TABLE 2. PRETREATMENT PATIENT PROFILES IN THE SNMC AND IFN GROUPS

	SNMC (n = 13)	*IFN (n = 27)*	P *value*
AFP (ng/ml)	29.970 ± 35.229	30.030 ± 39.643	0.9798
Age (yr)	54.308 ± 10.427	54.889 ± 10.685	0.8719
Gender (M/F)	9/4	17/10	0.6071
ALT (U/L)	147.846 ± 110.816	132.556 ± 272.702	0.6039
Platelets (×104/μl)	11.015 ± 6.244	13.441 ± 3.870	0.1387
Albumin (g/dl)	3.738 ± 0.568	3.867 ± 0.408	0.4185
PT (%)	72.615 ± 13.775	77.615 ± 10.887	0.2607
HCV-RNA (KIU/mL)	502.900 ± 299.403	455.500 ± 302.124	0.6752

Note. Mann-Whitney *U*-test or chi-square test was used. $P < 0.05$ was considered significant.
Values are expressed as mean ± SD.

decreased in the IFN group (53.0 ± 44.3 to 20.3 ± 26.7 ng/ml; $n = 14$; $P = 0.0023$). Interestingly, all 27 IFN-treated patients showed a decrease in AFP value regardless of response to treatment. However, there was no significant change in the AFP value after SNMC administration (31.1 ± 36.4 to 39.0 ± 46.5 ng/ml; $n = 9$; $P = 0.11$) (Figure 2). Mean AFP value was slightly increased in the SNMC group.

DISCUSSION

AFP is a fetal protein that is not normally present in the serum of adults and is commonly used as a tumor marker for HCC. However, serum AFP is also elevated during pregnancy and in chronic hepatitis patients (10, 11). In this study, a considerable number of type C chronic hepatitis and compensated cirrhosis patients demonstrated persistently elevated AFP levels in the absence of HCC. In addition, the AFP level decreased significantly after IFN administration. Furthermore, the AFP decrement was universally observed regardless of treatment response to IFN therapy. Transient AFP elevation has been observed after a rise in transaminase in acute hepatitis and fulminant hepatitis (12–14). This type of AFP elevation is explained as a result of hepatocyte regeneration accompanied by necroinflammatory change. In this study, AFP was not changed in the SNMC group despite significant improvement in transaminase, suggesting that the AFP elevation was not caused by hepatocyte regeneration in chronic hepatitis patients.

AFP production is supposed to regulate the transcription level of hepatocytes (15). Among HCV-infected patients, the HCV-coding core protein is regarded to be one of the proteins responsible for hepatocarcinogenesis, up-regulating several molecules resulting in activation of the cell cycle and cell proliferation at the transcriptional level in hepatocytes (16). The HCV-coding core protein may also upregulate AFP production at the transcriptional

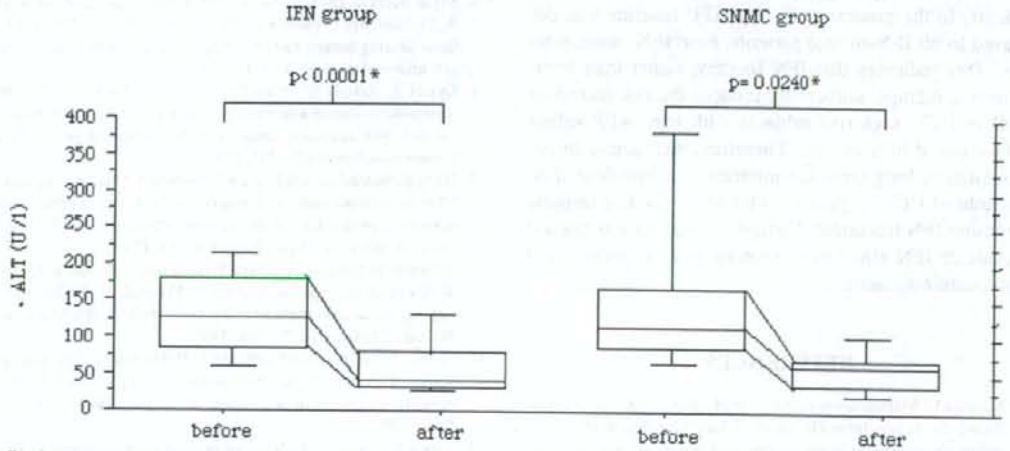


Fig 1. Changes in alanine aminotransferase (ALT) after IFN and SNMC administration. Paired *t*-test was used. *$P < 0.05$ was regarded as significant.

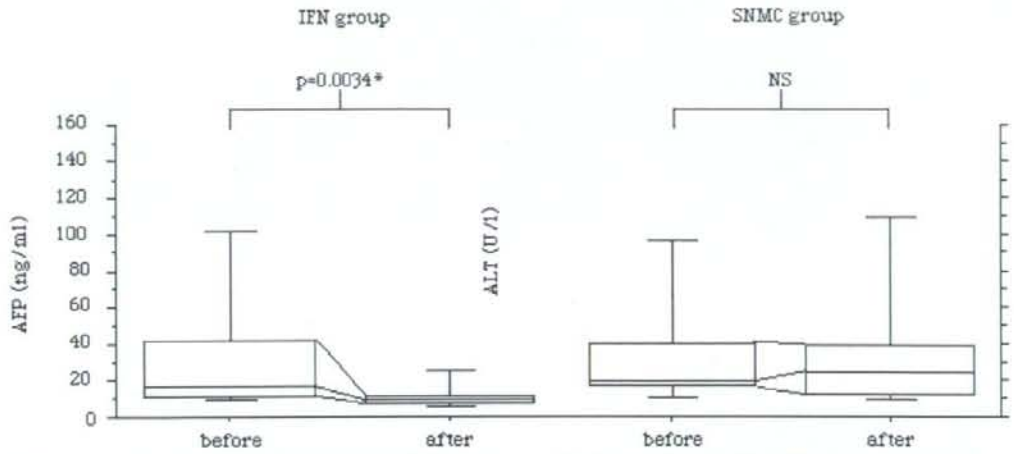


Fig 2. α-Fetoprotein (AFP) changes with IFN and SNMC administration Paired *t*-test was used. $*P < 0.05$ was regarded as significant. NS, not significant.

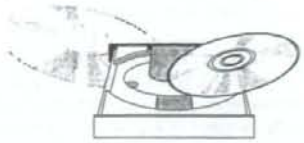
level. In contrast, IFN is considered to down-regulate cell cycle progression at the transcriptional level and induce apoptosis via the IFN receptor-mediated JAK-STAT signaling pathway (17). This competing action of IFN against HCV-related protein may be a direct anticancer mechanism that inhibits HCC. Actually, a clinical study has demonstrated anticancer effects of IFN administration against intrahepatic recurrence after resection of HCC (18), and IFN has also been used to treat HCC in combination with anticancer agents such as 5-fluorouracil (19).

Many reports have cited elevated AFP baselines as an independent HCC risk factor (8, 9) along with age, gender, liver histology stage, and ethnicity in HCV-infected patients. In the present study, the AFP baseline was decreased in all IFN-treated patients, even IFN nonresponders. This indicates that IFN therapy, rather than liver-protective therapy, universally reduces the risk factors of HCC in HCC high-risk subjects with high AFP values and advanced liver disease. Therefore, therapeutic strategies, such as long-term administration of low-dose IFN, may inhibit HCC in patients who have failed to respond to routine IFN treatment. Further investigation is needed to evaluate IFN effect in relation to AFP production and hepatocarcinogenesis.

