1.25 ± 0.26 (1.0–2.06)
Platelet (10 ³ /μl)	223.34 ± 56.48 (94–339)	210.07 ± 64.23 (97–453)	99.04 ± 64.14 (26–387)	170.37 ± 94.71 (21–451)
AFP (ng/ml)	3.15 ± 1.97 (0.95–10.21)	5.6 ± 15.93 (0.66–189.94)	26.9 ± 80.46 (1.48–570.18)	11898.96 ± 20535.03 (2.05–54001)
IL-27 (pg/ml)	31.12 ± 82.71 (0–770)	159.83 ± 352.62 (0–3225)	197.96 ± 652.99 (0–4500)	143.27 ± 295.55 (0–1225)
IL-6 (pg/ml)	1.16 ± 2.14 (0–12.3)	2.67 ± 6.03 (0–45)	13.71 ± 26.87 (0–121.5)	14.05 ± 17.35 (0–67.5)
Virological values				
HBeAg (+) (%)	0	44 (15.1%)	7 (12.3%)	5 (10.6%)
HBV DNA (copies/ml)	0	34057946 ± 53447126.19	6204054.8 ± 21529207.77	1957158.6 ± 5487694.159

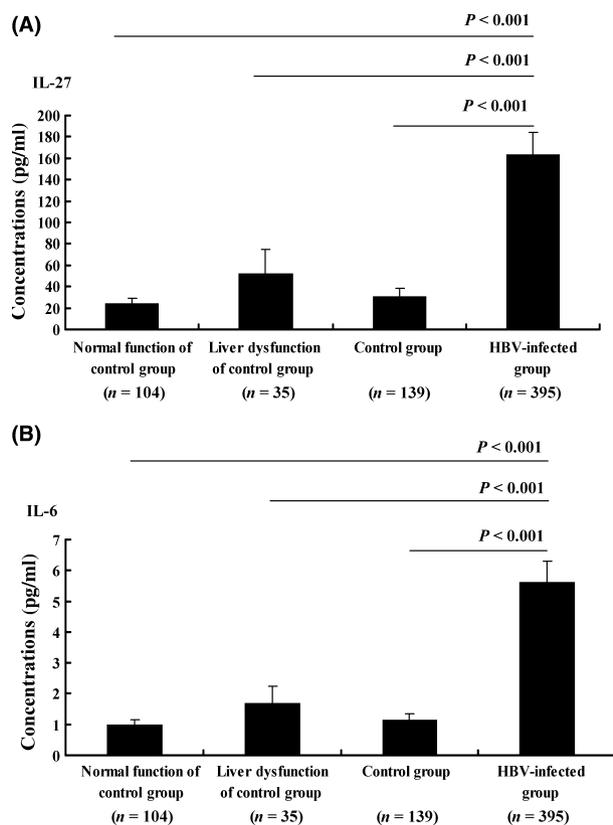


Fig. 1. (a) serum IL-27 concentrations (b) serum IL-6 concentrations in HBV-infected and control groups. * $P < 0.05$ was defined as statistically significant.

± 24.50 vs. $197.96 \text{ pg/ml} \pm 86.49 \text{ pg/ml}$ respectively). In contrast, IL-6 showed higher expression and significantly statistical difference in patients with advanced liver disease than those with mild or moderate to severe liver disease ($13.71 \text{ pg/ml} \pm 3.56 \text{ pg/ml}$ vs. $2.70 \text{ pg/ml} \pm 0.37 \text{ pg/ml}$, $P = 0.003$; $13.71 \text{ pg/ml} \pm 3.56 \text{ pg/ml}$ vs. $2.21 \text{ pg/ml} \pm 0.62 \text{ pg/ml}$, $P = 0.002$ respectively) (Fig. 3a,b). Similarly, there were no statistical differences in IL-27 expression between patients with early to intermediate ($n = 13$ cases), advanced ($n = 13$ cases) and terminal ($n = 21$ cases) stage HCC ($157.27 \text{ pg/ml} \pm 87.19 \text{ pg/ml}$ vs. $134.62 \text{ pg/ml} \pm 72.48$ vs. $139.59 \text{ pg/ml} \pm 69.16 \text{ pg/ml}$ respectively). In contrast, IL-6 showed higher expression and significantly statistical differences in patients with terminal stage HCC than those with early to intermediate stages or advanced stage HCC ($21.40 \text{ pg/ml} \pm 3.91 \text{ pg/ml}$ vs. $2.59 \text{ pg/ml} \pm 0.78$, $P < 0.001$; $21.40 \text{ pg/ml} \pm 3.91 \text{ pg/ml}$ vs. $13.63 \text{ pg/ml} \pm 5.36 \text{ pg/ml}$, $P = 0.05$ respectively) (Fig. 4a,b).

