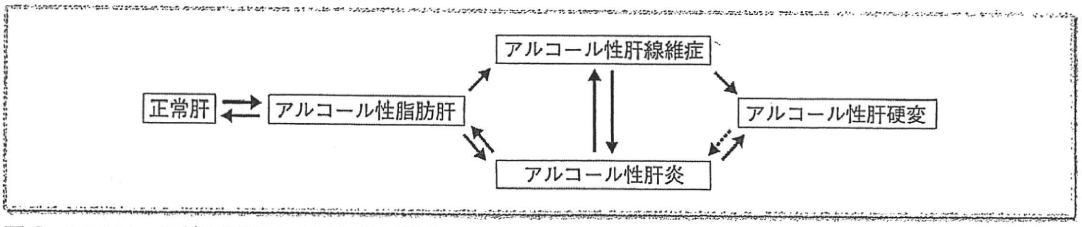

図2 アルコール性肝障害の病態と進展様式

り，アルコール性肝炎のような肝細胞の炎症や壊死がなくても，肝臓内の線維化が亢進する．

◉病態分類

病態分類には下記の4つがあり，その進展様式は，潜在性に脂肪肝から肝線維症を経て肝硬変にいたる場合と，多量・持続飲酒による急性肝細胞壊死が加わったアルコール性肝炎を経て肝硬変にいたる場合がある（図2）．わが国では欧米に比べて前者を多く認める．

a. アルコール性脂肪肝

NADH増加に伴う脂質代謝異常によりβ酸化が滞り，脂肪酸の増加とグリセロール三リン酸の合成が亢進，中性脂肪が産生過剰となり肝臓に蓄積し脂肪肝が生じる．

b. アルコール性肝線維症

アルコール性肝障害では炎症がなくても線維化が起こり，中心静脈周囲に進展する線維化と個々の肝細胞を囲むように進展する線維化がある．

c. アルコール性肝炎

多量のアルコール摂取が持続したときに発症する肝細胞の変性・壊死である．小葉中心部に肝細胞の膨化（風船化）と壊死が起こり，マロリー（Mallory）小体や多核白血球の浸潤を認める．

d. アルコール性肝硬変

初期は小葉中心性に変化が起こり，ウイルス性とは異なり比較的均一な小結節性肝硬変像を呈する．しかし，経過とともに大結節性肝硬変像もみられるようになる．

◉診断のすすめ方

本症は飲酒歴と肝障害があり，原因としてほかの肝疾患が否定されれば診断される．しかし，アルコール多飲者は飲酒期間，飲酒量ともに過小申告することが多く，飲酒歴の聴取時は本人ばかりでなく家族や知人からの聴取も重要である．また，実際の臨床の場では，ウイルス性肝炎などほかの肝疾患患者に飲酒歴が重なると診断は困難となる．そこで，アルコール性肝障害の診断基準が提唱されており，この基準をもとに診断を行う（表1）．

a. 症状・身体所見

脂肪肝や肝線維症では無症候性のことが多いが，アルコール性肝炎では急激な飲酒量の増大に伴い全身倦怠感や腹部膨満感が出現し，発熱，腹痛，嘔吐，下痢を認めることもある．肝硬変に進行すると，黄疸，浮腫，腹水，肝性脳症による意識障害を認める．

b. 血液検査

血液生化学検査の特徴はAST優位のトランスアミナーゼの上昇とγ-GTPの上昇である．これらの上昇は禁酒によりすみやかに改善する．末梢血では大赤血球症が高頻度に出現し，巨赤芽球性貧血や鉄芽球性貧血を認めることもある．肝硬変に進展すれば，アルブミンやプロトロンビン時間の低下，ビリルビン値の上昇を認める．

c. 画像検査

脂肪肝は，腹部超音波検査で肝腎コントラスト明瞭化，肝輝度の上昇などとして検出され(☞127頁，図1b)，腹部CTでは肝脾CT値比が低下する．アルコール性肝炎では肝腫大や脾腫を，肝硬変になると肝萎縮，腹水などを認める．

◉主な治療法

要因は過剰の飲酒であり栄養障害を伴っていることが多く，治療の基本は禁酒と食事療法である．アルコール性肝炎や肝硬変，ウイルスマーカー陽性の場合は絶対的な禁酒が必要となる

表1　アルコール性肝障害の診断基準

概念：「アルコール性」とは，長期（通常は5年以上）にわたる過剰な飲酒が肝障害の主な原因と考えられる病態で，以下の条件を満たすものを指す．
A. アルコール性
1. 常習飲酒家（日本酒に換算して1日平均3合以上），または大酒家（日本酒に換算して1日平均5合以上，5年間以上継続）である．ただし，女性の場合は上記飲酒量の2/3程度とする．また，ALDH2活性欠損者（ALDH2遺伝子のヘテロ接合者）では，3合以下の飲酒でも，アルコール性肝障害を生じ得る． 2. 禁酒により血清AST（GOT），ALT（GPT）活性がともに明らかな改善を示し，4週以内にほぼ正常値（80単位以下を目安とするが，禁酒前の値が100単位以下と50単位以下を目安とする）にまで下降する．ただし，重症型アルコール性肝炎，肝がん（細小肝がんは除く）合併例は例外とする． 3. 肝炎ウイルスマーカー（HBs抗原，HCV関連抗体）は陰性である．なお，HCV-RNAが陰性であれば確実である． 4. 次の検査のうち，少なくとも1つが陽性である． 1）禁酒により腫大していた肝臓の著明な縮小，4週間後にはほとんど肝腫大を認識できなくなる（肝下縁の確認は，弱打診か，超音波断層で行うことが望ましい）．ただし，重症型アルコール性肝炎と大きな肝がん合併例での肝腫大，および肝硬変例での正中線上での触知は例外とする．肝の縮小は禁酒後早期（1週以内）で著明なので，禁酒直後の検索が重要である． 2）禁酒による血清γ-GTP活性の明らかな低下（4週間後の値が正常上限の1.5倍以下，または禁酒前の値の40%以下までの下降を目安とする）． 5. なお，以下のアルコール性肝障害に特異的と考えられるマーカーが検索されて，そのいずれかが陽性の場合は，診断はより確実となる． 1）血清トランスフェリンの微小変異が陽性 2）CTスキャンで測定した肝容量が増加（単位体表面積あたり720 cm^3 以上）．ただし，非代償性肝硬変，肝がん合併例は除外 3）アルコール肝細胞膜抗体が陽性 4）血清GHDとOCT活性がともに異常高値を示し，その比（GDH/OCT）が0.6以上である．
B. アルコール＋ウイルス性
肝炎ウイルスマーカー（HBs抗原，HCV関連抗体，またはHCV-RNA）が陽性で，禁酒後AST，ALTの変化を除き上記の条件を満たす場合には，その病因は「アルコール＋ウイルス性」である．禁酒後のAST，ALTの値の明らかな低下については，禁酒4週間後の値がともに，120単位以下を目安とする．ただし，禁酒前の値が120単位以下の例では70単位以下を目安とする．
C. そのほか
上記の条件を満たさない場合には，大酒家であっても「アルコール性」，ないしは「アルコール＋ウイルス性」と診断することは現時点では困難である．ただし，禁酒後の変化が十分に追跡できなくとも，アルコール性肝障害に典型的な組織像が得られた場合には「アルコール性」，ないしは「アルコール＋ウイルス性」と診断する．

［文部省総合研究A高田班：アルコール性肝障害の診断基準．肝臓34：888-896, 1993より引用］

が，脂肪肝や肝線維症では，自己管理能力があれば1日1合程度の飲酒は許容されるという考えもある．

栄養障害に対しては，高タンパク食，高ビタミンが重要である．随伴症状として脱水があれば輸液を，低タンパク血症や腹水を認める場合はアルブミン製剤や利尿薬を投与する．

H. 薬物性肝障害

◎薬物性肝障害とは

薬物性肝障害（drug-induced hepatopathy）は，薬物によって肝細胞障害もしくは胆汁うっ滞が生じる病態である．

◎発生機序からの分類

一般に，薬物は主として肝臓内のミクロゾームに存在するチトクロームp-450等の代謝酵素系によって代謝される．

薬物性肝障害は，発生機序から中毒性肝障害と特異体質による肝障害に分類される．日常の臨床で遭遇する大部分は後者である．

a. 中毒性肝障害

薬物の直接作用ないし薬物代謝異常によるもので，投与量に比例した肝障害が起こり，発症は急激で動物実験にて再現することが可能である．

MAJOR ARTICLE

Association of Serum Cytokine Levels With Treatment Response to Pegylated Interferon and Ribavirin Therapy in Genotype 1 Chronic Hepatitis C Patients

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Background. We sought to clarify the associations among serum cytokines, amino acid substitutions in the interferon sensitivity–determining region (ISDR) and core region, and treatment outcome of pegylated interferon and ribavirin therapy in genotype 1 hepatitis C virus (HCV)-infected patients.

Methods. We quantified a total of 8 serum cytokines before, during, and after treatment in 79 genotype 1 chronic HCV patients. Viral ISDR and core region variants were determined by direct sequencing.

Results. High levels of interleukin (IL)-12 and IL-18 and more than 2 mutations in the ISDR were associated with a sustained virological response (SVR). Conversely, high baseline IL-10 levels and glutamine at amino acid 70 of the HCV core protein (Gln70) were significantly associated with a nonresponse to treatment, and patients with Gln70 had significantly higher IL-10 levels. In multivariate analysis, low IL-10, high IL-12, and high IL-18 levels were independently associated with an SVR. These 3 cytokine levels were decreased from baseline levels 4 weeks into treatment and remained low in patients with an SVR.