REFERENCES

1. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 358:958–965, 2001
2. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347:975–982, 2002
3. Takano S, Ito Y, Yokosuka O, Ohto M, Uchiumi K, Hirota K, Omata M: A multicenter randomized controlled dose study of ursodeoxycholic acid for chronic hepatitis C. Hepatology 22:1002, 1995
4. Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, Saitoh S, Kobayashi M, Kumada H: The long term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer 79:1949–1500, 1997
5. van Rossum TG, Vulto AG, Hop WC, Brouwer JT, Niesters HG, Schalm SW: Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial: J Gastroenterol Hepatol 14:1093–1099, 1999
6. Szymendera JJ, Zborzil J, Sikorowa L, Lenko J, Kaminska JA, Gadek A: Evaluation of five tumor markers (AFP, CEA, hCG, hPL and SP1) in monitoring therapy and follow-up of patients with testicular germ cell tumors. Oncology 40:1–10, 1983
7. Okuda K, Kotoda K, Obata H, Hayashi N, Hisamitsu T: Clinical observations during a relatively early stage of hepatocellular carcinoma, with special reference to serum alpha-fetoprotein levels. Gastroenterology 69:26–234, 1975
8. Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H: A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 18:47–53, 1993
9. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H: Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 428:1797–1801, 1993
10. Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ: Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 99:860–865, 2004
11. Alpert E: Serum alpha-fetoprotein (AFP) in benign and malignant gastrointestinal diseases: evaluation of an immunoenzymatic assay. Clin Chim Acta 58:77–83, 1975

12. Jagiello-Wojtowicz E, Rzeszowska G, Krawczuk G, Baran E, Surmaczynska B, Fijalka-Rymar M, Bielec D: Alpha-fetoprotein in acute viral hepatitis type A. Przegl Epidemiol 47:17–20, 1993
13. Francioni S, Pastore M: Alpha-fetoprotein and acute viral hepatitis type B. J Nucl Med Allied Sci 33(Suppl 3):103–106, 1989
14. Pastore G, Lapedota E, Dentico P, Buongiorno R, Mallardi M, Angarano G, Schiraldi: Prognostic value of alpha-foetoprotein in fulminant hepatitis. Quad Sclavo Diagn 15:14–21, 1979
15. Innis MA, Miller DL: alpha-Fetoprotein gene expression. Control of alpha-fetoprotein mRNA levels in cultured rat hepatoma cells. J Biol Chem 254:9148–9154, 1979
16. Yoshida T, Hanada T, Tokuhisa T, Kosai K, Sata M, Kohara M, Yoshimura A: Activation of STAT3 by the hepatitis C virus core protein leads to cellular transformation. J Exp Med 196:641–653, 2002
17. Yano H, Iemura A, Haramaki M, Ogasawara S, Takayama A, Akiba J, Kojiro M: Interferon alfa receptor expression and growth inhibition by interferon alfa in human liver cancer cell lines. Hepatology 29:1708–1717, 1999
18. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Yamazaki O, Shiomi S, Tamori A, Oka H, Igawa S, Kuroki T, Kinoshita H: Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. Ann Intern Med 134:963–967, 2001
19. Sakon M, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, Kawata S, Imai Y, Iijima S, Monden M: Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 94:435–442, 2002



学術

肝癌の発症予防－その対策と治療－

久留米大学医学部第二内科・消化器疾患情報講座
長尾由実子・佐田通夫

はじめに

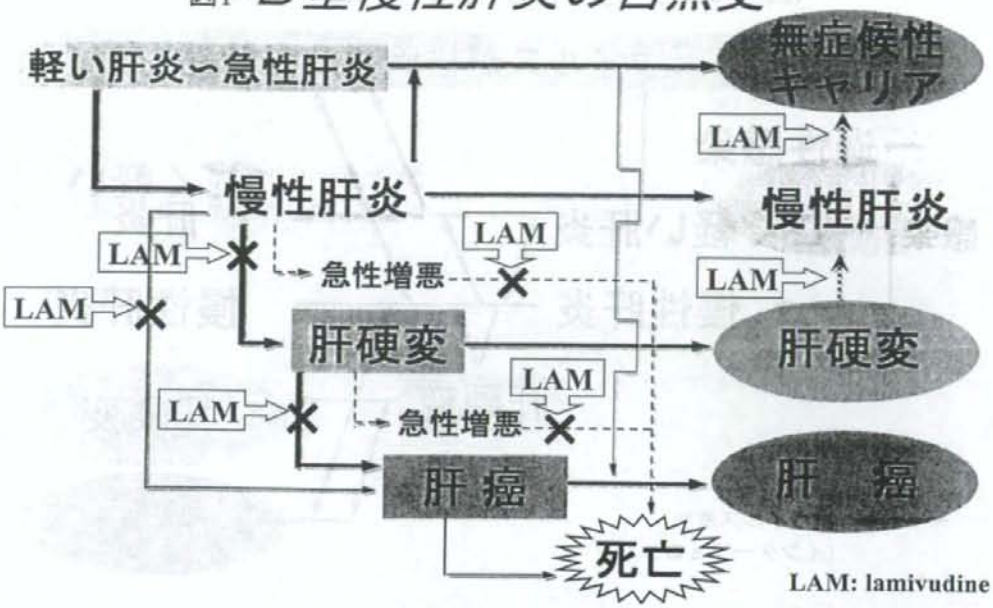
わが国の肝細胞がんによる死亡者数は増加の一途をたどり、この傾向は2015年まで続くと推測されている。よく知られているように肝細胞がん（以下肝がん）の原因の約80％がC型肝炎ウイルス（HCV）に起因するものであり、HCVによる肝がん患者の増加がわが国における肝がん死亡者数の増加の原因である。一方、B型肝炎ウイルス（HBV）による肝がんは約10％を占めているが、患者数は決して減少傾向を示してはいない。むしろ若年者で進行した状態で発見されるHBV起因の肝がん患者が、HCV起因の肝がんよりも多いことが問題となっている。

近年、C型慢性肝炎に対するインターフェロン（IFN）療法の導入が肝がんの発症予防と長期予後の改善に寄与することが明らかになった。C型慢性肝炎に対する新たな治療法としてリバビリンと持続型インターフェロン（Peg IFN）との1年間の併用投与が保険適応になり、治療効果の向上が期待されている。

B型肝炎の自然経過（図1）

治療の導入、治療法の選択に際してはHBVやHCV感染後の自然経過を十分に理解しておくことが肝要である。HBVは、HBVの母子感染、幼少児期の感染によって高率に持続感染状態を成立させる。即ちHBVキャリアに移行することが知られている。しかし、HBVワクチン、高力価HBs抗体含有グロブリン（HBIG）の投与や医療環境の整備により、母子間感染や幼少児期の感染によるキャリア化の例はあまりみられなくなった。一方、HBVキャリアの自然経過をみると、一般的に30－35歳までに80－90％のキャリアは生体の免疫反応によって“臨床的治癒”と呼ばれる状態に移行する。このような例の多くがウイルス健康保菌者として生涯を送ることになる。残りの10－20％が、慢性肝炎、肝硬変あるいは肝がんに移行するとされている。この移行を阻止する治療として経口の抗ウイルス薬であるラミブジンやインターフェロンの投与が行われている。

図1 B型慢性肝炎の自然史

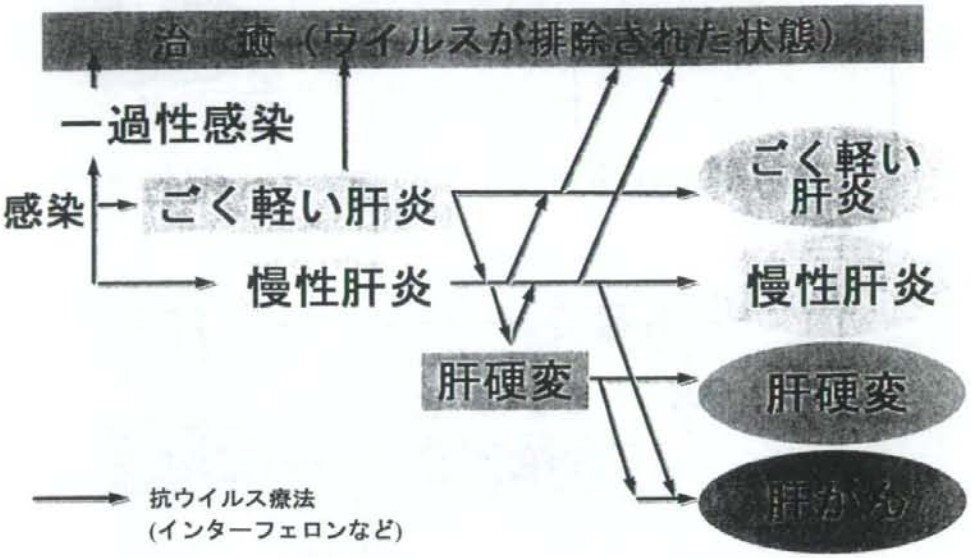


C型肝炎の自然経過（図2）

HCV感染の場合、B型肝炎ウイルス（HBV）の感染とは異なり、どの年代においても初感染例から約60－70％の確率でキャリア化が成立する。HCVワクチンや抗HCV抗体高力価γ-グロブリンがない現状では、C型急性肝炎の慢性化を阻止する治療法を確立する必要があるが、幸いにもインターフェロン療法が慢性化の阻止に有効であることが明らかになった。C型慢性肝炎から肝硬変への移行率は40－50％と推測されている。C型肝炎の場合、B型慢性肝炎にみられるような自然経過の中で肝炎の増悪を契機に起こる“臨床的治癒”と言われるような無症候性キャリアへの移行はみられない。またC型慢性肝炎の肝線維化の進行や肝硬変への進展によって起こる肝細胞がんの併発は、最も重大な合併症である。慢性肝炎の段階では年率1－3％の率で、肝硬変では年率7％の率で肝がんが発生することが知られている。一方、慢性肝炎でも線維化がみられないか、あっても軽度の状態そしてAST・ALT値が正常の状態からの肝がんの発症は稀であることがわかっている。我々の行ったHCV高感染地区における住民検診の結果からも肝がんや肝硬変死亡に寄与する重要な因子は、HCVの持続感染とALT値の異常であることも明らかになった。

