among patients with chronic hepatitis C virus infection. J Viral Hepat 2005 ; 12 : 125-9.
16) Morishima C, Polyak SJ, Ray R, et al. Hepatitis C virus-specific immune responses and quasi-species variability at baseline are associated with non-response to antiviral therapy during advanced hepatitis C. J Infect Dis 2006 ; 193 : 931-40.
17) Kaneko T, Moriyama T, Udaka K, et al. Impaired induction of cytotoxic T lymphocytes by antagonism of a weak agonist borne by a variant hepatitis C virus epitope. Eur J Immunol 1997 ; 27 : 1782-7.
18) Hiroishi K, Eguchi J, Ishii S, et al. Differential effect of cytotoxic T lymphocyte variant epitopes on generation and cytotoxicity in chronic hepatitis C virus infection. Hepatol Res 2002 ; 24 : 91-4.
19) Wang JH, Layden TJ, Eckels DD. Modulation of the peripheral T-Cell response by CD4 mutants of hepatitis C virus : transition from a Th1 to a Th2 response. Hum Immunol 2003 ; 64 : 662-73.
20) Hiroishi K, Eguchi J, Ishii S, et al. Differential effect of cytotoxic T lymphocyte variant epitopes on generation and cytotoxicity in chronic hepatitis C virus infection. Hepatol Res 2002 ; 24 : 91.
21) Urbani S, Amadei B, Cariani E, et al. The impairment of CD8 responses limits the selection of escape mutations in acute hepatitis C virus infection. J Immunol 2005 ; 175 : 7519-29.
22) Francavilla V, Accapezzato D, De Salvo M, et al. Subversion of effector $CD8^+$ T cell differentiation in acute hepatitis C virus infection : exploring the immunological mechanisms. Eur J Immunol 2004 ; 34 : 427-37.
23) Konan KV, Giddings TH Jr, Ikeda M, et al. Nonstructural protein precursor NS4A/B from hepatitis C virus alters function and ultrastructure of host secretory apparatus. J Virol 2003 ; 77 : 7843-55.
24) Mehal WZ, Azzaroli F, Crispe IN. Immunology of the healthy liver : old questions and new insights. Gastroenterology 2001 ; 120 : 250-60.
25) Sugimoto K, Ikeda F, Stadanlick J, et al. Suppression of HCV-specific T cells without differential hierarchy demonstrated *ex vivo* in persistent HCV infection. Hepatology 2003 ; 38 : 1437-48.
26) MacDonald AJ, Duffy M, Brady MT, et al. CD4 T helper type 1 and regulatory T cells induced against the same epitopes on the core protein in hepatitis C virus-infected persons. J Infect Dis 2002 ; 185 : 720-7.
27) Accapezzato D, Francavilla V, Paroli M, et al. Hepatic expansion of a virus-specific regulatory CD8 (+) T cell population in chronic hepatitis C virus infection. J Clin Invest 2004 ; 113 : 963-72.
28) Brady MT, MacDonald AJ, Rowan AG, et al. Hepatitis C virus non-structural protein 4 suppresses Th1 responses by stimulating IL-10 production from monocytes. Eur J Immunol 2003 ; 33 : 3448-57.
29) Tajimi M, Ugajin T, Ota M, et al. Immune responses of liver-infiltrating lymphocytes and peripheral blood mononuclear cells to hepatitis C virus core and NS3 antigens. Hepatol Res 2006 ; 35 : 250-5.

* * *

肝硬変患者

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● 肝硬変の死因の大部分は肝癌であり，C型肝硬変は年率7～8%の割合で発癌が認められる．
● C型代償性肝硬変（セロタイプ1型，高ウイルス量症例を除く）におけるウイルス血症の改善に対し，わが国ではインターフェロンβの投与が認可されている．
● 欧米では，C型肝硬変に対するペグ化インターフェロン，リバビリン併用療法が一般化している．
● C型肝硬変の発癌予防にインターフェロン治療がどの程度効果的であるかを今後検討していく必要がある．

Key Words: インターフェロンβ，ペグ化インターフェロン，リバビリン，発癌抑制

日本では，肝硬変をきたす疾患・病因のうち約70%以上がHCV感染である．肝硬変における死因は，70%が肝癌であり，20%が肝不全，消化管出血は10%以下である．C型肝硬変においては，抗ウイルス療法によりHCVを排除するかあるいは肝庇護療法によりALTを低下させて，肝炎を鎮静化させることにより肝機能を保つことが，肝不全への進展を防止することにつながると考えられる．また，肝硬変患者では年率が7～8%の割合で肝癌が合併するが[1]，抗ウイルス療法は肝発癌抑制にも期待がかかる．

一般に肝硬変患者は高齢で，合併症が多く，副作用の発現の可能性も高いことから，インターフェロン（IFN）による治療は困難をともなうことが予想される．しかしながら，肝硬変患者に対しIFNを投与しSVR（sustained viral response；IFN投与終了後6ヵ月間HCV RNA陰性化が持続すること）を得ることができれば，肝線維化の進展阻止や改善[2,3]，肝機能の改善[3]，また，肝発癌率が低下すること[1,3]が報告されており，C型肝硬変に対する安全で適切なIFN治療の実践が望まれる．

わが国では，2006年にセロタイプ1型以外または低ウイルス量の代償期のC型肝硬変症例に対しIFNβ（フェロン®）による抗ウイルス治療が保険認可された．本稿では，C型肝硬変に対するIFN治療の効果につき，海外からの報告やわが国での第Ⅲ相臨床試験について述べる．

□ 欧米での肝硬変におけるIFN治療の抗ウイルス効果

わが国ではいまだ治験段階であるが，欧米では，C型肝硬変に対する抗ウイルス療法はペグ化インターフェロン（Peg-IFN）とリバビリンの併用療法が一般化している．

海外での検討では，肝生検でF3，F4と診断された線維化が進行したC型肝炎患者を対象に，Peg-IFNα-2b 1.5μg/kg/weekまたは0.75μg/kg/weekと，リバビリン800mg/dayで48週間治療したところ，全体でのSVR率は，IFN 1.5μg/kg/week使用群では44.5%（45/101）で，0.75μg/kg/week使用群では37.2%（38/102）であった[4]．この検討では，①genotype 2，3に感染した線維化進行例ではPeg-IFN 0.75μg/kg/weekとリバビリンで治療可能である（SVR：73%），②高ウイルス量のgenotype 1，4，5に感染している例には，Peg-IFNα-2bの量でSVR達成率に優位差は認められないが（SVR；1.5μg：25%，0.75μg：17%），Peg-IFNα-2b 1.5μg/kg/weekとリバビリンの48週間投与が推奨される，③低ウイルス量のgenotype 1，4，5にはPeg-IFN 0.75μg/kg/weekで治療しても，SVRは1.5μgと比較しほぼ同様である（SVR；1.5μg：27%，0.75μg：26%，優位差なし），と

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表1　Peg-IFN とリバビリン併用療法での SVR 率

血小板数	線維化	
	Bridging fibrosis Ishak 3/4	Cirrhosis Ishak 5/6
12.5万/mm³	23%	10%
12.5万/mm³	17%	9%

(Everson GT, et al：Hepatology 44：1675-1684, 2006[5] より引用)

表2　C 型代償性肝硬変に対する IFNβ 治療の効果

投与群	SVR達成率	ALT 持続正常化率
I群（42回投与）	14.6%	16.7%
II群（84回投与）	28.9%	28.9%
III群（126回投与）	38.8%	40.8%

(鈴木　宏，他：医学と薬学 56 (2)：227-251, 2006[6] より引用)

表3　HCV セロタイプ，ウイルス量別の SVR 達成率

セロタイプ	ウイルス量	I群	II群	III群
1型	1Meq/ml未満	5.9%	20.0%	44.4%
1型以外	2Meq/ml未満	16.7%	50.0%	46.2%
1型以外	2Meq/ml以上	20.0%	0.0%	18.8%

(鈴木　宏，他：医学と薬学 56 (2)：227-251, 2006[6] より引用)

された．

また，Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) に登録された 1046 例での検討も報告された（表 1）[5]．この検討では，すべての症例が以前に受けた IFN 治療は無効例で，Ishak score 3 以上の線維化進行例であった．Peg-IFN とリバビリンの併用で SVR は，Ishak 3/4 (bridging fibrosis) で血小板数 12.5 万より多い群では 23%，Ishak 3/4 (bridging fibrosis) で血小板数 12.5 万以下群では 17%，Ishak 5/6（肝硬変）で血小板数 12.5 万より多い群では 10%，Ishak 5/6（肝硬変）で血小板数 12.5 万以下群では 9%であった．この結果から，線維化の進行は，IFN 治療が無効であることの独立した大きな要因であることが示された．

□ わが国での C 型代償性肝硬変に対する IFNβ 療法の治療成績

肝硬変に対する IFN 治療での SVR 達成率は，これまでのわが国と海外からの報告を含めると 0～20%となっている．肝硬変症例は高齢者が多く，血小板数も低下し，さらに高血圧や糖尿病などの合併もしばしばみられるため，IFN 投与については，安易な使用は重篤な副作用をきたす恐れもあり，適応をよく検討する必要がある．

2006 年に，C 型肝硬変患者に対する IFNβ 治療において，その投与回数の違いによる有効性および安全性を検討する目的で，全国 48 施設による多施設共同無作為化非盲検並行群間比較試験の結果が報告された[6]．対象は生検や判別式などにより C 型代償性肝硬変と診断された患者で，肝癌の合併がなく，HCV RNA 量が 1 Meq/ml 未満，または HCV セロタイプ 1 型以外である症例とされた．最初の 1 週間は 600 万 IU/日，2 週目から 6 週目は 300 万 IU/日を連日投与とした．この 42 回投与群を I 群（48 例）とし，II群はさらに投与期間を延長して 300 万 IU/日，週 3 回の間歇投与を 42 回（計 84 回投与：45 例），III群は同様に間歇投与を 84 回（計 126 回投与：49 例）行った．