Both IL-27 and IL-6 expression were not associated with the presence of HBeAg in HBV-infected group

Neither IL-27 nor IL-6 expression was influenced by the presence of HBeAg in patients in the HBV-infected

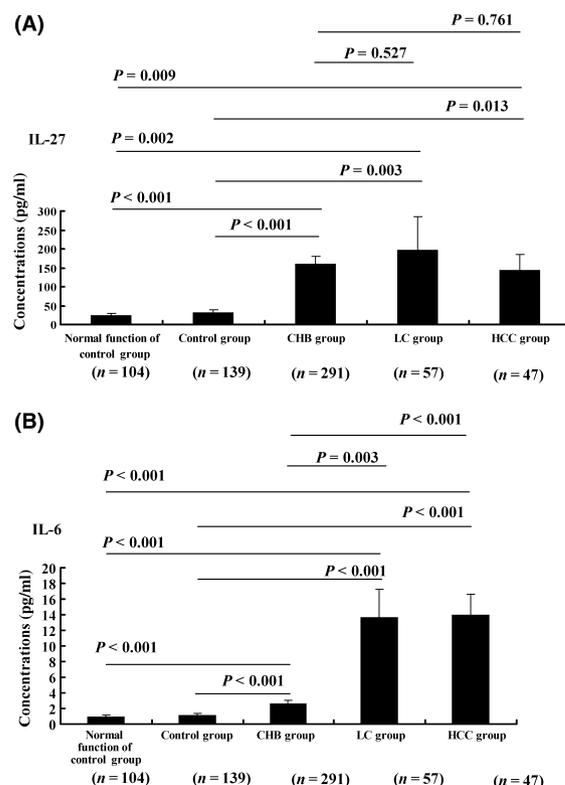


Fig. 2. According to different clinical-pathologic stages, the correlations (a) serum IL-27 concentrations (b) serum IL-6 concentrations in CHB, LC, HCC and control groups. * $P < 0.05$ was defined as statistically significant.

group ($176.45 \text{ pg/ml} \pm 41.23 \text{ pg/ml}$ vs. $161.19 \text{ pg/ml} \pm 22.65 \text{ pg/ml}$, $P = 0.794$; $4.92 \text{ pg/ml} \pm 1.55 \text{ pg/ml}$ vs. $5.73 \text{ pg/ml} \pm 0.76 \text{ pg/ml}$ vs., $P = 0.682$ respectively) or in those in non-HCC (CHB and LC) groups ($181.94 \text{ pg/ml} \pm 45.04 \text{ pg/ml}$ vs. $163.34 \text{ pg/ml} \pm 24.97 \text{ pg/ml}$, $P = 0.768$; $3.32 \text{ pg/ml} \pm 1.00 \text{ pg/ml}$ vs. $4.68 \text{ pg/ml} \pm 0.78 \text{ pg/ml}$ vs., $P = 0.483$ respectively) (Fig. 5a,b).

Increasing liver dysfunction and HBV infection compared with IL-27 influenced IL-6 expression

Furthermore, comparison of LD in the same groups, IL-27 or IL-6 showed no statistical differences in CG group [LD (+) vs. LD (-): $52.14 \text{ pg/ml} \pm 23.01 \text{ pg/ml}$ vs. $24.03 \text{ pg/ml} \pm 5.23 \text{ pg/ml}$, $P = 0.241$; $1.69 \text{ pg/ml} \pm 0.54 \text{ pg/ml}$ vs. $0.98 \text{ pg/ml} \pm 0.16 \text{ pg/ml}$, $P = 0.211$ respectively] and CHB group [LD (+) vs. LD (-): $163.36 \text{ pg/ml} \pm 34.91 \text{ pg/ml}$ vs. $157.62 \text{ pg/ml} \pm 25.61 \text{ pg/ml}$, $P = 0.893$; $2.93 \text{ pg/ml} \pm 0.61 \text{ pg/ml}$ vs. $2.51 \text{ pg/ml} \pm 0.43 \text{ pg/ml}$, $P = 0.563$ respectively]. However, the HBV-infected group showed significantly statistical difference in IL-6 [LD (+) vs. LD (-): $8.19 \text{ pg/ml} \pm 1.19 \text{ pg/ml}$ vs. $2.51 \text{ pg/ml} \pm 0.43 \text{ pg/ml}$, $P < 0.001$] not in IL-27 expression [LD (+) vs. LD (-): $168.12 \text{ pg/ml} \pm 30.48 \text{ pg/ml}$ vs. $157.62 \text{ pg/ml} \pm 25.61$