このような事実から推測すると、HCVの持続感染を絶つこと、そして肝炎を鎮静化させ肝硬変への進展を阻止し、慢性肝炎を治癒させることこそが、肝がんの発症予防や肝

図2 C型慢性肝炎の自然史



がんの撲滅手段として、如何に重要であるかがわかる。実際インターフェロン療法を受けた慢性肝炎や肝硬変患者では、HCVの駆除やALT値の正常化が得られた例において、明らかに肝がんの発生頻度は低く、長期生命予後も良好であることが明らかにされている。

わが国のC型肝炎ウイルスの抗体陽性率

わが国におけるHCV抗体陽性者は、一体どのくらい存在するのであろうか。このことを知ることは肝がん患者の今後の発生状況や予防、治療対策を立てる上で重要ある。広島県赤十字血液センター（1992.2月～2000.6月）の調査によると、出生年別にみた献血者のHCV抗体陽性率は平均1.05％、40歳未満では1.0％以下で、40歳以上になると抗体陽性率が上昇傾向を示し、60－64歳代では4.0％、70歳以上になると約7.0％の陽性率であることが示されている。これらの事実は40歳以降の年代にC型慢性肝疾患が多く、肝がん患者が高齢者層に多発するであろうということ、そしてこれらの中高齢者層を中心としたC型肝炎ならびに肝がん撲滅対策が急務であることを我々に示しているのである。我々の検診結果では、肝がん患者発生のピークは60－70歳代であり、10年前に比較すると患者発生のピークがさらに高齢者層にシフトしている現象が観察される。

治療

B型肝炎の治療

現在、「自然経過によって臨床的治癒状態に移行することが困難である」あるいは

「慢性肝炎から肝硬変に移行する可能性が高いと判断される例」「慢性肝炎や肝硬変の状況が長期に持続している例」に対しては、経口抗ウイルス剤であるラミブジン投与が実施されている。ラミブジンの効果がみられなくなった例に対しては、ラミブジンとアデフォビルの併用投与が実施されている。また最近では、インターフェロンの長期投与が効果的であるという報告やラミブジンやインターフェロンの投与による肝発がん阻止を示す報告も行われている。

C型肝炎の治療

C型肝炎の治療目的は肝組織進展と肝発がんの阻止である。この2つの目標を目指して肝炎の鎮静化とウイルス駆除をめざした治療が行われている。HCVの駆除が可能な治療法としてインターフェロン単独療法、インターフェロン・リバビリン併用療法が行われる。最近では、持続型インターフェロン・リバビリン併用療法が標準的な治療法になった。この併用療法を1年間施行すると、今まで難治例とされていたgenotype1b、高ウイルス量症例を含めた全ての治療対象者に対して半数に著効が得られることが明らかにされている。ただ最近、治療の対象となる患者の平均年齢が次第に高齢化しており、抗ウイルス療法を行う場合、副作用の発現や治療脱落例の増加など解決すべき問題点もいくつか指摘されるようになっている。HCV感染者は、肝疾患だけでなく肝臓以外の障害や疾患を合併していることが知られている（いわゆる肝外病変）。肝外病変の中には、皮膚や粘膜に出現する扁平苔癬や糖尿病、またシェーグレン症候群など種々の病変があるが、このような肝外病変合併者にインターフェロン治療を行うと、肝外病変がさらに増悪する場合があることが知られているため、治療前には全身疾患の把握と専門医との連携が重要である。

一方、インターフェロンには抗ウイルス効果と共に抗腫瘍効果があることもよく知られている。この効果に注目して肝がんの再発阻止あるいは肝発がん阻止を主体としたインターフェロンの少量長期投与が試みられている。一方、肝炎の鎮静化を主目的とする治療法として強力ネオミノファーゲンCの静注、ウルソや小柴胡湯の経口投与、さらに瀉血療法が行われている。肝臓に蓄積した鉄を取り除くために行われる瀉血療法では肝臓内の炎症所見が改善し、さらには肝がんの発生も抑えられる報告がなされている。この治療においては鉄の摂取を制限することも重要とされており、従来肝疾患患者に多く摂取することが勧められた食物中には鉄含有量が多いものがあり「昔常識、今非常識」という観点から療養の方法を見直すことの必要性が論じられている。

肝臓がん

肝臓がんの早期発見と早期治療が重要であり、そのためには専門医による定期画像検

査が重要である。種々の治療法があるが、肝がんの大きさ、発生部位、生物学的悪性度、背景肝病変などを十分に考慮した上での治療法の選択、集学的治療が最も重要である。また病－診、病－病連携のもとでの経過観察や治療を忘れてはならない。

まとめ

わが国では平成14年度より肝がん撲滅を目指したB型とC型肝炎に対する国家レベルでの検診事業が開始された。HCVやHBVキャリアとして発見された者に対して、どのような治療対策や指導を行っていくかが日本の肝がん死亡を減少するためのkey pointである。

もっと詳しくお知りになりたい方へ

「B型肝炎について（一般的なQ＆A）」「C型肝炎について（一般的なQ＆A）」「E型肝炎について（一般的なQ＆A）」は、下記ホームページに掲載されている。

参考資料

1．財団法人ウイルス肝炎研究財団　http://www.vhfj.or.jp/06.qanda/index.html
2．厚生労働省　http://www.mhlw.go.jp/
3．社団法人日本医師会　http://www.med.or.jp/kansen/
4．久留米大学医学部消化器疾患情報講座（佐田通夫）
http://www.med.kurume-u.ac.jp/med/joho/index.html

研究紹介

C型肝炎ウイルス持続感染者に対する薬物療法
－インターフェロン療法の普及とその現状－

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医薬産業政策研究所　主任研究員　野林　晴彦
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優れた新しい医薬品や薬物療法は、広く医療現場に普及し患者に用いられることによって、初めて大きな価値を持つ。しかし、それらが普及していくのは、必ずしも容易なことではない。

人口動態統計によれば、2003年時点で日本人の死因のトップは悪性新生物（がん）によるもので、その数は年々増加の傾向にある。1990年頃までは胃がんがトップを占めていたが、最近では肺がんや肝がんの増加が目立っている。わが国では、2003年に第３次対がん10ヵ年総合戦略が策定されるなど、これらのがんの予防や治療法の開発・普及が唱えられている。

肝がんと肝炎ウイルス

日本における肝がん患者は３万人を超えている。その死亡率は他のアジア諸国とほぼ同等だが、欧米と比較するとかなり高い水準にある[1]。この主な原因はB型あるいはC型肝炎ウイルス（それぞれHBV、HCV）といわれており、これらのウイルスに感染した患者は、将来肝がん発症のリスクを背負うことになる。

日本では肝がんの原因の約80％はHCVの持続感染に起因するものである。HCVに感染した場合、そのまま放置すると70％前後が持続感染の状態となり、さらに慢性肝炎へと進展する。その後20～30年を経て、さらにその半数が肝硬変、肝がんへと進展する。

かつては、持続感染したHCVを排除する手段はなく、慢性肝炎へ進行すると殆どの場合は自然治癒が望めず、肝がんへの進行を阻止することもできなかった。しかし、1980年代後半になり、HCVを患者の身体から排除し、肝炎の治癒、あるいは肝硬変への進展や肝がん発症に対しての抑制効果をもたらす画期的な薬剤として、インターフェロン（IFN）が使われはじめた。当初は、B型慢性肝炎だけの適応であったが、1989年にHCVが発見され、1992年にはC型肝炎の治療薬としても使用されるようになった。また、2001年には経口抗ウイルス薬リバビリンとIFNの併用、2003年には効果の持続性に優れるペグインターフェロンなど新たな治療法が保険適用とされており、これらの治療によるウイルス駆除例や肝炎鎮静例では、肝線維化の改善、肝がん発生の抑止、さらに生命予後の改善が明らかにされるなど治療成果をあげている。

現在わが国では、HCVに持続感染している人は、150万人以上存在すると推測されている。HCV持続感染者（HCVキャリア）は、自覚症状がない

1）国立がんセンター「がんの統計'05」における2001年の肝および肝内胆管の悪性新生物による死亡率（人口10万人当たり）は、日本で男性38.3、女性16.7、以下それぞれ米国：5.8、3.4、イギリス：2.0、0.8、フランス：13.9、3.1、ドイツ8.2、5.0、中国35.7、14.9、韓国32.5、10.0となっている。