IFNβ 治療による SVR の達成率は，I 群（42 回投与）で 14.6% (7/48)，II群（84 回投与）で 28.9% (13/45)，III群（126 回投与）で 38.8% (19/49) であり，IFNβ の投与回数が多くなるに従い，SVR 達成率の有意な増加がみられた（表 2）．また，肝機能改善度が著効を示した例（IFN 投与終了後 6ヵ月以内に ALT 値が正常化し，その後 6ヵ月以上正常が持続した症例）は，I 群（42 回投与）で 16.7% (8/48)，II群（84 回投与）で 28.9% (13/45)，III群（126 回投与）で 40.8% (20/49) であり，IFNβ の投与回数が多くなるに従い，肝機能改善度が著効を示した割合が有意に高かった（表 2）．

C 型代償性肝硬変に対する IFNβ 治療における HCV セロタイプ，ウイルス量別の SVR 達成率は，表 3 のようであった．III群（126 回投与）では，低ウイルス量症例に対し，セロタイプ 1 で 44.4% (8/18)，1 以外で 46.2% (6/13) と比較的高い SVR 達成率を示したが，セロタイプ 1 型

表4　HCV セロタイプ，ウイルス量別の ALT 持続正常化率

セロタイプ	ウイルス量	I群	II群	III群
1	1 Meq/ml 未満	11.8%	10.0%	33.3%
1以外	1 Meq/ml 未満	8.3%	40.0%	53.8%
1以外	1 Meq/ml 以上	26.7%	28.6%	31.3%

（鈴木　宏，他：医学と薬学 56（2）：227-251，2006[6]より引用）

以外の高ウイルス量症例では 18.8%（3/16）とSVR 率は低かった．IFNβ 投与量を増やしても，高ウイルス量の C 型肝硬変症例には効果が低いことがうかがわれる．一方，**表 4** のように，肝機能改善度が著効を示した例は，セロタイプ 1 型以外の高ウイルス量症例でも全体で 28.9%（13/45），III群（126 回投与）では，31.3%（5/16）にみられた．

IFNβ 治療の有害事象としては，全例にインフルエンザ症状などがみられ，好中球減少が 26 例（18.3%），血小板減少が 6 例（4.2%）に，その他，上部消化管出血，敗血症，関節炎，パーキンソン病，喘息が各 1 例にみられた．なお，これらの有害事象は投与中にのみ現れ，投与終了後すみやかに消失・回復した．

肝癌の発生は，I 群（42 回投与）4 例，II群（84 回投与）3 例，III群（126 回投与）で 2 例に認められており，II群および III群のカプラン・マイヤー法による生存関数の推定値は，年 7%の肝発癌を仮定した生存関数（指数分布）を上回っていた．

以上より，C 型代償性肝硬変に対する IFNβ 治療の際には，その患者背景から C 型慢性肝炎治療時と比較し，有害事象，特に臨床検査値の異常変動に注意する必要はあるが，長期の投与は可能であるとされた．

□ C 型代償性肝硬変に対する IFNβ の投与法

前述の通り，HCV セロタイプ 1 の血中 HCV-RNA 量が高い場合を除き C 型代償性肝硬変におけるウイルス血症の改善目的に，IFNβ（フェロン®）の使用が保険認可されている．標準的な使用法としては，1 日 600 万 IU を 1 週間，以後 1 日 300 万 IU を 5 週間連日，7 週目より 1 日 300 万 IU を週 3 回静脈内投与，あるいは点滴静注するとされている（**表 5**）．投与期間に制限は設けられていない．間質性肺炎，自殺企図に注意を促す警告のほか，自己免疫性肝炎患者，小柴胡湯投与中の患者には禁忌とされている．その他，IFN-β の副作用として，脳出血，糖尿病，網膜症，蛋白尿などにも注意を要する．また，白血球数 1500/mm^{3}未満，好中球数 750/mm^{3}未満，血小板数 5 万/mm^{3}未満となった場合には IFN の減量または投与間隔の延長を，白血球数 1000/mm^{3}未満，好中球数 500/mm^{3}未満，血小板数 2.5 万/mm^{3}未満となった場合には IFN の中止を考慮すべきとされている（**表 5**）．

表5　C 型代償性肝硬変に対する IFNβ（フェロン®）投与法と減量・中止基準

	開始4週間	5～6週目	7週目以降
投与量 投与間隔	600 万 IU/日 連日	300 万 IU/日 連日	300 万 IU/日 週 3 回

	減量または投与間隔の延長	中止
白血球数	1500/mm^{3}未満	1000/mm^{3}未満
好中球数	750/mm^{3}未満	500/mm^{3}未満
血小板数	5 万/mm^{3}未満	2.5 万/mm^{3}未満

（フェロン®効能・効果追加より）

□ C 型肝硬変への IFN 治療による肝合併症予防効果

IFN 3～6 MU 週 3 回，1 年間投与にて治療を行った C 型肝硬変患者 920 例のレトロスペクティブな検討では，13.5%（124/920）に SVR が認められている．約 4 年の経過観察期間において，SVR 例では肝関連合併症，肝癌合併，肝関連死をきたした割合は，それぞれ 0，0.66，0.19（/100 人/年）であったのに対し，SVR が得られなかった群ではそれぞれ 1.88，2.10，1.44（/100 人/年）であった．これらのことから，IFN 治療による SVR の達成は合併症や肝発癌を減少させ，肝関連死を低下させることが示された[7]．今後は，わが国でも同様に，C 型肝硬変に対する IFNβ 治療による肝発癌抑制効果を検討していく必要がある．

おわりに

HCV RNA 量が 1 Meq/m*l* 未満，または HCV セロタイプ 1 型以外である症例，つまり，比較的 IFN に感受性が高いと考えられる症例に対しては IFNβ の使用が認可され，今後臨床の場でも使用の増加が考えられる．今後，症例の集積により，さらに IFN の適切な投与量や投与期間の検討がなされるべきであろう．また，日本人に多いセロタイプ 1 型高ウイルス量の症例に対しては，現在のところ抗ウイルス療法の認可はなく，これらの症例に対する肝病変の進展，発癌予防も今後の課題である．

文　献

1) Yoshida H, Shiratori Y, Moriyama M, et al : Interferon therapy reduces the risk for hepatocellular carcinoma national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 131 : 174-181, 1999
2) Shiratori Y, Imazeki F, Moriyama M, et al : Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 132 : 517-524, 2000
3) Nishiguchi S, Shiomi S, Nakatani S, et al : Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. Lancet 357 : 196-197, 2001
4) Abergel A, Hezode C, Leroy V, et al : Peginterferon alpha-2b plus ribavirin for treatment of chronic hepatitis C with severe fibrosis : a multicentre randomized controlled trial comparing two doses of peginterferon alpha-2b. J Viral Hepat 13 : 811-820, 2006
5) Everson GT, Hoefs JC, Seeff LB, et al : Impact of disease severity on outcome of antiviral therapy for chronic hepatitis C : Lessons from the HALT-C trial. Hepatology 44 : 1675-1684, 2006
6) 鈴木　宏，西垣　克，熊田博光：低ウイルス量，または HCV セログループ 1 以外の C 型代償性肝硬変に対するインターフェロンベータ（IFN-β）療法．医学と薬学 56 (2) : 227-251, 2006
7) Bruno S, Stroffolini T, Colombo M, et al : Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis : a retrospective study. Hepatology 45 : 579-587, 2007

第2世代抗精神病薬の臨床

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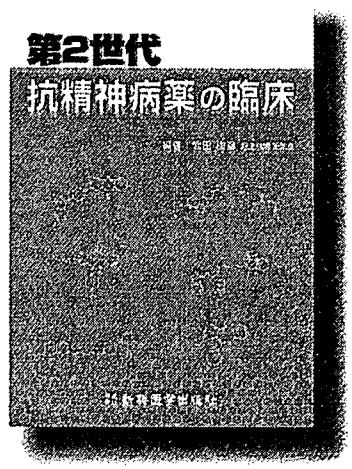

武田　俊彦　編著

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近年、統合失調症の薬物療法でもエビデンスに基づいた科学的、実証的な研究結果が集積されつつあるが、患者のQOLを最高レベルに保つためには、薬理学的知識を駆使するのはもちろんのこと、さらに患者自身や病気への深い理解や経験に裏打ちされた鋭い洞察が求められる。

本書は、第2世代抗精神病薬に関して科学的、実証的情報と、日常臨床における医術（art＝技術）としての薬物療法の架け橋となることを目指して企画された。

主要目次

Ⅰ．第2世代抗精神病薬の意義
Ⅱ．抗精神病薬の薬理学的特徴
Ⅲ．第2世代抗精神病薬 各論
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Original Article

Intrahepatic status of regulatory T cells in autoimmune liver diseases and chronic viral hepatitis

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Aim: Regulatory T cells (Tregs) maintain immunological tolerance and suppress autoreactive immune responses. We evaluated the intrahepatic status of Tregs in patients with autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), chronic hepatitis C (CH-C), or chronic hepatitis B (CH-B).

Methods: We analyzed 85 patients (20 AIH, 22 PBC, 27 CH-C, and 16 CH-B) and 14 controls. Using liver tissue samples obtained by needle biopsy or from marginal parts of resected metastatic liver tumors in the controls, immunohistochemical analyses of forkhead box $P3^+$, which is a specific marker for Tregs, $CD4^+$, and $CD8^+$ cells were performed.

Results: Intrahepatic Tregs were significantly more infiltrated in patients with liver diseases than in the controls. There were significantly fewer intrahepatic Tregs in the AIH patients than in the PBC patients ($P = 0.037$). Patients with a low frequency of intrahepatic Tregs were detected significantly more in the AIH and CH-B groups than in the PBC and CH-C groups ($P < 0.05$). In addition, the frequency of Tregs decreased in the liver of PBC patients as the pathological stage of the disease advanced. We found significantly less infiltration of $CD4^+$ T cells in AIH than in other diseases ($P < 0.05$). Liver-infiltrating $CD8^+$ T cells were detected more frequently in the CH-B group than in other groups ($P < 0.003$).

Conclusion: Intrahepatic Tregs were increased in both patients with autoimmune liver diseases and those with viral hepatitis. In autoimmune liver diseases, intrahepatic Tregs were fewer in the AIH patients than in the PBC patients.

