10^6 PBMCs; IQR, 13.3-149.7) than in recovered patients (15.3 SFCs/10^6 PBMCs; IQR, 3.3-142.7; $P = .05$) and normal controls (5.3 SFCs/10^6 PBMCs; IQR, 2.7-8.0; $P < .001$). The median numbers of the sum of anti-IgG–secreting B cells to structural antigens were not significantly higher in patients with chronic hepatitis C (108.3 SFCs/10^6 PBMCs) than in those who recovered (97.4 SFCs/10^6 PBMCs) (Fig. 4A). In contrast, the median numbers of the sum of anti-HCV IgG-secreting B cells to nonstructural antigens were significantly higher in patients with chronic hepatitis C (19.0 SFCs/10^6 PBMCs) than in patients who recovered (4.9 SFCs/10^6 PBMCs; $P = .018$), particularly for NS3 antigen (26.7 vs. 5.3 SFCs/10^6 PBMCs; $P = .032$) (Fig. 4B). Furthermore, patients with chronic hepatitis C had a significantly higher frequency of anti-HCV IgG-secreting B cells to the NS3 antigen than those who recovered (85% vs. 44%; $P = .02$) (Fig. 4C).

The median numbers of the sum of anti-HCV IgM-secreting B cells to all HCV antigens were similar in patients with chronic hepatitis C (22.0 SFCs/10^6 PBMCs; IQR, 8.2-49.3) and recovered patients (20.7 SFCs/10^6 PBMCs; IQR, 12.2-36.7) and were significantly higher than in the controls (8.0 SFCs/10^6 PBMCs; IQR, 0.0-10.7; $P < .001$) (Fig. 4A). When the responses were analyzed for structural and nonstructural antigens, the median numbers of the sum of anti-HCV IgM-secreting B cells were not significantly different in patients with chronic hepatitis C and recovered subjects for either structural antigens (30.7 vs. 31.6 SFCs/10^6 PBMCs) or nonstructural antigens (20.7 vs. 12.7 SFCs/10^6 PBMCs) (Fig. 4A).

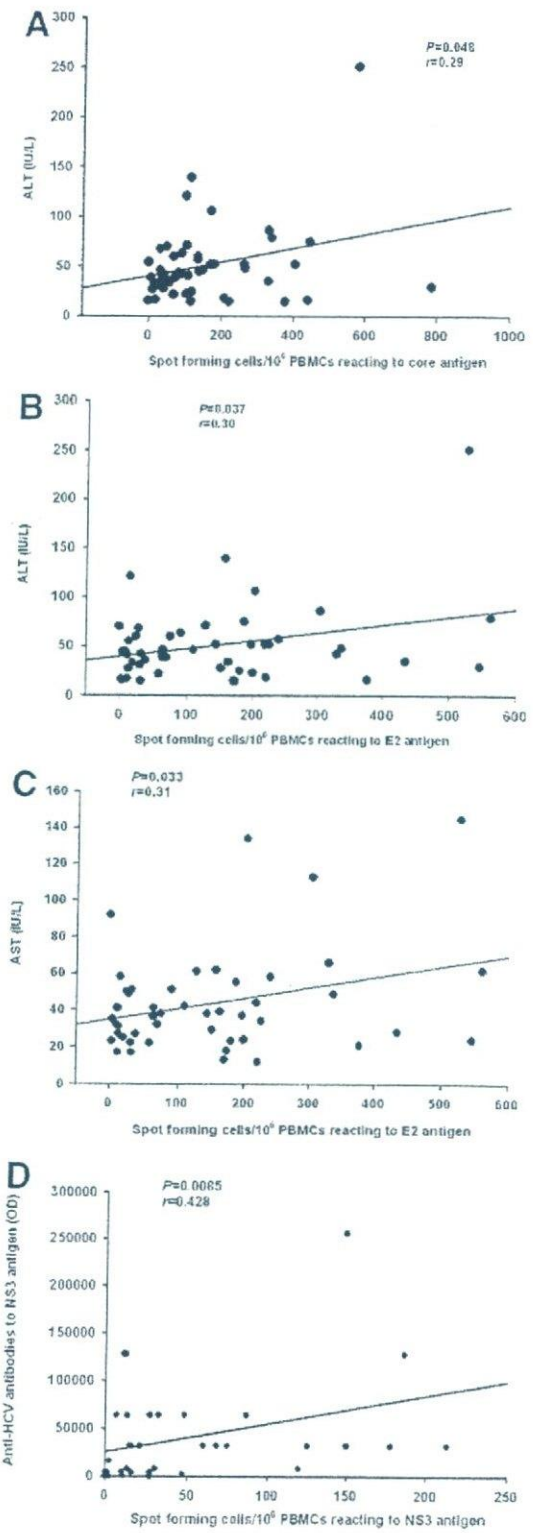

Fig. 3. Correlation of the number of anti-HCV IgG-secreting B cells and clinical characteristics in 48 patients with HCV infection. (A) Frequency of circulating anti-HCV IgG-secreting B cells to core antigen was significantly correlated with the value of ALT ($r = 0.29$, $P = .048$). (B-C) Frequency of circulating anti-HCV IgG-secreting to E2 antigen was correlated with the value of (B) ALT ($r = 0.30$, $P = .037$) and (C) AST ($r = 0.31$, $P = .033$), respectively. (D) Frequency of circulating anti-IgG-secreting B cells to NS3 antigen was correlated with the value of anti-HCV antibodies to NS3 antigen ($r = 0.43$, $P = .0085$). ALT, alanine aminotransferase; PBMCs, peripheral blood mononuclear cells; AST, aspartate aminotransferase; HCV, hepatitis C virus.

Discussion

We developed an ELISpot assay for sensitive quantitative assessment of anti-HCV antibody-secreting B cells in PBMCs from patients with HCV infection and used this technique to analyze the induction of humoral immune responses at the single-cell level. IgG and IgM anti-HCV antibody secreting B cells to core, E2, NS3, and NS5 were detected and quantified in patients with chronic HCV infection and compared with recovered patients and uninfected controls. The key findings were: (1) anti-HCV secreting B-cell responses were greater in chronically infected patients than in recovered patients, suggesting that antibody does not play a major role in recovery from acute