ことが多いため、感染していることを自覚しないままに慢性肝炎から肝硬変や肝がんに進展する例が多くみられ、適切な時期に治療を受ける機会のない感染者が存在することが問題となっている[2,3]。そのため、厚生労働省では、平成14年度より「C型肝炎等緊急総合対策」の一環として、地域住民を対象とした「肝炎ウイルス検診」(HCV並びにHBV)を開始した。この検診には、40歳から70歳までの老人保健法に基づく健康診査の受診者に対し5歳刻みに実施する節目検診と、過去に肝機能異常を指摘されたなど、早期に検査を受ける必要のある人を対象とした節目外検診[4]がある。

普及していないインターフェロン(IFN)療法

IFNがC型肝炎の治療薬として承認されてから13年が経過している。IFN療法は、インフルエンザ様症状やうつ症状が発現しやすいなどの副作用が伴うことによる制約があるものの、徐々に製剤の改良や治療法の改善が図られ、有効性が向上し、使用し易くなった結果、現在ではC型慢性肝炎の第一選択薬として肝臓専門医では高く評価されるに至っている。

ところが、平成15年度のアンケート調査から得られた全国調査(節目検診、節目外検診)によると、HCV陽性で要精密検者数20,364名のうち二次医療機関へ受診した者7,769名の中で、何らかの治療を受けた者の割合は24%であり、このうちIFN治療が行われた割合は13%であったと報告されている。約半数は専門医への受診をしておらず、専門医への受診率向上が課題と考えられている[5]。

現在われわれが行っている調査では、ある地域のHCV感染患者と医師を対象にアンケートを実施し、IFN療法が医療現場にどの程度普及しているのか、患者と医師とのIFN療法に対する認識はどの程度違いがあるのか、そしてあるべき治療と現実の間にギャップを生んでいる要因は何かを明らかにしようとしている。

本アンケート調査は現在実施中の段階にあるが、2006年1月6日までに回答の得られた患者および医師108例分のデータから、医療現場におけるIFN療法の実状の一部を紹介したい。

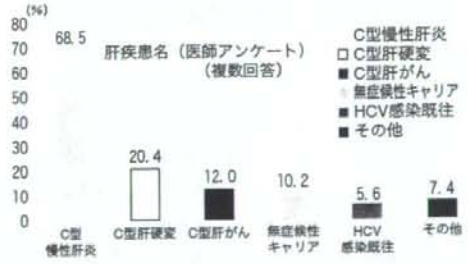
調査の対象と方法

ある地域の医療機関8施設(診療所7、病院1)において、通院患者の中でHCV感染者とその患者を診療している医師に対してそれぞれアンケート調査を実施した。

調査項目としては、患者、医師それぞれに、①患者背景、②IFN療法について、③IFN療法を行わなかった場合はその理由、などを尋ねている。

この度集計した段階では、対象患者108名の通院先は、診療所102名、病院6名であり、年代別では、約7割が60歳以上であった。図1に示すように、診断名はC型慢性肝炎が7割近くを占めていた。

図1　対象患者の診断名



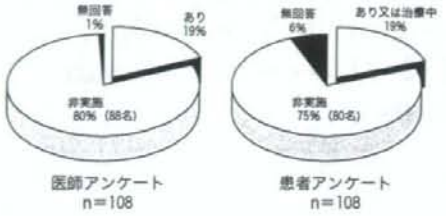
2)厚生労働省「C型肝炎について(一般的なQ&A)」(2003年8月)(改訂V版)
3)厚生労働省「C型肝炎対策等の一層の推進について」(2005年8月2日)C型肝炎対策等に関する専門家会議
4)節目外検診:上記以外の節目検診の対象とならない者のうち、以下の人に対して実施する検診
・過去に肝機能異常を指摘されたことのある者
・広範な外科的処置を受けたことのある者又は妊娠・分娩時に多量に出血したことのある者であって定期的に肝機能検査を受けていない者
・基本健康診査の結果、ALT(GPT)値により要指導とされた者
5)沖田極「厚生科学研究費補助金肝炎等克服緊急対策研究事業(肝炎分野)肝炎ウイルス検診要精検者の二次医療機関への受診状況に関する全国調査」

これまでの集計結果

(1) IFN 療法の実施状況

IFN 療法については、図2に示されるように、多くの患者で実施されていなかった。

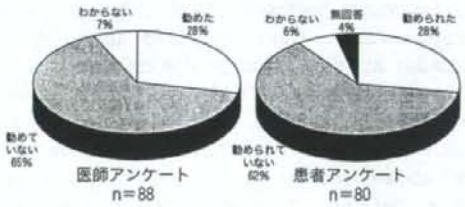
図2 IFN 療法の実施状況



(2) IFN 療法の推奨について

多くの患者に IFN 療法は実施されていなかったが、背景にどのような経緯があったのだろうか。図3は、IFN 非実施患者に対する、医師から患者への IFN 療法の推奨状況を示したものである。これによると、6割以上の患者に対し、医師は IFN 療法を勧めていなかった。

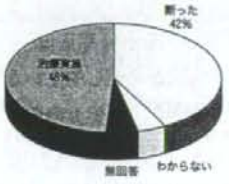
図3 IFN 療法非実施患者への IFN 推奨状況



また、図4には、IFN 療法を受けていない患者が、IFN 療法を推奨された際にどのような判断をしたかを示している。ここでは、IFN 療法が勧められているにも拘らず、8割を超える患者が受療を断っていた。なお、IFN 療法を受けた患者については医師からの推奨の有無を尋ねていないが、これらの患者は推奨を受けこの治療を受けるに至ったと考えられ、これを併せて集計すると、IFN 療法を断った患者は、それを勧められた患者全体の4割強であった（図5）。

図4 IFN 療法推奨に対する患者の判断
(IFN 療法非実施患者のみ)

図5 IFN 療法推奨に対する患者の判断
(IFN 療法実施者を含めた場合)



今後の集計・解析に向けて

以上はあくまで途中経過であり、今後回収が進む予定の病院（専門医）による回答があまり反映されていない。しかし、現時点まで得られたデータからも IFN 療法を実施された患者は少ないこと、また多くの場合、医師からも勧められていないことが明らかになっている。今後さらに集計を進め、何故医師が IFN 療法を勧めなかったのか、何故患者は勧められても断ったのか、IFN 療法についての理解が医師と患者の双方において進んでいるのか、などについて、医師が専門医か一般医か、患者の診断名は何か、といった様々な属性別分析も加えることにより、解析していく。新しい医薬品や薬物療法が普及しにくい原因を考察し、その対応策について何らかの示唆が得られるのではないかと考える。

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ORIGINAL INVESTIGATION

Serum levels of IgG to the peptide of HCV1b core at positions 35–44 correlated with persistent infection, while levels of IgG to the peptide of NS5A at positions 2132–2140 correlated with better prognosis in HCV-infected patients

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Abstract We previously reported that two IgG Abs to the hepatitis C virus (HCV), anti-core 35–44 (C35) and anti-NS5A 2132–2140 (NS5A2132), existed in the sera of the majority of patients with HCV infection. This study investigated if measuring the two Abs would facilitate the prediction of a patient's prognosis. The serum levels of anti-C35 were found to correlate with persistent infection, while those of anti-NS5A2132 correlated with a better prognosis in HCV-infected patients. These results suggest that sequential measurement of the two Abs together may provide new information for the prediction of prognosis.

Keywords HCV · Peptide · Antibody · Prognosis · CTL-epitope

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Introduction

The hepatitis C virus (HCV) infection is one of the most serious health problems worldwide, as 170 million people around the world are persistently infected with HCV and are thus at high risk of developing liver cirrhosis (LC) and hepatocellular carcinoma (HCC) at later stages of the disease [5]. Although many studies of the immunological mechanisms involved in patient prognosis have been conducted during the past 2 decades, no suitable immunological monitoring system to predict the prognosis of each patient is available at the present time [1, 6, 7, 13, 14, 16, 18, 19]. In acute resolving hepatitis, HCV RNA is cleared in some individuals, whereas the majority (>50%) do not clear the virus, but instead develop chronic hepatitis (CH). Some CH patients respond to interferon (IFN)-therapy with a disappearance of virus, but others develop further progressive diseases such as LC and HCC. Immunity is thought to be largely involved in these processes, but there is no suitable laboratory marker to arrive at a reliable prognosis. This lack of useful findings might in part be due to an insufficient understanding of the biological roles of antibodies (Abs) that are specific to HCV. There are several lines of evidence suggesting the involvement of cytotoxic T-lymphocyte (CTL) and T helper cell responses in viral eradication [1, 6, 7, 13, 14, 16, 18, 19]. However, it is difficult to carry out CTL and T helper cell assays, which in any case are not sensitive enough to be utilized as laboratory markers.