Key words: autoimmune hepatitis, chronic hepatitis, forkhead box P3, primary biliary cirrhosis, regulatory T cells

INTRODUCTION

T-CELL RESPONSES are implicated in host immune defense against microbes as well as immunopathogenesis of certain diseases, such as viral hepatitis. An appropriate T-cell response leads to the eradication of microbes, while a weak response may result in persistent infection. If the T-cell activation is too potent, however, severe inflammation or autoimmune disease may develop. The detailed mechanisms that lead to the breakdown of self-tolerance and the subsequent development of autoimmune disease are still unknown; however, the mechanisms are likely to involve the failure of homeostatic processes that keep the response against self-antigens under control.[1]

T-cell populations regulate and control the balance of immune responses. The $CD4^+$ and $CD25^+$ regulatory T cells (Tregs) are crucial for maintaining immunologic self-tolerance and negative control of various immune responses. The majority of Tregs are produced by the thymus as a functionally distinct T-cell subpopulation and are responsible for maintaining peripheral tolerance. Genetic abnormalities in the development and function of this Treg population can cause autoimmune disease, immunopathology, and allergy in humans.[2] In addition, there are different T-cell subpopulations with regulatory functions, such as natural killer T cells, T helper 3, T regulatory 1, $CD8^+$ and $CD28^-$, and $\gamma\delta$ T cells. These types of T cells may also prevent the activation of autoreactive T cells and be involved in the failure of homeostasis.[1]

Although several cell-surface molecules, such as CD25, glucocorticoid-induced tumor necrosis factor

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receptor family-related gene/protein, and cytotoxic T lymphocyte-associated molecule-4, have been reported as Treg markers, these molecules are also expressed on activated T cells derived from $CD4^+$ and $CD25^-$ naïve T cells.[3] Transcription factor forkhead box P3 (FOXP3) is expressed in $CD4^+$ and $CD25^+$ Tregs as a master control molecule for their development and function in mice and humans, thus, FOXP3 is thought to be a specific marker of Tregs.

Autoimmune mechanisms are involved in autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC). AIH is an inflammatory liver disease characterized by high levels of transaminases, circulating autoantibodies, hyper-γ-globulinemia, histological evidence of interface hepatitis, and response to immunosuppressive treatment.[4,5] PBC is an enigmatic liver disease characterized by the chronic non-suppurative destruction of small intrahepatic bile ducts, portal inflammation, and the presence of antimitochondrial antibodies (AMA).[6,7] The presence of AMA and autoreactive T and B cells, in conjunction with the co-occurrence of other autoimmune diseases, characterizes PBC as a typical autoimmune disease.[8] Although the etiology of PBC remains obscure, recent data suggest that autoreactive T-cell responses play a major role in its pathophysiology.[9–12]

Hepatitis C virus (HCV) infection is often asymptomatic, and approximately 80% of infected patients progress to chronic hepatitis.[13] After HCV infection, interaction between the innate and adaptive immune responses plays a pivotal role in perpetuation or clearance of HCV infection. T helper 1-type (Th1) cytokines, such as interferon (IFN)-γ and interleukin (IL)-2, are involved in cell-mediated immunity and play a crucial role in protection against intracellular pathogens.[14] A weak cellular immune response is thought to be one of the mechanisms of HCV persistence.

In hepatitis B virus (HBV) infection, a multispecific $CD4^+$ and $CD8^+$ T cell with a Th1 cytokine profile is also important for control of the infection.[15] These multispecific T-cell responses are maintained for decades after clinical recovery. However, these responses are lacking in patients with chronic HBV infection, and the mechanism of T-cell hyporesponsiveness or tolerance is still unknown.

The frequency of Tregs in the peripheral blood was decreased in patients with AIH and PBC and increased in patients with chronic hepatitis C (CH-C) and chronic hepatitis B (CH-B) compared with the healthy controls.[16] However, there are few reports investigating the intrahepatic status of Tregs. In the present study, we analyzed and compared the intrahepatic status of Tregs in patients with AIH, PBC, CH-C, and CH-B because liver-infiltrating immune cells should reflect the status of disease and pathogenesis more directly than peripheral cells.

METHODS

Patients and liver tissue

NEEDLE BIOPSIES WERE performed to obtain liver tissue from 85 patients, consisting of 20 AIH patients, 22 PBC patients, 27 CH-C patients, and 16 CH-B patients. All patients had a persistently increased level of serum alanine aminotransferase (ALT; >30 IU/L). The diagnosis of each case was based on reliable clinical and laboratory data and was independently confirmed histologically by two pathologists who specialize in liver diseases. All AIH patients were antinuclear antibody positive or antismooth muscle antibody positive, and all had histological features of interface hepatitis. Patients with morbid changes in bile duct were excluded individually by retrograde radiological cholangiography or magnetic resonance cholangiopancreatography. Patients with overlap syndrome were also excluded from this study. All PBC patients were AMA positive and fulfilled the diagnostic criteria of PBC based on internationally accepted standards. Livers from PBC patients were staged histologically by Scheuer's classification. Seventeen and five patients were of stage 1 and of stages 2/3/4, respectively. We included 14 patients with metastatic liver tumors as the controls. The control patients were not infected with HBV (negative for hepatitis B surface antigen) or HCV (negative for anti-HCV antibody), and they had no history of autoimmune diseases and were negative for autoimmune antibodies. Liver tissue from control patients was obtained from a marginal part of the resected liver in which the histological examination was normal. Table 1 shows the patients' characteristics. All patients gave written informed consent according to a protocol approved by the Ethical Committee of Showa University.

Immunohistochemical staining

Liver needle biopsies and resected tissues were obtained from the 99 patients, as described earlier. All tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin, and 3 μm-thick serial sections were cut from each paraffin block. Each specimen contained at least three portal tracts encompassing interlobular bile ducts, and a total of 297 portal tracts were counted. Antigen retrieval for CD4 and FOXP3 staining

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Table 1 Characteristics of the patients and controls analyzed in this study

Group (number)	AIH (20)	PBC (22)	CH-C (27)	CH-B (16)	Control (14)
Female (%)	95.0*,†,††	90.9†,‡‡,¶	40.7	18.8	28.6
Age (years)	55.8 ± 13.8†	55.5 ± 11.2‡‡	49.8 ± 11.9§,§§	38.1 ± 11.6$^{\|}$	59.1 ± 14.2
ALT (IU/L)	393 ± 462*,**,††	113 ± 139‡‡,¶	85 ± 57§,§§	295 ± 351$^{\|}$	17 ± 9
AST (IU/L)	306 ± 393*,**,††	88 ± 78¶	60 ± 40§,§§	148 ± 141$^{\|}$	22 ± 9
ALP (IU/L)	495 ± 300*,**,¶	842 ± 547†,‡‡,¶	268 ± 85	320 ± 96	316 ± 171
IgG (mg/dL)	2639 ± 1163*,**	1837 ± 584	1794 ± 290	n.d.	n.d.
IgM (mg/dL)	334 ± 355**	466 ± 231†	129 ± 67	n.d.	n.d.

Significance was assessed with Fisher's exact probability test. $P < 0.05$ (*AIH versus PBC, **AIH versus CH-C, †AIH versus CH-B, ††AIH versus Control, ‡PBC versus CH-C, ‡‡PBC versus CH-B, ¶PBC versus Control, §CH-C versus CH-B, §§CH-C versus Control, $^{\|}$CH-B versus Control). Values are mean ± standard deviation. AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH-B, chronic hepatitis B; CH-C, chronic hepatitis C; IgG, immunoglobulin G; IgM, immunoglobulin M; n.a., not determined; PBC, primary biliary cirrhosis.

was achieved by pressure cooking for 5 min in citrate buffer (pH 7.0), while antigen retrieval for CD8 staining was achieved by microwaving for 15 min in citrate buffer (pH 7.0). For CD4 or CD8 immunohistochemical staining, anti-CD4 monoclonal antibody (mAb; Nichirei Biosciences, Tokyo, Japan) or anti-CD8 mAb (Dako Cytomation, Tokyo, Japan) and biotinylated goat antimouse immunoglobulin G (IgG; Dako ChemMate Envision kit/HRP[DAB], Dako, Japan) were used. FOXP3 expression was analyzed by immunostaining with a goat antihuman FOXP3 polyclonal antibody (ab22510; Abcam, Cambridge, UK) and biotinylated rabbit antigoat IgG (Dako ChemMate Envision kit/HRP[DAB]). The slides were stained with hematoxylin following immunohistochemical staining.

Evaluation of frequency of FOXP3-, CD4-, and CD8-positive cells

To evaluate and compare the distribution and frequency of cells positive for FOXP3, CD4, and CD8, three small- to medium-sized portal tract areas were selected for investigation with an optical microscope. The same visual fields were chosen and examined using serial sections. The numbers of FOXP3-, CD4-, or CD8-positive cells contained within the three portal tract areas from each specimen were counted at a magnification of ×400 by two independent observers in a blinded fashion. To correct for differences in the sizes of the portal tracts, the proportion of FOXP3^{+} Tregs was determined as follows: %FOXP3 = (counts of FOXP3^{+} Tregs/counts of total mononuclear cells) × 100, which is a total mononuclear cell-corrected value for FOXP3^{+}. CD4^{+} and CD8^{+} T cells in total mononuclear cells were also calculated.

Statistical analyses

Significance was assessed with the Mann–Whitney *U*-test or Fisher's exact probability test. Differences between groups were considered statistically significant when the *P*-value was less than 0.05.

RESULTS

Intrahepatic Tregs were significantly more infiltrated in patients with liver diseases than in the controls

TO COMPARE THE frequencies of intrahepatic FOXP3^{+} Tregs between the liver diseases, we determined the percentage of FOXP3, as described in Methods. As shown in Figure 1, the frequency of FOXP3^{+} T cells in patients with AIH, PBC, CH-C, or CH-B was significantly much higher than that in the control patients. Interestingly, there were significantly fewer FOXP3^{+} T cells in the liver tissues of AIH patients than in those of PBC patients ($P = 0.037$). The frequency of intrahepatic FOXP3^{+} Tregs in the AIH patients was not different from that in the patients with CH-C or CH-B.