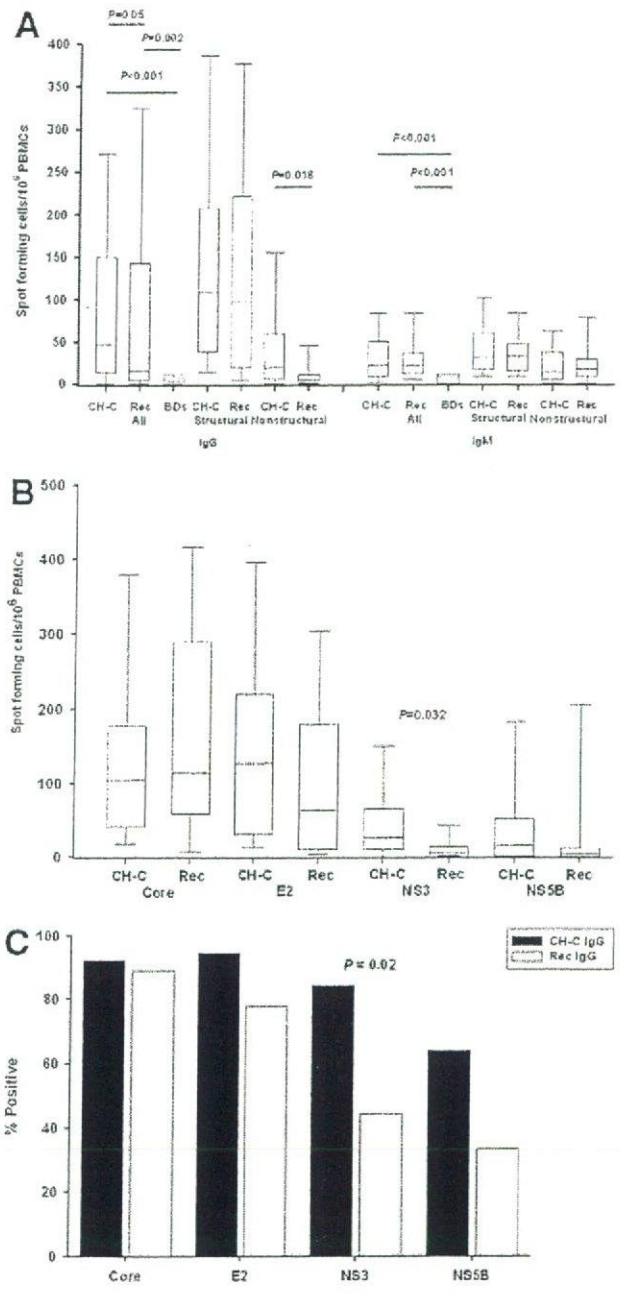

Fig. 4. Detection of anti-HCV antibody-secreting B cells in patients with chronic hepatitis C and in patients who had recovered from HCV. (A) Circulating anti-HCV IgG-secreting B cells were detected in 39 patients with chronic hepatitis C, 9 patients who had recovered from HCV infection, and 11 volunteer blood donors. Circulating anti-HCV IgM-secreting B cells were detected in 34 patients with chronic hepatitis C, 9 patinets who had recovered from HCV infection, and 6 volunteer blood donors. (B) Frequency of circulating anti-HCV IgG-secreting B cells to 4 HCV antigens were detected in 39 patients with chronic hepatitis C and in 9 recovered patients. (C) The prevalence of anti-HCV IgG-secreting B cells in 39 patients with chronic hepatitis C and 9 recovered patients. PBMCs, peripheral blood mononuclear cells; CH-C, chronic hepatitis C; BDs, blood donors; Rec, recovered; IgG, immunoglobulin G; IgM, immunoblobulin M.

HCV infection, as also indicated by recently developed pseudotype assays for HCV-neutralizing antibodies[24,25]; (2) the primary difference between chronically infected and recovered subjects was in the greater reactivity of the former to nonstructural antigens; (3) in chronic infection, HCV antibodies were cross-reactive against genotypes, again consistent with recent findings by neutralizing antibody assays[26,27]; (4) the ELISpot assay can measure IgM as well as IgG responses at the single-cell level, providing a new means to measure the more elusive IgM response; (5) IgM responses were surprisingly well maintained during chronic infection; and (6) IgG responses correlated positively with serum transaminase levels.

In this study, the B-cell ELISpot assay showed high specificity (91% to 100%) and sensitivity (58% to 92%) to all HCV antigens through analysis of the ROC curves and thus achieved high diagnostic accuracy. Although there was a general problem that raw numbers of SFCs were low, statistical analysis and prior publications[28,29] suggest that these small differences are consistent and relevant. Of note, individuals infected with nongenotype 1 variants were strongly positive in this assay, which used only genotype 1–derived antigens. This suggests that genotype 1 contains conserved epitopes that will allow the ELISpot assay to assess humoral immune responses to HCV irrespective of genotype (Fig. 2), with the caveat that we did not assess genotypes 4, 5, and 6, all of which are rare in the United States.

ELISpot assay has been used as a sensitive and specific tool to measure B-cell responses in autoimmune diseases[28,29] and viral infections such as cytomegalovirus,[15] rotavirus,[16] measles virus,[17] and hepatitis B virus,[18,19] as well as to evaluate responses to bacterial[30] and viral vaccines.[18,19] Other reports demonstrate that ELISpot is able to detect and numerate antigen-specific memory B cells in PBMCs after *in vitro* stimulation in both autoimmune diseases and viral infection.[31,32] Thus, the B-cell ELISPOT assay might be a useful tool to detect anti-HCV-specific memory B cells, and to monitor the efficacy of future HCV vaccines.

Interestingly, this study showed a strong correlation between the numbers of anti-HCV IgG-secreting B cells to the core and E2 antigens and the values of serum transaminases. The clinical significance of these observations is unknown, but raises the possibility that antibodies can contribute to liver cell injury. In addition, Ni et al.[33] recently reported that 10 of 36 hepatitis C patient samples showed increased B-cell frequencies that correlated with the degree of hepatic fibrosis. There are insufficient histological data in our study to assess whether the numbers of anti-HCV antibody–secreting B cells correlate with the degree of fibrosis as well as biochemical evidence of inflammation.

The median numbers of the sum of anti-HCV IgG-secreting B cells to nonstructural antigens were significantly higher in patients with chronic hepatitis C than in recovered patients. Similarly, an HCV-specific B-cell response was more frequently detected in patients with chronic hepatitis C than in recovered subjects (92% vs. 56%; $P = .017$) and was directed against a broader range of HCV antigens, particularly to NS3. In contrast, CD4 T-cell responses to NS3 epitopes are greatest in patients who recover from HCV infection.[34,35]

We have also developed and evaluated the ELISpot assay for detecting anti-HCV IgM-secreting B cells. It has been reported that IgM anti-HCV in serum might be predictive of viral clearance in acute hepatitis C or response to interferon therapy.[36-40] However, these results have been controversial and other studies have shown a significant correlation between IgM anti-HCV levels in serum and the recurrence of hepatitis C after liver transplantation.[41,42] In this study, we found that IgM-secreting B cells persisted during chronic infection so that the usefulness of IgM detection for assessing acute versus chronic HCV infection would have to depend on quantitative differences in IgM level rather than the simple presence or absence of IgM antibody. The fact that there are no standardized assays for measuring IgM anti-HCV in serum and the ready detection of IgM-secreting B cells in this study suggests that the ELISpot assay could be used to better define the clinical relevance of IgM antibody in acute and chronic HCV infection.

Overall, this study, as do studies of HCV-specific neutralizing antibodies,[26,27] suggest that the humoral arm of the HCV immune response is not a critical element of spontaneous viral clearance. However, because of the difficulty in obtaining serial acute-phase PBMC collections from recovering subjects, our study does not exclude a role for antibody-mediated viral clearance early in HCV infection. Sequential acute phase ELISpot IgM testing of PBMCs is planned in forthcoming chimpanzee infectivity studies. Nonetheless, studies of neutralizing and antienvelope antibodies that measured serial acute phase serum samples from recovering subjects[26,27] did not show that such antibodies correlated with viral clearance. Rather, it appears in those studies and the current study that antibodies to HCV increase in strength and broadness of reactivity during the course of chronic infection, presumably because of persistent antigenic stimulation. This is in contrast to cell-mediated immunity that is markedly diminished in chronically infected compared with recovered subjects. This dichotomy between the humoral and cellular immune response to HCV is intriguing and suggests T-cell tolerance in the absence of B- cell tolerance.

It is interesting to speculate on the role that antibodies might play in HCV infection. First, it seems reasonable that such antibodies complexed to virus would reduce the level of free virus and diminish transmission to others. This reduction in free virus in addition to lowered viral load might explain the relative rarity of sexual and perinatal transmission during chronic HCV infection. More intriguing is whether such antibodies establish the set point for viral load during chronic infection. It is known that viral loads are highest early in HCV infection prior to the appearance of antibody[43] and that chronically infected patients establish a lower and relatively constant level of viremia.[44] It appears that production and elimination of virus achieve a steady state. This steady state is probably multifactorial in origin, but antibody may play a key role. When patients in a steady state are immunosuppressed at the time of transplantation[45] or when coinfected with human immunodeficiency virus,[46] the viral load increases, supporting an immunological role for viral containment even in the absence of clearance. A deleterious function of anti-HCV is that it serves to drive quasispecies evolution making it increasingly hard for the immune system to achieve viral clearance. Farci et al.[47] have shown in both humans and chimpanzees that the appearance of antibody coincides with increasing viral diversity and complexity and predicts progression to chronic infection.

In conclusion, there is much to explore regarding the function and relevance of IgG and IgM antibodies in HCV infection, and we believe the ELISpot assay, by measuring antibody production at the single-cell level, provides a new and useful tool for understanding the complex interplay between HCV and the host immune response.

