表1　肝型糖原病の病型

病型		亜型	頻度(%)	責任酵素	遺伝形式	備考
I	von Gierke	Ia	90	グルコース-6-ホスファターゼ	常染色体劣性	
		Ib	10	グルコース-6-リン酸トランスロカーゼ	常染色体劣性	好中球減少，反復性細菌性感染症
III	Cori, Forbes	IIIa	85	脱分枝酵素	常染色体劣性	筋症状，高CK血症
		IIIb	15	脱分枝酵素（肝のみ）	常染色体劣性	
IV	Andersen	なし		分枝酵素	常染色体劣性	乳児期早期からの慢性肝不全，診断困難
VI	Hers	なし		ホスホリラーゼ	常染色体劣性	
IX（以前はVIII）		IXa	不明	ホスホリラーゼキナーゼ（α鎖）	X染色体劣性	
		IXb	不明	ホスホリラーゼキナーゼ（β鎖）	X染色体劣性	
		IXc	不明	ホスホリラーゼキナーゼ（γ鎖）	常染色体劣性	

（文献1）より引用）

糖原病の病型を表1に示した[1]．糖原病の病型番号は基本的に責任酵素が発見された順番につけられたものであり，ほぼ重症度と相関する．IV型（Andersen病）は，分枝酵素の異常によりアミロペクチン型の異常グリコーゲンが蓄積し，肝型糖原病のなかで最も予後が悪い．乳児期早期から慢性肝不全に至り，内科医がフォローすることはほとんどない．Ia型（von Gierke）がIV型を除くと，肝型糖原病では最も低血糖を起こしやすく，合併症も多い．合併症の多くは思春期以降に発症する[1]．合併症の発症機序を図1[2]に示した．内科医は，これらの合併症の発症の危険性を考慮しながら定期的な診察が重要である．

I型，IV型を除く肝型糖原病の多くは，予後良好とされているが，わが国における長期フォローのまとまった報告はない．筆者らは小児期に線維化をきたした肝型糖原病（I，IV型を除く）を経験している．肝型糖原病は肝腺種や肝がんの発症を見据えたフォローを内科医に依頼すべきと考える．

3．Wilson病

Wilson病では，ATP7B蛋白の異常により，肝内の余分な銅を胆汁中に排泄できず，肝細胞内に銅が過剰に蓄積し，その後他の臓器へ銅が沈着する[3,4]．小児期に診断されるWilson病の多くは，発症前型（血液検査で偶然トランスアミナーゼの高値が判明）や肝型（腹部症状や黄疸で判明）でキレート剤の服用で症状なく改善する場合が多い．そのために病識がなく，思春期以降にしばしば怠薬がおこる．怠薬後の転帰は様々で劇症肝炎型や神経型で発症する場合があり，生命学的予後や神経学的予後が著しく悪い．内科医にこの点を十分説明し，トランジションすることが重要である．

Wilson病の患者の平均寿命は伸びており，肝がんの発症が散見される[5]．内科医には発がんの危険性についても併せて紹介する．

4．シトリン欠損症（NICCD）

シトリンはミトコンドリア膜輸送蛋白質の一員で，内膜に存在するアスパラギン酸・グルタミン酸膜輸送体である[6]．シトリンはまた，リンゴ酸・アスパラギン酸シャトルを構成し，解糖系で生成された細胞質NADHをミトコンドリア内に輸送することにより，エネルギー産生・代謝に深く関与している．すなわち，シトリン欠損症では，尿素，蛋白質，

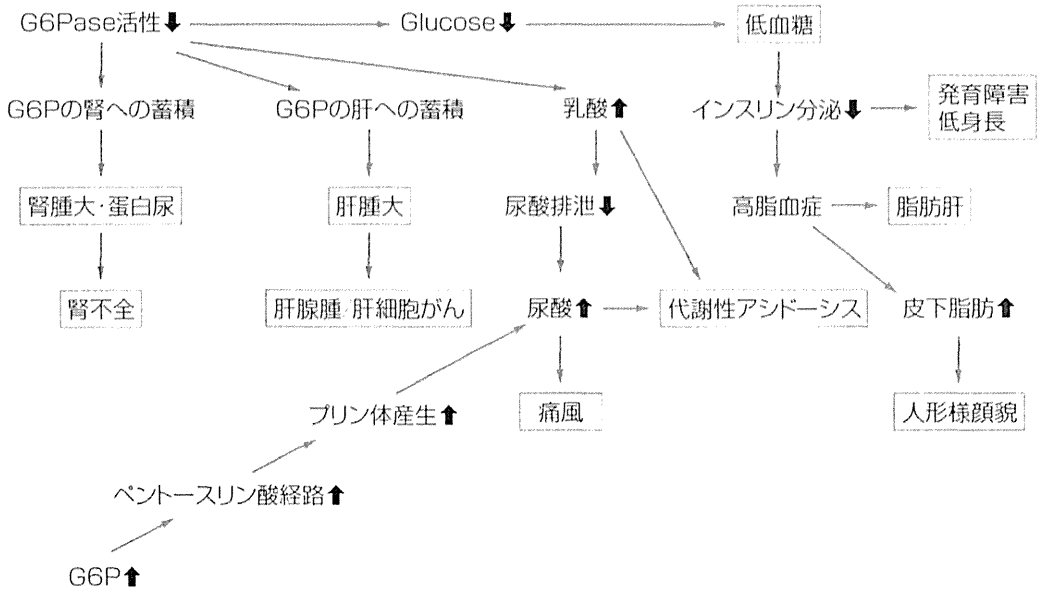

図1　I型糖原病の病態生理　（文献2）より引用一部改変）

図2　シトリン欠損症の年齢依存性臨床症状
NAFLD:non-alcoholic fatty liver disease, NASH:non-alcoholic steatohepatitis
（文献7）より引用一部改変）

核酸合成，糖新生，好気的解糖，エネルギー産生などに障害を受けるため，多彩な症状と複雑な病態を呈する（図2）[7]．

小児期はNICCD（neonatal intrahepatic cholestasis caused by citrin deficiency）として発見されることがほとんどで，その後適応・代償期に内科医にトランジションすることとなる．図3は「シトルリン血症の会」が作成した学校へのパンフレットである．成人のシトリン欠損症でもその生活形態はほぼ同様であり，これを活用して内科医に理解を深めてもらう．

おわりに

医学の進歩により，小児期発症の慢性肝疾患の予後は著しく改善した．われわれ小児科医の希望は，これらの患児が自立した成人となって社会貢献し，生きがいのある生活を送ることである．そのために，発症時期から将来を見据えた患家とのかかわりあいが重要であり，正確な診療情報を内科肝臓専門医へ提供することが今後の課題である．また，内科一般医は必ず内科肝臓専門医と連携をとることが必要である．

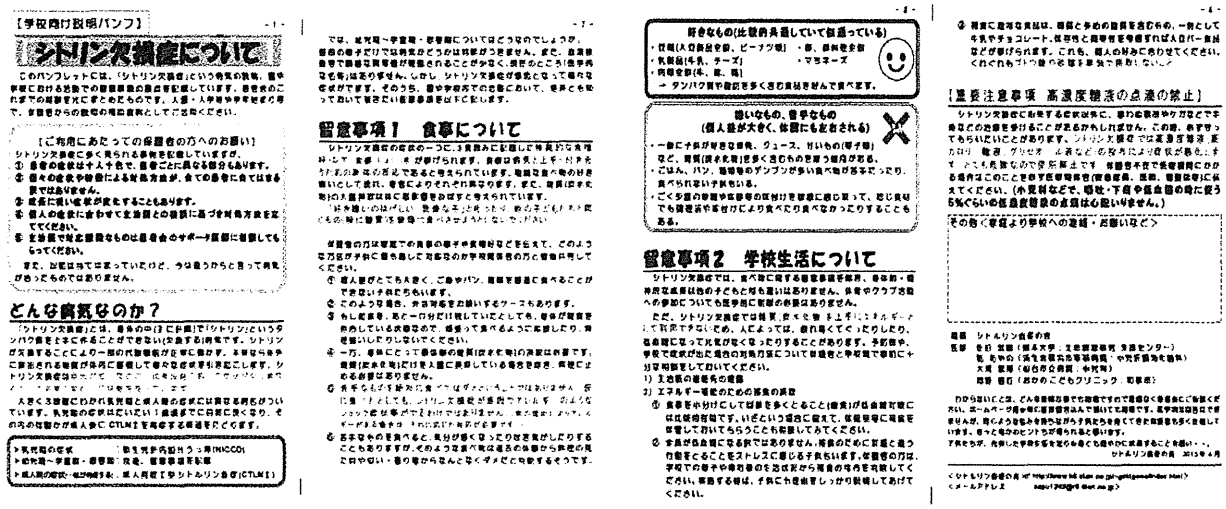

図3　「シトルリン血症の会」が発行している学校向けパンフレット

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Case Report

Neonatal liver failure owing to gestational alloimmune liver disease without iron overload