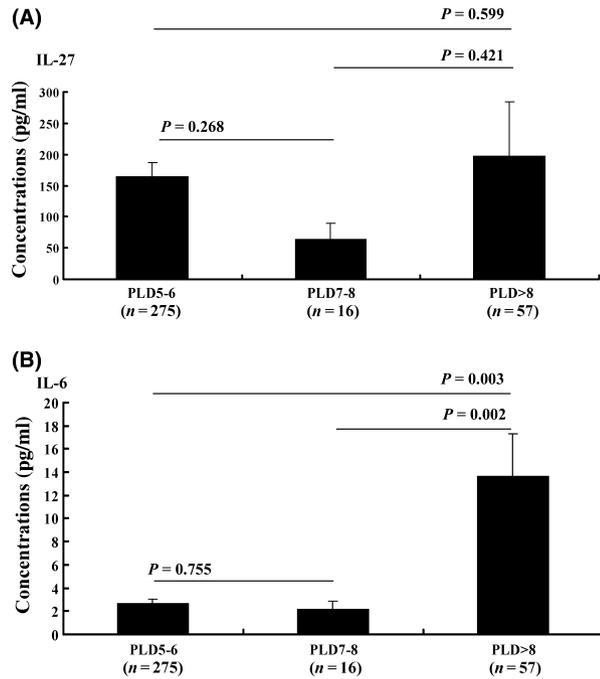


Fig. 3. According to the classifications of parenchymal liver disease, the correlations (a) serum IL-27 concentrations (b) serum IL-6 concentrations in CHB and LC patients. * $P < 0.05$ was defined as statistically significant.

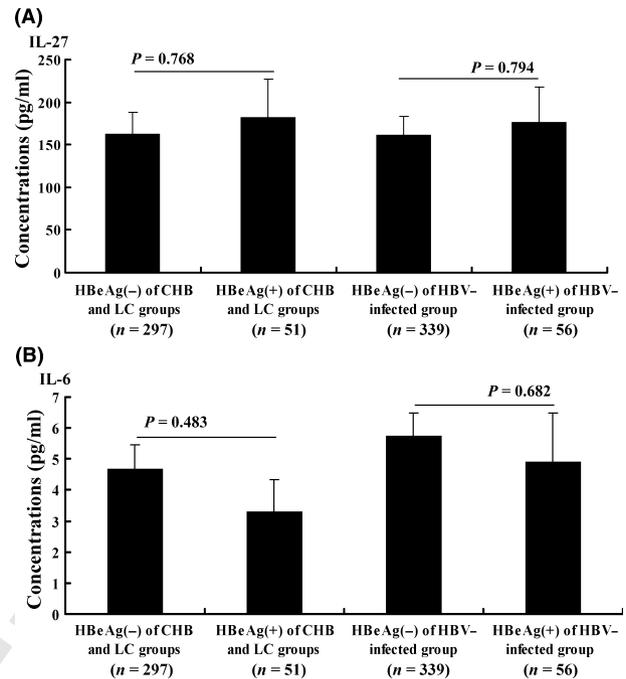


Fig. 5. (a) serum IL-27 concentrations (b) serum IL-6 concentrations in HBeAg-positive and HBeAg-negative patients.