Conclusion. Serum IL-10, IL-12, and IL-18 levels are predictive of the response to HCV treatment with pegylated interferon and ribavirin and are associated with amino acid substitutions in the ISDR and core region.

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. More than half of patients with acute HCV infections develop chronic hepatitis, which leads to liver cirrhosis or hepatocellular carcinoma (HCC) in at least 20% of cases [1, 2]. HCC is ranked fourth in men and fifth in women as a cause of death from malignant neoplasms in Japan [3, 4]. Because approximately 70%–80% of Japanese HCC patients are infected with HCV, viral eradication is important to decrease the incidence of HCC. Interferon-based therapy can reduce HCV to undetectable levels and improve prognosis. The primary aim of antiviral therapy in HCV patients is a sustained virological response (SVR), which is defined as undetectable serum HCV RNA 24 weeks after completion of therapy. Despite recent advances, however, approximately 50% of patients with genotype 1 HCV infection do not achieve an SVR by antiviral therapy [5, 6].

Cytokines play an important role in the pathogenesis, progression, and treatment outcome of HCV infection. Because the control of cytokine production is highly complex and the effects of cytokines are widespread throughout multiple regulatory networks, it would seem that screening for multiple biomarkers could best clarify the immunopathogenesis of the disease and predict responses to antiviral therapy. However, such analysis is difficult using enzyme-linked immunosorbent assay, which requires each biomarker be tested individually. In

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this study, we used a new broad-spectrum bead-based multiplex immunoassay to simultaneously test multiple factors in the sera of patients with chronic hepatitis C. Wan et al recently reported that some cytokines are elevated in non-SVR HCV patients using this bead system, but only 17 patients with genotype 1 were evaluated [7]. Thus, the association between multiple cytokines and treatment outcome are largely unknown.

The objective of this study was to determine which cytokines in patients with genotype 1 chronic hepatitis C relate to the clinical and virologic characteristics of hepatitis and how they affect the HCV response to pegylated interferon (PEG-IFN) and ribavirin therapy.

PATIENTS AND METHODS

Participants

We included 79 consecutive patients with genotype 1 chronic hepatitis C in this study. We based diagnosis of chronic hepatitis C on the following criteria, as reported previously [8]: 1) presence of serum HCV antibodies and detectable viral RNA; 2) absence of detectable hepatitis B surface antigen; and 3) exclusion of other causes of chronic liver disease. No patient had a history of or developed decompensated cirrhosis or hepatocellular carcinoma. The baseline characteristics of patients are shown in Table 1. We used a group of 26 healthy individuals with normal transaminase levels and negative serologic results for hepatitis B and hepatitis C as the control. All participants were negative for the antibody to the human immunodeficiency virus. The protocol of this study was approved by the ethics committee of the Shinshu University School of Medicine, and all patients provided written informed consent.

Laboratory Testing

We measured antibodies to HCV in serum samples via third-generation enzyme-linked immunosorbent assays (EIA-3; Abbott Laboratories). We determined serum levels of HCV RNA using the COBAS AMPLICOR assays (Roche Diagnostic Systems), which amplify HCV RNA by reverse transcriptase–polymerase chain reaction. The lower limit of the assay was 50 IU/mL. We determined HCV genotypes using INNO-LiPA HCV II (Innogenetics). We found that all patients in our test cohort were infected with genotype 1b. We performed alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical tests using standard methods [9].

Antiviral Therapy

All patients received body weight–adjusted PEG-IFNα-2b (PegIntron, Schering-Plough K.K.; ≤45 kg, 60 μg/dose; 46–60 kg, 80 μg/dose; 61–75 kg, 100 μg/dose; 76–90 kg, 120 μg/dose; ≥ 91 kg, 150 μg/dose), and ribavirin (Rebetol, Schering-Plough K.K.; ≤60 kg, 600 mg/day; 61 kg–80 kg, 800 mg/day; ≥81 kg, 1000 mg/day) for 48 weeks, as reported previously [10].

Definition of Viral Kinetic Response and Treatment Outcome

An early virological response (EVR) was defined as undetectable serum HCV RNA by 12 weeks of therapy. An SVR was classified as serum HCV RNA that was undetectable 24 weeks after completing therapy. Post-treatment relapse was defined as a reappearance of serum HCV RNA after treatment in patients whose HCV RNA level was undetectable during or at the completion of therapy. A nonresponse was defined as a decrease in HCV RNA of <2 log copies/mL at week 12 and detectable HCV RNA during the treatment course.

Detection of Amino Acid Substitutions in the Core and NS5A Regions

We determined the sequence of 1–191 amino acids (aa) in the core protein of genotype 1b HCV, and we evaluated substitutions at aa70 of arginine (Arg70) or glutamine (Gln70) [11] with the use of HCV-J as a reference [12]. We also determined the sequence of 2209–2248 aa in the NS5A region of genotype 1b HCV containing the interferon sensitivity–determining region (ISDR), and the number of aa substitutions in the ISDR was defined as wild-type (0), intermediate-type (1), or mutant-type

Table 1. Demographic and Clinical Characteristics of Patients with Hepatitis C Virus Infection

Characteristics	All (n = 79)	SVR (n = 31)	Non-SVR (n = 48)	P
Median age, y (range)	60 (17–74)	56 (28–72)	61 (17–74)	0.08
Male, n (%)	40 (51)	23 (74)	17 (35)	0.001
Median values (range)				
ALT, IU/L (range)	54 (22–389)	53 (24–172)	61 (22–389)	0.25
AST, IU/L (range)	44 (20–288)	36 (21–133)	48 (20–288)	0.012
HCV RNA, 10^5 IU/mL(range)	17 (1.1–51)	15 (1.1–50)	19 (2.2–51)	0.13
Substitutions				
Core aa 70(Arg70/Gln70)	47/28	22/6	25/22	0.028
ISDR of NS5A(wild/intermediate/mutant)	46/17/13	13/7/9	33/10/4	0.026

NOTE. HCV, hepatitis C virus; SVR, sustained virological response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; aa, amino acid; ISDR, interferon sensitivity–determining region.

(≥2) [13]. We determined all aa substitutions in the core region and ISDR by direct sequencing.

Detection of Cytokines

We quantified 8 cytokines (interleukin [IL]-2, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IL-18, and vascular endothelial growth factor [VEGF]) using Luminex Multiplex Cytokine Kits (Procarta Cytokine assay kit) for serum samples obtained before the start of treatment, 4 weeks after the start of treatment, and 24 weeks after treatment completion. All collected samples were immediately stored at −70°C and remained in storage until testing.

Statistical Analysis

We used the Mann–Whitney *U* test and Kruskal–Wallis test to analyze continuous variables where appropriate. We used the Friedman test to evaluate changes in serum cytokine levels over time. We used the Spearman rank correlations to evaluate the relationship between pairs of markers. We used the χ^2 test with the Yates correction for the analysis of categorical data. In cases where the number of participants was <5, we used the Fisher exact test. We considered a *P* value of ≤.05 statistically significant. To predict treatment outcome, cutoff points for continuous variables were decided by receiver-operating characteristic (ROC) curve analysis. Multivariate analysis was performed using a stepwise logistic regression model. Statistical analyses were performed using SPSS software version 18.0J.

RESULTS

Detection and Quantification of Serum Markers in Patients with Chronic Hepatitis C and Controls

Of the 79 patients receiving PEG-IFN and ribavirin therapy, 31 (39%) were sustained responders with accompanying normalization of ALT levels. Of the 48 patients without an SVR, 23 had a relapse and 25 did not respond to treatment. Patients with an SVR had a higher male ratio compared with patients without ($P = .001$) (Table 1). Before treatment, the median AST level in the SVR group was significantly lower than that in the non-SVR group (36 vs 48 IU/L; $P = .012$). Substitutions of aa 70 in the core region ($P = .028$) and in the ISDR ($P = .026$) were both significantly associated with treatment outcome.

Serum samples obtained prior to antiviral therapy were examined for the presence of 8 cytokines by multiplex assays. Of these, 6 could be reliably quantified in a large majority of samples. As shown in Figure 1, the median baseline serum concentrations of 4 cytokines [IL-10 (4.8 vs 4.3 pg/mL; $P = .032$), IL-12p40 (20.4 vs 8.5 pg/mL; $P < .001$), IL-12p70 (12.8 vs 1.0 pg/mL; $P < .001$), and IL-18 (21.9 vs 14.5 pg/mL; $P = .008$)] were significantly higher in patients with HCV infection than in healthy controls. Conversely, serum levels of IL-4 (7.3 vs 7.9 pg/mL; $P = .011$) and VEGF (57.5 vs 78.0 pg/mL; $P = .025$) were significantly lower in patients with HCV infection compared with those in controls.

Effects of Antiviral Therapy on Serum Cytokine Levels

The median baseline serum levels of 4 cytokines (IL-12p40 [24.1 vs 17.2 pg/mL; $P = .003$], IL-12p70 [15.9 vs 12.6 pg/mL; $P < .001$], IL-18 [27.9 vs 17.7 pg/mL; $P = .001$], and VEGF [93.0 vs 39.7 pg/mL; $P < .001$]) were significantly higher in patients who achieved an SVR than in those who did not (Figure 2). In contrast, SVR patients showed significantly lower baseline IL-10 concentrations (4.1 pg/mL) than non-SVR patients (7.3 pg/mL; $P = .002$).