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Although neonatal hemochromatosis (NH) is a well-known cause of liver failure during the neonatal period and iron deposition in extrahepatic tissues is considered essential in the diagnosis of NH, there is no consensus regarding the pathology or diagnostic criteria of NH. Recent studies of immunohistochemical assays have shown that the C5b-9 complex (the terminal membrane attack complement complex) is strongly expressed in the liver of NH cases, suggesting that a gestational alloimmune mechanism is the cause of liver injury. The patient was a low birthweight primiparous male born at 37 weeks of gestation by vaginal delivery. Blood tests 3 h after birth showed signs of liver failure, including high transferrin saturation, resembling the clinical characteristics of NH. However, magnetic resonance imaging and a lip biopsy showed no obvious iron deposition outside the liver. The patient was refractory to exchange transfusion and immunoglobulin therapy but was successfully treated by liver transplantation. Histologically, the explanted liver showed established cirrhosis, with large amounts of human C5b-9 in the residual hepatocytes, suggesting the alloimmune mechanism of liver injury was the cause of his liver failure. Liver failure caused by a gestational alloimmune mechanism should be considered in patients with antenatal liver failure, even without obvious extrahepatic siderosis.

Key words: C5b-9, gestational alloimmune liver disease, liver failure, liver transplantation, membrane attack complex, neonatal hemochromatosis

INTRODUCTION

THE ETIOLOGY OF neonatal hemochromatosis (NH) is often difficult to determine, resulting in high mortality rates. Although NH is an important cause of neonatal liver failure, its pathogenic mechanism remains unclear. Among the diagnostic criteria for NH is the demonstration of extrahepatic iron deposition.[1] Recent findings suggested that a maternal gestational alloimmune disease mechanism may cause NH.[2] We describe a patient with neonatal liver failure but without extrahepatic iron deposition resulting from maternal alloimmune liver injury.

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CASE REPORT

THE MOTHER OF the patient was a 26-year-old woman with no history of miscarriage. She and her husband were not consanguineous, with neither having a relevant past medical history, except that her husband had chronic hepatitis B. Fetal ultrasound showed intrauterine growth retardation, beginning at week 34 of gestation. The newborn, a male, was born at 37 weeks and 5 days of gestation by spontaneous delivery as a primiparous baby. He was of low birthweight (2122 g), with no abnormalities on physical examination. However, a blood test 3 h after birth revealed hypoglycemia (10 mg/dL), a low platelet count ($8.3 \times 10^4/\mu L$), cholestasis (5.3 mg/dL total bilirubin [T-Bil], 2.3 mg/dL direct bilirubin [D-Bil], 27 IU/L γ-glutamyltransferase

[γ-GT]) and mildly elevated transaminases (142 IU/L aspartate aminotransferase [AST], 15 IU/L alanine aminotransferase [ALT]). Cholestasis progressively worsened and, on day 5, he was referred to our hospital.

On admission, our 5-day-old patient showed mild lethargy and poor sucking. His liver and spleen were palpable 2 cm under the costal margin. Blood tests on admission showed 2.1 g/dL albumin, 8.3×10^4/μL platelets, 151 IU/L AST, 25 IU/L ALT, 15.2 mg/L T-Bil, 4.0 mg/dL D-Bil, 27 IU/L γ-GT, 53.9 μM total bile acids and 1.66 prothrombin time international normalized ratio. Transferrin saturation was high, at 95%, with ferritin and total iron-binding capacity concentrations of 1521 ng/mL and 152 μg/dL, respectively. Metabolic screening by tandem mass spectrometry was used to determine the composition of plasma/urine organic acids and amino acids and his acylcarnitine profile. The only abnormality detected was an elevated serum tyrosine concentration (684.3 nM/mL). Succinylacetone, however, was absent from urine, excluding a diagnosis of tyrosinemia. Cultures of blood and urine were negative. TORCH (toxoplasmosis, other [syphilis], rubella, cytomegalovirus, herpes simplex virus) and other viral infections (hepatitis B virus, hepatitis C virus, Epstein–Barr virus, human herpesvirus 6, enterovirus, adenovirus and parvovirus B19) were excluded serologically and/or by real-time polymerase chain reaction. Bone marrow examination was normal, excluding hemophagocytic syndrome and hematological malignancy. Bile acid assays of plasma and urine revealed increased levels of Δ^4-3-oxo-type bile acids in urine (53.5%) and their presence in serum, indicating reduced activity of the enzyme Δ^4-3-oxo-steroid 5 beta-reductase, consistent with findings in other patients with NH.[3] However, sequencing of the *SRD5B1* gene in genomic DNA from peripheral lymphocytes showed no known mutations.[4] Liver ultrasound showed slightly irregular surfaces and dull edges. The internal structure was rough and liver and kidney contrast was normal. Ascites was detected. Although NH was highly suspected, computed tomography and magnetic resonance imaging (MRI) failed to demonstrate iron deposition in the liver or extrahepatic tissue (Fig. 1). Lip biopsy revealed no iron deposition in the salivary glands. Liver biopsy could not be performed because of coagulopathy.

Although iron deposition in extrahepatic tissue was not demonstrated, evidence of antenatal liver failure, intrauterine growth retardation and high serum ferritin concentrations strongly suggested that NH was the cause of liver failure. Moreover, none of our findings suggested any other condition, such as infection, metabolic or hematological diseases. He was treated for 15 days with exchange blood transfusion, consisting of high-dose (1 g/kg) i.v. immunoglobulin (IVIG), 100 mg/kg acetylcysteine i.v., 3 μg/kg selenium, 0.4 μg/kg per h prostaglandin-E1 and 25 IU/kg vitamin E, but his liver

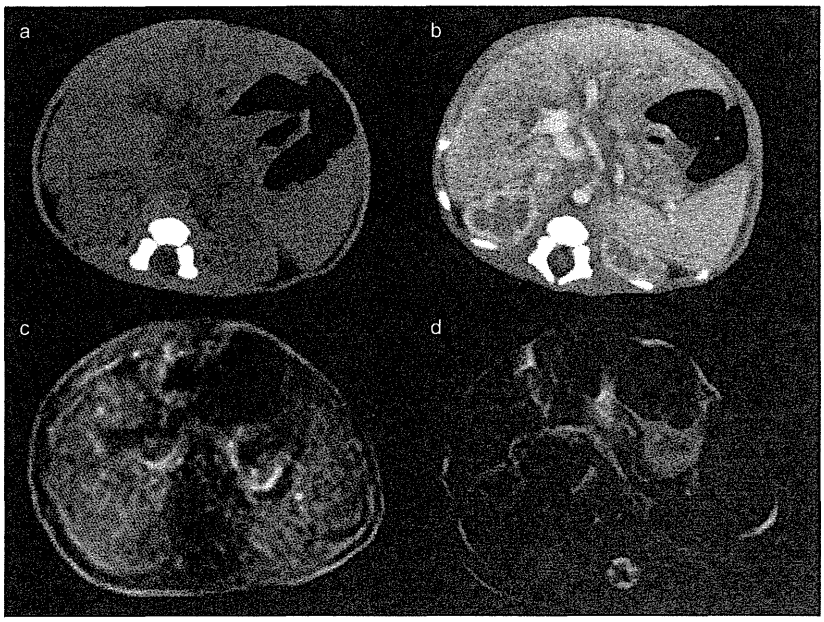

Figure 1 Computed tomography (CT) and magnetic resonance imaging (MRI) findings in our patient. Attenuation of the liver was not increased on CT and the intensity of the liver signal was not low on MRI T_2-weighted images. Neither suggested iron deposition in the liver or extrahepatic tissue. (a) CT plain. (b) CT contrasted. (c) MRI T_1-weighted image. (d) MRI T_2-weighted image.