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特集・C型肝炎の最新治療ー治療方針のたて方と治療効果ー

治療方針とその効果

高ウイルス量の患者への治療方針と効果

松本晶博*・田中榮司*1)・清澤研道*2)

Summary

C型慢性肝炎に対するインターフェロン治療が始まって15年以上が経過している。従来難治とされてきた1b型高ウイルス量の患者についても，ペグインターフェロンとリバビリンの併用48週間投与法の登場により約50%の著効率が得られるようになった。しかしながら，残りの50%の非著効例や，高齢者や血小板の低い症例など副作用の強いインターフェロン治療が難しい症例に対する治療法の工夫が望まれる。今後シクロスポリンA併用療法，新たな抗ウイルス剤の開発により治療効果がさらに改善されることを期待する。

Key Words C型肝炎ウイルス（HCV）／1b型高ウイルス量／ペグインターフェロン／リバビリン

はじめに

C型慢性肝炎患者に対するインターフェロン療法が始まって15年以上が経った。その間，治療効果に関係する因子について多くのことが解明され，初期の治療に比べ，投与方法の改良，新しいインターフェロンの開発により，治療効果は格段に高まってきた。さらに経口の抗ウイルス剤であるリバビリンの併用により，従来難治であったC型肝炎ウイルス（HCV）1b型高ウイルス量の患者についてもめざましく治療効果が改善されてきている。

HCVウイルス量とウイルス型

HCVの定量系には現在PCR法（ポリメラーゼ連鎖増幅法）を用いたアンプリコアモニター法，分岐鎖DNAプローブ法および，HCVコア抗原測定法がある。3者の相関は良く，ウイルス量からみたインターフェロン治療効果予測能はほぼ同等である。高ウイルス量の定義は，アンプリコアモニター法で100KIU/mL以上，分岐鎖DNAプローブ法で1.0Meq/mL以上，コア抗原測定法で300fmol/mL以上である。

現在わが国には主に3つの遺伝子型（1b，2a，2b型）のC型肝炎ウイルスが分布して

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いる。インターフェロンの治療効果はウイルス型によっても大きく異なり，同じウイルス量でも1b型に比べ2a，2b型では著効率が高い[1]。この3つの遺伝子型の中で1b型が約70％を占めており，インターフェロン抵抗性の1b型高ウイルス量患者の割合が約50％と最も多い。

インターフェロンの治療法とその効果

インターフェロンの治療効果は投与方法で異なっている。現在まで臨床の場で主に用いられてきた治療方法は以下の7通りである。①インターフェロン単独24週投与法，②コンセンサスインターフェロン単独24週投与法，③ペグインターフェロン単独48週投与法，④インターフェロン＋リバビリン併用24週投与法，⑤インターフェロン＋リバビリン48週投与法，⑥ペグインターフェロン＋リバビリン併用48週投与法，⑦インターフェロンまたはペグインターフェロン単独長期（2年以上）投与法である。①から⑥はこの順番にしたがって治療効果が上昇している。一般に，インターフェロン投与期間が長いほど，また，リバビリンを併用するほど効果が高くなる。特に1b型高ウイルス量患者についてはこの差が著明である。1b型高ウイルス量の症例に対する著効率は，インターフェロン単独24週間投与法で約5％程度，コンセンサスインターフェロン単独24週投与法で約10％，ペグインターフェロン単独48週投与法で約20％，インターフェロン＋リバビリン併用24週投与法で約20％，インターフェロン＋リバビリン併用48週投与法で約45％，ペグインターフェロン＋リバビリン併用48週投与法で約50％である（図1）[2]。

インターフェロン治療の問題点

インターフェロン治療の最大の問題点はその副作用の強さにある。副作用はペグインターフェロンに比べて従来のインターフェロンで強く，リバビリンを併用するとインターフェロンの副作用の増強効果が見られる。また，

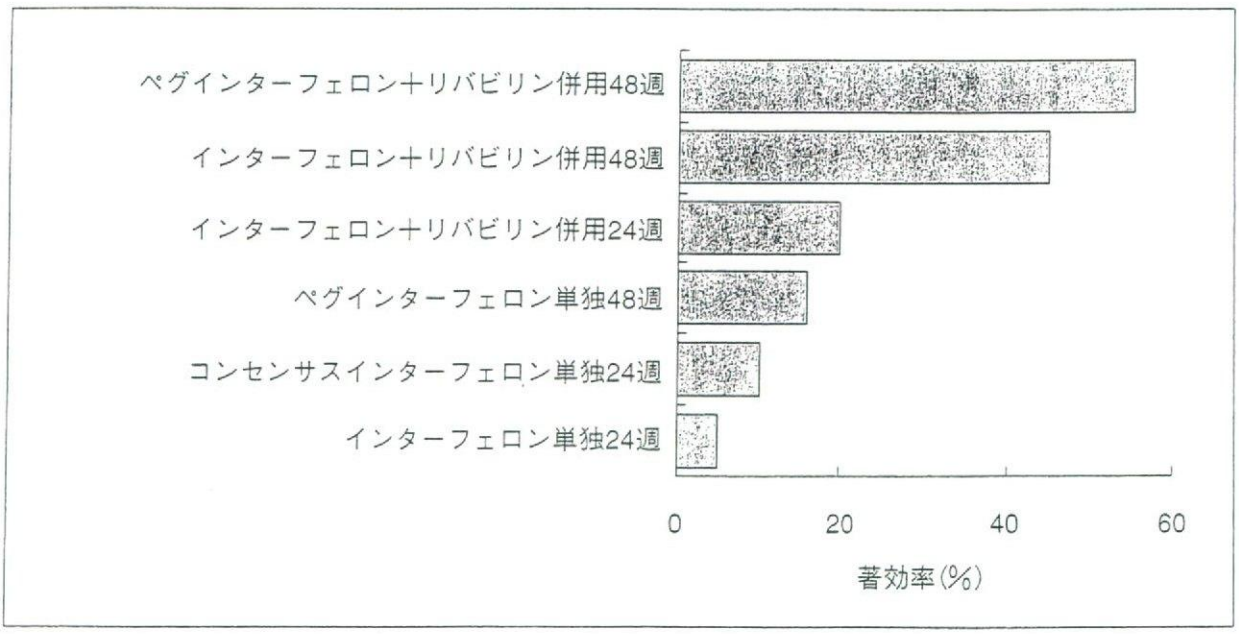

図1　インターフェロン投与法とHCV 1b高ウイルス量患者に対する効果

リバビリンはその代謝特徴により血中濃度依存性に溶血がおき，ヘモグロビン量の少ない患者では溶血性貧血が現れる。また，腎排泄のため腎機能の低下した高齢者では副作用が強く現れることがあり注意が必要である。ペグインターフェロンでは発熱や食欲不振などの副作用は従来のインターフェロンに比べて少ないが，血小板減少が強く現れる例も報告されている。

インターフェロンおよびリバビリンは高価であり，患者の経済的な負担も強く費用対効果も念頭にいれて治療する必要がある。

これらの副作用や治療費について患者に十分なインフォームド・コンセントを行い，代替治療についても説明し，治療に対しての理解を得てから治療を開始することが重要である。

ペグインターフェロン＋リバビリン併用療法

HCV 1b 型高ウイルス量患者に対する治療はやはり治療効果が高いペグインターフェロン＋リバビリン併用48週間投与法が第１選択となる。現在行われている方法はペグインターフェロン α-2b を週１回1.5μg/kg 皮下投与を行い，同時にリバビリンを１日12mg/kg 経口投与する。この治療法により約50%から60%の症例で著効が得られる。著効に関連する因子は現在のところまだ解明されていないが，従来著効率に関連のあったウイルス量や非構造領域５A の変異はその関連性が薄れている。しかしながらウイルス量がアンプリコアモニター法のハイレンジ法で5,000 KIU/mL を超えるような症例ではやはり著効率が低下する。また，高齢者では治療中止率が高く，著効率が低下する。インターフェロンやリバビリンを投与予定量の80%程度まで減量しても効果はそれほど変わらないが，中止すると著効率が低下するため，副作用が出た場合早めに減量して治療を継続することが重要である[2]。

血小板減少例に対する治療

ペグインターフェロンを使用する場合に注意すべき点は，作用持続期間が長いため副作用が現れた場合にすぐ血中濃度を下げるのが難しいことである。特に血小板減少には注意が必要である。C 型肝炎の場合，肝線維化が進んでいる症例では脾機能亢進により血小板が減少している。ペグインターフェロンを安全に使用できる血小板値は開始時に10万/μL 以上とされている。血小板の値がこれ以下で治療を開始した場合はすぐに減量したり中止したりしなければならず，十分な治療ができないことが多い。治療開始時の血小板が低い症例では，脾動脈塞栓術や脾臓摘出術を行って血小板値を上げてからインターフェロン療法を行う例もある[3]。血小板値がコントロールしにくい場合は，インターフェロンの体内濃度を調整しやすい従来のインターフェロン＋リバビリン併用48週間投与も考慮するべきであろう。