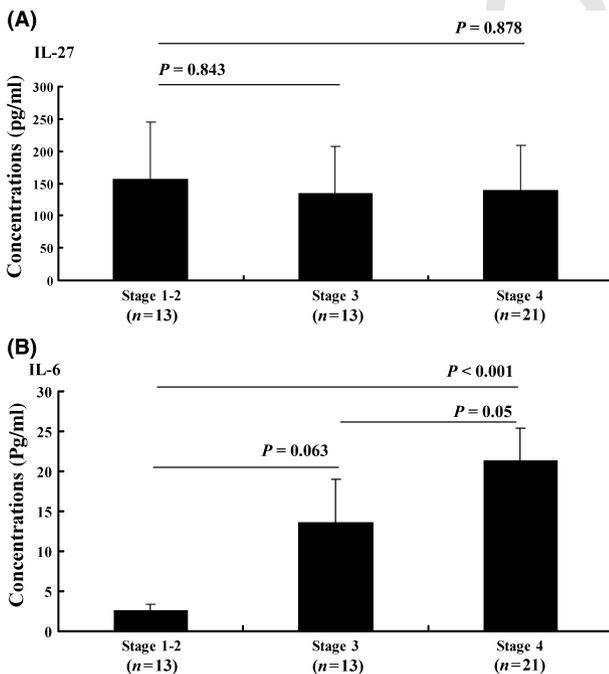


Fig. 4. According to the classifications of BCLC system, the correlations (a) serum IL-27 concentrations (b) serum IL-6 concentrations in different-stage hepatocellular carcinoma patients. * $P < 0.05$ was defined as statistically significant.

pg/ml, $P = 0.797$]. For comparison of LD in the different groups, CHB or HBV-infected groups showed significant differences in IL-27 expression compared with the CG. [LD (+): 163.36 pg/ml \pm 34.91 pg/ml vs. 52.14 pg/ml \pm 23.01 pg/ml in CHB group and CG, $P = 0.009$; 168.12 pg/ml \pm 30.48 pg/ml vs. 52.14 pg/ml \pm 23.01 pg/ml in HBV-infected group and CG, $P = 0.003$] [LD (-): 157.62 pg/ml \pm 25.61 pg/ml vs. 24.04 pg/ml \pm 5.23 pg/ml in CHB group and CG, $P < 0.001$; 157.62 pg/ml \pm 25.61 pg/ml vs. 24.04 pg/ml \pm 5.23 pg/ml in HBV-infected group and CG, $P < 0.001$] (Fig. 6a). In IL-6 expression, the HBV-infected group showed significant differences compared with the CG [LD (+): 8.19 pg/ml \pm 1.19 pg/ml vs. 1.69 pg/ml \pm 0.54 pg/ml in HBV-infected group and CG, $P < 0.001$] [LD (-): 2.51 pg/ml \pm 0.43 pg/ml vs. 0.98 pg/ml \pm 0.16 pg/ml in CHB group and CG, $P = 0.001$; 2.51 pg/ml \pm 0.43 pg/ml vs. 0.98 pg/ml \pm 0.16 pg/ml in HBV-infected group and CG, $P = 0.001$]. With deteriorating liver function (Symp), IL-27 showed no statistical differences in CG group [Symp (+) vs. Symp (-): 13.11 pg/ml \pm 10.27 pg/ml vs. 32.61 pg/ml \pm 7.51 pg/ml, $P = 0.547$], CHB group (Symp (+) vs. Symp (-): 273.91 pg/ml \pm 76.57 pg/ml vs. 138.96 pg/ml \pm 19.85 pg/ml, $P = 0.094$), and HBV-infected group [Symp (+) vs. Symp (-): 203.65 pg/ml \pm 42.54 pg/ml vs. 138.96 pg/ml \pm 19.85 pg/ml, $P = 0.17$]. However, IL-6 showed significant statistical dif-

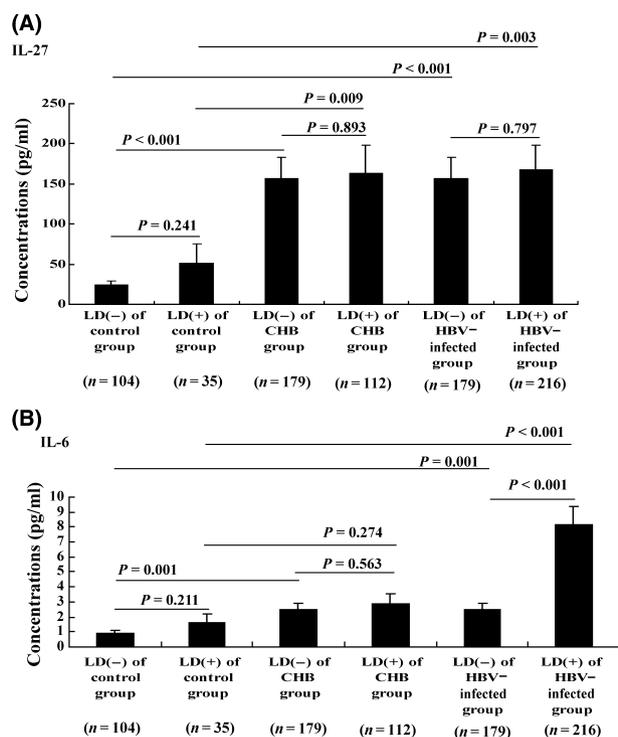


Fig. 6. According to the presence or absence of liver dysfunction, the correlations (a) serum IL-27 concentrations (b) serum IL-6 concentrations in CG, CHB and HBV-infected groups. * $P < 0.05$ was defined as statistically significant.