We recently found two unique Abs specific to HCV1b-derived peptides; one of these Abs was specific to the human leukocyte antigen (HLA)-A2-restricted

Table 1 Diagnoses of residents in 1995 and 2002

1995	*n*	2002	*n*	Gender (M/F)	Age ± SD
CH	17	CH	12	3/9	67.7 ± 11.9
		LC	3	2/1	59.0 ± 7.9
		HCC with CH	1	0/1	82
		HCC with LC	1	1/0	65
LC	1	LC	1	0/1	69
ASC	4	CH	1	0/1	75
		ASC	1	0/1	76
		Past history of HCV infection	2	0/2	57.7 ± 6.36
Past history HCV infection	11	Past history HCV infection	11	2/9	68.8 ± 10.71
Total	33		33	8/25	67.1 ± 10.6

CH chronic hepatitis, *LC* liver cirrhosis, *HCC* hepatocellular carcinoma, *ASC* asymptomatic healthy carrier

CTL epitope peptide of the HCV1b core protein at positions 35–44 (anti-C35 Ab), and the other Ab was specific to the HLA-A24-restricted CTL epitope peptide of the NS5A protein at positions 2132–2140 (anti-NS5A2132 Ab) [17]. These two Abs were detected in a majority of a sample of patients with HCV$^+$, regardless of differences in terms of their disease conditions, but the biological activities of these Abs remain unclear at the present time. To gain a better understanding of their biological features, we investigated in the present study whether or not the measurement of these Abs would facilitate the prediction of a patient's prognosis. Our approach primarily involved the analysis of sera from patients at various stages of disease, including patients from a cohort study of HCV individuals evaluated from 1995 to 2002. That cohort study has been ongoing since 1990 in H town of Fukuoka prefecture in Japan, where the prevalence of HCV infection is the highest in the country [10, 11]. Our present results showed that the levels of anti-C35 Ab correlated with persistent infection, while those of anti-NS5A2132 correlated with a better prognosis in the sera of HCV$^+$ patients.

Materials and methods

Subjects

Informed written consent was obtained from all subjects whose serum samples were used in this study. The samples were obtained from 33 inhabitants with HCV-related diseases as part of a cohort study conducted from 1995 to 2002. The results of our follow-up survey of 33 inhabitants are given in Table 1. Serum samples from patients who were not participants in the cohort study, but who were treated at Kurume University Hospital, were also provided for the analyses; these patients were diagnosed with CH ($n = 68$), LC ($n = 43$), and HCC ($n = 52$). Anti-HCV Ab was measured by a chemiluminescent enzyme immunoassay (CLEIA) kit (Lumipulse II HCV, Fujirebio Inc., Tokyo, Japan). A second- or third-generation enzyme immunoassay was performed by a clinical lab company, SRL, Tokyo, Japan. Measurement of HCV-RNA and genotyping was performed by SRL using RT-PCR and direct sequencing of HCV in the sera of patients. Serum samples were also obtained from subjects with diseases other than HCV infection, including 17 cases of hepatitis B virus (HBV) infection (positive for HBV-surface antigen), 3 patients with human immunodeficiency virus (HIV), and 10 cases with human T cell leukemia virus type I (HTLV-1) infection. All of the subjects used in this study did not contain multi-infected patients. As a negative control, the sera from 37 healthy donors (HDs) with no history of either viral hepatitis or HBV vaccination, and who also had normal liver function test results, were used for this study.

Peptides

The following two peptides with a purity of >90% were purchased from Bio Synthesis (Lewisville, TX, USA); HCV1b core protein at positions 35–44 (YLLPRRGPRL), HCV1b NS5A protein at positions 2132–2140 (RYAPACKPL). HIV derived-peptide with an HLA-A2 binding motif (SLYNTVATL) and an HLA-A24 binding motif (RYLRDQQLLGI) were used as negative controls.

Measurement of peptide-reactive Abs

The levels of peptide-specific IgG were measured by means of an enzyme-linked immunosorbent assay (ELISA) that was carried out according to a previously reported method [10]. In brief, serum samples were diluted at 1:100, 1:200, and 1:400 with 0.05% Tween20-Block Ace, and 100 µl/well of the sample was added to each well that was pre-coated with a peptide. After incubation at 37°C for 2 h, the plates were washed with 0.05% Tween20-PBS (PBST) three times and were incubated at 37°C for 2h with 100 µl/well of 1:1,000-diluted

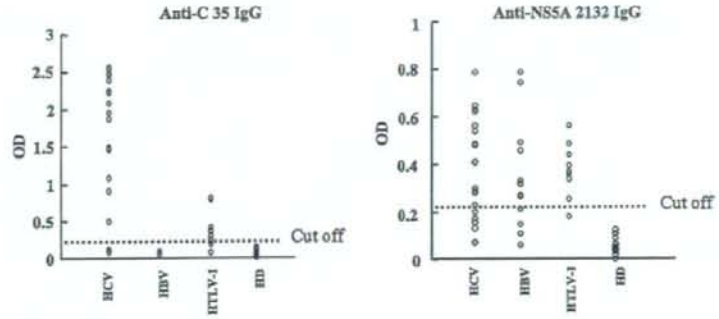

Fig. 1 Detection of anti-C35 and -NS5A2132 Abs in serum samples from patients with various diseases. Sera from patients with various viral infection-related diseases (HCV-related CH, HBV-related diseases, HTLV-1-related adult T-cell leukemia) along with HDs were measured for their reactivity to C35 and NS5A2132 peptides. The optical density (*OD*) values of each sample were assayed in serially diluted serum samples in order to estimate peptide-reactive IgG levels by ELISA. Representative OD results at a serum dilution of 100:1 are shown. The cut-off values were set as the mean + 2SD of the OD value of HDs (0.23 and 0.22 for anti-C35 and anti-NS5A2132, respectively). Statistical analyses were performed using the χ^2 test. Values of $P < 0.05$ were considered statistically significant

rabbit anti-human IgG (γ-chain-specific) (DAKO, Glostrup, Denmark). After the plates were washed three additional times, 100 µl/well of 1:100-diluted anti-rabbit IgG-conjugated horseradish peroxidase-dextran polymer (En Vision, DAKO) was added to each well, and the plates were incubated at room temperature for 40 min prior to stopping the reaction.

Statistics

The statistical analyses were performed using the χ^2 test. Values of $P < 0.05$ were considered statistically significant.

Results

Cross-reactivity, HLA-restriction, and genotype-specificity

We first investigated the cross-reactivity of the two Abs. Serum samples of 22 patients with HCV$^+$ CH were provided to test their reactivity to the HCV core protein at positions 35–44 (C35) and the NS5A protein at positions 2132–2140 (NS5A2132). Serum samples were also obtained from 17 patients with HBV infection and 10 patients with HTLV-1$^+$ adult T-cell leukemia. Sera from 37 HDs served as negative controls. As shown in Fig. 1, significant levels of anti-C35 IgG were detectable in 20 of 22 (90.9%) patients with HCV$^+$. Significant, but low, levels of anti-C35 IgG were also found in 8 of 10 (80%) patients with HTLV-1$^+$. In contrast, none of the patients with HBV$^+$ and none of the healthy donors possessed positive levels of anti-C35 Ab in their sera. Anti-NS5A2132 IgG was more widely found in patients with HCV, HBV, HTLV-1, but not in HDs.

We studied the relationship between the HLA-class I A-locus subtypes and serum levels of the two Abs, since the C35 and NS5A2132 peptides have been originally defined as HLA-class I A-restricted CTL-epitope peptides. Each of the Abs was detectable in the majority of HCV-infected patients, regardless of differences among the HLA-class IA phenotypes (Fig. 2a). We then examined the serum levels of anti-C35 Ab of patients with HCV1b ($n = 29$), HCV2a ($n = 16$), and HCV2b ($n = 3$) infection to determine the HCV-genotype specificity of anti-C35 Ab; as a result, we detected this Ab in 26 of 29, 16 of 16, and 3 of 3 patients, respectively. The results from all samples at a serum dilution of 1:100 are shown in Fig. 2b. Similarly, anti-NS5A2132 Ab was detectable in the sera from 23 of 29 patients infected with HVC1b, 8 of 16 patients with HCV2a, and all 3 HCV2b patients (Fig. 2b). These results suggest that both anti-C35 and -NS5A2132 Abs are detectable in HCV$^+$ patients, regardless of differences in HLA-class IA subtypes, and also with respect to differences in the genotypes of HCV.