Significantly higher baseline levels of 3 cytokines (IL-4 [7.8 vs 7.0 pg/mL; $P = .001$], IL-12p40 [24.1 vs 14.6 pg/mL; $P < .001$], and VEGF [65.5 vs 43.0 pg/mL; $P = .025$]) were observed in patients with a virological response compared with levels in those without. Conversely, IL-10 levels (4.3 vs 7.9 pg/mL; $P < .001$) were significantly lower in virological responders compared with that in nonresponders.

Several demographic (age and sex) and clinical (ALT level, AST level, and viral load) findings were examined for their correlation with serum cytokines in patients with HCV infection, but no significant associations were observed. However, serum IL-12p40 levels were significantly correlated with serum IL-18 ($P = .004$, $r = 0.325$) (Figure 3A) and VEGF ($P = .024$, $r = 0.253$) (Figure 3B). There was also a significant correlation between IL-18 and VEGF ($P < .001$, $r = 0.394$) (Figure 3C).

Prediction of Treatment Outcome in Patients with Chronic Hepatitis C

We performed ROC curve analyses to determine the optimal cutoff values for serum cytokines in predicting treatment outcome for genotype 1 HCV–infected patients. We obtained the ROC curve for serum IL-10 via calculations using the values obtained from 25 nonresponders and 54 patients with a virological response. The ROC curves for serum IL-12p40, IL-18, and VEGF were obtained from 31 patients who achieved an SVR and 48 non-SVR patients. We selected optimal cutoff point values based on the cytokine level at which accuracy was maximal. The optimal cutoff value, sensitivity, specificity, positive predictive value, negative predictive value, and calculated area under the curve (AUC) for the 4 cytokines are listed in Table 2. The AUC values were consistently high and ranged between.70 (IL-12p40) and.86 (IL-10).

In addition, ROC curves for serum IL-10, IL-12p40, IL-18, and VEGF at 4 weeks after the start of treatment were obtained (Table 2). The AUCs for these 4 cytokines (.62–.86) were also high, but lower than those at baseline.

Correlation Between Core Region and Interferon Sensitivity–Determining Region Amino Acid Substitutions and Cytokine Production.

Because core region and ISDR substitutions have been associated with treatment outcome both in this study and elsewhere, we analyzed whether substitutions in these regions were correlated with baseline serum cytokine concentrations as

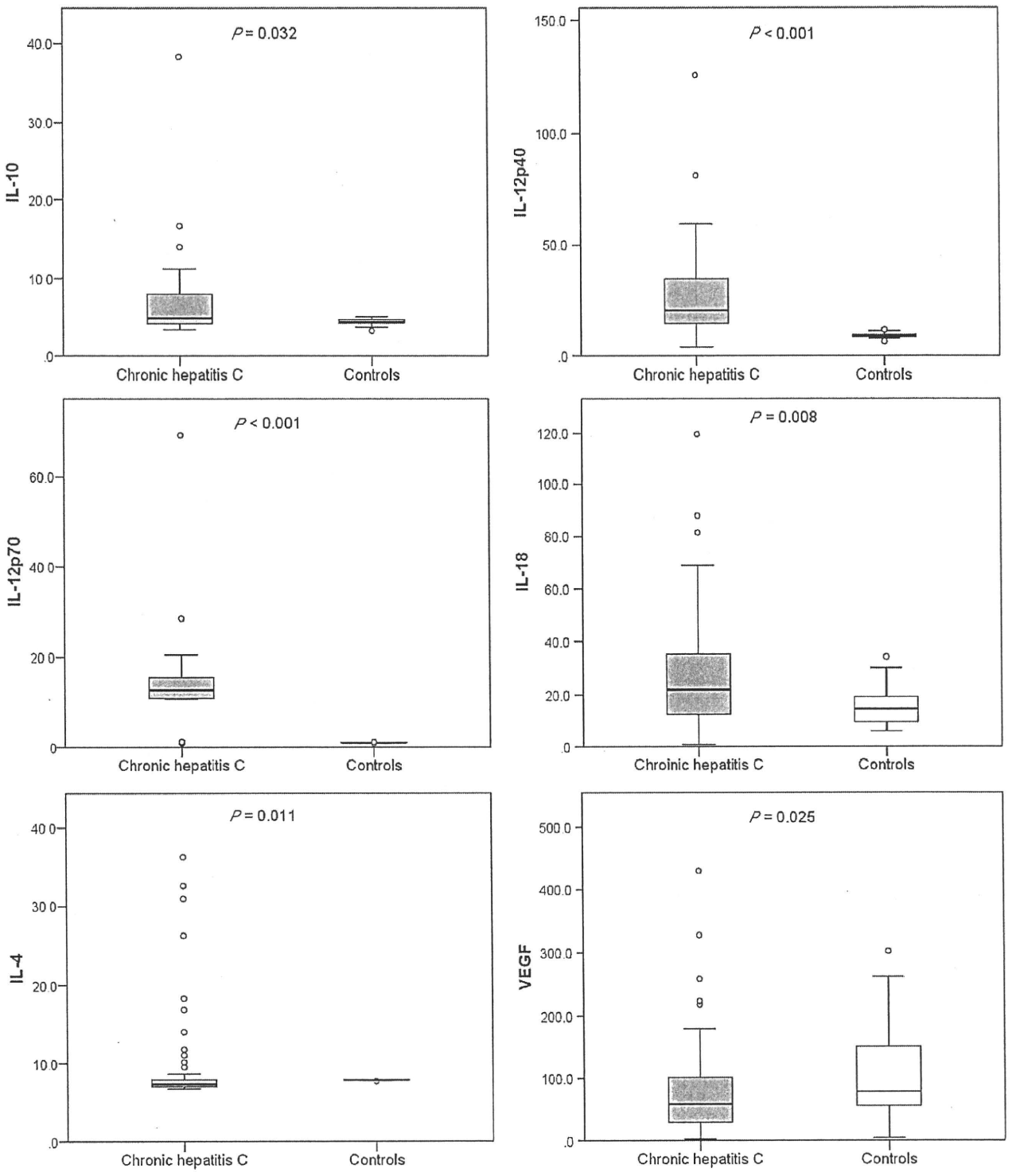

Figure 1. Detection of Serum Cytokines in Patients with HCV Infection and Healthy Subjects. Boxes represent the interquartile range of the data. The lines across the boxes indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. Serum IL-10, IL-12p40, IL-12p70, IL-18, IL-4, and VEGF levels were detected in 79 patients with HCV infection and 26 controls. **NOTE.** HCV, hepatitis C virus; IL, interleukin; VEGF, vascular endothelial growth factor.

well. Before treatment, median IL-10 levels in patients with Gln70 (7.5 pg/mL) were significantly higher than those in patients with Arg70 (4.3 pg/mL; $P = .045$). The prevalence of higher serum IL-10 (≥5.0 pg/mL at baseline) was significantly greater in the nonresponse group than in the response group (25 of 25 patients [100%] vs 11 of 50 [22%]; $P < .001$). The frequencies of the combination of higher IL-10 and HCV with and without core Gln70 were 14 of 25 patients (56%) and 3 of 50