ferences in CHB and HBV-infected groups [Symp (+) vs. Symp (-): 4.62 pg/ml \pm 1.37 pg/ml vs. 2.31 pg/ml \pm 0.33 pg/ml, $P = 0.031$; 11.07 pg/ml \pm 1.66 pg/ml vs. 2.31 pg/ml \pm 0.33 pg/ml, $P < 0.001$]. For comparison of Symp in the different groups, CHB or HBV-infected groups showed significant differences in IL-27 expression compared with the CG [Symp (+): 273.91 pg/ml \pm 76.57 pg/ml vs. 13.11 pg/ml \pm 10.27 pg/ml in CHB group and CG, $P = 0.002$; 203.65 pg/ml \pm 42.54 pg/ml vs. 13.11 pg/ml \pm 10.27 pg/ml in HBV-infected group and CG, $P = 0.023$] [Symp (-): 138.96 pg/ml \pm 19.85 pg/ml vs. 32.61 pg/ml \pm 7.51 pg/ml in CHB group and CG, $P < 0.001$; 138.96 pg/ml \pm 19.85 pg/ml vs. 32.61 pg/ml \pm 7.51 pg/ml in HBV-infected group and CG, $P < 0.001$] (Fig. 7a). In IL-6 expression, CHB or HBV-infected groups also showed significant differences as compared with the CG [Symp (+): 4.62 pg/ml \pm 1.37 pg/ml vs. 0.36 pg/ml \pm 0.23 pg/ml in CHB group and CG, $P = 0.004$; 11.07 pg/ml \pm 1.66 pg/ml vs. 0.36 pg/ml \pm 0.23 pg/ml in HBV-infected group and CG, $P < 0.001$] (Symp (-): 2.31 pg/ml \pm 0.33 pg/ml vs. 1.22 pg/ml \pm 0.19 pg/ml in CHB group and CG, $P = 0.005$; 2.31 pg/ml \pm 0.33 pg/ml vs. 1.22 pg/ml \pm 0.19 pg/ml in HBV-infected group and CG, $P = 0.005$]. (Fig. 7b).

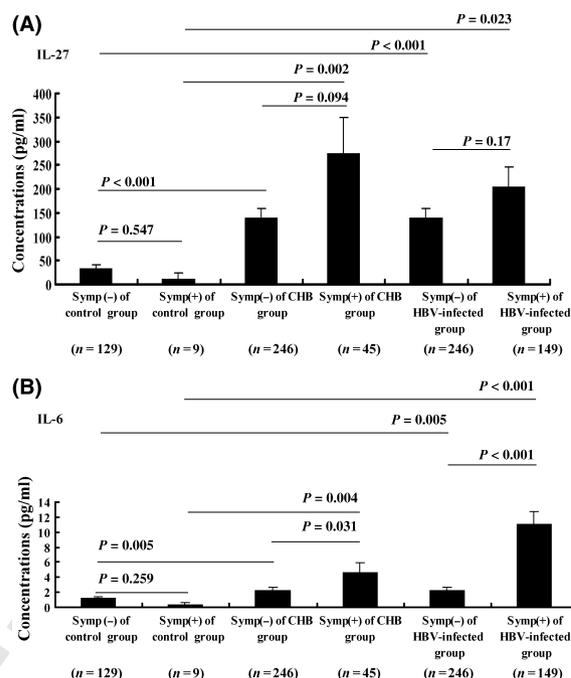


Fig. 7. According to the presence or absence of symptoms, the correlations (a) serum IL-27 concentrations (b) serum IL-6 concentrations in CG, CHB and HBV-infected groups. * $P < 0.05$ was defined as statistically significant.

Compare to IL-27, IL-6 showed significant correlation with impaired liver function in HBV-infected patients

The data are shown in Table 2.