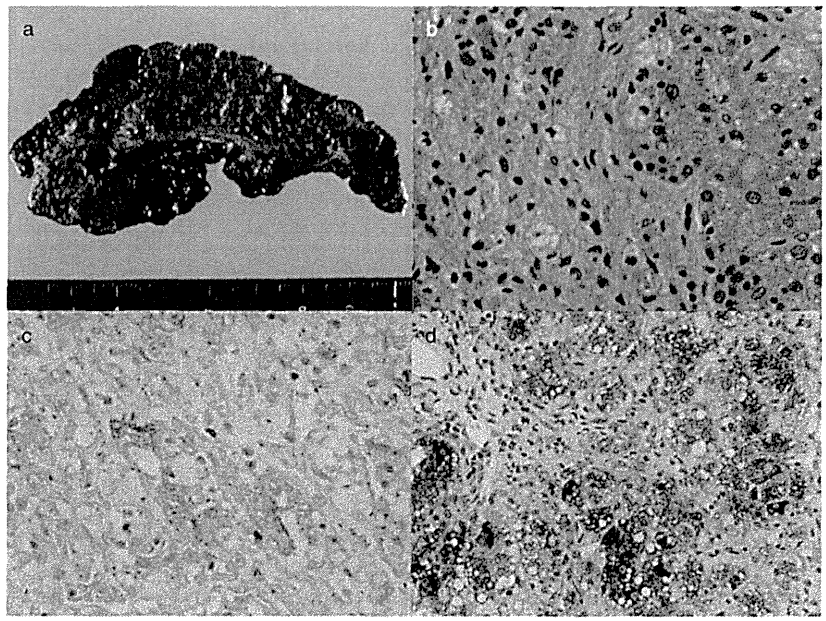

Figure 2 Histopathology of the explanted liver from our patient. (a) Multiple nodules on the liver surface and a divided face. (b) Disturbance of the lobular architecture, with severe fibrosis and marked loss of hepatocytes. Inflammation was minimal (hematoxylin–eosin, original magnification ×400). (c) Faint iron staining of hepatocytes (Berlin blue, ×400). (d) Residual hepatocytes were strongly positive for the Cb5-9 complex immunohistochemically (×400).

function did not improve. At age 19 days, a liver transplant was performed. The liver was obtained from a deceased 30-year-old male, with 138 g (graft/recipient weight ratio, 5.1 %) of the split allograft transplanted to the patient.

Pathological examination of the explanted liver showed cirrhosis with the formation of multiple micro- and macronodules. The liver architecture was deformed with marked pseudo-lobular formation and smaller numbers of hepatocytes were isolated with massive fibrous septa. Regenerative nodules were present. Inflammatory cell infiltration was minimal. Bile pigments and plugs were observed in all areas, indicating severe cholestasis. Granules faintly positive for Berlin blue staining were scattered among a few hepatocytes. Immunohistochemical staining with murine monoclonal anti-human SC5b-9 (QUIDEL, San Diego, CA, USA) showed strong staining for the C5b-9 complex in 80% of the residual hepatocytes (Fig. 2d). The activities of the mitochondrial respiratory chain complexes in the explanted liver tissue were normal, and congenital mitochondrial respiratory chain disorders were excluded.

Postoperatively, the patient received immunosuppressive therapy with prednisolone and tacrolimus. Acute cellular rejection was not observed. His liver chemistry tests were normal, and he was discharged 82 days after transplantation.

DISCUSSION

ACUTE LIVER FAILURE in the neonatal period is rare, but its etiology is diverse. Early diagnosis and treatment is essential but limitations of examinations due to neonatal physical immaturity can be an obstacle for adequate evaluation and management, and therefore the mortality rate is still high. Causes of neonatal liver failure include infections, metabolic defects, NH and hematological disorders. NH is a rare antenatal liver failure, associated with secondary iron deposition in liver and extrahepatic tissues such as the pancreas and heart. Its unique pattern of high recurrence in siblings of the same mother, not the father, suggests a maternofetal alloimmune mechanism rather than a hereditary condition.[5] Hereditary hemochromatosis (HH) also causes iron deposition in tissue but HH is caused by defects of iron metabolism with autosomal recessive inheritance, and is therefore an entirely different disease from NH. Treatments for NH are IVIG, exchange transfusion and cocktail therapy containing an iron chelator and antioxidants.[1,6] Liver transplant is performed on those who fail to respond to medical therapy. Recent accumulating data demonstrates that IVIG administration for women during pregnancy with proven NH of recent gestation can prevent severe recurrence of NH.[7] This preventive method is based on the hypothesis of a gestational

alloimmune mechanism of NH; therefore, the efficacy of this treatment supports the hypothesis.

At birth, our patient showed signs of liver failure, including cholestasis, hypoglycemia and thrombocytopenia, suggesting that the onset of disease was antenatal. NH is one well-known cause of antenatal liver failure.[8] One of the most important diagnostic criteria for NH is the demonstration of extrahepatic iron deposition.[1] However, the mechanism underlying iron deposition remains unclear. Recent findings have shown that iron overload does not result from an inborn error of iron metabolism but from fetal liver injury.[9,10]

Whitington *et al.* proposed an alloimmune mechanism of NH.[11] A maternal immunoglobulin G alloantibody against an alloantigen expressed by fetal hepatocytes may pass through the placenta, with the subsequent antigen–antibody reaction activating the classical complement pathway in fetuses. This, in turn, produces the membrane attack complex (C5b-9 immune complex), which causes hepatocyte injury. Immunohistochemical staining with antibody against the C5b-9 immune complex is strongly positive in the hepatocytes of patients with NH, indicating that congenital alloimmune hepatitis is the etiology in most or all patients with NH.[2]

In our patient, neither MRI nor lip biopsy demonstrated extrahepatic tissue siderosis. The MRI and lip biopsy each have approximately 60% sensitivity for detecting extrahepatic siderosis and negative tests do not exclude NH.[5] However histopathology of liver showed only weak iron deposition in hepatocytes, atypical in patients with NH. In contrast, the finding of the C5b-9 complex in hepatocytes strongly indicates that an alloimmune disease process was the cause of liver failure. Debra *et al.* reported a similar case of neonatal liver cirrhosis.[12] This case showed no iron deposition in both liver and extrahepatic tissue, C5b-9 was strongly positive in hepatocytes. C5b-9 complex was initially demonstrated as a specific histological marker of NH and proposed the gestational alloimmune mechanism of its pathogenesis.[2] Therefore, these cases without extrahepatic iron deposition suggested that siderosis of the liver or extrahepatic tissue may be a phenotypic difference within the same spectrum of gestational alloimmune liver diseases.

Whitington *et al.* suggested a term "gestational alloimmune liver disease" (GALD) for liver injury due to this alloimmune mechanism and the term "gestational alloimmune liver injury" should replace the term "neonatal hemochromatosis".[5,12] Our case also demonstrated that GALD can occur even in cases of neonatal liver failure without extrahepatic iron deposition. The iron deposition in extrahepatic tissue is just a phenotypic difference of GALD and those who show strong iron deposition in the extra hepatic tissue may have been diagnosed with NH among GALD cases. Therefore, as Whitington *et al.* pointed out, NH is a phenotype-based terminology and neonatal liver injury due to a gestational alloimmune mechanism is to be comprehensively called GALD.

In patients who present with antenatal liver failure but who do not meet the criteria for NH, gestational alloimmune liver disease should be differentiated as a cause of liver failure. C5b-9 staining of liver tissue is helpful for diagnosis but often difficult because of coagulopathy. Treatment should be initiated concurrently with diagnosis and exchange transfusion or immunoglobulin is theoretically effective for alloimmune diseases.[5,6] Our patient did not respond well to exchange transfusion or immunoglobulin therapy but was successfully treated with liver transplantation.

In conclusion, gestational alloimmune liver disease should be suspected in neonates with antenatal liver disease, even in the absence of obvious iron deposition. Liver transplantation is indicated in a patient refractory to exchange transfusion and immunoglobulin therapy. Additional studies are needed to understand how fetal hepatocytes are recognized as an antigen in the maternal immune system and to identify the causes of phenotypical clinical differences.