非著効例，副作用が強い例，高齢者に対する治療

ペグインターフェロンまたはインターフェロン＋リバビリン併用48週療法非著効例や副作用による治療継続困難例に対してはウイルス消失効果を得ることが難しいため，発癌抑制を狙ってインターフェロン少量長期投与を考慮する。特に肝線維化進行例で，ウルソデオキシコール酸やグリチルリチン製剤によるトランスアミナーゼ抑制効果が低い場合に導

入することが望ましい。インターフェロン療法の年齢的な限界は70歳程度と考えられており，それ以上の高齢者の場合は患者個々の状態により判断すべきであろう。高齢者や肝線維化の軽い例では，ウルソデオキシコール酸やグリチルリチン製剤の投与を行いトランスアミナーゼを抑制する。また，瀉血および鉄制限食による除鉄療法も有効である。

今後の展開

近年，免疫抑制剤であるシクロスポリンAの抗ウイルス効果が注目されている[4]。C型肝炎関連の肝移植例では術後高率に肝炎が再燃し，ウイルス量が高値となり，肝線維化の進行が早いため，移植後のインターフェロン療法が必要であると考えられている[5]。肝移植後に免疫抑制剤としてシクロスポリンAが用いられることがあるため，インターフェロンとリバビリンとの3者併用療法を行うことが検討されている。また，シクロスポリンAの類似体で免疫抑制効果が無く，抗ウイルス効果のみを持った薬剤の臨床応用が検討されている[6]。

おわりに

新しいインターフェロン製剤の導入により1b型高ウイルス量の難治性C型肝炎に対する治療効果は著しく向上してきている。今後開発される薬剤の導入によりさらなる治療効果が期待される。しかしながらこれらの治療抵抗性のウイルスもおり，今後ウイルス側のみでなく宿主側の条件も解析し，より効果的な治療法の開発が望まれる。

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特集II　C型肝炎の自然経過と治療介入

C型肝炎の自然経過*

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清澤研道**

Key Words：fibrosis, interferon therapy, hepatocellular carcinoma(HCC), hepatitis C virus(HCV)

はじめに

本邦ではC型肝炎ウイルス(HCV)感染者は約150～200万人いると推定され，国民の健康を脅かす社会問題となっている．C型慢性肝炎は，肝線維化が緩徐に進行し肝硬変，肝癌に至る．わが国では，毎年3万人を超える人が肝癌で亡くなっており，近年急激にその数が増加している．肝癌患者の8割以上はHCV感染者が占めている．C型慢性肝炎の根本的治療としてインターフェロンが用いられており，近年ではリバビリンとの併用，さらにペグインターフェロンの導入によって，ウイルス駆除率は劇的な改善を認めている．その治療効果，ことにウイルス駆除効果と長期的治療効果が明らかにされてきており，本稿ではC型肝炎の自然史について概説する．

自然史を明らかにすることは難しい

通常，病気の自然史を明らかにするためにはいくつかの事柄が正確に把握されなければならない．これらは感染，病気の発症時期がはっきりとし，治療によって修飾されず，前向きコホートで観察されている，罹病率，死亡率は判明していることなどである．しかし，アルコールの多飲，HBVやHIVの重複感染など他の因子の影響が関与する場合や抗ウイルス療法が行われている状況では，C型肝炎の自然史を正確に観察することは非常に困難である．輸血などの感染時期が明らかな症例を除けば感染時期も特定することは難しい状況であり，C型肝炎の自然史は完全に理解されてはいない．

C型肝炎の自然史

急性C型肝炎を発症した患者のうち倦怠感や黄疸などの症状を呈するものは少なく，大多数のHCV感染者は無症状であり気づかずにいる．急性C型肝炎のうち54～86%の症例で慢性化することが報告されている(図1)[1)～9)]．急性肝炎から慢性肝炎，さらに肝硬変へと進展する場合も症状が出現しないことが多い．症状が出現したときは非代償性肝硬変になり，時には肝癌の合併を認めている時である．しかし，ここまでに何十年という経過を経ているのが実際のところである．よって，C型肝炎の自然史を確定するのは非常に難しいため，さまざまな研究デザインを用いて明らかにする努力がなされている．ここでは代表的な後ろ向きコホート(retrospective study)，前向きコホート(prospective study)，これらを合わせた後ろ向き－前向きコホート

* Natural history of hepatitis C.

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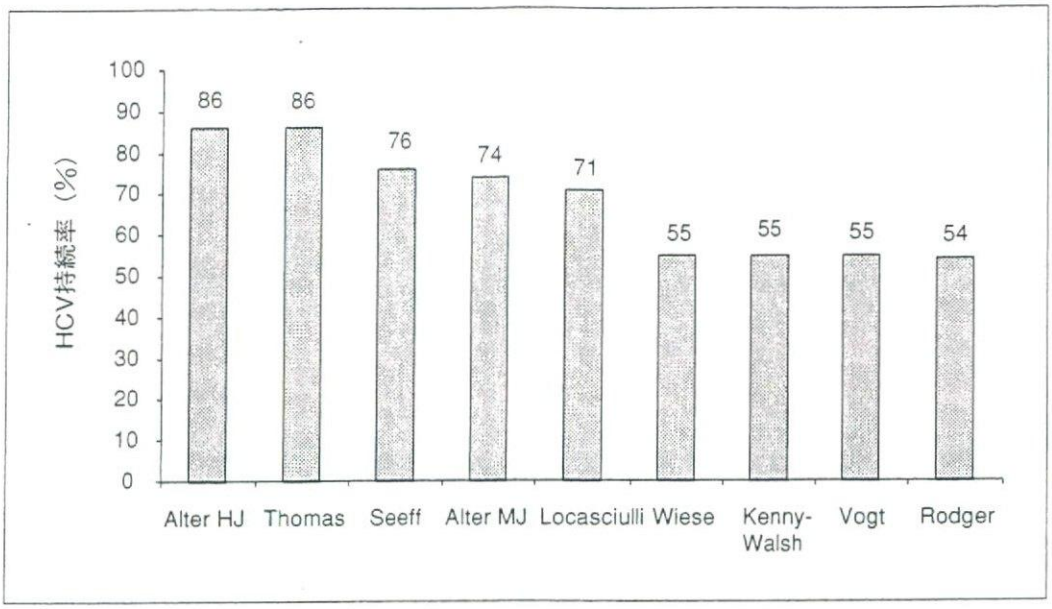

図1　急性C型肝炎後のHCV感染の持続率（文献[10]より引用改変）

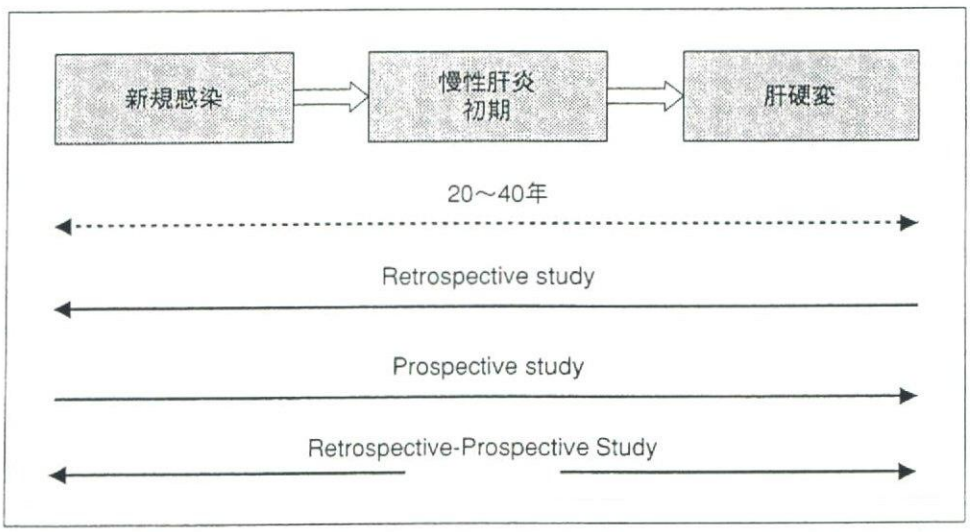

図2　C型肝炎の自然史を明らかにするための研究デザイン（文献[10]より引用改変）

(retrospective-prospective study)での研究について解説する(図2)[10].