Discussion

Although anti-HBV agents have been developed (2, 3), the therapeutic response remains limited. Of most importance, cytokine-mediated immunity linking innate and adaptive immunities in host may play a crucial role in determining the outcome of HBV infection (4–7). IL-6, a well-recognized inflammatory cytokine, might reflect more active hepatic necro-inflammation and be associated with the presentation and severity in HBV-infected or HCC studies (27–31). Compared with IL-6, IL-27 is a newly identified cytokine and can be stimulated earlier to present anti-inflammation and anti-tumour ability *in vitro* or *vivo* studies (25, 26, 32, 33). It has been shown that IL-27 acts on hepatocytes and hepatic stellate cells and that it contributes to the antiviral response in these cells (11,12). To clarify the real role and relationship of IL-27 and IL-6 in HBV-infected patients, we adopted this study in different clinical-pathologic stages of naïve HBV-infected patients.

Compatible with previous studies (29, 33), both serum IL-27 and IL-6 showed significantly higher expression in the HBV-infected group or separately

Table 2. Correlations between IL-27 and IL-6 with biochemical data in HBV infection patients ($N = 395$)

	CHB and LC groups ($n = 348$)				HBV-infected group ($n = 395$)			
	IL-27		IL-6		IL-27		IL-6	
	γ	P	γ	P	γ	P	γ	P
Albumin (g/dl)	-0.062	0.436	-0.453	<0.001	-0.038	0.654	-0.436	<0.001
Bilirubin (mg/dl)	0.194	<0.001	0.338	<0.001	0.181	<0.001	0.327	<0.001
Creatinine (mg/dl)	-0.017	0.791	0.067	0.309	-0.03	0.689	0.053	0.481
ALT (IU/L)	-0.013	0.769	0.157	<0.001	-0.024	0.642	0.155	0.002
INR	0.152	0.044	0.337	<0.001	0.134	0.08	0.32	<0.001
Platelet ($10^3/\mu\text{l}$)	-0.026	0.697	-0.159	0.018	0.015	0.848	-0.019	0.116
AFP (ng/ml)	-0.037	0.423	0.192	<0.001	-0.05	0.335	0.183	<0.001

* $P < 0.05$ was defined as statistically significant.

HBV-infected groups than in the CG or subgroups of CG (Figs 1a and 2b). However, our study found IL-27 expression did not show significantly statistical differences with deteriorating liver condition when compared LC or HCC with CHB groups (Fig. 2a). In turn, IL-6 showed significant differences with deteriorating liver condition when compared LC or HCC with CHB groups (Fig. 2b). This provides new clinical evidence that IL-27 can trigger the immune response to prevent liver from damage in an earlier phase than IL-6 in the different clinical-pathologic stages of HBV-infected patients (9, 34, 35), but IL-6 may play an important role with the progression of liver diseases. Evidence also provided by IL-6, rather than IL-27, showed significantly statistical differences in patients of advanced liver disease compared with those of mild or moderate to severe liver disease (Fig. 3a,b) and in patients of terminal stage HCC compared with those of early to intermediated or advanced stage HCC (Fig. 4a,b).

Furthermore, regardless of the LD existence or not, neither the CG, nor CHB group, or HBV-infected groups showed statistical differences in IL-27 expression in the same group. (Fig. 6a) With impairing liver function (Symp), the results were also found in IL-27. (Fig. 7a) However, IL-6 showed obvious differences not only in the HBV-infected group with LD existence but also in CHB and HBV-infected groups with deteriorating liver function (Symp) in the same group. (Figs 6b and 7b) In addition, both IL-27 and IL-6 expressions all showed significantly statistical differences in the different groups in the comparison of LD or Symp. This was compatible to our finding that IL-27 and IL-6 concentrations were induced after HBV infection as shown in Figs 1 and 2; IL-6, compared with IL-27, increased expression with the progression of liver diseases as shown in Figs 3 and 4. This also supported our finding that IL-6 was highly associated with severity of liver condition in patients of HBV infection.