Serum Ab levels and clinical stages of HCV infection

We next examined the sera of HCV-related CH ($n = 24$), LC ($n = 22$), HCC ($n = 26$), and HCV$^-$ HD ($n = 9$) subjects, and measured the levels of anti-C35 and anti-NS5A 2132 IgG in each of these groups (Fig. 3a). No significant difference was observed

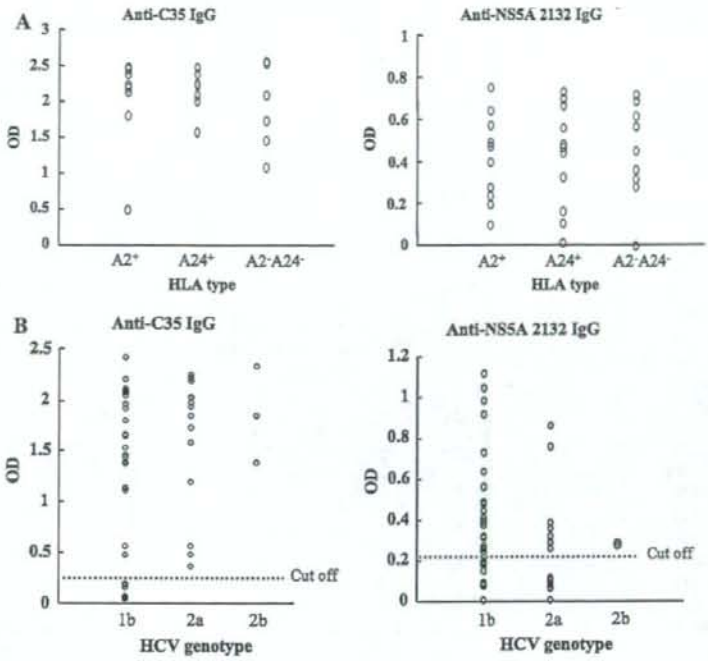

Fig. 2 No restriction of anti-peptide Abs with HLA or HCV genotypes. **a** The relationship between HLA-class IA phenotypes and the levels of anti-C35 or -NS5A2132 IgG were studied in 29 patients with HCV-related diseases. HLA-class IA phenotypes were determined by standard serological methods, and 9 patients were HLA-A2$^+$, 13 patients were HLA-A24$^+$, and the remaining 7 patients were HLA-A2$^-$/A24$^-$. Anti-C35 and -NA5A2132 were measured by standard ELISA as described above, and the OD value of each patient at a serum dilution of 1:100 was plotted. **b** The relationship between HCV genotypes and the levels of anti-C35 or -NS5A2132 IgG was studied in a double-blind manner using sera from patients with HCV1b ($n = 29$), HCV-2a ($n = 16$), and HCV-2b infection ($n = 3$). HCV genotypes were determined by the direct sequencing of HCV in the sera of HCV-infected patients. The results obtained from the sera of all subjects at a dilution of 1:100 are shown. Anti-C35 and -NA5A2132 Abs were measured by standard ELISA as described above, and the OD value of each patient's serum sample at a dilution of 1:100 was plotted. The cut-off value was set at 0.23 and 0.22 for anti-C35 and anti-NS5A2132, respectively

between the anti-C35 Ab levels of these three groups. In contrast, the anti-NS5A 2132 Ab levels of CH were higher than those of LC or HCC, i.e., the mean ± SD values of the Ab were 0.51 ± 0.24, 0.27 ± 0.20, 0.26 ± 0.23, and 0.08 ± 0.07, respectively. The levels of anti-NS5A 2132 Ab in the LC and HCC patients were significantly ($P < 0.05$) lower than those of the CH patients, but still higher than those of the HDs. Statistic analysis among the subgroups classified by disease severity, such as inflammatory activity or inactivity, suggested no difference between the subgroups of each clinical stages (data not shown). The levels of anti-HCV Ab measured by the third-generation assay did not significantly differ between the three groups (CH, 10 ± 2.7; LC, 11 ± 1.4; HCC, 11 ± 1.1) (Fig. 3b). The HCV RNA levels of the HCC patients (360 ± 269.6) were lower ($P < 0.05$) than those of the CH patients (610 ± 347.3) and the LC patients (610 ± 246.4) (Fig. 3c). However, there was no apparent correlation between the levels of anti-NS5A Ab and the HCV RNA levels (Fig. 3c).

Results of the cohort study

To further investigate the individual levels of correlation between anti-NS5A2132 Ab and the prognosis of HCV$^+$ patients, we investigated the serum levels of the two Abs in the samples from a cohort study that was conducted in H town in Fukuoka prefecture, where HCV-infected residents received an annual health screening from 1990 to 2002 [10, 11]. For this study, a total of 66 serum samples from 33 residents were used; here, the samples were harvested twice from the same individual, once in 1995 and once in 2002. The diagnosis of these 33 residents in 1995 were CH ($n = 17$), LC ($n = 1$), asymptomatic carrier (ASC) ($n = 4$), and a past

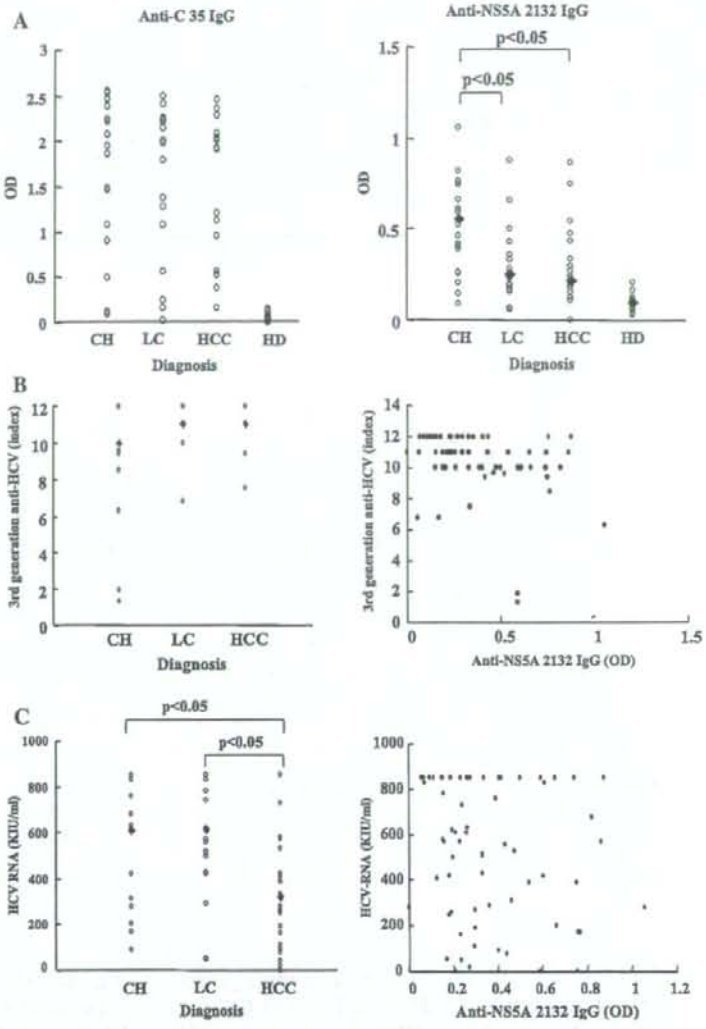

Fig. 3 Measurement of anti-NS5A 2132 Ab. **a** The sera of patients with CH ($n = 24$), LC ($n = 22$), HCC ($n = 26$) and that of HDs ($n = 8$) were provided for the measurement of the anti-C35 and anti-NS5A 2132 IgG levels. The levels of anti-NS5A 2132 Ab in the LC and HCC patients were significantly ($P < 005$) lower than those of the CH patients, but were still higher than those of the HDs. **b** All of the current serum samples were used for the measurement of the anti-HCV Ab levels by means of a commercially available kit (third-generation assay, SRL). These levels were given as the index (*left column*). The relationship between the levels of anti-HCV, as shown in the Index, and those of anti-NS5A2132 are given in the *right column*. **c** All of the present serum samples were also used for the measurement of the HCV RNA levels by means of a commercially available kit (Roche). The HCV RNA levels in the HCC patients (360 ± 269.6) were lower ($P < 0.05$) than those of the CH patients (610 ± 347.3) and the LC patients (610 ± 246.4). The relationship between the levels of HCV-RNA and those of anti-NS5A2132 is given in the *right column*

history of HCV infection (spontaneously recovered individuals; anti-HCV Ab^+ and HCV RNA^-) ($n = 11$), whereas in 2002, the diagnoses were as follows: CH ($n = 13$), LC ($n = 4$), HCC ($n = 2$), asymptomatic carrier (ASC) ($n = 1$), and a past history of HCV infection ($n = 13$). Namely, disease progression was not observed in 26 inhabitants, but was observed in the remaining 7 inhabitants during the 7 years (Table 1). As expected, the levels of anti-C35 Ab measured in the year 1995 were for the most part equal to those measured in 2002 in the majority of these 33 cases, regardless of the condition of the disease (Fig. 4a). In contrast, the levels of anti-NS5A Ab measured in the year 1995 had decreased in all seven residents with disease progression when they were measured again in the year 2002, whereas these levels were for the most part equal to those measured in the year 2002 in the majority of the 25 subjects who had no disease progression (Fig. 4a). The mean ± SD of anti-NS5A 2132 Ab (0.67 ± 0.13) in 1995 in the sera of the seven patients who had disease progression was significantly ($P < 0.05$) higher than that (0.27 ± 0.11) measured in 2002.