1．後ろ向きコホート

1990年にKiyosawaらが231名の非A非B型慢性肝疾患においてHCV抗体を測定することによってC型肝炎の長期経過を明らかにした[11]．さらに輸血歴のあるC型肝炎症例の経過から感染から慢性肝炎，肝硬変，肝癌へ至るまでの平均年数をそれぞれ，10年，21年，29年であることを初めて示した．同様のデータがアメリカのTongらのグループからも報告された[12].

C型肝炎の20年，30年にわたる長期の経過の後には，肝硬変，肝癌へと進展することが明らかになった(図3)．ただし，この数字についてはあくまでも平均であり，輸血年齢(感染時期)が若年と高齢では病状進展度が相違することがわかっている[13][14].

2．前向きコホート

欧米では輸血後急性非A非B肝炎患者についての経過観察を行うことでその大部分を占める急性C型肝炎患者の自然史が明らかになっている．これらの結果から肝硬変，肝癌の発生はそれぞれ7～16%(平均11%)，0.7～1.3%であり，後ろ向きコホートと比較すると低いことが判明している[15]~[18].

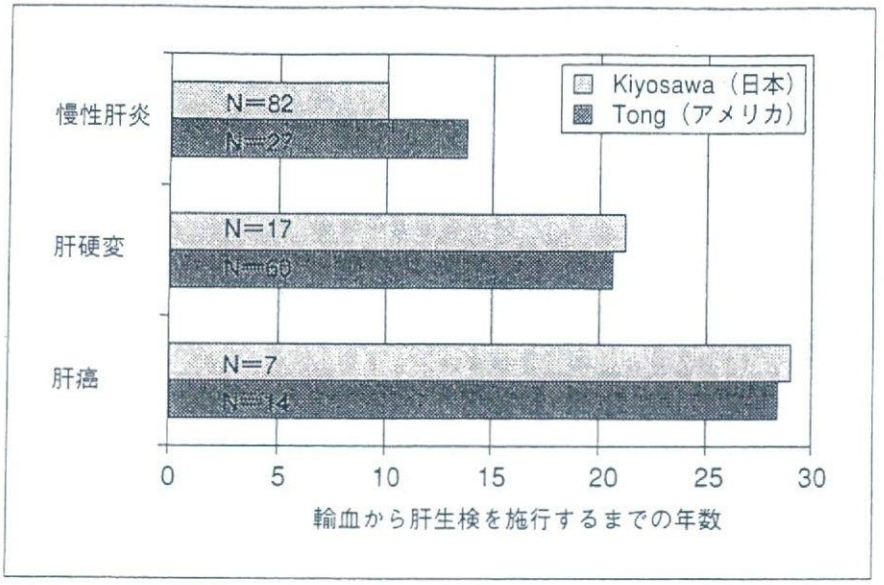

図３　輸血によるHCV感染から慢性肝疾患発症までの期間

表１　C型肝炎の後ろ向き－前向きコホートの研究

研究	集団	期間(年数)	肝硬変(%)	肝癌(%)	肝蔵死(%)
Vogt	子供	17	0.3	0	0
Kenny-Walsh	若い女性	17	2.0	0	0
Wiese	若い女性	20	0.4	0	0
Seeff	若い男性	45～50	5.9	0	0
Thomas	薬物濫用者	9～15	1.0	0	2.1
Rodger	Community	25	4.0	0	1.0
Seeff	輸血後肝炎	23	15.0	1.9	2.8

（文献[10]より引用改変）

3．後ろ向き－前向きコホート

過去に急性C型肝炎を発症した患者群を集め，その群を前向きに経過観察しなおしたものである．代表的な７つの研究を表１に示す．それぞれの背景を簡単に説明するとVogtら[8]は３歳までに心臓手術を施行された458名の子供達について，Kenny-Walshら[7]，Wieseら[6]はHCVに感染した免疫グロブリンを使用された若い女性についてそれぞれ62,667名，2,867名について検討している．Thomasら[2]は1,667名の薬物濫用者，Rodgerら[9]もほとんどが薬物濫用者である95名について，Seeffら[19]は連鎖球菌の抗体を測定した約9,000例の兵士についてHCVを測定している．これらの結果からは肝硬変，肝癌への進展はそれぞれ0.3～5.9%（平均2.1%），０%といずれの群の中でもっとも低率であった．

肝硬変の出現する頻度は後ろ向きでは42%，前向きでは11%，後ろ向き－前向きでは2.1%であった．後ろ向きコホートの研究はすでに症状が出現し，病状が進行している患者たちが集中しやすく，急性肝炎が自然に治癒した例や，比較的軽度な肝障害の患者は集まりにくい．前向きコホートは輸血後に発症したC型肝炎を観察しているが，経過観察期間が8～16年と短いため，まだ結論を導き出すには至らない．しかし，後ろ向き－前向きコホートではさまざまな性，年齢の人々を対象としており，ほとんどの例で急性発症の時期が判明しており経過観察の期間も前向きコホートより長期である．

最近ではオーストラリアのグループがC型肝炎の自然史について過去に発表された論文を解析し，報告している[20]．著者らは過去に報告のあった145編の論文のうち20例未満の報告，年齢もしくは感染期間が明言されていない論文を除いた57編について解析している．著者らははこれらの論文を４つの群

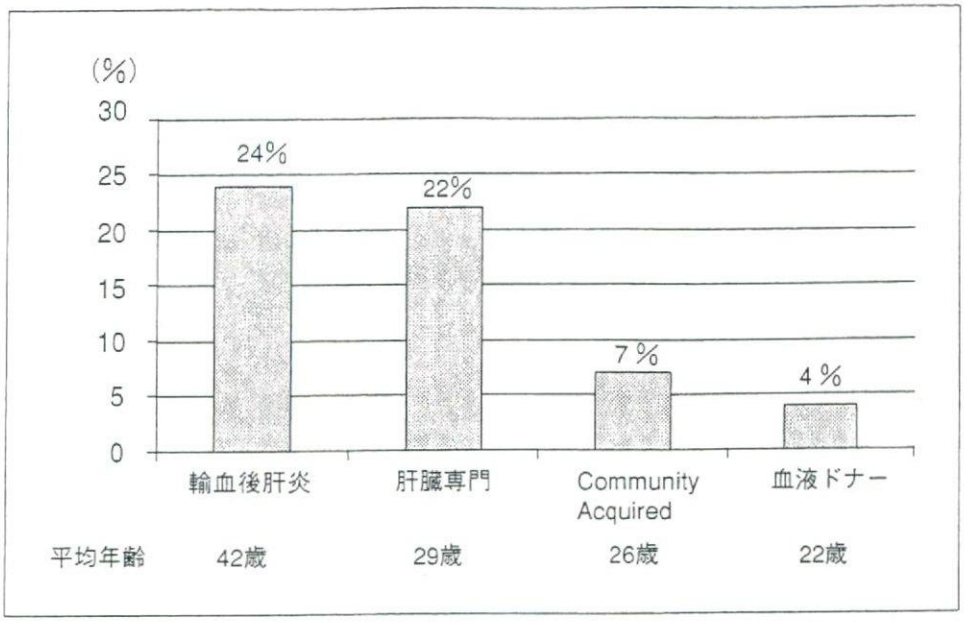

図4　HCV感染後20年で肝硬変に進行する割合(文献[10]より引用改変)

1)肝臓病専門クリニックにおける断面調査(cross-sectional)
2)輸血後肝炎の長期経過観察
3)血液ドナーのスクリーニングの際にHCV感染を指摘された断面調査
4)Community-basedの長期経過観察