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新生児肝不全

虫明 聡太郎

はじめに

新生児の肝不全は稀な病態であるが，いかなる基礎疾患においても発症から肝不全へは急性かつ重篤な経過をとり，肝移植が行われてもその救命率は70％程度にとどまる。急性肝不全(acute liver failure：ALF)は，一旦この病態に陥ると，失われていく肝細胞機能を特異的に回復させる治療法はなく，成因となる疾患自体の治療が必要不可欠である。新生児ALFの原因疾患スペクトラムは成人とは異なっており，最も頻度の高い成因は肝臓の代謝疾患と周産期の後天性感染症であるが，成因の特定できない症例も多く存在する。一部の代謝疾患や単純ヘルペス感染症などでは的確に原因治療を行うことによって自己肝を守って救命することが可能である一方，肝移植が唯一の救命手段となることが多い。そのため，成因の如何にかかわらず，新生児ALFの治療は新生児科医とともに発症初期から小児肝臓疾患の専門家，および肝移植が可能な施設と連携して行うことが望ましい。

疾患概念

肝不全とは，肝細胞の変性・壊死，あるいは代謝的機能障害により，肝臓の合成，代謝，および浄化能が低下した状態である。急性肝不全ではトランスアミナーゼ値の上昇，高ビリルビン血症，血液凝固能低下が短期間に進行し，重症化に伴って腹水，栄養障害，さらに意識障害(肝性脳症)などの症状をきたす。このうち初発症状出現から8週以内に高度の肝機能障害に基づいてプロトロンビン時間が40％以下，ないしはINR値1.5以上を示すものがALFと定義される[1]。さらに，ALFは肝性脳症が認められない，ないしは昏睡度がⅠ度までの「非昏睡型」と，昏睡Ⅱ度以上の肝性脳症を呈する「昏睡型」(いわゆる劇症肝不全)に分類される。しかし，小児，特に新生児では肝性脳症の判断が容易でなく，肝臓の予備能が小さいため，発症後は昏睡の有無にかかわらず急速な重篤化を十分予測して全身状態の観察を行うことが重要である。

新生児肝不全の病態的特性

新生児期には肝不全による症候はその原因によって異なる。先天性感染症や新生児ヘモクロマトーシス，一部のミトコンドリア関連疾患などでは子宮内からの病態が生後すぐに症状となって現れる。細菌感染やウイルス感染，あるいはミルク摂取の開始後に発症する代謝異常症(ガラクトース血症，遺伝性果糖不耐症，高チロシン血症)では，出生直後には異常がみられない。血族婚や流産，周産期死亡歴や同胞の肝疾患の有無などについての詳細な病歴聴取が重要である。

以下に，新生児における肝不全の病態的特性を記述する[2,3]。

1．一般状態と神経症状

初期には活気がない，哺乳量が少ない，嘔吐しやすい，体重増加不良といった非特異的な症状しかみられないことも多い。黄疸の程度はさまざまで，特に代謝異常症では軽度かほとんど問題にならない場合もある。脳症の発現はむしろ遅れて現れる症候で，刺激に対する反応が乏しい，易刺激

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性や昼夜逆転といった傾向が脳症を疑わせるが，新生児期に肝性昏睡の進行度を客観的に把握することは困難である。痙攣を呈する場合は，脳症よりまず髄膜炎，あるいは低血糖を疑うべきである。

2．低血糖

新生児・乳児ではブドウ糖・グリコーゲン蓄積量が少なく，糖新生能も低いため肝不全状態では低血糖に陥りやすい。低血糖は治療経過中に栄養やグルコース投与が減少した際に突然生じるので注意が必要である。

3．血液凝固障害

新生児において血液凝固能の異常をみた場合には，まずビタミンK欠乏を疑うべきであり，ビタミンKの補充による改善が認められなければ肝不全と考えて原因診断を急がなければならない。一方，肝不全では血液凝固因子の低下とともに抗凝固系因子(アンチトロンビン，プロテインC，プロテインS)の低下も潜在的に進む。そのため，新生児では侵襲的な処置や外傷による出血がなければ出血傾向の存在に気づかれにくいことに注意が必要である。

4．免疫力低下と高サイトカイン血症

肝での補体産生の低下と好中球機能の低下により，液性，細胞性免疫ともに低下するため細菌感染が重篤化しやすい。一方，肝不全状態では産生されたサイトカインの肝での処理能が低下しているため，重症感染から高サイトカイン血症が誘導される。また，重症感染は肝移植の適応禁忌となり，敗血症に至ると多臓器障害への進展から救命率は著しく低下する。

5．腎機能低下(肝腎症候群)

いわゆる肝腎症候群は，門脈圧亢進に伴う臓器血管の拡張が高度となり，それに対してレニン-アンギオテンシン，バソプレシンやカテコラミン系が反応することによる腎血流量低下がその主たる原因であるが，腎機能低下は重症感染に伴う全身性の血圧低下や薬剤の腎毒性も原因となる。

6．脳浮腫と脳症

高アンモニア血症，低Na血症，炎症性サイトカインの上昇を伴った脳血流の増加が脳浮腫と脳症の中心的な成因となる。脳アストロサイトはグルタミン酸からグルタミンを合成することによってアンモニアを処理する。高アンモニア血症の環境下ではアストロサイト内のグルタミン濃度とともに浸透圧が上昇して細胞浮腫をきたすと考えられている。新生児や乳児では血管内皮の透過性が亢進しやすいためにサイトカインの影響を受けやすく，肝不全状態における脳症の進行は年長児や成人に比して急速である。

7．黄疸

黄疸の程度はさまざまで，特に代謝異常症では軽度かほとんど問題にならない場合もある。一方，新生児ヘモクロマトーシスなどでは数十 mg/dL に達することもある。重篤な肝不全では代謝障害の一部としてビリルビン抱合能が低下するため，総ビリルビンに占める直接ビリルビンの比率(D. bil/T. bil比)が低下することが肝不全の進行の指標となる。しかし，新生児では生理的に間接ビリルビン優位の黄疸があるため，生後早期には判断が難しい。

8．肝腫大

発症時に腫大していた肝臓の萎縮は，小児でも最も重要な劇症化の徴候のひとつであるが，**表1**にあげた新生児の肝疾患では肝萎縮をきたすものは少ない。脾腫や腹水といった門脈圧亢進に伴う徴候は早期に肝硬変に向かう新生児ヘモクロマトーシスや高チロシン血症で認められる。

9．その他

三桁後半から四桁以上に至る高度のトランスアミナーゼ値の上昇は，ウイルス感染や薬剤(中毒)性，あるいは循環障害による広範囲な肝細胞傷害，壊死を示唆する。逆に，多くの代謝疾患ではトランスアミナーゼ上昇は中等度以下にとどまることが多い。

表1　新生児肝不全の原因疾患と検査

疾患名	検査
一般検査 (スクリーニング，および病状評価)	血算，Na，K，Cl，Ca，P，Mg，BUN，クレアチニン，総ビリルビン，直接ビリルビン，AST，ALT，LDH，ALP，γGTP，乳酸，アンモニア，血液ガス，血型，クームステスト，各種培養検査，α-フェトプロテイン，血液凝固能(プロトロンビン時間，プロトロンビン活性(INR)，フィブリノーゲン，D-ダイマー)，アンチトロンビンⅢ
高チロシン血症Ⅰ型	尿中サクシニルアセトン，血漿アミノ酸分析，α-fetoprotein，骨X線
ガラクトース血症	新生児マススクリーニング検査(ボイトラー法，ペイゲン法，酵素法)
遺伝性果糖不耐症	*ALDOB*遺伝子解析
α_1アンチトリプシン欠損症	血清α_1アンチトリプシン
新生児ヘモクロマトーシス	血清鉄，UIBC，フェリチン，唾液腺生検(鉄染色)，腹部MRI
ミトコンドリア関連疾患	乳酸/ピルビン酸比，静脈血ケトン体分画，尿中有機酸分析，ミトコンドリアDNA解析，ミトコンドリア呼吸鎖関連酵素活性(肝生検，筋生検)，髄液中乳酸，CK，CK-MB，CK-BB
尿素サイクル異常症	血漿アンモニア，尿中オロット酸(尿中有機酸分析)
シトリン欠損症	血漿アミノ酸分析，SLC25A13遺伝子解析，肝生検
胆汁酸代謝異常症	尿中胆汁酸分析
先天性グリコシル化異常症	トランスフェリン等電点電気泳動
感染症	HAV，HBV，HHV-1，2，6，CMV，EBV，HSV，VZV，echovirus，adenovirus，enterovirus，parbovirus 19，paramyxovirusの血清抗体価(母児とも)，血清保存，便培養，梅毒(VDRL)
血球貪食症候群，マクロファージ活性化症候群	血清トリグリセライド，コレステロール，フェリチン，骨髄穿刺
新生児ループス	母体自己抗体(抗SS-A，抗SS-B，抗U1RNP抗体)
薬剤性・中毒性	被疑薬剤血中濃度，DLST
循環不全	心臓超音波検査，BNP

成因と鑑別のための検査

ALFの成因は代謝異常，感染，中毒，自己免疫，循環不全，および腫瘍関連の六つに分けることができる。このうち新生児では代謝異常と感染の占める割合が高い。我が国の全国調査(1995～2010年)では1歳未満の急性肝不全症例63例中，成因が特定された症例は確診・疑診を含めて34例(54%)で，そのうちウイルス感染が16例，代謝疾患が12例であった[4,5]。