These 33 subjects were then subdivided into five different groups (CH, LC, HCC, past history of HCV-infection recovered individuals, and ASC), and the levels of the two Abs in the year 2002, measured at a serum dilution of 1:100, are plotted in Fig. 4b. The levels of anti-C35 Ab were consistently high in CH, LC, and HCC residents, and became very low or were

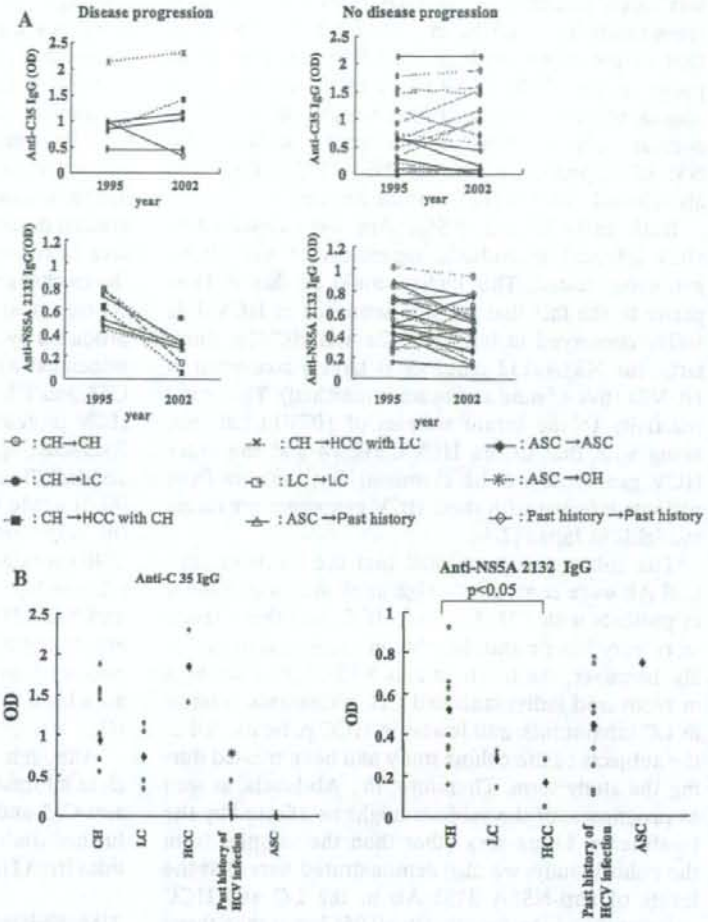

Fig. 4 Detection of anti-C35 and -NS5A2132 Abs in the cohort study. **a** A total of 66 serum samples from 33 inhabitants (harvested twice, once in 1995 and once in 2002) were simultaneously tested for their levels of anti-C35 and -NS5A2132 IgG. The diagnoses of these 33 patients in 1995 and 2002 are shown in Table 1. The patients were subdivided into two groups as follows: no disease progression (25 inhabitants) and disease progression ($n = 8$) groups. The measurement was repeated twice with consistent results, and the representative results of one experiment are shown. The OD values of anti-C35 Ab and anti-NS5A2132 IgG measured in 1995 and again in 2002 at a serum dilution of 1:100 are plotted. **b** Thirty-three residents were then subdivided into five different groups (CH, LC, HCC, past history of HCV-infection cured individuals, and ASC), and the results of the two tests of Ab levels measured in the year 2002 at a dilution of 1:100 are plotted. Statistical analyses were performed using the χ^2 test. Values of $P < 0.05$ were considered statistically significant

undetectable in cured individuals, whereas the levels of anti-NS5A 2132 Ab were high in CH residents, recovered individuals, and ASC residents, moderate in LC patients, and lowest ($P < 0.05$ vs. CH) in the HCC patients.

Discussion

The cross-reactivity of anti-C35 IgG was observed in the sera of HTLV-1$^+$ patients, which could in part be due to the fact that the HTLV-1 envelope protein also possesses the same previously reported fine epitomic sequence (LPRR) as the C35 peptide at positions 37–40 [17]. The HIV tat protein also shares five amino acids (PRRGP) with the C35 peptide at positions 38–42; furthermore, the HIV envelope protein shares five amino acids (RRGPR) with the C35 peptide at positions 39–43. We therefore used the sera from three HIV$^+$ subjects to examine the cross-reactivity to the C35 peptide. As a result, significant levels of anti-C35 IgG were found in the sera from all three subjects (data not shown). However, it should be noted that this cross-reactivity needs to be confirmed by the analysis of a greater number of samples from HIV$^+$ individuals. HBV core protein, as well as HBV envelope protein, also shares four amino acids (PRRG) with the C35 peptide at positions 38–41, and thus we used the sera of 24 HBV$^+$ subjects to investigate the cross-reactivity to the C35 peptide; here, it was observed that none of the serum samples tested had detectable levels of anti-C35 Ab. These results suggest that the sequence homology with the C35 peptide alone cannot account for this type of cross-reactivity. Further studies will therefore be conducted in order to resolve this issue.

In contrast to anti-C35 Ab, anti-NS5A2132 Ab was detected in patients with other diseases, including HBV$^+$ individuals and HTLV-1$^+$ subjects, whereas it

was rarely detected in the HDs. This wide range of cross-reactivity could be in part due to the fact that four of ten amino acids of the NS5A2132 peptide at positions 2133–2136 (YAPA) are identical to those of oligodendrocyte-related protein, which is expressed in normal cells [3]. Four of ten amino acids of the NS5A2132 peptide at positions 2134–2137 (APAC) are also shared with the HBV surface protein.

Both anti-C35 and -NS5A Abs were observed in HCV-infected individuals, regardless of the HCV-genotypes tested. This finding could be due at least partly to the fact that the C35 sequence of HCV1b is 100% conserved in both HCV2a and HCV2b. Similarly, the NS5A2132 sequence is largely conserved in HCV2a (five of nine amino acids matched). The cross-reactivity to the serum samples of HCV1a patients, along with that to the HCV3, HCV4 and the other HCV genotypes will be examined, but the sera from patients infected with these HCV genotypes are rarely available in Japan [2, 8].

The cohort study revealed that the levels of anti-C35 Ab were consistently high at all stages of disease in patients with CH, LC, and HCC, and these levels were very low or undetectable in recovered individuals; however, the levels of anti-NS5A2132 were high in recovered individuals and CH inhabitants, modest in LC inhabitants, and lowest in HCC patients. All of the subjects of the cohort study had been treated during the study term. Therefore, the Ab levels, as well as prognoses, of the subjects might be affected by the treatments. Using sera other than the samples from the cohort study, we also demonstrated here that the levels of anti-NS5A 2132 Ab in the LC and HCC patients were significantly ($P < 0.05$) lower than those of the CH patients, but still higher than the levels observed in HDs. Collectively, these results demonstrated that the serum levels of anti-C35 correlated with persistent infection, while those of anti-NS5A2132 correlated with better prognosis in HCV-infected patients. Anti-C35 Ab was detectable in HCV, HTLV-1, and is most likely also detectable in HIV-infected individuals, but not in patients with the other diseases tested, whereas anti-NS5A Ab was detectable in the sera from patients with variable diseases. The third-generation assay utilizes four recombinant HCV proteins from the core, NS3, NS4, and NS5 regions as a source of antigens, and yet this type of assay cannot provide conclusive information with regards to either a patient's prognosis or to immunological responses to CTL epitope peptides. Therefore, from a clinical perspective, the sequential measurement of these two Abs together may provide new information for predicting a patient's prognosis, although follow-up studies with a larger number of samples are needed.

However, if one considers the possible mechanisms responsible for the different patterns of these two Abs, no information is available at the present time. Anti-C35 IgG might be required, either directly or indirectly, for the elimination of HCV, primarily because it has been shown to decrease in recovered individuals. A gradual decrease in the Ab to HCV core protein in the sera of recovered individuals was reported, although the mechanisms of this decrease remain obscure [6]. In contrast to anti-C35 IgG, anti-NS5A2132 IgG might be produced by long-lasting memory B cells, because it is associated with good prognosis. Long-lasting memory CTL and T helper cells reactive to certain recombinant HCV proteins besides the NS5A protein at positions 2003–2267 appear to be present in persons who were accidentally exposed to HCV1b, but not in CH patients [6]. It would be of interest to study T helper activity to the NS5A protein in the vicinity of the NS5A 2132–2140 peptides. It might also be of interest to study the relationship between these two Abs, and in particular, anti-NS5A2132 and autoimmune hepatitis, since a variety of antibodies reactive to nuclei, smooth muscle cells and liver/kidney microsomes type 1 (LKM-1) have been found in patients with HCV-related diseases [4].