Patients with a low frequency of intrahepatic Tregs were detected significantly more in the AIH and CH-B groups than in the PBC and CH-C groups

Since the patients with numerous intrahepatic FOXP3^{+} Tregs were observed in the PBC and CH-C groups, we separated the patients into two groups according to the frequency of intrahepatic Tregs. The patients were divided into those with FOXP3^{+} cells of less than 9% and those with FOXP3^{+} cells of 9% or more: this level

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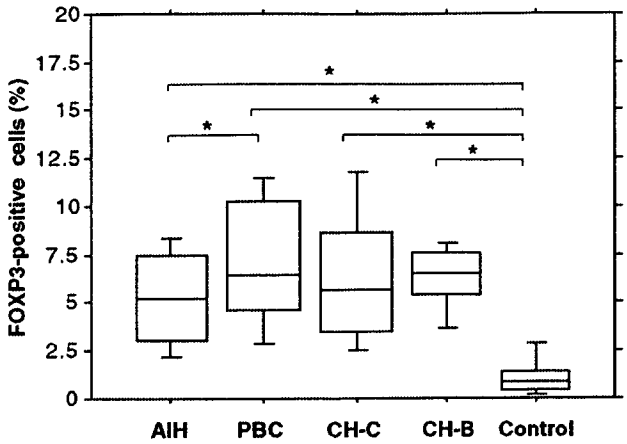

Figure 1 Intrahepatic forkhead box P3⁺ (FOXP3⁺) T cells in autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), chronic hepatitis C (CH-C), chronic hepatitis B (CH-B), and controls. To compare intrahepatic FOXP3⁺ cell status, intrahepatic FOXP3⁺ cells in patients with AIH, PBC, CH-C, and CH-B were stained. For the enumeration of positive mononuclear cells, mononuclear cells were counted in three high-powered fields (×400) by two independent observers in a blinded fashion. For each sample, the mean percentage of positive cells was chosen. Results are expressed as the median and range of all tested patents in each group. $P < 0.05$.

was decided arbitrarily as follows: (mean percentage of FOXP3 of controls + 3 standard deviation) × 2. When the number of patients with a high frequency of intrahepatic Tregs (%FOXP3 ≥9%) and that with a low frequency (%FOXP3 <9%) were compared for each liver disease, patients who had a low frequency of intrahepatic Tregs were detected significantly more in the AIH group than in PBC and CH-C groups as shown in Table 2. In addition, more patients with low frequency Treg infiltration were found in the CH-B group than in the PBC and CH-C groups. Thus, PBC is characterized by higher frequency of FOXP3⁺ cells compared to AIH, whereas the number and profiles of liver-infiltrating T cells are comparable. In viral hepatitis, a higher frequency of FOXP3⁺ cells is observed in CH-C, while higher frequency of CD4⁺ or CD8⁺ cells is characteristic for HBV-infected liver.

Table 3 Comparison of the intrahepatic Tregs frequency with histological stages in PBC patients

	<9%	≥9%	Total
Early stage	8	9	17
Advanced stage	5	0	5
Total	13	9	22

$P = 0.034$. PBC patients were divided into two groups as early stage (Scheuer's classification stage 1) and advanced stage (stages 2/3/4). Number of patients with a high frequency of intrahepatic regulatory T cells (Tregs; %FOXP3 ≥9%) and that with a low frequency (%FOXP3 <9%) were compared for each stage. PBC, primary biliary cirrhosis.

When the PBC patients were divided into two groups as early stage (Scheuer's classification stage 1) and advanced stage (stages 2/3/4), the frequency of Tregs was higher than 9% in nine of 17 (53%) PBC patients with early histological stage, while that of Tregs was below 9% in all patients with advanced stage ($P = 0.034$), as shown in Table 3. Furthermore, as shown in Figure 2, more FOXP3⁺ T-cell infiltration was seen in the early stage than in the advanced stage (8.03 ± 3.50 *vs* 4.47 ± 1.40, $P = 0.041$). Therefore, it was thought that the frequency of Tregs decreased in the liver of PBC patients as the pathological stage of the disease advanced.

Frequency of intrahepatic CD4⁺ T cells was lower in AIH patients, while the frequency of intrahepatic CD8⁺ T cells was higher in CH-B patients

We evaluated the intrahepatic frequencies of CD4⁺ cells as well as CD8⁺ cells to investigate whether these

Table 2 Comparison of the number of patients with high frequency of intrahepatic Tregs with those with low frequency

	<9%	≥9%	Versus PBC*	Versus CH-C**
AIH ($n = 20$)	20	0	$P = 0.001$	$P = 0.014$
PBC ($n = 22$)	13	9	-	$P = 0.266$
CH-C ($n = 27$)	20	7	$P = 0.266$	-
CH-B ($n = 16$)	16	0	$P = 0.003$	$P = 0.026$
Control ($n = 14$)	14	0	$P = 0.006$	$P = 0.036$

Significance was assessed with Fisher's exact probability test. *P*-values are shown as VS PBC group(*) and VS CH-C(**) group. AIH, autoimmune hepatitis; CH-B, chronic hepatitis B; CH-C, chronic hepatitis C; PBC, primary biliary cirrhosis; Tregs, regulatory T cells

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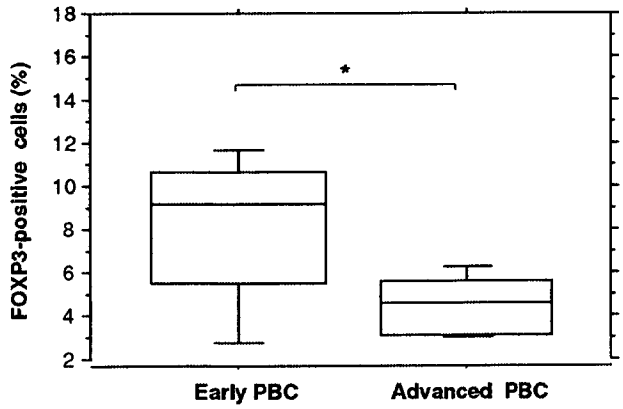

Figure 2 Frequency of regulatory cells (Tregs) in the liver of primary biliary cirrhosis (PBC) patients in terms of pathological stage of the disease advances. Intrahepatic forkhead box P3$^+$ (FOXP3$^+$) T cells in the PBC patients were divided into two groups as early stage (Scheuer's classification stage 1) and advanced stage (stages 2/3/4). Results are shown as the mean percentage of Tregs frequency ± standard deviation in each stage. $P < 0.05$.

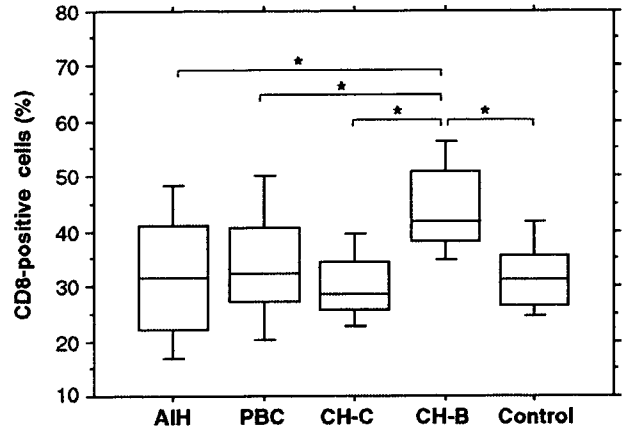

Figure 4 Intrahepatic CD8$^+$ T cells in autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), chronic hepatitis C (CH-C), chronic hepatitis B (CH-B), and controls. To compare intrahepatic CD8$^+$ cell frequency, intrahepatic CD8$^+$ cells in patients with AIH, PBC, CH-C, and CH-B were stained. CD8$^+$ cells were counted with the same procedure used for forkhead box P3$^+$ cells. Results are expressed as the median and range of all tested patents in each group. $P < 0.05$.

immune cells were involved in the immunopathogenesis of each liver disease. As shown in Figure 3, the frequency of CD4$^+$ T cells infiltrating the liver tissue was significantly higher in CH-B patients than in the controls. We found significantly less infiltrating CD4$^+$ T cells in the AIH patients than in the PBC patients ($P = 0.007$), CH-C patients ($P = 0.045$), and CH-B patients ($P < 0.001$). As shown in Figure 4, the frequency of CD8$^+$ T cells was significantly higher in the CH-B patients than in the controls. There were also significantly higher CD8$^+$ T cells in the liver tissues of the CH-B patients than in those of the AIH patients ($P = 0.003$), PBC patients ($P = 0.002$), and CH-C patients ($P < 0.001$).

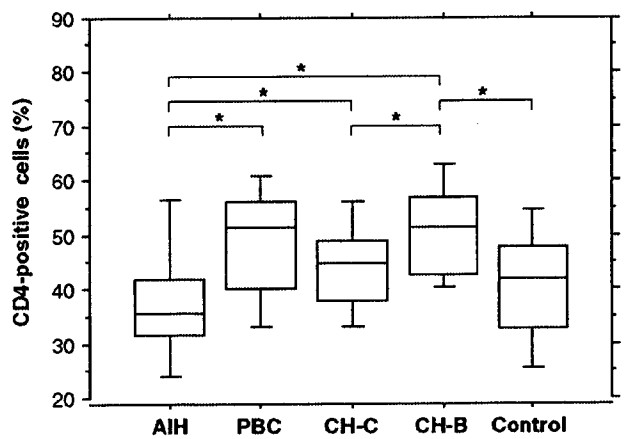

Figure 3 Intrahepatic CD4$^+$ T cells in autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), chronic hepatitis C (CH-C), chronic hepatitis B (CH-B), and controls. To compare intrahepatic CD4$^+$ cell frequency, intrahepatic CD4$^+$ cells in patients with AIH, PBC, CH-C, and CH-B were stained. CD4$^+$ cells were counted with the same procedure used for forkhead box P3$^+$ cells. Results are expressed as the median and range of all tested patients in each group. $P < 0.05$.

