Analysis of Serum Cytokine Levels in Primary Biliary Cirrhosis Patients and Healthy Adults

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By using commercially available ELISA kits, serum IL-6 and TNF- α levels in healthy adults, and the levels of various cytokines in patients with primary biliary cirrhosis or chronic viral liver diseases, were investigated. IL-6 levels of healthy subjects were distributed in a wide range, and the distribution pattern was similar to those of the patients. TNF- α levels tended to be low in females in their 30s, but there were no abnormalities in the patients. Characteristic findings, in the primary biliary cirrhosis pa-

tients, were an increase of IFN- γ and IL-2 levels, and a decrease of GM-CSF levels ($P < 0.05$). IL-8 levels were higher in the patients than in the healthy subjects ($P < 0.05$), and the increase was remarkable in chronic viral liver disease patients. We believe that measurement of serum cytokine levels as a clinical immunological test is highly useful. Further development of simpler, more rapid, and more sensitive analysis methods is desired. *J. Clin. Lab. Anal.* 12:77–82, 1998. © 1998 Wiley-Liss, Inc.

Key words: cytokine; serum cytokine level; primary biliary cirrhosis; chronic liver diseases; IL-6; TNF- α

INTRODUCTION

A decade has passed since the technical term *cytokine* was first introduced into immunology. Since then, new cytokines have been identified one after another, and more than 30 cytokines have been reported. Cytokines (1) are glycoproteins; (2) act at a quite low level (pg~ng/ml); (3) mainly act in situ on the produced area; (4) individually express several physiological activities; (5) are produced by various cells; (6) express common activities; (7) sometimes act on each other synergistically or antagonistically; (8) are part of a cytokine network; (9) have inhibitors; and (10) are essential for maintaining the homeostasis of the body, but are also overexpressed under specific conditions such as inflammation, and are related to the development and progression of a disease.

In liver disease patients, cytokine levels in the serum (1–3), cytokine production levels in peripheral blood mononuclear cells (4–8), and localization of cytokines in a lesion (9,10) are quite important not only for elucidating the pathogenesis and for understanding the pathophysiology, but also for developing new therapies. Even though measurement methods are not complicated, serum cytokines have not been regarded as immunological markers for use in general laboratory tests (11).

Recently, serum interleukin 6 (IL-6) and tumor necrosis

factor alpha (TNF- α) levels are presumed to be useful markers for clinical diagnosis of inflammation at an early stage. Immune markers often change by age or gender (12). In the present study, we examined the distribution pattern of serum IL-6 and TNF- α levels in healthy subjects according to age and gender, and investigated the clinical meaning of serum cytokine measurement by examining the levels of various primary biliary cirrhosis, which is an autoimmune liver disease and is associated with various immunological abnormalities.

MATERIALS AND METHODS

Control subjects (Table 1) were all employees of Mie University School of Medicine (Controls), and consisted of 99 healthy adults for IL-6 measurements, and 96 healthy adults for TNF- α . In the measurements of other cytokines, 15–30 serum samples from the controls were examined. Peripheral blood samples were also obtained from 66 patients with chronic viral liver diseases (CVLD; disease control) and 64

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TABLE 1. Number of Healthy Subjects by Age and Gender

Age group (years)	Male	Female
20~29	14	7
30~39	11	11
40~49	14	12
50~58	9	10
60~69	8	3

patients with primary biliary cirrhosis (PBC, Table 2). Histological diagnosis of CVLD or PBC was made on liver biopsy specimens during the patients' clinical courses. All 66 patients with CVLD had experienced an increase of alanine transaminase levels for 6 months or longer. Fifty-one patients were positive for hepatitis C virus antibody and 15 patients were positive for hepatitis B surface antigen. Twenty-five patients with PBC were symptomatic (PBC-s) showing either itching or increased serum total bilirubin level (more than 2 mg/dl). Thirty-nine patients with PBC were asymptomatic (PBC-a) and did not demonstrate any objective symptoms or jaundice (13). Sera were obtained by centrifuging blood samples, and then stored at -80°C until use. Informed consent was obtained from each subject prior to participation in this study. Because each measurement required a relatively large volume of blood, subjects were excused from blood samplings when their physical condition was not good.