Although the present results do not provide any clear information for elucidating the biological roles of anti-C35 and -NS5A Abs, the results may encourage further studies of Abs reactive to CTL epitope peptides from HCV.

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References

1. Battegay M, Fikes J, Di Bisceglie AM, Wentworth PA, Sette A, Celis E, Ching WM, Grakoui A, Rice CM, Kurokohchi K, Berzofski JA, Hoofnagle JH, Feinstone SM, Akatsuka T (1995) Patients with chronic hepatitis C have circulating cytotoxic T cells which recognize hepatitis C virus-encoded peptides binding to HLA-A2.1 molecules. J Virol 69(4):2462–2470
2. Blatt LM, Mutchnick MG, Tong MJ, Klion FM, Lebovics E, Freilich B, Bach N, Smith C, Herrera J, Tobias H, Conrad A, Schmid P, McHutchison JG (2000) Assessment of hepatitis C virus RNA and genotype from 6807 patients with chronic hepatitis C in the United States. J Viral Hepat 7(3):196–202
3. Bronstein JM, Popper P, Micevych PE, Farber DB (1996) Isolation and characterization of a novel oligodendrocyte-specific protein. Neurology 47(3):772–778

4. Cassani F, Cataleta M, Valentini P, Muratori P, Giostra F, Francesconi R, Muratori L, Lenzi M, Bianchi G, Zauli D, Bianchi FB (1997) Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile. Hepatology 26(3):561–566
5. Chander G, Sulkowski MS, Jenckes MW, Torbenson MS, Herlong HF, Bass EB, Gebo KA (2002) Treatment of chronic hepatitis C: a systematic review. Hepatology 36(5 Suppl 1):S135–S144
6. Hoofnagle JH (2002) Course and outcome of hepatitis C. Hepatology 36(5 Suppl 1):S21–S29
7. Hunziker IP, Zurbriggen R, Glueck R, Engler OB, Reichen J, Dai WJ, Pichler WJ, Cerny A (2001) Perspectives: towards a peptide-based vaccine against hepatitis C virus. Mol Immunol 38(6):475–484
8. Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K (1990) Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 87(24):9524–9528
9. Mine T, Sato Y, Noguchi M, Sasatomi T, Gouhara R, Tsuda N, Tanaka S, Shomura H, Katagiri K, Rikimaru T, Shichijo S, Kamura T, Hashimoto T, Shirouzu K, Yamada A, Todo S, Itoh K, Yamana H (2004) Humoral responses to peptides correlate with overall survival in advanced cancer patients vaccinated with peptides based on pre-existing, peptide-specific cellular responses. Clin Cancer Res 10(3):929–937
10. Nagao Y, Tanaka K, Kobayashi K, Kumáshiro R, Sata M (2004) A cohort study of chronic liver disease in an HCV hyperendemic area of Japan: a prospective analysis for 12 years. Int J Mol Med 13(2):257–265
11. Nagao Y, Tanaka K, Kobayashi K, Kumashiro R, Sata M (2004) Analysis of approach to therapy for chronic liver disease in an HCV hyperendemic area of Japan. Hepatol Res 28(1):30–35
12. Noguchi M, Kobayashi K, Suetsugu N, Tomiyasu K, Suekane S, Yamada A, Itoh K, Noda S (2003) Induction of cellular and humoral immune responses to tumor cells and peptides in HLA-A24 positive hormone-refractory prostate cancer patients by peptide vaccination. Prostate 57(1):80–92
13. Pawlotsky JM (2002) Use and interpretation of virological tests for hepatitis C. Hepatology 36(5 Suppl 1):S65–S73
14. Rodriguez-Lopez M, Rieze-Boj JI, Ruiz M, Berasain C, Civeira MP, Prieto J, Borras-Cuesta F (1999) Immunogenicity of variable regions of hepatitis C virus proteins: selection and modification of peptide epitopes to assess hepatitisa C virus genotypes by ELISA. J Gen Virol 80(Pt3):727–738
15. Shomura H, Shichijo S, Matsueda S, Kawakami T, Sato Y, Todo S, Itoh K (2004) Identification of epidermal growth factor receptor-derived peptides immunogenic for HLA-A2 (+) cancer patients. Br J Cancer 90(8):1563–1571
16. Takaki A, Wiese M, Maertens G, Depla E, Seifert U, Liebetrau A, Miller JL, Manns MP, Rehermann B (2000) Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. Nat Med 6(5):578–582
17. Takao Y, Yamada A, Yutani S, Sata M, Itoh K (2004) Antibody reactive to a hepatitis C virus (HCV)-derived peptide capable of including HLA-A2 restricted cytotoxic T lymphocytes is detectable in the majority of HCV-infected individuals without HLA-A2 restriction. Microbiol Immunol 48(7):507–517
18. Vertuani S, Bazzaro M, Gualandi G, Micheletti F, Marastoni M, Fortini C, Canella A, Marino M, Tomatis R, Traniello S, Gavioli R (2002) Effect of interferon-alpha therapy on epitope-specific cytotoxic T lymphocyte responses in hepatitis C virus-infected individuals. Eur J Immunol 32(1):144–154
19. Ward S, Lauer G, Isba R, Walker B, Klenerman P (2002) Cellular immune responses against hepatitis C virus: the evidence base 2002. Clin Exp Immunol 128(2):195–203

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Clearance of HCV Improves Insulin Resistance, Beta-Cell Function, and Hepatic Expression of Insulin Receptor Substrate 1 and 2

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OBJECTIVES: **Hepatitis C virus (HCV) infection is linked to greater insulin resistance. Although HCV itself is a candidate for the development of insulin resistance, the effects of antiviral treatment on impaired glucose metabolism remain unclear. The aim of this study is to examine the effects of clearance of HCV on insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate (IRS)1/2, central molecules for insulin signaling.**

METHODS: **We analyzed 89 biopsy-proven patients with chronic HCV infection. Patients received interferon-α or interferon-α plus ribavirin for 6 months and were classified into three groups at 6 months after the conclusion of antiviral therapy according to their response to antiviral therapy: sustained responders (N = 29), relapsers (N = 12), and nonresponders (N = 48). Insulin resistance and beta-cell function were assessed by the homeostasis model assessment method (HOMA-IR and HOMA-%B, respectively). Hepatic expression of IRS1/2 was evaluated by immunoblotting and immunostaining in 14 sustained responders.**

RESULTS: **In nonresponders and relapsers, there were no significant changes in HOMA-IR and HOMA-%B values after antiviral therapy. On the other hand, in sustained responders, HOMA-IR values significantly decreased to 1.7 ± 0.8 from 3.1 ± 1.1 ($P < 0.05$) after antiviral therapy. Similarly, HOMA-%B values significantly decreased to 90.6 ± 10.0 from 113.7 ± 15.3 ($P < 0.05$). Immunoblotting showed a threefold increase in IRS1/2 expression after clearance of HCV. Immunostaining revealed that greater IRS1/2 expression was seen in hepatocytes.**

CONCLUSIONS: **We showed that clearance of HCV improves insulin resistance, beta-cell function, and hepatic IRS1/2 expression.**

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection is associated with a greater risk for the development of insulin resistance (1). Greater insulin resistance is more prevalent among patients with HCV infection compared with those with other liver diseases and with the general population (2). In patients with HCV infection, insulin resistance is involved in progression of hepatic fibrosis (3), the development of hepatocellular carcinoma (4, 5), extrahepatic manifestations (6), and prognosis (7). Thus, insulin resistance plays a crucial role in patients with HCV infection.

Insulin resistance can be caused by many factors. In general, obesity, inflammation, and various kinds of metabolic disorders are common factors for the development of insulin resistance. Similarly, body mass index (BMI), serum tumor necrosis factor-α (TNF-α) and hepatic iron concentrations, and hepatic steatosis are reported to be possible causative factors for the development of insulin resistance in patients with HCV infection (8–11). In addition to these factors, HCV itself is also known to have a variety of biological effects (12).

In HCV core transgenic mice, the development of insulin resistance is seen by 1 month of age, in the absence of either overt liver injury or excessive body weight gain (12, 13). Furthermore, even if liver function is restored by transplantation, postliver transplantation diabetes mellitus occurs more frequently among patients who undergo transplantation for HCV than for other conditions (14). Although precise mechanisms for HCV-associated insulin resistance have not been fully elucidated, we recently demonstrated the involvement of insulin receptor substrate 1 and 2 (IRS1/2), central molecules in insulin signaling. Downregulation of IRS1/2 is seen in