Furthermore, the CD4$^+$/CD8$^+$ ratio was lower in the CH-B patients than in the PBC patients (1.18 ± 0.26 *vs* 1.56 ± 0.66, $P = 0.037$) and CH-C patients (1.18 ± 0.26 *vs* 1.49 ± 0.39, $P = 0.007$). There was no difference in the total infiltration of mononuclear cells between patients with AIH, PBC, CH-C, and CH-B. Intrahepatic CD4$^+$ T cells and CD8$^+$ T cells in the control patients were significantly less than in the CH-B patients ($P = 0.013$ and $P < 0.001$, respectively), although we did not detect any differences between the control group and the other liver disease groups. There was no relationship between the biochemical data or histological activities and infiltration of the immune cells.

Since intrahepatic immune cells may directly affect inflammation in the liver, we compared the biochemical data, such as the serum ALT level, and histological activities with the intrahepatic frequencies of FOXP3$^+$, CD4$^+$, and CD8$^+$ cells. There was no relationship between the ALT, alkaline phosphatase, IgG, immuno-

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globulin M level, or histological activities and the frequency of infiltrating immune cells other than described above (data not shown).

DISCUSSION

TREGS ARE THOUGHT to play roles in immune regulation, such as the suppression of severe inflammation and autoimmune diseases. The removal or reduction of Tregs can also enhance immune responses against infectious microbes, thus, Tregs affect the elimination of infectious microbes.[17-24] A higher proportion of $CD4^+$ and $CD25^+$ T cells in peripheral blood was found in patients with chronic HCV infection as compared to recovered patients and normal controls.[25] Tregs secrete transforming growth factor-β_1 and IL-10, and these cytokines may attenuate the function of macrophages. IL-10 also inhibits HCV-specific immunity when administered exogenously in patients with chronic HCV infection.[26] Thus, Tregs may disturb the eradication of HCV and lead to chronic infection. Chronic HBV patients harbor an increased frequency of Tregs in peripheral blood as compared to control patients, and Tregs have an immunosuppressive effect on HBV-specific T helper cells.[27] This may be one of the mechanisms that leads to chronic infection.

Several recent studies have focused on Tregs in patients with autoimmune liver diseases, such as AIH and PBC. Since Tregs prevent the proliferation and effector function of autoreactive T cells[16] and downregulate the production of IFN-γ by $CD8^+$ T cells in a murine model and in humans,[28,29] Tregs may be implicated in the pathogenesis of AIH and PBC. In fact, the relative frequencies of Tregs are decreased in peripheral blood samples of patients with PBC,[30] and Tregs are few in patients with AIH.[16] However, there are only a few reports regarding the status of intrahepatic Tregs.

Tregs maintain the ability to suppress IFN-γ production by $CD4^+$ and $CD25^-$ T cells in AIH, and circulating Tregs are significantly less in AIH patients than in controls.[16] However, few details regarding the roles of Tregs in the pathogenesis of AIH have been revealed.

In the present study, we demonstrated that intrahepatic Tregs were significantly more infiltrated in patients with liver diseases than in the controls. Indeed there are significantly fewer intrahepatic Tregs in AIH patients and CH-B patients than in PBC patients and CH-C patients, but as a whole, there is more infiltration of $FOXP3^+$ Tregs than in the controls, and there is not a great difference. In addition, we found significantly fewer infiltrating $CD4^+$ T cells in AIH patients than in the patients with other diseases, whereas $CD8^+$ T cells infiltrating liver tissue were detected with a significantly greater frequency in CH-B patients than in the other patients.

Although both AIH and PBC are representative autoimmune liver diseases, we identified differences in immune cell infiltration between these two autoimmune diseases in the present study. The results indicate that different mechanisms are involved in the pathogenesis of AIH and PBC. However, there are significantly more ratios of Tregs than control, and it seems that only a ratio of Tregs does not relate to the pathogenesis of these diseases.

We found that the frequency of Tregs decreased in the liver of PBC patients as the pathological stage of the disease advanced. A previous report demonstrated that there were few liver-infiltrating Tregs in PBC patients,[30] although it has not been confirmed by other researches. Sasaki *et al.* recently reported findings similar to ours. They found that the extent of $FOXP3^+$ Tregs in inflamed portal tracts with chronic non-suppurative destructive cholangitis in early stage (Scheuer's classification 1 and 2) of PBC was higher than that in late stage (Scheuer's classification 3, 4) of PBC.[31]

It is not clear whether this decrease of Tregs is a cause or a result of disease progression. Although we cannot explain the reason for these differences in Tregs' infiltration, the race of the study patients may be one of the factors. Functional investigations of intrahepatic Tregs in these autoimmune liver diseases may clarify this issue.

Since the frequency of intrahepatic Tregs in CH-C groups is diverse widely, we could not detect a significant difference in Tregs' accumulation between the CH-C and AIH groups. However, several CH-C patients had a large number of intrahepatic Tregs. When we divided the patients in each group into those with $FOXP3^+$ cells of less than 9% and those with $FOXP3^+$ cells of 9% or more, a significant difference was confirmed. In addition, patients who had a high frequency of intrahepatic Tregs were detected significantly more often in the CH-C group than in the CH-B group. In HCV infection, it has been suggested that HCV itself, especially in the NS3 region, induces Tregs in patients with HCV infection as well as in healthy donors,[32] and these Tregs are involved in the development of viral persistence, which occurs usually in acute HCV infection and rarely in acute HBV infection in adults. Thus, in chronic hepatitis, the pathogenesis of HCV should be different from that of HBV.

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There were only a few Tregs in the pathologically normal tissue that surrounded metastatic liver tumors. The same phenomenon has been described in other reports.[30,33] The decreased frequency of Tregs was not likely to be the effect of metastatic tumors, because it has been reported that malignant tumors often induce Tregs.[34–39] In normal liver tissue, Treg infiltration may be suppressed because it is necessary to induce immunity against many pathogens flowing into the liver, rather than prevent inflammation or induction of autoimmunity.

Intrahepatic Tregs may be involved with immunopathogenesis and play a crucial direct role in the development of each liver disease. However, since immune systems in liver diseases are complicated, further investigations are needed to clarify the detailed relationship between Tregs and immunopathogenesis.

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REFERENCES

1 Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002; **2**: 389–400.
2 Sakaguchi S, Setoguchi R, Yagi H, Nomura T. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in self-tolerance and autoimmune disease. *Curr Top Microbiol Immunol* 2006; **305**: 51–66.
3 Sugimoto N, Oida T, Hirota K *et al.* Foxp3-dependent and – independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis. *Int Immunol* 2006; **18**: 1197–209.
4 Gregorio GV, Portmann B, Reid F *et al.* Autoimmune hepatitis in childhood: a 20-year experience. *Hepatology* 1997; **25**: 541–7.
5 Mieli-Vergani G, Vergani D. Progress in pediatric autoimmune hepatitis. *Semin Liver Dis* 1994; **14**: 282–8.
6 Selmi C, Invernizzi P, Keeffe EB *et al.* Epidemiology and pathogenesis of primary biliary cirrhosis. *J Clin Gastroenterol* 2004; **38**: 264–71.
7 Kaplan MM, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med* 2005; **353**: 1261–73.
8 Ichiki Y, Shimoda S, Ishibashi H, Gershwin ME. Is primary biliary cirrhosis a model autoimmune disease? *Autoimmun Rev* 2004; **3**: 331–6.
9 Kita H, Lian ZX, Van de Water J *et al.* Identification of HLA-A2-restricted CD8 (+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. *J Exp Med* 2002; **195**: 113–23.
10 Kita H, Imawari M, Gershwin ME. Cellular immune response in primary biliary cirrhosis. *Hepatol Res* 2004; **28**: 12–17.
11 Shimoda S, Nakamura M, Ishibashi H, Hayashida K, Niho Y. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. *J Exp Med* 1995; **181**: 1835–45.
12 Matsumura S, Kita H, He XS *et al.* Comprehensive mapping of HLA-A0201-restricted CD8 T-cell epitopes on PDC-E2 in primary biliary cirrhosis. *Hepatology* 2002; **36**: 1125–34.
13 Huang Z, Murray MG, Secrist JA 3rd. Recent development of therapeutics for chronic HCV infection. *Antiviral Res* 2006; **710**: 351–62.
14 Bertoletti A, D'Elios MM, Boni C *et al.* Different cytokine profiles of intrahepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997; **112**: 193–9.
15 Bertoletti A, Naoumov NN. Translation of immunological knowledge into better treatments of chronic hepatitis B. *J Hepatol* 2003; **39**: 115–24.
16 Longhi MS, Ma Y, Bogdanos DP, Cheeseman P, Mieli-Vergani G, Vergani D. Impairment of CD4 (+) CD25 (+) regulatory T-cells in autoimmune liver disease. *J Hepatol* 2004; **41**: 31–7.
17 Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. *Nature* 2002; **420**: 502–7.
18 Aseffa A, Gumy A, Launois P, MacDonald HR, Louis JA, Tacchini-Cottier F. The early IL-4 response to Leishmania major and the resulting Th2 cell maturation steering progressive disease in BALB/c mice are subject to the control of regulatory CD4+CD25+ T cells. *J Immunol* 2002; **169**: 3232–41.
19 Xu D, Liu H, Komai-Koma M *et al.* CD4+CD25+ regulatory T cells suppress differentiation and functions of Th1 and Th2 cells, Leishmania major infection, and colitis in mice. *J Immunol* 2003; **170**: 394–9.
20 Iwashiro M, Messer RJ, Peterson KE, Stromnes IM, Sugie T, Hasenkrug KJ. Immunosuppression by CD4+ regulatory T cells induced by chronic retroviral infection. *Proc Natl Acad Sci USA* 2001; **98**: 9226–30.
21 Montagnoli C, Bacci A, Bozza S *et al.* B7/CD28-dependent CD4+CD25+ regulatory T cells are essential components of the memory-protective immunity to *Candida albicans*. *J Immunol* 2002; **169**: 6298–308.
22 Raghavan S, Suri-Payer E, Holmgren J. Antigen-specific in vitro suppression of murine Helicobacter pylori-reactive