Serum levels of various cytokines were measured with commercially available ELISA kits, and measurements were performed according to the manufacturer's instruction. The kits for IL-6 and IL-8 were purchased from Toray Industries (Tokyo, Japan); for TNF- α , from Endogen Inc. (Cambridge, MA); for IL-1 β , IL-10, interferon (IFN)- γ , and granulocyte-macrophage colony-stimulating factor (GM-CSF), from Medgenix Diagnostics (Fleutus, Belgium); for IL-2, from Genzyme Diagnostics (Cambridge, MA); for IL-3, IL-4, IL-7, granulocyte colony-stimulating factor (G-CSF), and TNF- β , from Amersham International Plc (Buckinghamshire, UK); and for IL-5, from R&D Systems (Minneapolis, MN). Optical absorbance was measured with S-Jeia Auto-reader System (Sanko Junyaku Co., Ltd., Tokyo, Japan).

All measurements were expressed as mean $\pm$ SD. Significant analyses between age groups in the healthy controls, and between the healthy controls and each disease group, were conducted using one-way analysis of variance. When there

TABLE 2. Number of Patients with Primary Biliary Cirrhosis (PBC) by Age and Gender

Age group (years)	Asymptomatic PBC		Symptomatic PBC	
	Male	Female	Male	Female
30~39	0	1	0	3
40~49	1	7	1	8
50~59	0	11	1	7
60~69	1	14	0	4
70~79	1	3	0	1

was a significant difference, between-group analyses were performed with the Bonferroni/Dunn test (14).

RESULTS

IL-6 levels of the 99 healthy subjects were distributed in a wide range, and the mean level was 34.1 pg/ml (Fig. 1). According to age and gender, there were no significant group differences. Six male subjects, with no age relationships, showed quite high levels, over 100 pg/ml. TNF- α levels were measured in 96 healthy subjects, and the mean level was 21.3 pg/ml (Fig. 2). According to age and gender, the TNF- α levels of healthy females in their 30s tended to be lower than the others.

In comparison of cytokine levels in the 4 groups (i.e., Control, CVLD, PBC-a and PBC-s), IL-6 levels in the PBC-a group were significantly lower than in the Control ($P<0.05$, Fig. 3), while IL-6 levels were distributed in a wide range in the PBC-s group. TNF- α levels did not differ significantly between the groups, but some PBC patients had quite high levels (Fig. 4). IL-1 β levels in PBC patients varied widely, and were far lower than the minimum detectable level in approximately half of the patients (Fig. 5). IL-2 levels were abnormally high in 30~50% of PBC patients, and the PBC-a and PBC-s groups had significantly higher levels than the Control ($P<0.05$, Fig. 6). IL-8 levels in the 3 disease groups were significantly higher than in the Control: the increase in the CVLD group was remarkable, and there was a significant difference between the CVLD and PBC-a groups ($P<0.05$, Fig. 7). IFN- γ levels in the 3 disease groups were significantly higher than in the Control, and the increase was remarkable in PBC patients ($P<0.05$, Fig. 8). GM-CSF levels were undetectable in many PBC patients, and a significant difference was observed between the PBC-a and the Control, or between the PBC-s and the Control or CVLD ($P<0.05$, Fig. 9).

Serum IL-3, IL-4, IL-5, IL-7, IL-10, TNF- β , and G-CSF levels were lower than the minimum detectable level in the majority of the subjects.

Fig. 1. Distribution of serum IL-6 levels in healthy adult subjects.

Fig. 2. Distribution of serum TNF-α levels in healthy adult subjects.