に分けて分析した．図4に示すようにHCV感染20年後に肝硬変が出現する頻度は輸血後肝炎のコホートでは24%(感染者の平均年齢は42歳)，肝臓専門クリニックにおけるコホートでは22%(平均年齢29歳)であった．一方，community-basedでは7%(平均年齢26歳)，血液ドナーでは4%(22歳)と低率であった．

肝線維化進展速度

慢性肝炎では肝障害の持続，すなわち肝組織の壊死・炎症の持続とともに線維化が進展し，肝硬変さらには肝癌発症へと至る．慢性肝炎から肝硬変に至るまでの進展度を正確に評価するための指標として，肝組織所見が重要である．肝炎の活動性は肝組織における壊死・炎症の程度を表し，進展度は肝線維化の程度すなわちF因子で表され，F0～F4の5段階に分類されている．F0は肝線維化がなく，慢性肝炎では通常軽度の線維化であるF1ないしは高度線維化のF3と表し，肝硬変はF4に相当する．つまり肝線維化の程度で慢性肝炎の進展度を判断しており，肝線維化の程度の評価は重要である．

Poynardらは感染時期を輸血や薬物使用の時期と推定したうえで，肝生検の線維化の程度から年間の肝線維化の進展率を計算したところ，C型慢性肝炎全体では0.133単位/年，男性；0.154単位/年，女性；0.111単位/年，アルコール飲酒者；0.167単位/年，非飲酒者；0.125単位/年であった．これらの値は1回の肝生検と病歴からの推定の感染期間に基づいている[21]．一方，Shiratoriらは，同一症例で2回の肝生検の線維化の程度の違いを両肝生検の期間で除して計算し，本邦における肝線維化進展率は0.10単位/年と報告している[22]．

肝線維化と発癌率

信州大学第二内科の検討から，C型慢性肝炎における肝線維化の進展度別に年間発癌率を計算したところF1では0.5%，F2で1.5%，F3で2.6%，F4で5.8%であった(図5)[23]．また，YoshidaらもC型慢性肝炎490例の検討からもそれぞれ0.4%，2%，5%，8%とほぼ同様の成績が報告されており[24]，C型肝炎ウイルス群では肝硬変がもっとも強い危険因子であることが分かる．

現在までに慢性肝疾患の進展や肝発癌に影響

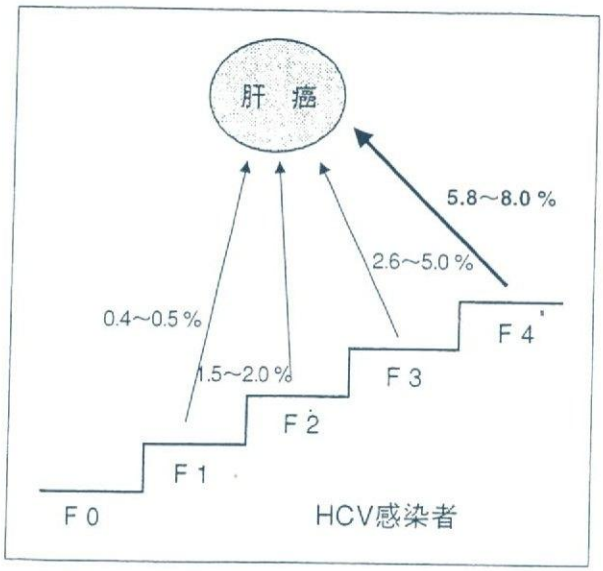

図5 C型慢性肝炎の自然経過と肝線維化

を及ぼしている因子がいくつか報告されている[14]．

宿主側の要因：高齢者や感染時年齢が高齢の場合は進展が早い．性差(男性)，人種差(黒人)も重要な要因である．糖尿病，肥満の合併なども進展を早める．肝線維化の進展度(肝硬変)も発癌に影響を与える．

ウイルス側の要因：われわれは以前，genotype 1に感染した群はgenotype 2に感染した群より肝癌患者が多いと報告したがgenotype 1と慢性C型肝炎の進展の関連性には否定的な報告もあり明らかとなっていない．ウイルス量，超可変領域(Hypervariable region)のquasispeciesが線維化の進展に関与するという報告は現在までにない．HBVやHIVとの重複感染は慢性肝炎の進展に関与するという報告もされている．

環境因子：大量のアルコール摂取は進展を早めることが報告されている．

おわりに

C型肝炎の自然史としてC型慢性肝炎の経過，肝線維化の進展，肝発癌の危険因子について概説した．従来，線維化の評価は肝生検によって行われてきた．しかし，肝生検は危険が0でないこと，時にサンプリング・エラーや読み違いなどが起こる可能性もある．感度の高い線維化の血清マーカーは存在しないため，簡単に線維化の状態を推定する計算式や最近では肝臓の"硬さ"を非侵襲的に測定できるFibroscan®の開発も行われてきている[25][26]．しかし，感度，特異度，コストなどに問題がある．肝線維化の進展を正確に評価できるマーカーの開発は今後の重要な課題である．2004年，アメリカではNational Institutes of Healthが中心となって"Action Plan for Liver Disease Research"(http://www.niddk.nih.gov/fund/divisions/ddn/ldrb/ldrb_action_plan.htm)を発表している．肝臓病を16の分野に分けておのおのの専門家たちがその分野において現在までに明らかになっていること，今後明らかにしなくてはならないことについて優先順位を示している．肝線維化の分野では血清マーカーの開発が最重要プロジェクトとしてあげられている．また，今後の研究はC型肝炎の進展に関する病態を明らかにし，線維化の進展に寄与しているかもしれない未知の因子の同定などにも力を入れる必要がある．

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SURGERY TODAY

Improved Quality of Life and Unchanged Magnetic Resonance Brain Imaging After Living Donor Liver Transplantation for Late-Onset Ornithine Transcarbamylase Deficiency: Report of a Case

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Abstract
We report the case of a 7-year-old girl with ornithine transcarbamylase deficiency whose quality of life (QOL) improved greatly after a living donor liver transplantation (LDLT). Ornithine transcarbamylase deficiency had been diagnosed when she was 2 years old and she finally underwent LDLT, with her father as the donor, when she was 7 years old. The patient had suffered episodes of hyperammonemic encephalopathy ranging from lethargy to coma, treated by hemodialysis twice before LDLT, and her intelligence quotient was borderline for her age. Preoperative magnetic resonance imaging (MRI) showed an atrophic area in the subcortical white matter of the frontal lobe. After LDLT, the patient suffered acute rejection with hyperamylasemia, but not hyperammonemia. Postoperative MRI and quantitative MR spectroscopy showed no changes in the subcortical lesion. She has been followed up carefully for 16 months and has had no further complications or any sign of hyperammonemia.

Key words Living donor liver transplantation · Ornithine transcarbamylase deficiency · Magnetic resonance imaging · Brain atrophy · Quality of life

Introduction

Ornithine transcarbamylase deficiency (OTCD) is one of the most common inherited disorders of the urea cycle in Japan.[1] It is an X-linked disorder characterized by signs and symptoms of encephalopathy caused by the accumulation of precursors of urea, principally ammonia and glutamine, in the blood. The incidence of OTCD is 1:80000 and it can manifest as the early-onset type or the late-onset type. In boys, the early-onset type of OTCD manifesting during the neonatal period is often fatal, although the late-onset type is milder. On the other hand, heterozygous girls may be normal or have episodes of hyperammonemic encephalopathy with a consequent decline in cognitive function.[1] The treatment for this disease is directed at minimizing the requirement for urea biosynthesis by decreasing dietary nitrogen intake and by increasing waste nitrogen excretion with sodium phenylbutyrate.[2] However, this conservative therapy does not completely prevent hyperammonemic coma and deterioration on cognition, and liver transplantation is necessary if the hyperammonemic attacks are frequent.[3] We report a case of living donor liver transplantation (LDLT) for OTCD which resulted in improved quality of life (QOL) and no further sign of deterioration on cranial magnetic resonance (MR) images 16 months after transplantation.