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immunopathological T cells by CD4CD25 regulatory T cells. *Scand J Immunol* 2004; **60**: 82–8.
23 Hori S, Carvalho TL, Demengeot J. CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyper-inflammation driven by Pneumocystis carinii in immunodeficient mice. *Eur J Immunol* 2002; **32**: 1282–91.
24 Singh B, Read S, Asseman C *et al.* Control of intestinal inflammation by regulatory T cells. *Immunol Rev* 2001; **182**: 190–200.
25 Cabrera R, Tu Z, Xu Y *et al.* An immunomodulatory role for CD4 (+) CD25 (+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004; **40**: 1062–71.
26 Nelson DR, Tu Z, Soldevila-Pico C *et al.* Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. *Hepatology* 2003; **38**: 859–68.
27 Alvarez F, Berg PA, Bianchi FB *et al.* International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929–38.
28 Piccirillo CA, Shevach EM. Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells. *J Immunol* 2001; **167**: 1137–40.
29 Camara NO, Sebille F, Lechler RI. Human CD4+CD25+ regulatory cells have marked and sustained effects on CD8+ T cell activation. *Eur J Immunol* 2003; **33**: 3473–83.
30 Lan RY, Cheng C, Lian ZX *et al.* Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. *Hepatology* 2006; **43**: 729–37.
31 Sasaki M, Ikeda H, Sawada S, Sato Y, Nakanuma Y. Naturally-occurring regulatory T cells are increased in inflamed portal tracts with cholangiopathy in primary biliary cirrhosis. *J Clin Pathol* 2006; **60**: 1102–7.
32 Tajimi M, Ugajin T, Ota M, Hiroishi K, Nakamura I, Imawari M. Immune responses of liver-infiltrating lymphocytes and peripheral blood mononuclear cells to hepatitis C virus core and NS3 antigens. *Hepatol Res* 2006; **35**: 250–5.
33 Xu D, Fu J, Jin L *et al.* Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739–47.
34 Yang XH, Yamagiwa S, Ichida T *et al.* Increase of CD4+ CD25+ regulatory T-cells in the liver of patients with hepatocellular carcinoma. *J Hepatol* 2006; **45**: 254–62.
35 Woo EY, Chu CS, Goletz TJ *et al.* Regulatory CD4 (+) CD25 (+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001; **61**: 4766–72.
36 Liyanage UK, Moore TT, Joo HG *et al.* Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol* 2002; **169**: 2756–61.
37 Viguier M, Lemaitre F, Verola O *et al.* Foxp3 expressing CD4+CD25 (high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. *J Immunol* 2004; **173**: 1444–53.
38 Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A. CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. *Cancer* 2003; **98**: 1089–99.
39 Ichihara F, Kono K, Takahashi A, Kawaida H, Sugai H, Fujii H. Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers. *Clin Cancer Res* 2003; **9**: 4404–8.

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Original Article

Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts

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Aim: We aimed to identify the candidates for antiviral therapy, among patients who are hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT), focused on the inhibition of hepatocellular carcinoma (HCC).

Methods: Four hundred and sixty-four HCV carriers with normal serum ALT and 129 HCV carriers with persistently normal ALT (PNALT) and platelet (PLT) counts≥150 000/μL who received liver biopsies were enrolled. HCV carriers with normal serum ALT were divided into four groups according to their ALT levels (≤30 U/L or 31–40 U/L) and PLT counts (≥150 000/μL or <150 000/μL).

Results: In 129 HCV carriers with PNALT, the rate of progression of fibrosis stage was 0.05/year and no HCC was detected during the follow up for 10 years. Approximately 20% of patients with ALT ≤40 U/L and PLT counts ≥150 000/μL were at stage F2–3; however, approximately 50% of patients with ALT ≤ 40 U/L and PLT counts <150 000/μL were at stage F2–4. An algorithm for the management of HCV carriers with normal serum ALT was advocated based on ALT and PLT counts.

Conclusion: The combination of ALT and PLT counts is useful for evaluating the fibrosis stage in HCV carriers with normal serum ALT. Most patients with PLT counts <150 000/μL are candidates for antiviral therapy, especially those with ALT levels ≥31 U/L when we focus on the inhibition of the development of HCC.

Key words: antiviral therapy, chronic hepatitis C, hepatitis C virus carriers, normal serum aminotransferase, platelet count

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) caused by hepatitis C virus (HCV) infection usually develops in patients with advanced chronic hepatitis (CH) or liver cirrhosis. The antiviral treatment for chronic hepatitis C (CH-C) is useful for inhibiting hepatic inflammation and progression of hepatic fibrosis, and consequently the development of HCC.[1–6]

Serum aminotransferase (ALT) levels are within the normal ranges in 20–40% of patients with chronic HCV infection,[7–11] defining the upper limit of normal serum ALT as ≤40 U/L. Significant hepatic fibrosis (≥F2 by the METAVIR classification) has been demonstrated in 5–30% of such patients.[9,12–16] We reported previously

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that HCV carriers with persistently normal ALT (PNALT) had histological features ranging from normal to minimal CH[17,18]; they showed slow progression of liver fibrosis and were at very low risk of developing HCC.[18]

The National Institute of Health Consensus Development Conference reported that HCV carriers with normal serum ALT are candidates for antiviral therapy.[19] A controlled study for the treatment of HCV carriers with PNALT with pegylated interferon alpha and ribavirin (PEG-IFN/Riba) for 48 weeks led to the eradication of HCV RNA in 40% of patients with genotype 1 and high viral load,[20] which is similar to the results of CH-C patients with elevated ALT levels.[21,22] However, it remains controversial whether these patients are candidates for antiviral therapy because of the limited efficacy of treatment, post-treatment flare-up, various side-effects, high cost of treatment, and their good prognoses.

In many Western countries, the upper limits of normal serum ALT are below 40 U/L;[23] however, a recent report from Italy demonstrated that the upper limit in healthy individuals was less than 30 U/L for men and 19 U/L for women.[24] We attempted to draft therapeutic guidelines for the treatment of HCV carriers with normal serum ALT. The biochemical and histological analyses were performed in HCV carriers with serum ALT levels below 40 U/L. These patients were divided into two groups based on ALT levels and then further divided into two subgroups according to their platelet (PLT) counts. We proposed an algorithm for the treatment of HCV carriers with normal serum ALT, taking into consideration the risk of progression to cirrhosis and the development of HCC. The present study demonstrated that the ranges of serum ALT and PLT counts are useful for deciding the indication of antiviral therapy for HCV carriers with normal serum ALT.

METHODS

Eligibility and definition

TWELVE HEPATOLOGISTS BELONGING to the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis, supported by the Ministry of Health, Labour and Welfare of Japan, which was settled on April 2004, participated in the study. Hiromitsu Kumada (Toranomon Hospital, Tokyo, Japan) serves as a chief and Takeshi Okanoue served as a researcher responsible for drafting the guidelines for the treatment of HCV carriers with normal serum ALT. In the present study, we tentatively defined the upper limit of the normal serum ALT as ≤40 U/L.

Patients with hepatitis B virus surface antigen, previous IFN treatment, history of heavy alcohol abuse, antinuclear antibody or antismooth muscle antibody, overt diabetes mellitus, or obesity (body mass index; ≥25 kg/m^2) were excluded from the study.

All of the patients underwent liver biopsy (≥2.0 cm in length) within 6 months prior to antiviral therapy, at which time their serum ALT levels were ≤40 U/L. Informed consent was obtained from every patient prior to liver biopsy and antiviral therapy.

Another study was conducted from January 1990 to August 2004 at Kyoto Prefectural University of Medicine (Kyoto, Japan). HCV carriers with PNALT were defined by serum ALT levels ≤30 U/L on at least three different occasions over a 12-month period and PLT counts ≥150 000/μL as reported previously.[18]

Study design

Among the 580 HCV carriers with normal serum ALT (≤40 U/L), 116 patients were excluded from the study because of insufficient data. Thus, 464 patients who received antiviral therapy from 1995 to 2004 were enrolled in this study (Table 1). Formalin-fixed liver specimens were stained with hematoxylin–eosin, and with Masson's trichrome. The liver specimens ($n = 262$) were also stained with Perls' Prussian blue to study hepatic iron loading. The histological findings were scored according to the classification proposed by Desmet *et al.*[25] and Ishak *et al.*[26] Steatosis was defined as fat droplets in >10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0–4+, based on the scoring system of MacSween *et al.*[27]

The serum ALT, blood glucose level, immunoreactive insulin (IRI), serum ferritin, PLT count, serum hyaluronic acid, amount of serum HCV RNA, and the HCV genotype were examined. The homeostasis model assessment–insulin resistance was calculated as follows: plasma fasting glucose (mg/dL) × IRI (ng/mL) ÷ 405. The serum HCV RNA levels were determined using an Amplicor GT HCV monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotype 1 (G1) and 2 (G2) were determined by a serologic genotyping assay.[28] G1 and G2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds *et al.*[29]

All the patients received IFN monotherapy or IFN/Riba combination therapy for 12–36 weeks. The average

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Table 1 Baseline of hepatitis C virus patients with normal serum aminotransferase (ALT) received antiviral therapy

	ALT ≤ 30 U/L (group A)	ALT 31–40 U/L (group B)	*P*-value
No. patients	255	209	
Age	51.6 ± 13.0	53.5 ± 13.2	0.548*
Sex (male/female)	112/143	117/92	0.01**
BMI (kg/m^2)	21.6 ± 2.9	22.8 ± 3.0	<0.001*
HOMA-IR	2.5 ± 3.2	5.2 ± 6.5	0.093*
Genotype: 1/2/others	127/127/1	112/96/1	0.881**
Viral load: low/high	138/117	99/110	0.203**
G1 (low/high)	114/125		
G2 (low/high)	161/62		
Histology			
F stage (0/1/2/3/4)	29/166/48/11/1	22/122/57/6/2	0.169**
Grade (0/1/2/3)	25/187/41/2	7/159/43/0	0.046**
Fatty change† 0–1/2–4	232/23	161/48	0.033**
Iron load‡ 0/1–4	101/15	97/19	0.458**
Ferritin (ng/mL)	83.9 ± 103.7	118.8 ± 135.3	0.006*
PLT count (/μL)	19.2 ± 5.4	18.4 ± 6.1	0.059*
≥150 000/<150 000	204/51	141/68	0.002**
Hyaluronate (ng/mL)	60.8 ± 73.7	69.1 ± 73.0	0.249*
Duration of antiviral therapy (weeks)	25.6 ± 12.0	26.1 ± 12.1	0.297*
Effects of therapy			
SVR/non-SVR	142/113	99/110	0.075**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in sex distribution ($P = 0.01$), BMI ($P = 0.01$), frequency of steatosis ($P = 0.033$), serum ferritin level ($P = 0.006$), grade of hepatic inflammation ($P = 0.046$), incidence of fatty change ($P = 0.033$), serum ferritin level ($P = 0.006$), and the incidence of low PLT counts ($P = 0.002$) between groups A and B. Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet; SVR, sustained viral responders.

duration of therapy between 1995 and 2003 was 26 weeks for IFN monotherapy and 24 weeks for IFN/Riba combination therapy. In principle, 6–10 MU IFN was administered daily for 2 weeks and three times per week subsequently. The daily dosage of ribavirin was 600–1000 mg depending on body weight. Sustained viral responders (SVR) were defined as patients who were negative for serum HCV RNA 6 months after the completion of antiviral therapy.