表1に新生児ALFの原因となり得る疾患とそれらの診断に有用な臨床検査を示す[6,7]。シトリン欠損症の発症は新生児期を過ぎてからのことが多く，一般に予後良好であるが，乳児期早期に肝の線維化が進行して肝移植の適応となる場合がある。高チロシン血症Ⅰ型は我が国では報告例が少なく，明らかな血漿チロシン値の上昇と胆汁うっ滞や肝の線維化を呈するものの尿中サクシニルアセトンの上昇や肝組織でのフマリルアセト酢酸ヒドラーゼの低下が証明されない非Ⅰ型高チロシン血症が経験されるが，詳細な原因や疫学は不明である。

対応と治療

肝機能異常や胆汁うっ滞性黄疸に気づいたら必ず血液凝固能をチェックする。プロトロンビン時間の延長が認められれば，まずビタミンKの投与を行い，投与後12時間で有意な改善がない，またはさらなる低下が認められる場合には，急性肝不全を強く疑って小児肝疾患の集学的治療が可能な医療機関へ移送すべきである。同時に，下記の項目に配慮した初期対応をとる[7]。

表2　原疾患に対する特異的治療

疾患名	検査
高チロシン血症Ⅰ型	NTBC〔2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione〕，フェニルアラニン・チロシン除去ミルク
ガラクトース血症	ガラクトース除去(乳糖除去ミルク)
遺伝性果糖不耐症	果糖，ショ糖除去
新生児ヘモクロマトーシス	デスフェラール®，セレン，プロスタグランジンE_1，ビタミンE，ガンマグロブリン大量療法
アセトアミノフェン肝障害	N-アセチルシステイン*
尿素サイクル異常症	安息香酸ナトリウム，アルギニン，血漿交換，または腹膜透析，蛋白除去ミルク
単純ヘルペス感染症	アシクロビル
B型肝炎	ラミブジン，エンテカビル，アデフォビル
血球貪食症候群，マクロファージ活性化症候群	化学療法(エトポシド，デキサメサゾン，シクロスポリン)，末梢血幹細胞移植

N-アセチルシステイン*はその他の成因によるALFにも有効との報告がある。

・ガラクトース血症が否定できるまで乳糖除去ミルクに変更する。
・血糖と電解質を頻回にチェックし，補正に努める。
・広域ペニシリン，またはセフェム系抗菌薬を投与する。
・単純ヘルペスの診断を急ぎ，結果を得る前からアシクロビルを治療量で開始する。
・母体のHB抗体価をチェックする(特にHBe抗体陽性母体からの出生児は要注意)。
・消化管出血予防を目的として制酸剤の静注投与を開始する。
・鎮静剤は人工呼吸を行わない限り投与しない。

診断に基づく特異的治療法を**表2**に示す。我が国の全国調査では，B型肝炎や単純ヘルペスの母児感染例に対してラミブジンやアシクロビルの投与を行って肝性昏睡Ⅳ度から救命・治癒し得た症例が報告されている。また，血球貪食症候群による肝不全では免疫抑制療法や化学療法が奏功する場合が少なくない。小児ALFの原因として欧米で多くを占めるアセトアミノフェン肝障害は我が国ではほとんど報告がないが，これに対する特異的治療薬として用いられるN-アセチルシステイン(ミトコンドリアと肝細胞質内のグルタチオン量を高めることによる抗酸化物質として作用する)は，そのほかの原因によるALFにおいても有効かつ安全で，非移植救命率と移植後の生存率を改善させたとの報告がある[8]。

人工肝補助療法と肝移植

有効な原因治療法がない，あるいは行っても改善が期待できない場合の最も有効な治療は人工肝補助療法と肝移植である。我が国では欧米より人工肝補助療法(血液浄化療法)が進歩しており，近年，小径(6 Fr)ダブルルーメンカテーテルや新生児・小児用の血漿交換，持続濾過透析カラムが開発され使用可能となっている。血液浄化療法では安定した脱血ルートを確保することが重要であるが，新生児では一旦血液凝固能が著しく低下した状態に陥ると，ルート確保自体が困難となって出血や循環動態変動のリスクが高まるため，小児外科医に依頼してできるだけ早めのルート確保に努めるべきである[9]。

一般に，人工肝補助療法としては急速に低下する血液凝固能を補う目的で血漿交換(plasma exchange：PE)を行い，終了後に継続して持続的濾過透析(continuous hemodiafiltration：CHDF)が行われることが多い。血液凝固能は保たれていながら高アンモニア血症の回避が求められるOTC欠損症ではHFDまたは腹膜透析(peritoneal dialysis：PD)が選択される。

人工肝補助療法の継続によって昏睡型ALF(劇症肝不全)からの回復もあり得る。しかし，新生児や乳幼児では中枢神経障害を遺さず救命するためにはやはり肝移植が最も有効な治療手段である。小児全体を対照とした調査では，英国，北米と日本における小児ALFに対する肝移植施行率は，それぞれ41%，57%，74%と日本が最も高いが，これには保護者の側から「自らドナーとなって患児を救命したい」という申し出があることが多いこと，また健康保険制度が整っていることが一因となっていると考えられる。近年では外科的技術の進歩に伴って新生児に対する肝移植成績が向上し，2008～2010年の3年間を対象とした我が国での調査では，生後2カ月未満発症のALF8症例中5例に対して生体肝移植が施行され，うち4例が救命されており，すでに複数の施設において生後2週未満，体重2,700g未満の児に対する生体部分肝移植が実施されている。

ただし，生体肝移植はドナーを得て初めて成立し得る治療である。そのため，短期間に移植が必要となる可能性を判断し，家族に対して的確な説明と情報提供が行われなければならない。特に新生児・乳児では発症から中枢神経障害の進行が速いため，原因診断と治療を進めると同時に肝移植へのワークアップを考慮した医療連携が必要である。

おわりに

新生児発症の重篤な肝障害については，近年多くの原因疾患の鑑別が可能となり，一部の感染症や代謝疾患では原因治療によって救命・治癒が可能となってきた。一方，未だ成因不明の症例も少なくなく，原因治療のない疾患に対しては多くの場合肝移植が唯一の救命的治療とならざるを得ない。しかし，厳密には新生児の肝移植適応と禁忌を客観的に判定する基準はなく，原因診断に基づいて予測される一般的な予後と，個々の症例における肝予備能，中枢神経障害の程度，感染や他臓器の合併症から総合的に判断するしかない。今後はさらに，ALFの新たな成因が解明されることと，より客観的な肝移植適応と成績に関する情報を患者家族に提供できるよう，症例数と知見が重ねられていくことが期待される。

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Original Article

Influence of splenectomy in patients with liver cirrhosis and hypersplenism

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Aim: Splenectomy improves hypersplenic thrombocytopenia in cirrhotic patients with hypersplenism. However, the long-term influence of splenectomy has not been clarified. We examined whether splenectomy improved liver fibrosis and caused immunological changes.

Methods: We collected liver and spleen specimens and peripheral blood (PB) from 26 patients with hepatitis C virus-related liver cirrhosis. An immunohistochemical examination of CD4, CD8, forkhead box P3, granzyme B and transforming growth factor-β1, and Masson-trichrome stain were performed in spleen and liver tissues and in seven cases of follow-up liver biopsy sections obtained after splenectomy. We obtained PB before and at various intervals after splenectomy. We also examined the ratio of $CD4^+$ and $CD8^+$ lymphocytes in PB using flow cytometry.

Results: We observed improvements in liver fibrosis in four biopsy specimens obtained after splenectomy, in which fibrotic areas significantly decreased from 19.5% to 8.2% ($P < 0.05$). Increases were also observed in the ratio of $CD8^+$ cells in PB after splenectomy, which resulted in a significant decrease in the $CD4^+/CD8^+$ ratio ($P < 0.001$). The carcinogenic rate in patients with a $CD4^+ : CD8^+$ ratio that decreased by more than 0.5 at 1 month after splenectomy was significantly lower than that in patients with a ratio that decreased by less than 0.5 ($P < 0.05$).

Conclusion: Splenectomy may improve liver fibrosis and cause beneficial immunological changes in cirrhotic patients with hepatitis. Improvements in antitumor mechanisms can be also expected.