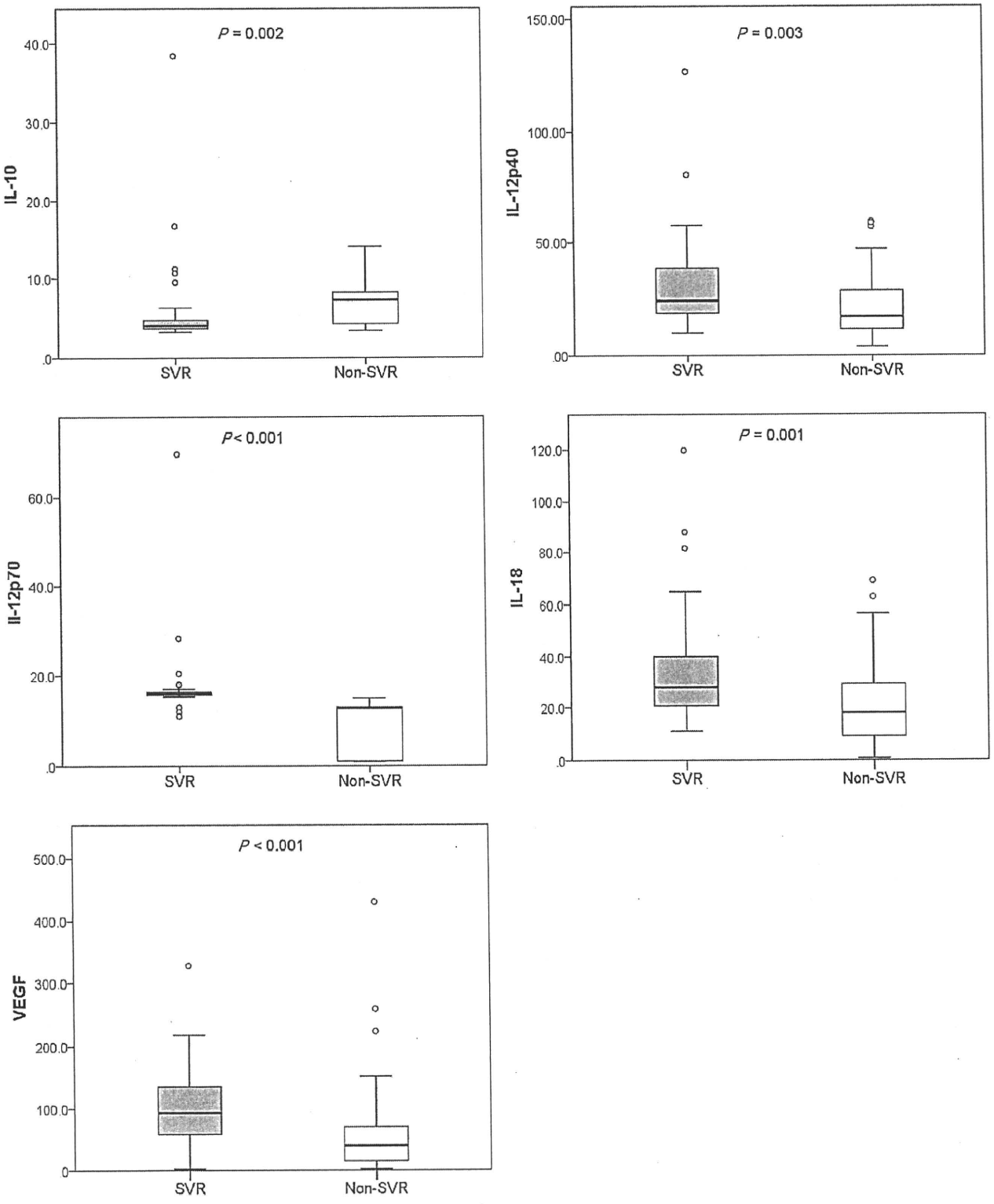

Figure 2. Serum Cytokines Related to Antiviral Therapy Outcome. Boxes represent the interquartile range of the data. The lines across the boxes indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. (A) Serum IL-10, IL-12p40, IL-12p70, IL-18, and VEGF were detected in 31 patients who achieved a sustained virological response and 48 patients who did not. **NOTE.** SVR, sustained virological response; IL, interleukin; VEGF, vascular endothelial growth factor.

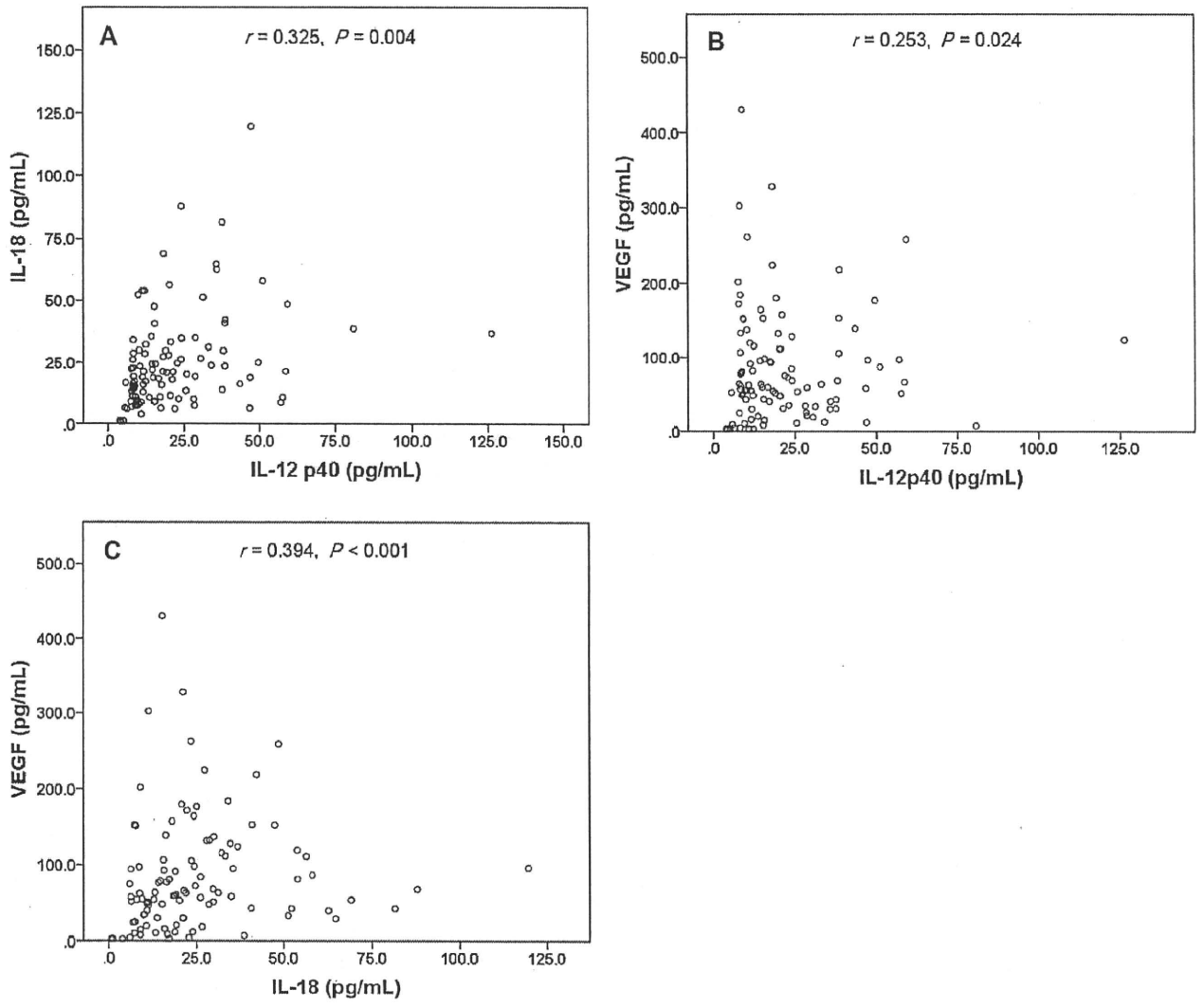

Figure 3. Correlation Between Serum Cytokines in 79 Patients with HCV Infection. (A–B) Serum IL-12p40 was significantly correlated with the level of (A) IL-18 ($r = .325$; $P = .004$) and (B) VEGF ($r = .253$; $P = .024$). (C) Serum IL-18 was correlated with the level of VEGF ($r = .394$; $P < .001$). **NOTE.** HCV, hepatitis C virus; IL, interleukin; VEGF, vascular endothelial growth factor.

Table 2. Optimal Cutoff Value, Sensitivity, Specificity, Area Under The Curve, and Predictive Values of Serum IL-10, IL-12p40, IL-18, and VEGF at Baseline and After 4 Weeks of Treatment in 79 Patients with Chronic Hepatitis C

Cytokine	Collection Time	Cutoff Value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	AUC (95% CI)	PPV (%)	NPV (%)
IL-10	baseline	5.0	100 (86–100)	80 (67–89)	.86 (.84–.98)	69	100
	4 wk	6.8	82 (69–91)	100 (86–100)	.86 (.78–.95)	100	71
IL-12p40	baseline	17.4	81 (63–93)	52 (37–67)	.70 (.59–.82)	52	81
	4 wk	21.3	81 (63–93)	60 (45–74)	.69 (.57–.81)	57	83
IL-18	baseline	15.4	97 (83–100)	46 (31–61)	.72 (.61–.83)	54	96
	4 wk	24.6	87 (70–96)	42 (28–57)	.62 (.50–.75)	49	83
VEGF	baseline	57.6	77 (59–90)	69 (54–81)	.74 (.63–.86)	62	83
	4 wk	62.6	74 (55–88)	67 (52–80)	.70 (.58–.82)	59	80

NOTE. CI, confidence interval; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; IL, interleukin

All AUC values were significantly higher than a 0.50 nonpredictive value ($P < .01$ for all comparisons). Cutoff values were determined by constructing receiver operating characteristic curves and are expressed as pg/mL. IL-10 is predictive of a nonresponse. IL-12p40, IL-18, and VEGF are predictive of a sustained virological response.

Table 3. Multivariate Analysis of Factors Independently Associated with a Sustained Virological Response to Pegylated Interferon and Ribavirin Therapy in Patients Infected with Hepatitis C Virus Genotype 1

Factors	OR	95% CI	*P*
Gender: male	10.932	2.178–54.780	.004
AST ⩾ 40 IU/L	.946	.906–.989	.013
IL-10 ⩾ 5.0 pg/mL	.823	.704–.962	.014
IL-12p40 ⩾ 17.4 pg/mL	1.071	1.009–1.137	.024
IL-18 ⩾ 15.4 pg/mL	1.085	1.024–1.150	.006

NOTE. OR, odds ratio; CI, confidence interval; AST, aspartate aminotransferase;IL, interleukin.

Only variables that achieved statistical significance ($P < .05$) in multivariate logistic regression analysis are shown.

patients (6%), respectively, which was statistically significant ($P < .001$).

Serum levels of IL-12p70 were significantly correlated with the number of substitutions in the ISDR (Kruskal–Wallis; $P = .027$). In addition, median baseline serum IL-12p70 levels were significantly higher in patients with mutant-type ISDR than in those with wild or intermediate types (15.6 vs 12.7 pg/mL; $P = .009$).

Factors Independently Associated with a Sustained Virological Response

We evaluated several factors found in association with an SVR from PEG-IFN and ribavirin therapy for their independence by multivariate analysis (Table 3). Male (odds ratio 10.93 [95% confidence interval 2.18–54.87], $P = .004$), AST ⩾40 IL/L (.95 [.91–.99], $P = .013$), IL-10 ⩾ 5.0 pg/mL (.82 [.70–.96], $P = .014$), IL-12p40 ⩾ 17.4 pg/mL (1.07 [1.01–1.14], $P = .024$), and IL-18 ⩾ 15.4 pg/mL (1.09 [1.02–1.15], $P = .006$) were independent risk factors related to an SVR. Conversely, core region or ISDR substitutions were not significant independent associations in this study.