Previous studies have demonstrated that serum ALT levels are decreased in IL-6 knockout mice (28), and increased IL-6 expression with impaired liver condition (30). Our study also found a positive correlation between IL-6 and ALT levels in CHB and LC groups

($r = 0.157$; $P < 0.001$) or in the HBV-infected group ($r = 0.155$; $P = 0.002$). Furthermore, our study found the data associated with impaired liver function, including Albumin ($r = -0.453$; $P < 0.001$), Bilirubin ($r = 0.338$; $P < 0.001$), INR ($r = 0.337$; $P < 0.001$), Platelet ($r = -0.159$; $P = 0.018$), and AFP levels ($r = 0.192$; $P < 0.001$) in CHB and LC groups or Albumin ($r = -0.436$; $P < 0.001$), Bilirubin ($r = 0.327$; $P < 0.001$), INR ($r = 0.330$; $P < 0.001$), and AFP levels ($r = 0.183$; $P < 0.001$) in the HBV-infected group were significantly correlated to IL-6 expression. In contrast, IL-27 expression presented weak correlations with biochemical examinations associated with impaired liver function. It again provides clinical evidence that IL-6 was highly associated with the progression of liver disease as compared with IL-27 in HBV patients.

HBeAg, an accessory protein of HBV, is regarded as probably responsible for host immunomodulation during CHB infection (36, 37). However, we observed that neither IL-27 nor IL-6 expression was influenced by the presence of HBeAg in the HBV-infected group or in CHB and LC groups (Fig. 5a,b). This might be complicated and could be contributed to by the exam timing, liver situation and viral loads.

The current study fails to demonstrate the microenvironment of liver tissues. However, this is difficult to perform, particularly for patients with normal liver condition as it needs to be based on ethical considerations and those with decompensated cirrhosis usually have a high haemorrhagic risk.

To date, many studies have demonstrated that antiviral therapy could delay progression of liver disease (38–41), but the therapeutic response remains limited and influenced by the interaction between the virus and host's immune system (5–7). However, our findings have clearly demonstrated that serum IL-27 can stimulate immune response to prevent hepatic damage earlier than IL-6 in the different clinical-pathologic stages of HBV-infected patients, but IL-6 may play an extremely important role to determine the progression of liver diseases, which may be contributive factors to modify the host's immune response and be attractive candidate agents in application of HBV-infected immunotherapy.