Key words: $CD4^+$ cytotoxic T lymphocytes, $CD8^+$ cytotoxic T lymphocytes, liver cirrhosis, liver fibrosis, splenectomy

INTRODUCTION

SPLENECTOMY IS A common treatment used to improve hypersplenic thrombocytopenia in cirrhotic patients with splenomegaly in Japan.[1–7] Splenectomy has recently been applied as another option to cure hepatocellular carcinoma (HCC) and for cirrhotic patients with no potential donor for liver transplantation. Thus, the clinical application of splenectomy has been expanded; however, the immunophysiology of the spleen in cirrhotic patients and the long-term outcome after splenectomy have not been clarified.[8–14] This study was designed to clarify the long-term changes and prediction of HCC development following splenectomy, with a focus on hepatic fibrosis and immunology. Regarding hepatic fibrosis, Akahoshi *et al.* reported that transforming growth factor (TGF)-β1 derived from the spleen could have an inhibitory role in healing liver cirrhosis by inhibiting the regeneration of the damaged liver[15] and we experimentally confirmed that splenectomy significantly reduced liver fibrosis and decreased TGF-β1 in the serum of a dimethylnitrosamine-induced cirrhotic rat model.[16] However, no studies have yet described a reduction in hepatic fibrosis following splenectomy in humans.

The spleen plays an important role in the immune response; however, the functional aspects of the spleen in cirrhotic patients with hepatitis C virus (HCV) infection are largely unknown.[2,17] Hashimoto *et al.* reported that splenectomy was followed by an increased ratio of interferon (IFN)-γ to interleukin (IL)-10 and a reduction in programmed death (PD)-1-expressing $CD4^+$ T cells in peripheral blood (PB).[7] In order to clarify chronological changes in immunity after splenectomy, we examined liver and spleen tissues and sera to assess $CD4^+$ and

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$CD8^+$ cytotoxic T lymphocytes (CTL) and regulatory T (Treg) cells.[18,19] TGF-β1 was also examined as it is a multifunctional cytokine that inhibits the growth of tumor cells[20–23] and liver regeneration by facilitating tissue fibrosis in the liver.[16]

Host immunoreactions against cancer were shown to be closely related to cellular immunity by $CD8^+$ CTL and Treg cells, produced by T lymphocytes, and $CD8^+$ CTL in particular.[19] The level of Treg cells, characterized by the expression of forkhead box P3 (FOXP3) transcription factor in the PB and tumor tissues of patients with HCC, was elevated and appeared to be negatively correlated with prognosis.[21,24,25]

In the present study, we examined whether splenectomy could improve liver fibrosis, cause immunological changes, especially in CTL, or be used to predict the risk of carcinogenesis.

METHODS

Patients and samples (Table 1)

AT THE DEPARTMENT of Surgery, Kurume University Hospital, 26 patients (Child A, 16 cases; Child B/C, 10 cases) with HCV-related liver cirrhosis (with HCC, seven cases; without HCC, 19 cases) and hypersplenism underwent splenectomy (splenectomy group). The purpose of splenectomy was to improve hypersplenic thrombocytopenia and introduce IFN for clearance of the HCV virus. Forty-eight patients who underwent hepatectomy due to liver tumors were recruited as controls (control group 1). PB samples from 10 healthy adult volunteers (control group 2) and spleen tissues obtained by splenectomy from seven patients because of trauma (control group 3) were also used as controls. In addition, all patients were HIV negative. Patients received no medical treatment except splenectomy during the study period. All samples were studied after obtaining the appropriate institutional informed consent. We also obtained permission from the ethical review board.

Liver tissue

A total of 26 pieces from the resected liver specimens of patients with HCV-related liver cirrhosis and hypersplenism who underwent splenectomy were also examined for the immunohistochemical expression of $CD4^+$

Table 1 Subject characteristics

Variables	Results
Splenectomy group: splenectomy (26 cases, seven with HCC, 19 without HCC)	
Age, median (range)	60.4 ± 1.36 (46–75)
Sex (male/female)	12/14
Virus infection (HCV^+)	26
Fibrosis (F0/F1/F2/F3/F4)	0/0/0/0/26
Child–Pugh classification (A/B/C)	16/8/2
Tumor nodules (presence/absence)	7/19
Weight of the spleen (g)	510.4 ± 55.6 (125–1065)
Control 1: hepatectomy with HCC (48 cases)	
Age, median (range)	70.5 ± 1.33 (42–82)
Sex, male/female	29/19
Virus infection (HCV^+)	40
Fibrosis (F0/F1/F2/F3/F4)	8/10/10/10/10
Tumor nodules (presence/absence)	48/0
Control 2: healthy adult volunteers (10 cases)	
Age, median (range)	40.1 ± 2.97 (32–57)
Sex (male/female)	3/7
Control 3: splenectomy control (seven cases; trauma)	
Age, median (range)	59.8 ± 6.27 (36–82)
Sex (male/female)	6/1

Continuous variables are expressed as the mean ± standard deviation.
Fibrosis: F0, no fibrosis in the portal tract; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.
HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

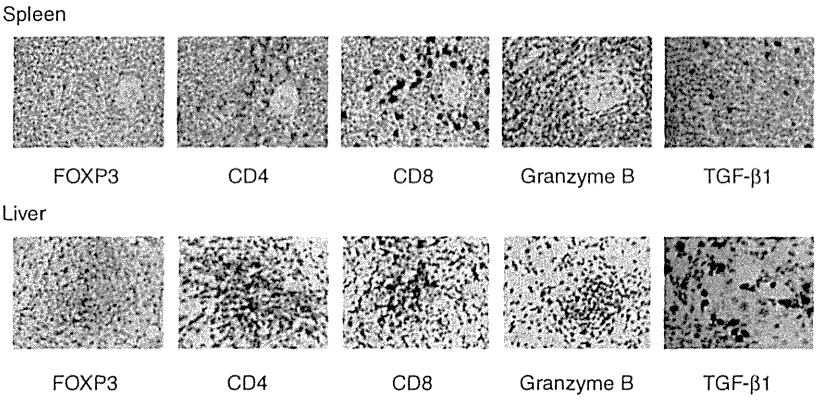

Figure 1 Immunohistochemical staining of spleen and liver specimens with forkhead box P3 (FOXP3), CD4, CD8, granzyme B and transforming growth factor (TGF)-β1 in the spleen and liver.

lymphocytes, $CD8^+$ lymphocytes, FOXP3, granzyme B and TGF-β1 positive cells (Fig. 1). We classified liver specimens into five stages according to the degree of fibrosis as follows: F0, no fibrosis in the portal tract; F1, portal fibrosis without septa; F2, portal fibrosis with a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. We collected resected liver specimens from 10 cases each of F1, F2, F3 and F4 with HCV-related liver disease. We also collected specimens from eight cases of liver hemangioma of F0 with both negative hepatitis B surface antigen and HCV antibody. Follow-up liver biopsy sections were obtained from the same part of the liver if possible from seven of the 26 patients at various intervals after splenectomy (Table 2). These sections were used for CD4 and CD8 immunostaining and Masson-trichrome staining for the morphometric evaluation of fibrotic areas.

Spleen tissue

A total of 26 spleens with HCV-related liver cirrhosis and hypersplenism were examined for the immunohistochemical expression of CD4 positive lymphocytes, CD8 positive lymphocytes, FOXP3, granzyme B and TGF-β1 positive cells. We measured the same parameters in spleens from the seven control cases in control group 3 as a non-cirrhotic control (Fig. 1). Spleen and liver tissues were pathologically assessed by two pathologists (Y. N. and M. K.).

Peripheral blood cells

Peripheral blood samples were serially collected from 26 patients with HCV-related liver cirrhosis and hypersplenism just before and 14 days, 1 month, 3 months, 6 months and 1 year after splenectomy. We examined the ratio of $CD4^+$ T cells to all lymphocytes, $CD8^+$ T cells to all lymphocytes, and the $CD4^+/CD8^+$ ratio in PB samples using flow cytometry. TGF-β1 levels in PB were also measured using enzyme-linked immunoassays in the sera just before and 14 days, 1 month, 3 months, 6 months and 1 year after splenectomy. Patients were excluded from the protocol if IFN or other therapeutics were introduced for the liver disease. Ten healthy adult

Table 2 Clinical and pathological findings of 7 patients who underwent follow-up liver biopsies

Case	Age	Sex	Activity	Child–Pugh (score)	CD4/8	Follow-up range (days)	Before (%)	After (%)	Rate of change
1	63	M	1	A (5)	1.73	581	6.59	18.31	2.78
2	58	M	2	A (5)	1.22	24	7.38	8.99	1.22
3	58	M	2	B (7)	1.57	333	9.92	12.02	1.21
4	52	M	2	A (5)	1.08	431	16.71	5.10	0.30
5	74	M	2	A (6)	0.63	353	20.02	6.31	0.32
6	53	F	2	A (6)	0.93	248	30.03	13.34	0.44
7	59	M	2	A (5)	0.95	42	11.27	8.05	0.71

Activity: A0, none; A1, portal inflammation only; A2, mild interface hepatitis; A3, moderate interface hepatitis; A4, severe interface hepatitis.
Before, the rate of fibrotic areas before splenectomy; after, the rate of fibrotic areas after splenectomy.

volunteers in control group 2 without a history of liver disease or splenomegaly were also recruited as controls, and samples were collected only once.