Serum Cytokine Changes During and After Treatment

We next measured cytokine levels 4 weeks after the initiation of therapy and 6 months after its completion (Table 4). The levels of IL-10 ($P < .001$, Friedman test), IL-12p40 ($P = .008$), and IL-18 ($P < .001$) were significantly decreased in samples collected from patients who achieved an SVR. The reduction in serum cytokine levels from baseline to 4 weeks of treatment was determined and compared between SVR and non-SVR groups, and showed that the ratio of IL-10 had a significant negative association with both an EVR ($P = .024$) and an SVR ($P = .001$).

DISCUSSION

In this study, we measured the levels of 8 cytokines in patients with genotype 1 chronic hepatitis C and analyzed their association with the outcome of PEG-IFN and ribavirin therapy using a newly developed bead-array multiplex system. Serum IL-10, IL-12p40, IL-12p70, and IL-18 were higher in patients with HCV infection than in healthy participants. In addition, cytokines IL-10, IL-12p40, and IL-18 all decreased during treatment and remained low in patients with an SVR. These findings suggest that cytokines may in fact compromise host immune responses to the virus.

A strong association between high baseline serum IL-10 and a nonresponse to PEG - IFN and ribavirin therapy was found in our cohort, which is consistent with previous studies [7, 14, 15]. We found achievement of an EVR or SVR to be diminished in patients who had a lower IL-10 ratio between baseline and 4 weeks of treatment. In addition, using ROC curve analysis, we found sensitivity, specificity, and AUC were all high for IL-10, suggesting that serum IL-10 values at baseline and 4 weeks of treatment are predictive markers for treatment nonresponse (Table 2). Although humoral immunity is said to play a minor role in recovery from HCV infection and B-cell immunity is strongest in those with persistent infection [8, 16], a strong natural killer cell–mediated and Th1 cell–mediated immune response seems to be a key factor in protection from HCV infection. IL-10 was originally described as a cytokine synthesis inhibitory factor [17, 18], but recent studies have demonstrated that IL-10 produced by Th17 cells restrains the pathologic effects of Th17 [19, 20]. Furthermore, there is strong evidence of a substantial genetic component to IL-10 production [21, 22]; the –1082 G/G genotype is known to be related to increased IL-

Table 4. Serum Cytokine Levels Changes During and After Treatment of Pegylated Interferon Plus Ribavirin

Cytokines	Treatment Outcome	Baseline	Week 4	Week 72	*P*
IL-10	SVR	4.1 (3.3–25.4)	3.7 (3.1–19.9)	3.5 (2.9–9.0)	< .001
	Non-SVR	7.3 (3.7–10.8)	7.5 (3.9–8.8)	7.4 (3.9–10.9)	0.962
IL-12p40	SVR	24.1 (11.3–99.0)	22.1 (11.6–75.2)	18.4 (7.8–76.5)	0.008
	Non-SVR	17.2 (4.6–57.9)	19.2 (8.1–50.1)	21.6 (5.8–77.0)	0.281
IL-18	SVR	27.9 (13.8–100.6)	25.1 (13.2–95.2)	23.3 (6.6–48.5)	< .001
	Non-SVR	17.7 (1.1–59.9)	31.3 (10.3–90.6)	17.4 (5.4–52.0)	< .001

NOTE. Data are median (5th–95th percentile) values. IL, interleukin; SVR, sustained virological response.

10 production and is associated with a high risk of inefficient HCV clearance [23, 24] and resistance to IFN treatment [25–28].

In agreement with our findings, recent studies have indicated that Gln70 substitutions in the HCV core region are associated with treatment failure [11, 29–32]. Additionally, patients with Gln70 had higher IL-10 levels compared with those with Arg70. Among the 28 HCV patients who had Gln70, all 14 nonresponders had higher IL-10 (≥5.0 pg/mL), whereas 11 of 14 responders had lower IL-10 levels ($P < .001$). This association between Gln70 and elevated IL-10 levels is intriguing. Dolganiuc et al reported that HCV core and NS3 proteins in monocytes and dendritic cells induce IL-10 [33], so further studies are needed to clarify the relationship between IL-10 and core region amino acid substitutions.

This report demonstrates the beneficial role of IL-12 in achieving an SVR during PEG - IFN and ribavirin therapy. IL-12 is a proinflammatory cytokine that promotes the differentiation of Th1 cells, suppresses Th2 function, and amplifies the cytotoxicity of cytotoxic T lymphocytes and natural killer cells [34]. Thus, production of IL-12 is directed toward the elimination of intracellular pathogens and viruses. Elevated serum IL-12 has been noted in patients with chronic HBV or HCV infection, and is even more prominent among responders to IFN-α treatment [35, 36]. In our study, we noted significantly higher serum IL-12p70 in participants carrying mutant-type ISDR than in those with intermediate- or wild-type ISDR. This correlation between IL-12 and ISDR substitutions is striking and requires further study to verify its favorable effect during PEG-IFN and ribavirin therapy.

It is believed that the dynamics of the Th1/Th2 response determine the outcome of antiviral therapy to chronic hepatitis C [10] and that IL-18 is an important mediator of the Th1/Th2 balance. IL-18 plays a critical role in host defense against infection by intracellular microbes but also induces autoimmune diseases and propagates inflammation [37]. IL-18 is significantly upregulated in patients with chronic HCV infection and is correlated with hepatic injury [38, 39], indicating a key role in disease pathogenesis. However, the effect of IL-18 on antiviral therapy for chronic hepatitis C is still unclear. We found that IL-18 levels were significantly higher in patients with chronic HCV infection compared with healthy controls, but they were also higher at baseline in patients who achieved an SVR than in those who did not. In addition, there was a significant correlation between IL-18 and IL-12; in the presence of IL-12, IL-18 stimulates *IFNG* expression, thus promoting the Th1-mediated immune response. Without IL-12, IL-18 stimulates Th2 responses [37]. In this study, because serum IFN-γ levels were below detection thresholds, we could not assess the association of such cytokines.

Lastly, we observed that pretreatment serum VEGF levels were associated with an SVR. A previous study showed no association between baseline VEGF and treatment outcome, but only 36 patients, including 19 with genotype 1, were studied [40]. Hence, it is still unclear if this angiogenesis marker plays a critical role in response to antiviral therapy in chronic HCV infection. Furthermore, we correlated VEGF with IL-12 and IL-18 in our study. In particular, IL-18 enhances the production of VEGF in rheumatoid arthritis synovial fibroblasts, suggesting that IL-18 could be an angiogenic mediator with triggering effects on VEGF production [41]. Although the preoperative serum VEGF level was found to be a significant predictor of tumor recurrence and overall survival in patients with HCC [42], there have been no reports regarding treatment response in patients with chronic hepatitis C during antiviral therapy.

In multivariate analysis of our cohort, low IL-10, high IL-12p40, and high IL-18 were independent factors related to an SVR in patients treated with PEG-IFN and ribavirin. Our results indicate that such 3-cytokine profiling may offer clinicians another tool in predicting treatment outcome of HCV infection. Further investigation must be done in vitro and using many samples to validate the significance of our findings.

In conclusion, several cytokines were seen to be elevated in patients with chronic hepatitis C using the multiplex bead assay. Serum IL-10 levels and amino acid substitutions at the 70 aa core region of HCV are useful for predicting a nonresponse to PEG-IFN and ribavirin therapy in patients with chronic hepatitis C genotype 1. A higher level of serum IL-12 is considered to be favorable for response to antiviral therapy, and is correlated with substitutions in the ISDR. Lastly, IL-18 is notably high in patients with chronic HCV infection, and is correlated with IL-12.

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INTERNATIONAL FORUM

Clinical significance of immunoglobulin G4-associated autoimmune hepatitis

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Abstract
Background Immunoglobulin (Ig) G4-associated autoimmune hepatitis (AIH) is a recently identified and possibly new disease entity. However, the epidemiology and clinical features of IgG4-associated AIH remain uncertain. The aim of this study was to determine the prevalence and the clinical, serological, and histological characteristics of IgG4-associated AIH.

Methods We examined the clinical features, serum IgG4 concentration, liver biopsy histology, and IgG4-bearing plasma cell infiltration of 60 patients with type 1 AIH and 22 patients with autoimmune pancreatitis.

Results High serum IgG4 concentration (≥135 mg/dL) and IgG4-bearing plasma cell infiltration in the liver (≥10/high-power fields [HPFs]) were found in 2 of the 60 (3.3%) patients with type 1 AIH. These patients had high serum levels of IgE, giant cell change, and rosette formation in the liver. Although corticosteroid therapy reduced the serum IgG4 concentration and normalized liver enzymes and histology, one patient developed IgG4-related sclerosing cholangitis after 5 years of follow-up.

Conclusions Because IgG4-associated AIH was found in over 3% of Japanese patients with type 1 AIH in our cohort, further studies are needed on this possible new disease entity and its impact on the diagnostic guidelines of AIH.

Keywords Autoimmune hepatitis · Autoimmune pancreatitis · IgG4 · Histology · Sclerosing cholangitis

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Introduction

Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver, elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies, histological evidence of interface hepatitis, and a favorable response to immunosuppressive treatment [1–3]. Type 1 AIH is the major form of AIH in Japanese and Caucasoid adults, and can be distinguished by the presence of circulating anti-nuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA). The diagnosis of type 1 AIH is based on the

revised scoring system developed by the International Autoimmune Hepatitis Group (IAIHG) [4].