Case Report

A girl born after an uncomplicated gestation and delivery was taken to a nearby hospital at the age of 2 years 3 months for investigation of general fatigue, vomiting, and emotional instability. She was diagnosed as having OTCD at the age of 2 years 9 months based on the following findings: hyperammonemia, orotic aciduria, and elevation of plasma glutamine. Protein restriction and medication with sodium benzoate, citrulline, lactulose, carnitine, and arginine were begun, despite with she suffered several episodes of hyperammonemia, which developed into fulminant hyperammonemia,

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Table 1. Peak levels of orotate and uracil (mmol/g creatinine) in the urine of the patient and her parents after an allopurinol load

	Orotate (μmol/g Cr)	Uracil (μmol/g Cr)
Patient		
Before LDLT	1691.6	1220.2
1 year after LDLT	29.5	86.0
Father	54.5	59.0
Mother	90.4	119.8
Normal range	<98 (child), <211 (adult)	<254

After oral allopurinol (5 mg/kg), four consecutive 6-h urine collections were taken over the next 24 h.[4] Normal range data were obtained from previous studies[5,6]
LDLT, living donor liver transplantation

Table 2. Changes in serum ammonia, glutamate, glutamine, urine orotate, and urine uracil in the patient

	NH_3 (μg/dl)	Glutamate (μmol/l)	Glutamine (μmol/l)	Orotate (μmol/g Cr)	Uracil (μmol/g Cr)
1 day before LDLT	74	35	371	29	614
Start of LDLT	86	41	358	749	543
Ahepatic period	78	14	332	5	571
Declamping	138	50	376	3	351
End of LDLT	47	NA	NA	4	94
POD 1	94	15	196	3	34
POD 3	82	25	213	14	98
POD 6	44	14	178	15	112
POD 31	63	14	126	NA	NA
1 year after LDLT	34	4	172	4	51
Normal range	<85	15–72	420–700	5–31	<105

Normal range data were obtained from previous studies[4,5]
LDLT, living donor liver transplantation; NH_3, ammonia; POD, postoperative day; NA, not available

twice necessitating hemodialysis. At the age of 7, she was referred to our hospital for LDLT. Her parents were evaluated as potential volunteer donors. The allopurinol loading test, which is used to determine latent carriers of OTCD, showed no abnormalities in either parent (Table 1).[4–6] Thus, the genetic origin of the disease in the patient was considered to be a new mutation. The father decided to be the donor because his blood type was identical to that of the patient.

At the time of admission, the patient was alert and asymptomatic. Physical examination revealed no abnormalities in her abdomen and the superficial lymph nodes were not palpable. The palpebral conjunctivae were not anemic and the bulbar conjunctivae were not stained yellow. The patient showed a normal growth pattern and her intelligence quotient (IQ) was at the borderline of normal for her age (IQ = 76: Tanaka Binet Scale of Intelligence). Laboratory data showed a slightly low prothrombin time (51.0%, international normalized ratio 1.73), an increased serum ammonia level (174 μg/dl), and normal aspartate aminotransferase (AST, 33 IU/l), alanine aminotransferase (ALT, 36 IU/l), and albumin (4.3 g/dl) levels. Her serum was positive for anti-cytomegalovirus and anti-Epstein–Barr virus antibodies. Contrast-enhanced computed tomography (CT) of the abdomen showed a normal-sized liver and a spleen within the normal range of vessel variation. Doppler ultrasonography showed normal blood flow through the intrahepatic portal vein and hepatic vein. Magnetic resonance imaging (MRI) of the brain revealed spotty T1 and T2 prolongations in the subcortical white matter of the frontal lobe. Based on the results of these preoperative examinations we concluded that there were no contraindications to LDLT, which we performed using the left lobe of her father's liver.

During the operation we monitored her serum ammonia levels and had prepared for hemodialysis in case hyperammonemia developed. However, as her serum ammonia levels decreased after declamping, we did not need to carry out acute blood purification during or after transplantation (Table 2). The graft weight/recipient weight ratio was 1.4. The operation time was 12 h and the total blood loss was 889 ml. The patient was given tacrolimus, methylpredonisolone, and mycophenolate mofetil (MMF) as immunosuppressive

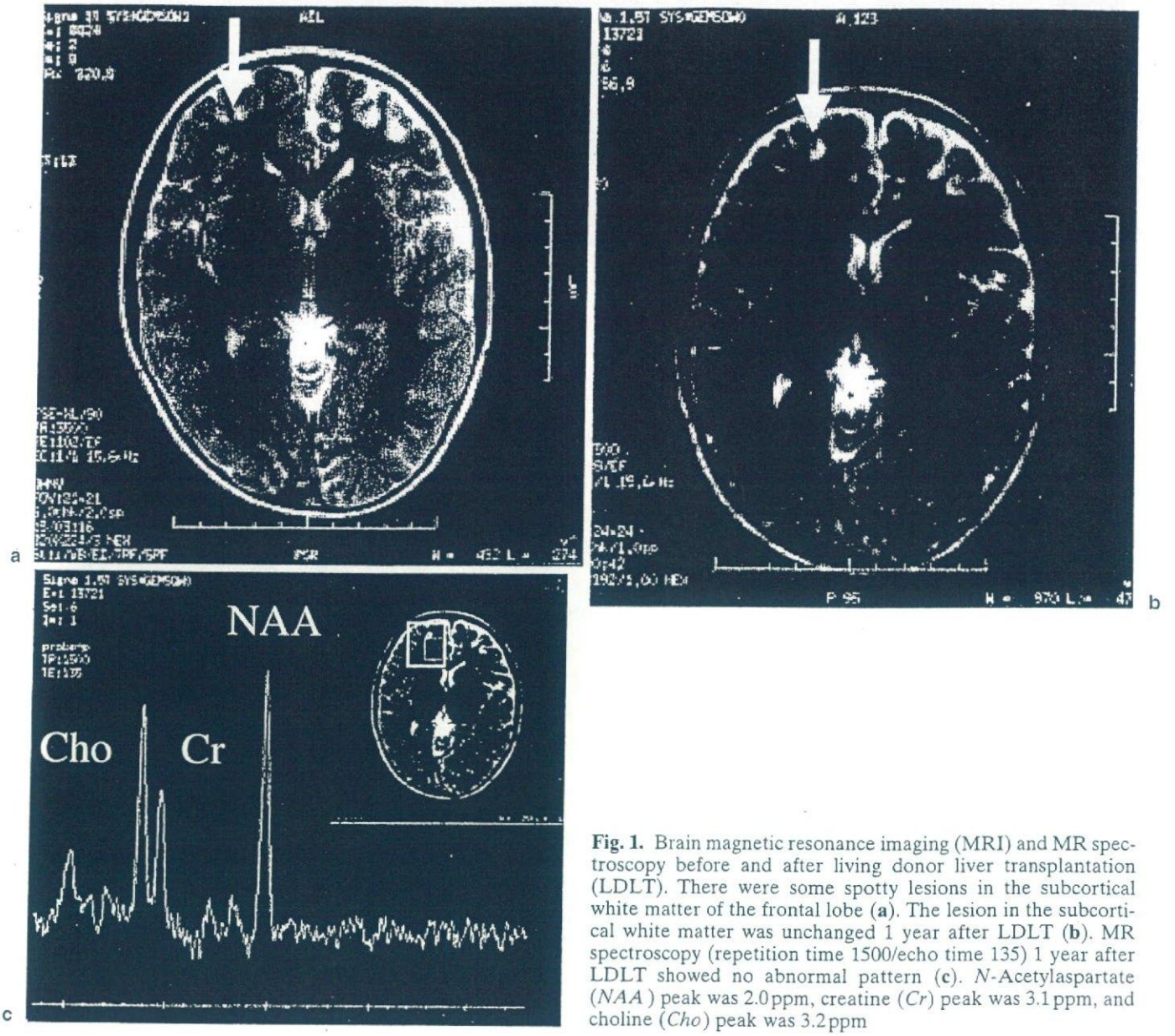

Fig. 1. Brain magnetic resonance imaging (MRI) and MR spectroscopy before and after living donor liver transplantation (LDLT). There were some spotty lesions in the subcortical white matter of the frontal lobe (**a**). The lesion in the subcortical white matter was unchanged 1 year after LDLT (**b**). MR spectroscopy (repetition time 1500/echo time 135) 1 year after LDLT showed no abnormal pattern (**c**). *N*-Acetylaspartate (*NAA*) peak was 2.0 ppm, creatine (*Cr*) peak was 3.1 ppm, and choline (*Cho*) peak was 3.2 ppm

therapy. Her post-transplant course was unremarkable, apart from symptoms of acute rejection and mild acute pancreatitis, and she was discharged from hospital on the 76th postoperative day.