Immunohistochemical analysis

All fresh specimens were fixed by 10% formalin, and paraffin-embedded tissue samples were cut at a thickness of 4 μm, examined on a coated slide glass, and labeled with the following antibodies using the Bond-Max autostainer (Leica Microsystems, Newcastle, UK) and DAKO autostainer (DakoCytomation, Glostrup, Denmark): CD4 (×200; Leica Microsystems), CD8 (×200; Leica Microsystems), granzyme B (×50; Leica Microsystems), TGF-β1 (×300; Santa Cruz Biotechnology, Heidelberg, Germany) and FOXP3 (×600; Abcam, Cambridge, MA, USA).

Immunohistochemical examinations with CD4, CD8, granzyme B and TGF-β1 were performed on the same fully automated Bond-Max system using onboard heat-induced antigen retrieval with ER2 for 10 min and the Refine polymer detection system (Leica Microsystems). 3,3′-Diaminobenzidine-tetrachloride (DAB) was used as the chromogen for all immunostaining. FOXP3 immunostaining was carried out using the DAKO autostainer with the ChemMate ENVISION method (DakoCytomation). Briefly, specimens were boiled in a microwave for 30 min in 1 mmol/L ethylenediaminetetraacetic acid, pH 9.0, and target retrieval solution (DakoCytomation) to recover antigens, and the specimens were then incubated with the antibody at 4°C overnight. After washing in Tris-buffered saline (TBS), slides were incubated with the labeled polymer-horseradish peroxidase secondary antibody for 30 min at room temperature. After washing in TBS, slides were visualized using DAB.

Detection of immune function using flow cytometry

T-lymphocyte subsets in PB such as CD4, CD8 and CD4/8 were determined by flow cytometry, and the monoclonal antibodies of CD4 and CD8 (labeled CD4-FITC, CD-8-RD1) were purchased from Beckman Coulter (Danvers, MA, USA).

Result assessment

For assessment criteria for lymphocytes and other positive cell counts, the number of lymphocytes and other positive cells were counted in 20 areas within a specimen under high-power fields (×40 objective, ×10 eyepiece). Ten areas of white and red pulp were assessed in the spleen, and 10 periportal areas and 10 hepatic lobule areas (Fig. 1) were assessed in a non-tumor area of the liver.

Morphometric analysis (computer image analysis) was performed in the following manner on specimens stained with Masson-trichrome. The equipment used to assess morphometry consisted of a light microscope, a three-color charge-coupled device camera, and a high resolution computer image analysis system (WinRooF software package version 6.1; Mitani, Fukui, Japan). The magnified images (×40) of specimens captured by the camera mounted on the microscope were sent to the image analyzing computer. Collagen fibers stained with Masson-trichrome were then selected. In this study, this scanning procedure was repeated 10 times in random areas. The area of fibrosis (AF) was defined as the ratio (%) of the whole area of collagen fibers to that of the liver tissue scanned.

Statistical analyses

Statistical analysis was performed using Student's *t*-test. A *P*-value of less than 0.05 was considered to be significant.

The follow-up time was calculated as the interval between the date of surgery and intervention of the medical treatment, last follow up or recognition of HCC. Survival rates or failure rates were analyzed with the Kaplan–Meier method using the log-rank test to assess differences between curves. A *P*-value of less than 0.05 was considered to be significant. Statistical calculations were performed using the JMP software package (release 10, SAS Institute, Cary, NC, USA).

RESULTS

Liver

IN THE SEVEN follow-up liver biopsy sections (Table 2) available for histological examination, liver fibrosis in the hepatic lobules improved from F4 to F3 in four cases (cases 4–7: average, 268.5 ± 168.6 days; range, 42–431 days) (Fig. 2a). Improvements were not observed in the remaining three cases (cases 1–3: average, 312 ± 279.1 days; range, 24–581 days) (Fig. 2b). There were no statistical differences in the duration between the improvement cases and non-improvement cases ($P = 0.80$). Conducting an evaluation was difficult because only a few specimens were available; however, no significant differences in clinical profiles were observed among the seven patients. In four of these cases (cases 4–7), the ratio significantly

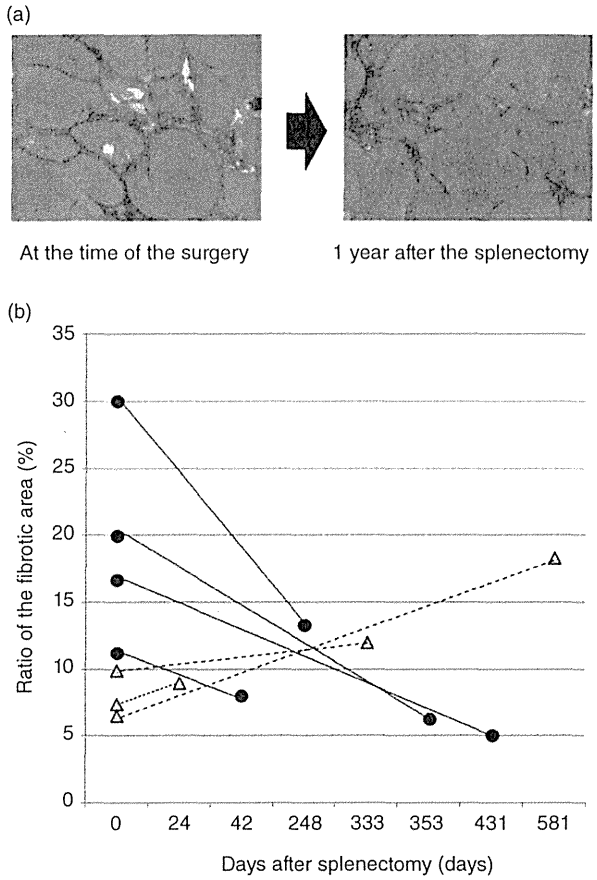

Figure 2 (a) Improvements in liver fibrosis. Distortions in hepatic lobules improved in the liver biopsy sections of four cases after splenectomy, and fibrotic areas significantly decreased from 19.5% to 8.2% in these sections. (b) Changes in the fibrotic areas of seven patients at various intervals. •—• shows patients in whom the fibrotic area significantly decreased after splenectomy. △---△ shows patients in whom fibrosis deteriorated.

decreased from 19.5% to 8.2% ($P < 0.05$) (Fig. 2b), while the average AF in the remaining three cases (cases 1–3) increased from 8.0% to 13.1% ($P = 0.15$). The four cases of improved fibrosis were all Child–Pugh A, and one of the three cases that showed no improvement was Child–Pugh B. In addition, AF before splenectomy was slightly higher in the improvement cases than in the non-improvement cases, while the $CD4^+/CD8^+$ ratio before splenectomy was lower in the improvement cases than in the non-improvement cases ($P < 0.05$). Histopathologically, $CD4^+$ and $CD8^+$ lymphocytes were mainly seen in the periportal area, and $CD4^+$ lymphocytes were rarely seen in the hepatic lobules. The epithelial cells, fibroblasts, monocytes and macrophages also produced TGF-β1.[4,21,26] However, we picked up and counted the TGF-β1 positive cells that were seen in the lymphocytes and found that these cells were distributed diffusely in the hepatic lobules and periportal area. The distribution pattern of Treg and granzyme B was the same as that of $CD4^+$ and $CD8^+$ lymphocytes, respectively. No significant differences were observed in the $CD4^+/CD8^+$ ratio ($P = 0.21$) in liver specimens, regardless of the association of HCC. The $CD4^+/CD8^+$ ratio ($P < 0.05$) and FOXP3/$CD4^+$ ratio ($P < 0.001$) significantly increased with the progression of liver fibrosis (from F0 to F4). However, the granzyme B/$CD8^+$ ratio was approximately constant, and was unrelated to the progression of liver fibrosis ($P = 0.32$).

The number of TGF-β1 positive cells in livers with HCC was slightly higher than that in livers without ($P = 0.06$), and the number of TGF-β1 positive cells also significantly increased with the progression of liver fibrosis ($P < 0.001$) (Fig. 3).