We recently reported a case suggesting the existence of a new disease entity termed "immunoglobulin G4 (IgG4)-associated AIH" [5]. Although the revised IAIHG disease score of this patient was 18 and representative of definite AIH, a high serum IgG4 concentration was detected before the administration of corticosteroid therapy. Moreover, immunostaining of liver tissues prior to treatment showed abundant plasma cells with strong immunohistochemical reactivity to IgG4. Abdominal computed tomography, endoscopic retrograde cholangiography, and magnetic resonance cholangiopancreatography showed no abnormalities of the extrahepatic bile ducts or pancreas. Raised serum IgG4 concentration and IgG4-bearing plasma cell infiltration have high sensitivity and specificity for the diagnosis of autoimmune pancreatitis (AIP) [6–8] and IgG4-related sclerosing cholangitis or IgG4-associated cholangitis [9, 10]. Thus, we suggested that IgG4-associated AIH was in fact an IgG4-related disease [5]. Since then, the epidemiology and clinical significance of IgG4-associated AIH have remained largely unknown. Chung et al. [11] recently conducted IgG4 immunostaining of liver biopsies from 26 patients with AIH. Although they reported that 9 out of the 26 patients had infiltration of more than 5 IgG4-positive plasma cells per high power field (HPF) in the liver, no cases in their cohort showed high serum IgG4. We have also found that patients with AIP had histological liver findings that included portal inflammation, large bile duct damage, portal sclerosis, lobular hepatitis, and cholestasis and/or IgG4-bearing plasma cell infiltration in the liver, and have accordingly proposed that histological liver changes in AIP be considered as an IgG4 hepatopathy as well [8]. In the present study, we investigated serum IgG4 concentrations and IgG4 immunostaining of the liver in patients with type 1 AIH, compared the findings with those in AIP, and proposed diagnostic criteria for IgG4-associated AIH.

Methods

Subjects

Between June 1985 and December 2006, 70 consecutively treated type 1 AIH patients (12 men and 58 women; median age 57 years, interquartile range [IQR] 45–63 years)were seen at Shinshu University Hospital, Japan. All patients were diagnosed as having probable or definite AIH according to the scoring system of the IAIHG. Among them, 60 (86%) subjects from whom serum samples and paraffin-embedded liver biopsy samples before administration of corticosteroids were available were registered for this study; their characteristics are summarized in Table 1. No patients had a pancreatic mass or common bile duct strictures detectable by ultrasonography, computed tomography, and/or magnetic resonance imaging. All 60 patients were treated with corticosteroids, and none were given azathioprine during follow-up. All patients were negative for hepatitis B surface antigen, antibody to hepatitis B core antigen, antibody to hepatitis C virus, and antibody to human immunodeficiency virus. No patients were positive for antimitochondrial antibody-M2. No patients had potential drug-induced liver injury. Twenty-two patients with AIP were enrolled as control cases. All were biopsied. The diagnosis of AIP was based on criteria released by the Japan Pancreas Society [12]. Serum samples were obtained at the time of liver biopsy and stored at −70°C until testing. The protocol of this study conformed to the Declaration of Helsinki, and was approved by the ethics committee of Shinshu University School of Medicine.

Laboratory tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical tests were performed using standard methods [13]. ANA and SMA were determined by indirect immunofluorescence on murine tissue sections as reported previously [8]. A serum titer of 1:40 or greater was considered positive for ANA and SMA. Patterns of ANA reactivity were also recorded. Anti-double-stranded DNA (<12 IU/mL), anti-Ro (SS-A), anti-La (SS-B), and antimitochondrial antibody-M2 were tested by enzyme-linked immunosorbent assay (ELISA) as reported previously [8]. Serum IgG4 concentrations were determined by single radial immunodiffusion (IgG4-SD RID kit, RN109.3; Binding Site, Birmingham, UK) as reported previously [6] or by a nephelometric assay (IgG4 BN^{TM}II kit, NK009.T; Binding Site) (normal value < 135 mg/dL).

Genomic DNA from the patients was isolated by phenolic extraction of sodium dodecyl sulfate-lysed and proteinase K-treated cells as described previously [14]. HLA class I and II alleles were determined using a Micro SSPTM DNA Typing Kit (One Lambda, Canoga Park, CA, USA). DNA typing of DRB1 and DQB1 alleles was performed by polymerase chain reaction-restriction fragment length polymorphism analysis as previously described [14, 15].

Histological evaluation and immunohistochemistry of IgG and IgG4

Liver biopsies were performed by percutaneous sampling of the right lobe with a 14-gauge needle in all but one

Table 1 Demographic and clinical characteristics of AIH and AIP patients

Characteristics	AIH ($n = 60$)	AIP ($n = 22$)	P value	AIH	
				IgG4-AIH ($n = 2$)	Classical AIH ($n = 58$)
Median age, years (IQR)	58 (46–64)	63 (57–66)	0.06	48 (42–54)	58 (46–65)
Female, *n* (%)	49 (82)	3 (14)	<0.001	1 (50)	48 (83)
AIH score, median (IQR)	17 (16–19)	9 (6–10)	<0.001	17 (16–19)	17 (16–19)
Definite, *n* (%)	48 (80)	0 (0)	<0.001	2 (100)	46 (79)
Probable, *n* (%)	12 (20)	11 (50)		0 (0)	12 (21)
Median values (IQR)					
ALT (n.v. 7–45 IU/L)	573 (153–1090)	66 (27–140)	<0.001	497 (253–739)	573 (150–1103)
AST (n.v. 12–37 IU/L)	437 (150–907)	36 (24–59)	<0.001	425 (199–650)	437 (145–907)
ALP (n.v. 124–367 IU/L)	418 (295–548)	481 (322–753)	0.429	539 (472–605)	401 (294–507)
Bilirubin (n.v. 0.3–1.2 mg/dL)	2.2 (1.0–8.5)	1.1 (0.6–1.8)	0.002	5.6 (2.5–8.6)	2.1 (1.0–8.2)
Alb (n.v. 4.2–5.1 g/dL)	3.6 (3.0–3.9)	3.7 (3.4–4.0)	0.314	3.0 (2.8–3.2)	3.6 (3.1–3.9)
IgG (n.v. 870–1700 mg/dL)	2940 (2330–3493)	2494 (1598–3268)	0.096	4015 (2403–5627)	2940 (2328–3464)
IgG4 (n.v. <135 mg/dL)	23 (18–50)	699 (346–1335)	<0.001	560 (557–642)	22 (18–47)
IgG4:IgG	0.009 (0.006–0.015)	0.253 (0.189–0.410)	<0.001	0.173 (0.114–0.232)	0.009 (0.006–0.014)
IgE (n.v. <361 IU/mL)	64 (20–381)	246 (105–700)	0.010	1490 (1330–1650)	58 (18–306)
High IgG4 levels, *n* (%)	4 (7)	21 (95)	<0.001	2 (100)	2 (3)
ANA-positive, *n* (%)	60 (100)	17 (77)	<0.001	2 (100)	58 (100)
SMA-positive, *n* (%)	30/54 (56)	0 (0)	<0.001	1 (50)	29/52 (56)
ds-DNA-positive, *n* (%)	4 (7)	1 (5)	0.593	1 (50)	3 (5)
SS-A-positive, *n* (%)	5 (8)	0 (0)	0.200	0 (0)	5 (9)
SS-B-positive, *n* (%)	1 (2)	0 (0)	0.732	0 (0)	1 (2)
HLA-DR4, *n* (%)	38/56 (68)	9/15 (60)	0.557	1 (50)	37/54 (69)

AIH autoimmune hepatitis, *AIP* autoimmune pancreatitis, *IgG4-AIH* IgG4-associated autoimmune hepatitis, *IQR* interquartile range, *n.v.* normal value, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *Alb* albumin, *ANA* anti-nuclear antibody, *SMA* anti-smooth muscle antibody, *ds-DNA* anti-double stranded DNA

patient, whose liver specimen was obtained during surgery. All biopsy specimens were 1.5 cm or more in length. Liver specimens were taken before the administration of corticosteroid therapy in the 60 patients with AIH and the 22 patients with AIP. Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, Azan–Mallory, silver impregnation reticulin, or silver impregnation orcein, with the remaining material being used for immunohistochemical analysis. The following [11] histological features were assessed by two experienced pathologists (Y.Z. and Y.N.), who analyzed each feature under code and independently of other data: [1] portal fibrosis (0, absent; 1, periportal fibrosis; 2, bridging fibrosis; 3, bridging fibrosis with lobular distortion; 4, cirrhosis); [2] portal inflammation (0, absent; 1, inflammation in ≤1/3 of periportal areas; 2, inflammation in 1/3 to 2/3 of periportal areas; 3, inflammation in ≥2/3 of periportal areas); [3] interface hepatitis (0, absent; 1, interface hepatitis in ≤1/3 of periportal areas; 2, interface hepatitis in 1/3 to 2/3 of periportal areas; 3, interface hepatitis in ≥2/3 of periportal areas); [4] lobular hepatitis (1, 0–2 focal necrosis/high-power field (HPF); 2, ≥3 focal necrosis/HPF; 3, zonal necrosis); [5] plasma cell infiltration (1, 0–9 cells/HPF in portal area; 2, 10–19 cells/HPF; 3, ≥20 cells/HPF); [6] eosinophil infiltration (0, 0–4 cells/HPF in portal area; 1, ≥5 cells/HPF); [7] syncytial giant cell change (0, absent; 1, present); [8] rosette formation (0, absent; 1, present); [9] bile duct loss (0, absent; 1, present); [10] bile duct damage (−, absent; +, irregularity of cellular and nuclear arrangement of the biliary epithelium with or without narrowing of the bile duct lumen); and [11] cholangitis (0, absent; 1, present).