All of the patients were divided into two groups (group A: ALT ≤ 30 U/L, group B: 31 U/L ≤ ALT ≤ 40 U/L) which were further divided into two subgroups based on PLT counts: group A-1 and B-1 (PLT counts ≥150 000/μL) and groups A-2 and B-2 (PLT counts <150 000/μL).

One hundred and twenty-nine HCV carriers with PNALT were enrolled to determine their long-term prognosis. These patients showed normal serum ALT levels (≤30 U/L) over a 12-month period on least three different occasions (PLT counts ≥150 000/μL, and body mass index [BMI] <25 kg/m^2). Thirty-nine patients received serial liver biopsies. The mean follow-up period of the 129 patients was 7.2 ± 3.2 years on 15 November 2006.

Statistical analyses

Data are expressed as mean ± SD. We compared continuous variables using the Mann–Whitney *U*-test. A frequency analysis and comparison between the groups were performed using the χ^2-test or Fisher's exact test and the Mann–Whitney *U*-test. ANOVA and Tukey's HSD procedure was used to determine the difference between multiple groups. All tests were two-tailed and *P*-values of less than 0.05 were considered significant. All statistical analyses were performed using Statistical Package of Services Solutions software, version 11.0 (SPSS, Chicago, IL, USA).

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Table 2 Baseline of hepatitis C virus patients with less than 30 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/mL (group A-1)	PLT < 150 000/mL (group A-2)	*P*-value
No. patients	204	51	
Age	48.4 ± 12.7	58.7 ± 7.5	<0.001*
Sex (male/female)	90/114	22/29	1.000**
BMI (kg/m^2)	21.6 ± 3.0	21.3 ± 2.4	0.514*
HOMA-IR	2.8 ± 3.5	1.2 ± 0.8	0.598*
Genotype: 1/2/others	101/101/2	25/26/0	0.952**
Viral load: low/high	112/92	26/25	0.574**
Histology			
F stage (0/1/2/3/4)	29/142/27/6/0	1/25/21/3/1	<0.001**
Grade (0–1/2,3)	179/25	33/18	<0.001**
Fatty change† 0–1/2–4	188/16	44/7	0.582**
Iron load‡ 0/1–4	82/12	17/3	0.762**
Ferritin (ng/mL)	86.0 ± 112.1	73.9 ± 46.6	0.204*
PLT count (/μL)	21.0 ± 4.4	12.1 ± 2.5	<0.001*
Hyaluronate (ng/mL)	41.8 ± 56.1	112.5 ± 109.9	<0.001*
Duration of antiviral therapy (weeks)	25.7 ± 10.3	27.0 ± 9.9	0.503*
Effects of therapy			
SVR/non-SVR	115/89	27/24	0.66**

*P-values were calculated by Mann–Whitney-*U*-test. **Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), grade of inflammatory activity ($P < 0.001$), PLT count ($P < 0.001$), and serum-hyaluronic acid ($P < 0.001$) between groups A-1 and A-2. Frequency of F2–4 patients was 16.2% in group A-1 and 51.6% in group A-2. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet counts; SVR, sustained viral responders.

RESULTS

Demographic, clinical, and histological features of 464 HCV carriers with normal serum ALT

THE CHARACTERISTICS OF the 464 HCV carriers with normal serum ALT are shown in Table 1. There were significant differences in sex, frequency of steatosis, serum ferritin levels, BMI, and the incidence of low PLT counts (<150 000/μL) between groups A and B.

There were significant differences in age, fibrosis (F) stage, inflammatory activity, PLT counts, and serum hyaluronate between groups A-1 and A-2 (Table 2). The frequency of stage F2–4 patients was 16.2% in group A-1, and 49.0% in group A-2 (Table 2). In group B, there were significant differences in age, F stage, PLT counts, and serum hyaluronate between groups B-1 and B-2 (Table 3). There were no F4 patients in group A-1 and B-1, and the frequency of F3 patients was very low compared with those in groups A-2 and B-2 (2.6% *vs* 7.6%). The PLT counts decreased in proportion to the progression of liver fibrosis as follows; F0 ($n = 51$); 20.7 ± 5.2 × 10^4/μL, F1 ($n = 288$); 19.8 ± 5.6 × 10^4/μL, F2 ($n = 105$); 16.9 ± 5.3 × 10^4/μL, F3 ($n = 17$); 15.9 ± 4.6 × 10^4/μL, and F4 ($n = 3$); 11.3 ± 3.8 × 10^4/μL.

Of the 464 patients, the frequency of the F0–1 stages was 80.1% and that of the F2–4 stages was 19.9% in patients with PLT counts ≥150 000/μL, and it was 50.4% and 49.6%, respectively, in patients with PLT counts <150 000/μL. In patients with PLT counts ≥17.0 × 10^4/μL, 80.8% were in stages F0–1 and 19.2% were in stages F2–4, and in patients with PLT counts <17.0 × 10^4/μL, 60.1% were in stages F0–1 and 39.9% were in stages F2–4.

The SVR rates of IFN therapy were 52.4% in F0–1 patients, 49.5% in F2–4 patients ($P = 0.896$ by Fisher's exact test), and 58.0% and 43.8% ($P = 0.592$) in IFN/Riba therapy, respectively.

In patients with genotype 1b and high viral load, the SVR rate was 12.5%. The SVR rate in genotype 2 patients was 60.4% in the IFN group and 67.7% in the IFN/Riba combination therapy group.

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Table 3 Baseline of hepatitis C virus carriers with 31–40 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/mL (group B-1)	PLT < 150 000/mL (group B-2)	*P*-value
No. patients	141	68	
Age	48.2 ± 11.9	57.9 ± 7.5	<0.001*
Sex (male/female)	80/61	37/31	0.751**
BMI (kg/m^2)	22.9 ± 3.1	22.7 ± 2.6	0.08*
HOMA-IR	3.0 ± 2.0	8.2 ± 9.5	0.8.8*
Genotype: 1/2/others	82/58/1	30/38/0	0.095**
Viral load: low/high	64/77	35/33	0.542**
Histology			
F stage (0/1/2/3/4)	17/91/31/2/0	4/30/26/6/2	<0.001**
Grade (0–1/2,3)	116/25	50/18	0.114**
Fatty change† 0–1/2–4	111/30	50/18	0.10**
Iron load‡ 0/1–4	67/12	30/7	0.762**
Ferritin (ng/mL)	114.4 ± 116.1	127.2 ± 167.8	0.869*
PLT count (/μL)	21.5 ± 4.9	12.2 ± 2.1	<0.001*
Hyaluronate (ng/mL)	46.9 ± 35.4	100.7 ± 0.98.1	<0.001*
Administration of IFN (weeks)	26.1 ± 11.9	27.7 ± 11.4	0.983*
Effects of therapy			
SVR/non-SVR	64/77	35/33	0.409**

*P-values were calculated by Mann–Whitney-*U*-test. **Fisher-exact-test. †0: no fatty change, 1. ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. In group B, there were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), PLT count ($P < 0.001$), and hyaluronic acid ($P < 0.001$) between B-1 and B-2. Frequency of F2–4 was 23.4% in B-1and 50.0% in B-2, respectively. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; IFN, interferon; PLT, platelet counts; SVR, sustained viral responders.

Demographic, clinical, and histological features of 129 HCV carriers with PNALT

The demographic and clinical features of the 129 HCV carriers with PNALT who were followed up for 7.2 years are shown in Table 4. Normal liver histology was noted in 17 patients, 102 showed minimal to mild CH, and 10 had moderate CH. Steatosis was seen in 7% and iron loading was noted in 12%.[18]

Of the 78 patients followed longer than 7 years (mean follow-up period; 10.4 ± 3.1 years), 11 (14%) had continuously normal ALT (G-1), 43 (55%) showed a transient elevation of ALT (G-2), and 24 (31%) changed to CH with continuously elevated ALT (G-3).

Thirty-nine patients received repeated liver biopsies (2–4 times). Of the 39 patients, six were in G-1, 17 were in G-2, and 16 were in G-3. The intervals between the first biopsy and the last biopsy in these three groups were 7.1, 7.8, and 7.2 years, respectively. The progression of the F stage was noted in two of six in G-1, six of 17 in G-2, and seven of 16 in G-3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.05, and 0.08 fibrosis unit. HCC was not detected in any patients during the follow-up periods.

Guidelines for the antiviral therapy of HCV carriers with normal serum ALT focused on the inhibition of the development of HCC

Considering the risk of progression to liver cirrhosis and the development of HCC, as well as the expected efficacy and various side-effects of antiviral therapy, an algorithm is needed for the management of HCV carriers with normal serum ALT. The progression rate of liver fibrosis stage was 0.05/year in HCV carriers with PNALT. The annual incidence of HCC in CH-C patients has been reported to be 0.5% at stages F0–F1, 1–2% at stage F2, 3–5% at stage F3, and 7% at stage F4.[4]

In principle, follow up without antiviral treatment is recommended for HCV carriers with PNALT (ALT ≤30 U/L) and PLT counts ≥150 000/μL, particularly in older patients (i.e. >65 years old), because over 90% show normal or minimal liver damage with good prognoses. However, antiviral therapy is not contraindicated for such patients since roughly 40% are infected with HCV genotype 2,[18] which suggests a high rate of SVR to the therapy with PEG-IFN/Riba.