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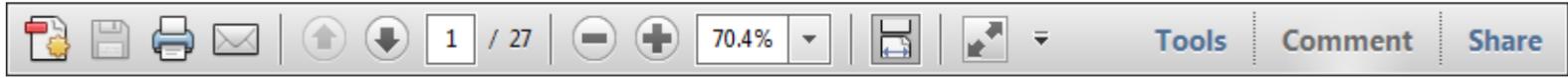
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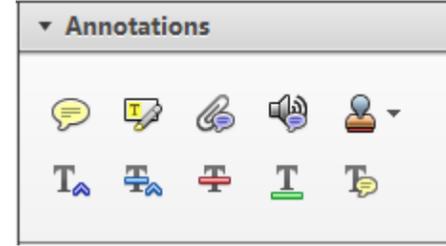
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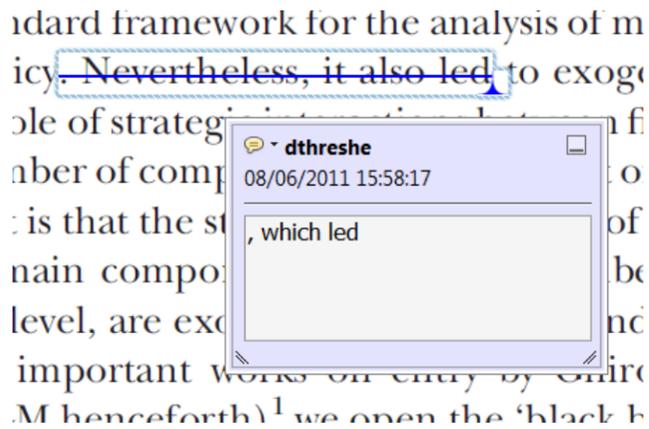
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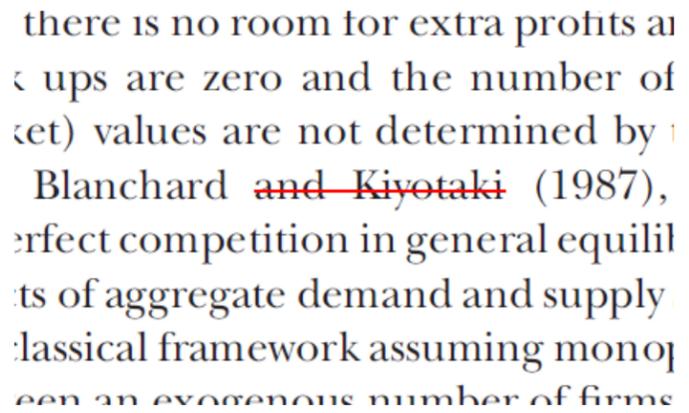
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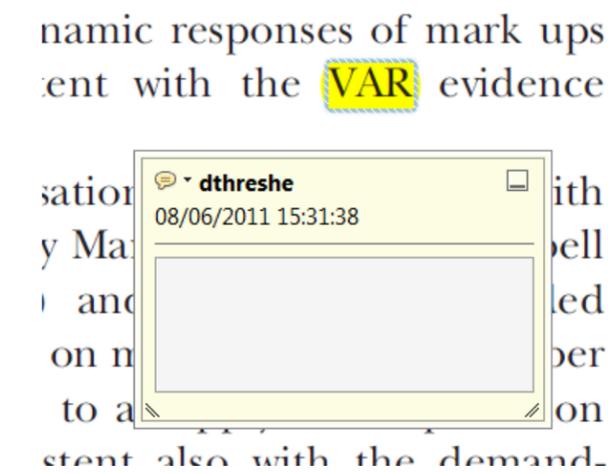
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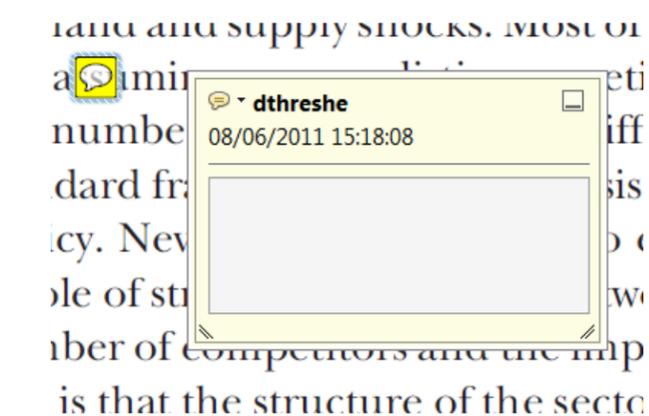
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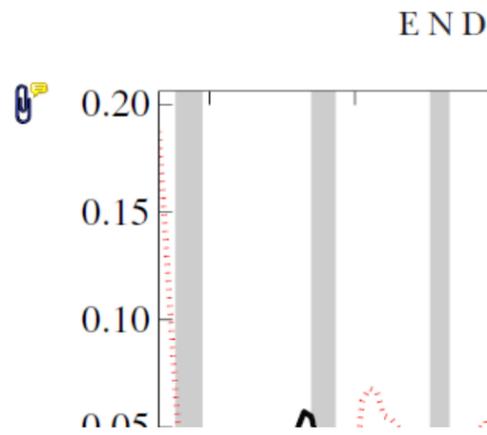
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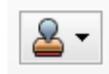
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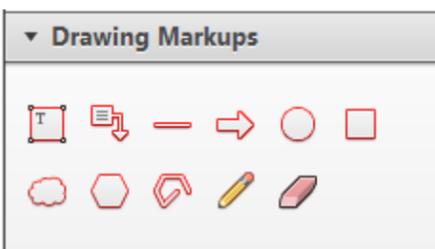


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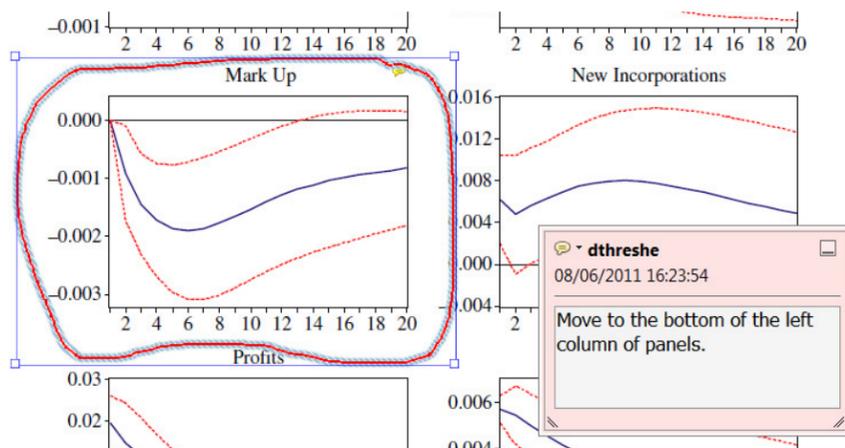


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