Immunostaining for IgG and IgG4 was performed using mouse monoclonal antibodies against human IgG (Dako Cytomation, Glostrup, Denmark), and human IgG4 (ZYMED Laboratory, San Francisco, CA, USA) as reported previously [9]. Counts were tallied for IgG- and IgG4-bearing plasma cells per HPF, and the number of positive cells was expressed as the mean of triplicates and compared. The ratio of IgG4-positive to IgG-positive cells was also calculated for each case.

Statistical analysis

The Mann–Whitney *U*-test was used to analyze continuous variables where appropriate. The χ^2 test with Yates's correction was used for the analysis of categorical data. In cases where the number of subjects was less than 5, Fisher's exact test was used. $P \leq 0.05$ was considered to be significant. Statistical analysis was performed using SPSS software (version 15.0J; SPSS, Chicago, IL, USA).

Results

Clinical characteristics of patients

The clinical profile of the experimental patient cohort is shown in Table 1. Forty-nine (82%) were women, and the median age at presentation was 58 years. All patients were graded by the IAIHG scoring system prior to treatment. Of them, 48 (80%) patients satisfied the criteria for a definite diagnosis of AIH and the remaining 12 patients met the requirements for a probable diagnosis. Although no AIP patients fulfilled the definite criteria for AIH, 11 of the 22 (50%) patients met the probable criteria for AIH as well. The median IAIHG score was significantly higher in patients with AIH than in those with AIP. AIH patients also had significantly higher median serum levels of ALT, AST, and total bilirubin. There were significant differences in positivity for ANA and SMA in AIH versus AIP, but the prevalence of HLA DR4 did not differ between the two groups.

The median serum IgG4 concentration, IgG4: IgG ratio, and serum IgE level were significantly higher in AIP patients than in the patients with AIH. However, an elevated serum IgG4 concentration (≥135 mg/dL) was detected in 4 of the 60 (6.7%) patients with AIH, and the serum IgG4 concentrations and IgG4: IgG ratios in each of these patients were 146, 215, 557, and 642 mg/dL and 0.041, 0.072, 0.232, and 0.114, respectively. We previously performed receiver operating characteristic curve analysis to determine the optimal cutoff value of the serum IgG4: IgG ratio to best differentiate patients with AIP from those with other diseases [8]. This value was 0.073, meaning that 2 of the 60 (3.3%) AIH patients had an extraordinarily high serum IgG4 concentration and IgG4: IgG ratio.

Histopathology of liver biopsies in patients with AIH and AIP

As shown in Table 2, portal and periportal fibrosis was present in all 60 patients with AIH, and 33 of them (55%) showed bridging fibrosis. Twenty-two patients (37%) had cirrhosis. Portal inflammation was evident in all but one patient, and 55 (92%) also showed associated interface hepatitis. Lobular hepatitis, which was defined as focal necrosis ≥3/HPF, was found in 39 patients (65%). Marked plasma cell infiltration (≥20/HPF) and eosinophil infiltration (≥5/HPF) in portal tracts were present in 30 (50%) and 18 patients (30%), respectively. Giant cell change and rosette formation were found in 7 (12%) and 9 patients (15%), respectively. Only 3 patients had both giant cell change and rosette formation. Bile duct damage was found in 10 patients (17%), but chronic nonsuppurative destructive cholangitis and bile duct loss were not found in any of the patients.

Immunostaining for IgG and IgG4 demonstrated many IgG- and IgG4-bearing plasma cells in the portal tract. The number of IgG4-bearing plasma cells in patients with AIP (6.0/HPF; IQR, 2.3–12.3) was significantly higher than that in patients with AIH (0.0/HPF; IQR, 0.0–3.8; $P < 0.001$), but the number of IgG-bearing plasma cells did not show a significant difference between the two groups. More than 5 IgG4-positive cells (/HPF) were found in 5 of the 60 (8%) patients with AIH. The numbers of IgG4-positive cells and ratios of IgG4: IgG bearing plasma cells in each of these

Table 2 Histological characteristics of patients with AIH and IgG4-AIH

	AIH ($n = 60$)	IgG4-AIH ($n = 2$)	Classical AIH ($n = 58$)
Bridging fibrosis	33 (55%)	2 (100%)	31 (54%)
Cirrhosis	22 (37%)	0 (0%)	22 (38%)
Portal inflammation (≥2/3 of periportal areas)	43 (72%)	2 (100%)	41 (71%)
Interface hepatitis (≥2/3 of periportal areas)	23 (38%)	2 (100%)	21 (36%)
Lobular hepatitis (zonal necrosis)	15 (25%)	2 (100%)	13 (22%)
Plasma cells (≥20/HPF)	20 (33%)	2 (100%)	18 (31%)
Eosinophils (≥5/HPF)	18 (30%)	1 (50%)	17 (29%)
Giant cell change	7 (12%)	2 (100%)	5 (9%)
Rosette formation	9 (15%)	2 (100%)	7 (12%)
Bile duct damage	10 (17%)	0 (0%)	10 (17%)

AIH autoimmune hepatitis, *IgG4-AIH* IgG4-associated autoimmune hepatitis, *HPF* high-power field

patients were 6, 6, 7, 24, and 29/HPF and 0.082, 0.102, 0.071, 0.282, and 0.528, respectively. The latter 2 patients were also the patients with elevated serum IgG4 and elevated IgG4: IgG ratios.

All patients with AIP in this study had relevant histological liver findings and/or IgG4-bearing plasma cell infiltration in the liver, and were thus considered to have an IgG4 hepatopathy.

Clinical significance of IgG4-associated autoimmune hepatitis

In our cohort, a high serum IgG4 concentration and more than 10 IgG4-positive cells (/HPF) were found in 2 patients. As shown in Table 1, the 2 patients with IgG4-associated AIH had higher median serum levels of IgG4, higher IgG4: IgG ratios, and higher IgE levels compared with the 58 patients with classical AIH. The HLA DRB1*0405 allele was found in one patient with IgG4-associated AIH. Liver biopsies of the 2 patients showed similar chronic active hepatitis with bridging fibrosis. Hepatic activity was high, and both patients showed interface hepatitis and zonal necrosis. Interestingly, giant cell change and rosette formation were simultaneously observed in both patients with IgG4-associated AIH (Table 2). The median numbers of IgG- and IgG4-bearing plasma cells in the patients with IgG4-associated AIH (70.3/HPF and 26.5/HPF) tended to be higher than those for the patients with classical AIH (24.5/HPF and 0.0/HPF) and AIP (14.5/HPF and 6.0/HPF).

Clinical course of IgG4-associated autoimmune hepatitis

Case 1 in this study was previously reported as the first proposed case of IgG4-associated AIH [5]. The patient's serum IgG4 concentrations and liver enzymes eventually normalized with continuous low-dose corticosteroid therapy (5.0–10.0 mg/day). However, after 5 years of follow-up, her serum alkaline phosphatase and γ-glutamyl transpeptidase rose to 622 and 936 IU/L, respectively. Although no abnormalities of the bile duct were found before administration of corticosteroid therapy, distal and proximal biliary strictures became evident by endoscopic retrograde cholangiography (Fig. 1) and magnetic resonance cholangiopancreatography. In addition, abundant IgG4-bearing plasma cell infiltration was found in a biopsy of the common bile duct, despite serum IgG4 concentrations remaining normal at 75 mg/dL. Hence, she was the first patient diagnosed with IgG4-associated AIH who later developed IgG4-related sclerosing cholangitis.

Case 2 was a 42-year old man who was admitted to our hospital because of elevated liver enzymes [16]. Serum IgG and IgE concentrations prior to corticosteroid therapy were extremely high. His ANA antibody, anti-double-strand DNA, and SMA were all positive. A first liver biopsy showed changes associated with typical AIH: interface hepatitis, lobular hepatitis, rosette formation, syncytial multinucleated giant cell change, and marked plasma cell infiltration. No biliary epithelial changes were found. Imaging modalities showed no abnormalities of the extrahepatic bile ducts or pancreas. A serum IgG4 concentration of 642 mg/dL was detected in stored serum, and immunostaining of liver tissue showed abundant plasma cells with strong immunohistochemical reactivity to IgG4. He fulfilled the criteria for definite AIH and was administered corticosteroids at 60 mg/day. Four weeks after the initiation of the corticosteroid treatment, serum IgG4 and IgE concentrations were decreased to 452 mg/dL and 909 IU/L, respectively. Although a second liver biopsy performed 7 months after the first showed remaining portal sclerosis, almost all the other histological findings were improved except for mild portal inflammation. Serum IgG4 and IgE concentrations were normalized. Furthermore, IgG4-bearing plasma cell infiltration in the liver was absent (0/HPF). After 13 years of follow-up, his transaminases are still elevated but below 100 IU/L with continuous low-dose corticosteroid therapy (5.0 mg/day), and image modalities show no abnormalities. His ANA antibody titer (1: 1280) and anti-double-strand DNA (>400 IU/mL) are still abnormal.