At the time of writing this report, 16 months after LDLT, her serum ammonia level and allopurinol loading tests were within the normal range (Tables 1 and 2) and there has been no further evidence of hepatic encephalopathy. Magnetic resonance imaging of the brain still showed the same spotty lesions in the subcortical white matter of the frontal lobe observed before LDLT, and the size of these lesions was also unchanged (Fig. 1a,b). Magnetic resonance spectroscopy after LDLT showed on abnormal peak of *N*-acetylaspartate, creatine, and choline (Fig. 1c). The patient's IQ was still at the borderline of normal for her age (IQ = 75: Tanaka Binet Scale of Intelligence). The patient attends a normal elementary school and is not on a restricted diet. She is followed up in our outpatient clinic every 6 weeks.

Discussion

We reported a case of late-onset OTCD initially treated by protein restriction with sodium benzoate, carnitine, and arginine, and finally cured by LDLT after the development of hyperammonemic encephalopathy, when the patient was 7 years old. Before LDLT, this patient was frequently in hospital and she had to follow a strict diet;

however, after LDLT she could eat what she wanted and her QOL was greatly improved. The patient also had focal brain atrophy caused by the continuous hyperammonemia, and MRI showed no further progression of this after LDLT. Up until 2003, only 14 cases of LDLT for OTCD had been reported in Japan,[7] but these cases constitute important evidence of the usefulness of LDLT to cure OTCD.

Ornithine transcarbamylase deficiency is an X-linked genetic disorder of the urea cycle, which is not uncommon in Japan.[1] The signs and symptoms of encephalopathy are related to the accumulation of the precursors of urea, principally ammonia and glutamine, in the blood.[2] Ornithine transcarbamylase deficiency occurs at an incidence of 1:50000 to 80000,[1,8] and it is a life-threatening disease in hemizygote male neonates with lethargy, vomiting, coma, and serious hyperammonemia and hyperglutaminemia soon after birth.[9] These babies have a poor prognosis. Mild forms of OTCD may present later, even during adulthood. The symptoms and signs include coma, mental retardation, protein avoidance, headache, bizarre behavior, and episodic hyperammonemia.[3,10] The clinical manifestations in girls carrying an OTC mutation vary, and are a reflection of both allelic heterogeneity and the variable pattern of X-chromosome inactivation in hepatocytes.[11] Partial OTCD and the ever rarer partial recessive urea cycle disorders may be difficult to diagnose in girls because they have few, if any, biochemical changes while they are asymptomatic.

Ornithine transcarbamylase deficiency is diagnosed by hyperammonemia, hyperglutaminemia, hypoargininemia, hypocitrullinemia, mild liver dysfunction, and increased urinary orotic acid levels. Performing a liver biopsy to measure the enzymatic activity of hepatocytes is also informative, but there may be a variety of activity in each hepatic cell. Provocative testing, such as the allopurinol challenge, has been used in girls.[12,13] This test measures the excretion of orotic acid and orotidine derived from a cytosolic pool of carbamyl phosphate, thereby monitoring a pathway of pyrimidine synthesis that is only indirectly influenced by metabolic events within the urea cycle itself. Moreover, this challenge test is a safe diagnostic method even for infants.[13] Our patient had a high level of urinary orotic acid before LDLT, which decreased to within the normal range after LDLT.

The pathophysiological mechanism of central nervous system injury in urea cycle disorders is not completely understood. It has been reported that high levels of ammonium result in the conversion of large amounts of glutamate to glutamine by glutamine synthetase, mainly in astrocytes, which causes swelling of the astrocytes, leading to brain edema, intracranial hypertension, and cerebral hypoperfusion.[14] Magnetic resonance spectroscopy has made it possible to analyze the biochemistry of the brain and accurately identify and quantify metabolites in well-localized regions.[15] It is important to monitor brain damage quantitatively in patients with OTCD, because hepatic encephalopathy can be very harmful and is one of the crucial predictors of their prognosis. In our patient, MRI of the brain before LDLT showed spotty atrophic lesions in the subcortical white matter of the frontal lobe, but this lesion was unchanged 16 months after the operation, and MR spectroscopy showed no abnormal peak of *N*-acetylaspartate, creatine, or choline.

According to one report, patients with late-onset OTCD who were treated with drugs that activate new pathways of waste-nitrogen excretion had fewer hyperammonemic episodes and a reduced risk of further cognitive decline.[2] However, despite remarkable residual activity, because of the heterozygote status of the liver these patients are always at risk of severe hyperammonemia. Thus, liver transplantation is the only complete treatment even for late-onset OTCD. Our patient and her family acknowledged her remarkable improvement and had no regrets about the LDLT, because they are now enjoying a normal life. From the hereditary point of view, deceased donor liver transplantation is feasible for this type of disease, but in Japan deceased donor sources are very limited, making LDLT the most reliable form of transplantation. Thus, all LDLT donors should be selected using the allopurinol challenge test, analysis of gene mutations, and the usual liver function tests.

In summary, we reported the case of a 7-year-old girl who underwent LDLT for late-onset OTCD. The patient has been followed up by measuring her serum levels of ammonia, liver function tests, the allopurinol loading test, and brain MRI. This case report shows that LDLT can cure and improve the QOL of patients with late-onset OTCD.

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ELSEVIER

New Strategy for ABO-Incompatible Living Donor Liver Transplantation With Anti-CD20 Antibody (Rituximab) and Plasma Exchange

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ABSTRACT

It is more difficult to control humoral rejection in living donor liver transplantations (LDLT) across the ABO blood group barrier than in matched or compatible combinations. We achieved excellent results in ABO-incompatible transplantation with novel immunosuppressive regimens and plasma exchange (PE). Among 82 LDLT were 10 cases of ABO-incompatible recipients, including three who were administered rituximab for rescue or prophylactic therapy. Pretransplantation PE was performed as necessary to maintain hemagglutinin titers below 1:16 and posttransplantation PE was performed when there were signs of hyperacute rejection associated with high titers. Induction immunosuppression consisted of FK506, steroid, mycophenolate mofetil (MMF), and rituximab. The first patient was administered rituximab with deoxyspergualin (DSG), steroid pulse therapy, and PE on postoperative day (POD) 7, because of biopsy-proven humoral acute rejection. The titers and LFTs improved drastically. The second and third patients were administered rituximab just after the operation with other routine immunosuppressants for prophylaxis of hyperacute rejection. The second patient showed a slight deterioration in LFTs with an elevated titer, which normalized after steroid pulse therapy and PE. The third patient had no episodes of rejection. At present, that is 27, 17, and 6 months after the operations respectively, the 3 transplant recipients are in stable condition.

THE DONOR SHORTAGE encouraged us to perform living donor liver transplantation (LDLT) across the ABO blood group barrier. The survival rate among ABO-incompatible recipients used to be much poorer than for ABO-compatible recipients, but the introduction of novel immunosuppressive regimens and plasma exchange (PE) has yielded excellent results in ABO-incompatible transplantation. We have successfully performed ABO-incompatible LDLT in 3 patients using rituximab and PE.

PATIENTS AND METHODS

Among 82 LDLT, 10 cases were ABO-incompatible recipients including three administered rituximab for rescue or prophylactic therapy. The age of the 10 recipients ranged from 7 months to 54 years (median age, 12.8 years). IgM and IgG hemagglutinin titers were measured before and after transplantation by serial dilution in saline. Pretransplantation PE was performed as necessary to maintain hemaggultinin titers below 1:16, and posttransplantation PE was performed when the patient showed signs of hyperacute rejection with high titers. Induction immunosuppression in the 10 recipients consisted of FK506, steroid and mycophenolate mofetil (MMF) or azathioprine (AZ), with MMF or AZ administered for 3 days prior to transplantation.

RESULTS

Nine of the 10 recipients and the 3 recipients who were administered rituximab survived (Table 1). The mean follow-up period of the 10 recipients was 4 years.

Case 1

A 22-month-old girl, whose primary disease was biliary atresia, underwent LDLT using her father as the donor (A to O). She also underwent PE twice before transplantation to reduce the titer to 16 or below. From postoperative day

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