DISCUSSION

In the healthy subjects, serum IL-6 levels did not differ according to age and gender, but 6 male controls presented abnormally high levels. Including these 6 males, we had monitored 20 healthy adults for 2 years following HB vaccination, and found that their IL-6 levels irregularly fluctuated in the levels below 150 pg/ml. The abnormally high levels should be interpreted carefully because IL-6 is considered to appear at a quite early stage of inflammation, and to have a circadian

Fig. 3. Serum IL-6 levels (mean ± SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD). ●: the levels of subjects. Distribution in the control group is shown in Fig. 1.

Fig. 4. Serum TNF-α levels (mean ± SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD). ●: the levels of subjects. Distribution in the control group is shown in Fig. 2.

Fig. 5. Serum IL-1β levels (mean ± SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD).

Fig. 6. Serum IL-2 levels (mean $\pm$ SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD).

rhythm such as the T lymphocyte subset does. IL-6 levels in the PBC-a group were significantly lower than in the other groups, and this was unexpected before this study. The immunological meaning of these low levels should be investigated in future studies.

Fig. 7. Serum IL-8 levels (mean $\pm$ SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD).

Fig. 8. Serum IFN- γ levels (mean $\pm$ SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD).

TNF- α levels did not differ according to age and gender, but many female controls in their 30s showed abnormally low levels. The meaning of this finding is also unknown, but it might be related to changes in hormone balance due to delivery or other reasons. With the kits used in the present study, the TNF- α levels of the healthy controls and the patients did not differ significantly; however, when we tried TNF- α measurement using ELISA kits from Medgenix in patients with acute viral hepatitis, chronic viral hepatitis, viral liver cirrhosis, or hepatocellular carcinoma (HCC), and in healthy controls; TNF- α levels in all the patients were significantly higher than in the controls (data not shown), and remarkably and abnormally high levels in HCC patients were significantly higher than the other disease patients. Even though we previously reported that the TNF- α production capacity in peripheral blood mononuclear cells of HCC patients increased (8), it is unknown whether our present and previous findings demonstrate the activation of the defense mechanism of the body against cancer cells.

In the examination of other cytokines, PBC patients showed significantly high IFN- γ and IL-2 levels. The re-

Fig. 9. Serum GM-CSF levels (mean $\pm$ SD) in patients with asymptomatic primary biliary cirrhosis (PBC-a), symptomatic primary biliary cirrhosis (PBC-s), or chronic viral liver diseases (CVLD).

relationship between IFN- γ and autoimmune diseases has been well reported (15,16). IFN- γ and IL-2 are not produced in monocyte/macrophage, but mainly in T lymphocyte. Because the pathology of PBC is presumed to be related to abnormal activation of T lymphocyte (7,17,18), increased serum levels of these cytokines would have an important meaning.

IL-1 β and GM-CSF levels were lower in PBC patients. These cytokines are produced mainly in macrophage. In PBC, it is possible that T lymphocyte function is abnormally activated, while macrophage function is suppressed.

IL-8 levels were significantly higher in the PBC patients than in the controls, but the levels in the CVLD patients were even higher. We also examined the IL-8 levels in patients with acute viral hepatitis, chronic viral hepatitis, viral liver cirrhosis, or HCC, as we did for TNF- α , and found significantly higher levels in all patients as compared to the healthy controls (data not shown). Therefore, IL-8 and TNF- α in the serum would be useful markers for HCC diagnosis, such as AFP and abnormal prothrombin (19).

In the present study, serum levels of IL-3, IL-4, IL-5, IL-7, IL-10, TNF- β and G-CSF were lower than the minimum detectable levels. However, with the continuous progress in analysis technology, novel and highly sensitive kits for quicker examinations have been introduced. Because serum cytokine measurement is useful both clinically and immunologically, we believe that the meaning of these cytokine measurements will be established in the near future with the development of new test methods.

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