As for the indication of antiviral therapy for HCV carriers with normal serum ALT (≤40 U/L), the PLT

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Table 4 Characteristics of 129 HCV carriers with persistently normal ALT who received liver biopsy

	$n = 129$	Follow up over 5 years ($n = 78$)
Follow-up period (years)	7.2 ± 3.2	10.4 ± 3.1
Age (years)	48 (21–77)	45 (29–71)
Male ($n = 24$)	49.8 ± 16.4	42.3 ± 14.9
Female ($n = 105$)	47.2 ± 12.5	46.6 ± 11.6
Sex (male/female)	24/105	10/68
ALT (U/L)	8–30	9–30
Male ($n = 24$)	22.5 ± 5.7	21.1 ± 5.4
Female ($n = 105$)	21.6 ± 4.8	22.3 ± 5.1
PLT ($\times 10^4$/mL)	15–31	15–31
Ferritin (ng/mL)	5–225	5–225
Male ($n = 24$)	76.2 ± 53.5	84.6 ± 59.2
Female ($n = 105$)	60.0 ± 43.3	66.6 ± 52.5
HCV genotype	G1 ($n = 58$), G2 ($n = 45$) Mixed and unclassified ($n = 16$)	
BMI (kg/m^2)	16–27	16–27
Male	22.2 ± 1.7	21.9 ± 1.9
Female	21.3 ± 2.2	21.0 ± 2.4

Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; PLT, platelet.

count is a good indicator for discriminating as to whether or not they have minimal to mild fibrosis or moderate to advanced fibrosis. Serum hyaluronate levels were significantly higher in HCV carriers with 31–40 U/L ALT having less than 150 000/μL PLT (Table 3). Advanced hepatic F stage, an elevated ALT level, old age (>65 years old), and sex (male) are important risk factors for the development of HCC.[6,18,30] We advocated an algorithm for such patients (Fig. 1) taking into consideration the risk of the progression to cirrhosis and the development of HCC. Therapy with PEG-IFN/Riba is the first-line treatment; therapy for 48 weeks is recommended for genotype 1 patients with high viral load and 12–24 weeks therapy for genotypes 2 and 1 with low viral load.

DISCUSSION

OUR PREVIOUS STUDY in 129 HCV carriers with PNALT demonstrated a predominance of females, higher frequency of genotype 2, minimal to mild liver histology, and very slow progression of hepatic fibrosis.[18] However, over 30% of these patients advanced to CH-C with elevated ALT levels during the 7-year follow up.

There are many reports concerning the natural course of liver fibrosis in CH-C patients, including those who are HCV carriers with normal serum ALT.[19,31–39] More than half of CH-C patients show progression of F stage from F1 to F2–4 within 10 years, and it was reported that the progression of liver fibrosis in HCV carriers with normal serum ALT was more rapid than was observed in the present study.[23] The main reason for the discrepancy between the report by Puoti *et al.*[23] and our results might be due to the definitions used for the normal range of serum ALT. In our previous study, the patients were HCV carriers with PNALT (ALT ≤ 30 U/L) and PLT counts ≥150 000/μL. On the other hand, the patients in the study by Puoti *et al.* had ALT levels ≤40 U/L, irrespective of PLT counts, in which cirrhotic patients might be included.[23] However, recent studies have demonstrated that normal ALT levels are less than 30 U/L[24] or 25 U/L in men[40] and less than 19 U/L[24] or 22 U/L in women.[40]

The present study demonstrated that the different distribution of hepatic F stage became remarkable when the A and B groups were divided into two subgroups according to their PLT counts. In HCV carriers with ALT levels ≤30 U/L, the frequency of stages F2–3 was 16.2% among those with PLT counts ≥150 000/μL; however, the frequency of stages F2–3 was 49.0% in those with PLT counts <150 000/μL. Conversely, in HCV carriers with ALT levels between 31 and 40 U/L, the frequency of stages F2–4 was 23.4% among those with PLT counts ≥150 000/μL and 50.0% in those with PLT counts <150 000/μL. The PLT count is a useful marker in dis-

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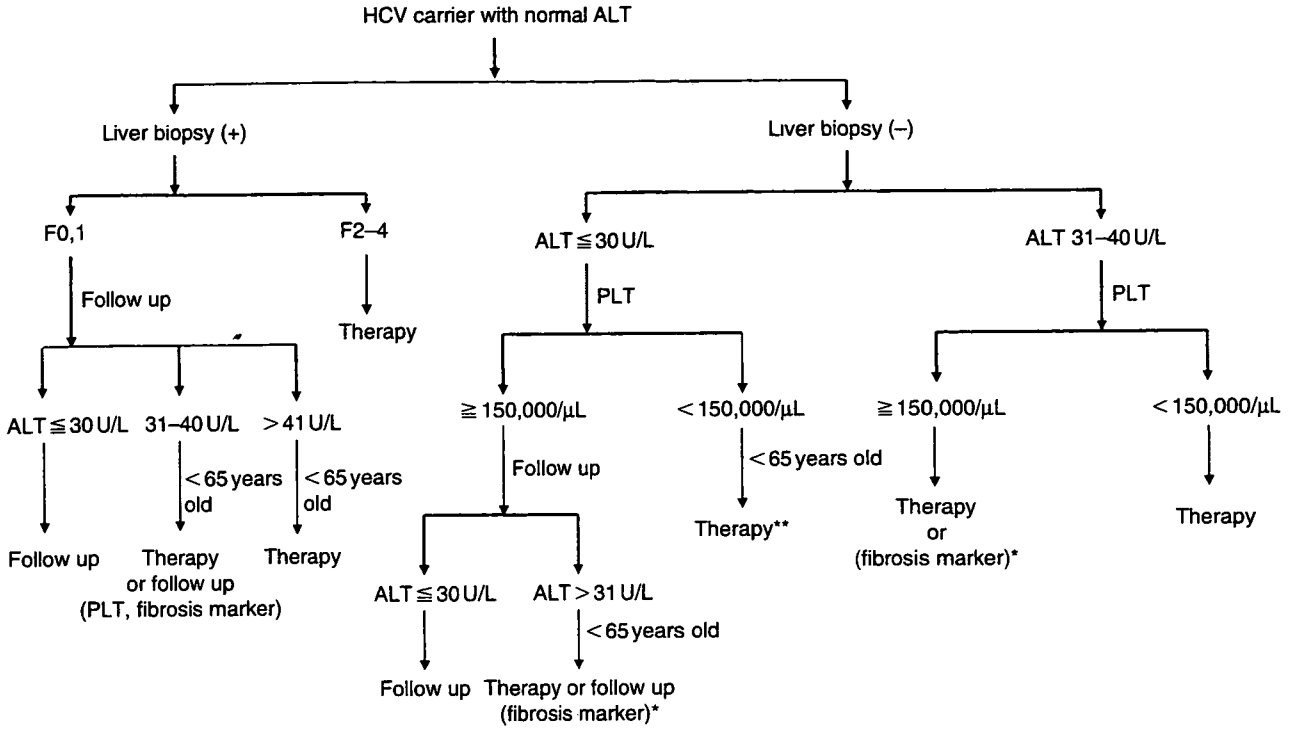

Figure 1 Algorithm for the management of hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT, ≤40 U/L) focused on the inhibition of the development of hepatocellular carcinoma. In patients who underwent liver biopsy, F0 and F1 patients younger than 65 years are candidates for antiviral therapy, especially those with genotype 2 after the elevation of serum ALT levels. In patients who did not undergo liver biopsy, ALT and platelet (PLT) levels are good indicators for determining candidates for antiviral therapy. Older patients (>65 years) and/or patients having uncontrolled hypertension, diabetes mellitus, or anemia should not be treated with pegylated interferon and ribavirin. Combination therapy with pegylated interferon and ribavirin for 48 weeks is recommended for patients with genotype 1 and high viral load, and 12–24 weeks therapy is suggested for patients with genotype 2 and genotype 1 with low viral load. *,**Serum fibrosis markers, such as hyaluronate, might be useful to decide whether patients are candidates for antiviral therapy or not.

criminating between stages F0–1 and F2–4 F in HCV carriers with normal serum ALT (≤40 U/L). In the present study, the mean PLT count in F2 and F3 patients was 16.9 ± 5.3 ($\times 10^4/\mu L$) and 15.9 ± 4.6 ($\times 10^4/\mu L$), respectively. The distribution of the F stage was not significantly different between patients with PLT counts $\geq 15 \times 10^4/\mu L$ versus $< 15 \times 10^4/\mu L$ and $\geq 17 \times 10^4/\mu L$ versus $< 17 \times 10^4/\mu L$.

The SVR rate for genotype 1 patients with high viral load treated with either IFN monotherapy or IFN/Riba were 12.5% and 37.7%, respectively. In genotype 2 patients with high viral load, the SVR rate in the present study was better than the data of Japanese CH-C patients with elevated ALT levels in our previous paper.[6] It was not reasonable to compare the SVR rates between HCV carriers with normal serum ALT and CH-C with elevated ALT in the present study, because the total dosage of IFN and the duration of treatment were significantly different.

The annual incidence of HCC is correlated with the progression of liver fibrosis, that is, the stage of liver disease.[2–4,6] Sustained low serum ALT levels are also associated with a lower incidence of HCC.[2,6,41] PEG-IFN/Riba therapy is expensive and induces various side-effects. The present results indicate that most HCV carriers with normal serum ALT (≤40 U/L) and PLT counts ≥150 000/μL have minimal to mild liver damage, indicating a low risk for the progression to cirrhosis and the development of HCC. This was more remarkable in patients with ALT levels ≤30 U/L and PLT counts ≥150 000/μL. However, nearly half of the patients with PLT count <150 000/μL have F2 or F3 F stages, indicating a certain risk for the progression to cirrhosis and the development of HCC. Fibrosis

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