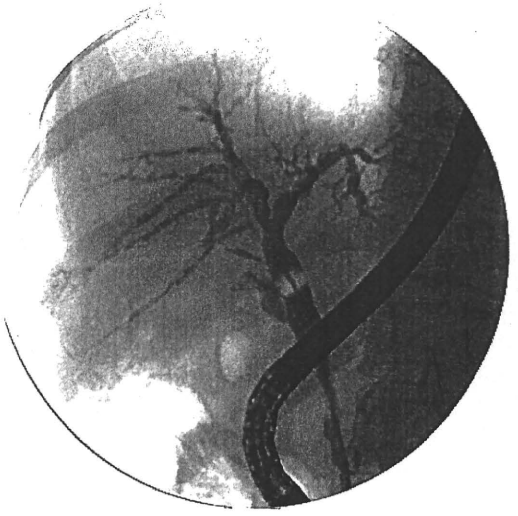

Fig. 1 Endoscopic retrograde cholangiography after 5 years of follow-up, showing smooth narrowing of the common bile duct and intrahepatic biliary strictures

Discussion

In an earlier report, a strong and unexpected association was seen between serum IgG4 concentration and IgG4-

bearing plasma cell infiltration in the liver in one patient with type 1 AIH, raising the possibility of a new disease entity, termed 'IgG4-associated AIH' [5]. In the present study, we investigated serum IgG4 concentration and IgG4 immunostaining of the liver in 60 Japanese patients with type 1 AIH and looked for correlations with liver histology and clinical features in comparison with AIP.

Based on our findings, we have provisionally set the diagnostic criteria for IgG4-associated AIH as follows: (1) having definite AIH according to the IAIHG scoring system, (2) serum IgG4 concentration ≥135 mg/dL, and (3) immunostaining of IgG4 showing infiltration of ≥10/HPF IgG4-bearing plasma cells in the portal tract. We ultimately identified 2 patients (3.3%) among our AIH cases in the present cohort who fulfilled these criteria. This rate was quite low, as we had expected. Conversely, Chung et al. [11] recently reported that 9 of 26 patients with AIH showed more than 5 IgG4-positive plasma cells (/HPF) in the liver. Although serum IgG4 concentration in all patients was normal at less than 80 mg/dL, these cases were classified as having IgG4-associated AIH, thus being distinct from IgG4-related disease. In general, the concept of an IgG4-related disease is high serum IgG4 concentration and abundant infiltration of IgG4-bearing plasma cells in the affected organs. Although it is possible that several of their patients had IgG4-positive cell infiltration without high serum IgG4 concentration, it is difficult to draw the conclusion that all of their cases can be classified as having an IgG4-associated disease. Koyabu et al. [17] have recently reported that an IgG4/IgG1-bearing plasma cell ratio of >1 in the liver is specific for IgG4-related diseases. In our 2 cases, the ratio of IgG4: IgG1-bearing plasma cells in the liver was >1. Hence, this ratio might be a useful marker for the diagnosis of IgG4-related diseases.

We recently reported IgG4 hepatopathy in AIP patients because almost all the patients had liver dysfunction and histological changes such as interface and lobular hepatitis [8]. Our patients with IgG4-associated AIH in the present study had high serum IgG4 and abundant infiltration of IgG4-bearing plasma cells in the liver, and therefore we believe this disease entity should be considered as an IgG4-associated disease (IgG4 hepatopathy) rather than AIH. Using the IAIHG scoring system, 50% of our 22 AIP patients had probable AIH. Hence, we excluded all AIH cases listed as probable for a more precise evaluation of IgG4-associated AIH. We also selected 135 mg/dL as the cutoff value for serum IgG4 because of its high accuracy for AIP diagnosis [6]. Because IgG4-bearing plasma cell infiltration is a characteristic finding in AIP [6, 18], IgG4-related sclerosing cholangitis, and IgG4-associated cholangitis [9, 10] we included the criteria of the infiltration of more than 10 IgG4-bearing plasma cells (/HPF) in the liver as well. Moreover, we believe that AIH patients with pancreatic abnormalities should be excluded from the diagnosis of IgG4-associated AIH, because a prior study showed that interface hepatitis was found in 24% of patients with AIP [8]. Although all the AIP patients in the present study had relevant histological liver changes and/or IgG4-bearing plasma cell infiltration in the liver, no patients fulfilled the criteria for definite AIH. Hence, they were considered to have AIP with histological damage in the liver, not IgG4-associated AIH. A relatively small number of patients with AIH were studied in this report, because the number of patients having both stored sera and paraffin-embedded liver biopsy samples is small. As such, a larger sample group will be needed to compare IgG4-associated AIH with classical AIH.

IgG4-related diseases are primarily found in the pancreatobiliary system and hepatic parenchyma [6–9]. Our patients were mainly affected in the liver and their disease resembled AIH both clinically and pathologically [5, 16]. The differences between IgG4-associated AIH and comorbid hepatic injury in AIP are considered to be: (1) patients with IgG4-associated AIH have a much higher degree of IgG4-bearing plasma cell infiltration in the liver compared not only with classical AIH, but also with AIP; (2) giant cell change and rosette formation are obvious; and (3) bile duct damage or loss is not found. Taken together, it appears that the histological liver findings of IgG4-associated AIH seem to be more severe compared with the findings in classical AIH and AIP.

Interestingly, one patient with IgG4-associated AIH who was administered low-dose corticosteroid therapy developed IgG4-related sclerosing cholangitis after 5 years of follow-up. Because we did not perform a bile duct biopsy prior to treatment, we cannot exclude the possibility that she had sclerosing cholangitis at that time. However, no bile duct abnormalities were seen by imaging modalities or in liver biopsy samples before therapy. AIH/primary sclerosing cholangitis overlap syndrome has been reported with an increasing incidence in a number of studies [19–24]. Although these studies did not examine serum IgG4 concentration or IgG4-immunostaining of liver biopsies, it is possible that a case of IgG4-associated AIH with sclerosing cholangitis might have been included in their populations.

The fundamental nature of IgG4-related diseases is enigmatic. Because IgG4 and IgE immune responses to antigens often occur in allergic disorders [25, 26], damage to pancreatic acinar cells, cholangiocytes, and hepatocytes via an allergic type of reaction to target tissue cells initially induced by a dietary or bacterial antigen is plausible. In addition, Zen and colleagues [27], who are coauthors of the present study, recently reported that IgG4-related diseases were characterized by the overproduction of T-helper 2 and regulatory cytokines, both of which are closely involved in the pathogenesis of allergic disorders. Our present patients

with IgG4-associated AIH had high serum IgG4 and IgE concentrations and lobular hepatitis with marked IgG4-bearing plasma cell infiltration, suggesting that an allergic reaction to hepatocytes or molecules in the liver parenchyma might be a possible pathogenesis of this disease. However, such a notion is highly speculative and would require additional in-depth studies to show mast cell involvement in the liver tissues of AIP and IgG4-associated AIH patients. At present, we can only describe the observation of high serum IgE concentrations and cannot provide a sound scientific basis for its occurrence.

Susceptibility to AIH and AIP is influenced by genetic factors, specific HLA alleles, amino acid sequences at the presentation site of the HLA molecule [28, 29], and Fc receptor-like gene 3 and cytotoxic T-lymphocyte antigen 4 single nucleotide polymorphisms [28, 30–33]. These genetic markers should be investigated in our 2 patients.

In conclusion, IgG4-associated AIH was found in over 3% of classical AIH cases in a Japanese cohort and is characterized by high serum IgG4 and IgE concentrations and IgG4-bearing plasma cell infiltration in the liver. Because IgG4-associated AIH is clearly an IgG4 hepatopathy, this disease should be differentiated from classical AIH. Further studies are needed to clarify the epidemiology and pathogenesis of this possible new disease entity.

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Review Article

Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009

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Recently, much progress has been made in the field of hepatitis B, such as natural history of the disease in relation to the amount of hepatitis B virus (HBV) DNA, genotypes of HBV influencing the natural course and treatment effects, mutations of HBV influencing the severity of the disease and development of hepatocellular carcinoma, and antiviral treatment such as nucleos(t)ide analogues and pegylated interferon. To make the consensus for the diagnosis, management and treatment of hepatitis B, a meeting was held during 45th annual meeting of Japan Society of Hepatology (JSH) in June 2009. In the meeting, recommendations and informative statements were discussed on the following subjects: (i) natural history of HBV infection; (ii) clinical implication of HBV genotypes; (iii) HBV mutations and their potential impact on pathogenesis of HBV infection; (iv) indications for antiviral treatment of chronic hepatitis B; (v) nucleos(t)ide analogues for chronic hepatitis B; and (vi) interferon therapy for chronic hepatitis B. The presenters reviewed the data on these subjects and proposed the consensus statements and recommendations. These statements were discussed among the organizers and presenters, and were approved by the participants of the meeting. In the current report, the relevant data were reviewed and the 12 consensus statements and nine recommendations on chronic hepatitis B were described.

Key words: genotype, hepatitis B virus, interferon, mutation, natural history, nucleotide analogue

Hepatitis B virus (HBV) is one of the most distributed viruses which infect humankind. More than 3 billion people, one half of the world's population, have been exposed to HBV during their life.[1] Acute infection in adults is self-limited in general whereas infection during early childhood will develop into persistent chronic infection in most individuals.[2] More than 400 million people worldwide are chronically infected with HBV and are at risk of developing life-threatening complications including liver cirrhosis and hepatocellular carcinoma (HCC).[1] HBV is a major public health problem worldwide especially in East Asia and Africa. In Japan, approximately 1.5 million people are infected with HBV and it is one of the major causes of HCC and chronic hepatic failure. Other complications of HBV infection include fulminant hepatitis and acute liver failure.

The consensus meeting for diagnosis, management and treatment for hepatitis B was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M Kudo), where the recommendations and informative statements were discussed. Although the JSH consensus meeting of hepatitis B had been held four times so far, recommendations were hitherto published only in Japanese and this is the first report in English. Established

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