Spleen

Histopathologically, $CD4^+$ and $CD8^+$ lymphocytes were found more in the white pulp than in the red pulp. The results of the clinicopathological analysis showed that the $CD4^+/CD8^+$ ratio in spleens with HCV-related liver cirrhosis and hypersplenism was higher than that in the spleens of control group 3 ($P = 0.06$). The FOXP3/$CD4^+$ ratio in control group 3 was higher than that in cases of hypersplenism ($P < 0.05$), and no significant differences

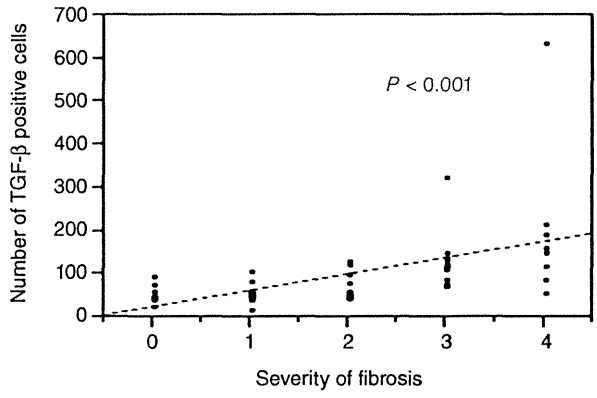

Figure 3 Correlation between transforming growth factor (TGF)-β1 positive cells and fibrosis in the liver. The number of TGF-β1 positive cells also significantly increased with the progression of liver fibrosis.

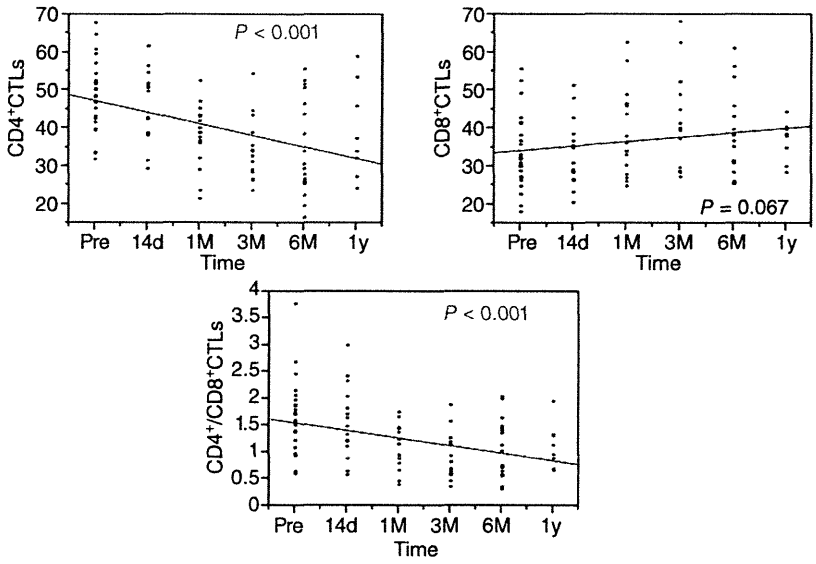

Figure 4 Changes in peripheral blood after splenectomy. pre, preoperative; d, days; M, months; y, year. The ratio of $CD4^+$ T cells to all lymphocytes significantly decreased 1 year after splenectomy, while the ratio of $CD8^+$ T cells to all lymphocytes slightly increased, resulting in a significant decrease in the $CD4^+/CD8^+$ ratio.

in the granzyme B/$CD8^+$ ratio ($P = 0.82$) were observed between the splenectomy group and control group 3 (data not shown).

Peripheral blood

The ratio of $CD4^+$ T cells to all lymphocytes and the $CD4^+/CD8^+$ ratio in PB samples obtained from 26 patients before splenectomy were significantly higher than those from control group 2 ($P < 0.01$, $P < 0.05$). In contrast, the ratio of $CD4^+$ T cells to all lymphocytes significantly decreased 1 year after splenectomy ($P < 0.001$), while the ratio of $CD8^+$ T cells to all lymphocytes slightly increased ($P = 0.07$), resulting in a significant decrease in the $CD4^+/CD8^+$ ratio ($P < 0.001$) (Fig. 4).

Transforming growth factor-β levels were higher in PB samples from patients with HCC than in those without. TGF-β1 levels slightly increased in PB samples 1 month after splenectomy, then decreased, and subsequently returned to the level measured before splenectomy in 1 year.

Relationship of the $CD4^+/CD8^+$ ratio between PB and the spleen or liver

In the splenectomy group, the $CD4^+/CD8^+$ ratio in PB had a significant positive correlation with the $CD4^+/CD8^+$ ratio in the spleen ($P < 0.05$), and was also positively associated with the liver ($P = 0.07$). As a result, a significant positive correlation was observed between the $CD4^+/CD8^+$ ratio in the spleen and that in the liver ($P < 0.05$) (Fig. 5).

Correlation between the $CD4^+/CD8^+$ ratio and clinical prognosis

We compared the $CD4^+/CD8^+$ ratio between PB obtained pre-splenectomy and 1 month after splenectomy ($n = 19$). The median of differences between pre-splenectomy and 1 month after splenectomy was 0.5. The occurrence of HCC was significantly lower in cases in which the difference in the $CD4^+/CD8^+$ ratio between the perioperative period and 1 month later was over 0.5 (≥0.5 vs <0.5, $P < 0.05$) (Fig. 6a).

A positive correlation in PB was observed between the $CD4^+/CD8^+$ ratio before splenectomy and differences in the $CD4^+/CD8^+$ ratio between pre-splenectomy and 1 month after splenectomy ($P < 0.001$). As the median of the preoperative $CD4^+/CD8^+$ ratio was 1.7, the postoperative (1 month after splenectomy) $CD4^+/CD8^+$ ratio significantly decreased in groups in which the preoperative value was larger than 1.7 (Fig. 6b,c).

DISCUSSION

PREVIOUS STUDIES HAVE shown that splenectomy was effective in improving pancytopenia, the decompression of portal hyperpressure and liver function.[1,2,27,28] Morinaga *et al.* reported that splenectomy

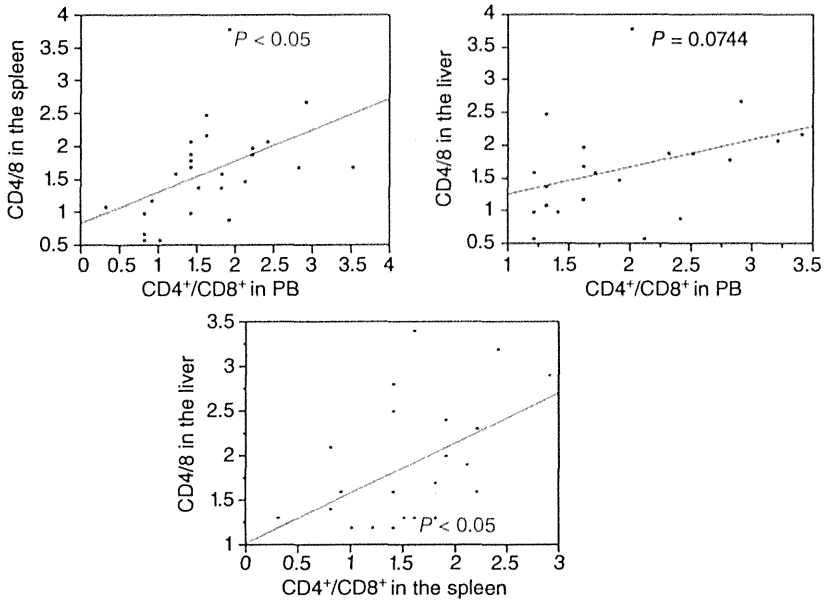

Figure 5 Correlations between the $CD4^+/CD8^+$ ratios in the spleen, liver and peripheral blood (PB). A significant positive correlation was observed between the $CD4^+/CD8^+$ ratio in the spleen and that in the liver.

significantly improved liver fibrosis with a reduction in plasma TGF-β1 levels in the rat. However, all these reports of hepatic fibrosis were conducted in animal models[1,16,29,30] whereas the present study described improvements in liver fibrosis after splenectomy in humans. Interestingly, the $CD4^+/CD8^+$ ratio changed after splenectomy without other treatment. However, many confounding factors may be implicated in this change. It is likely that patients with a high fibrotic area in their liver specimens had a high $CD4^+/CD8^+$ ratio;

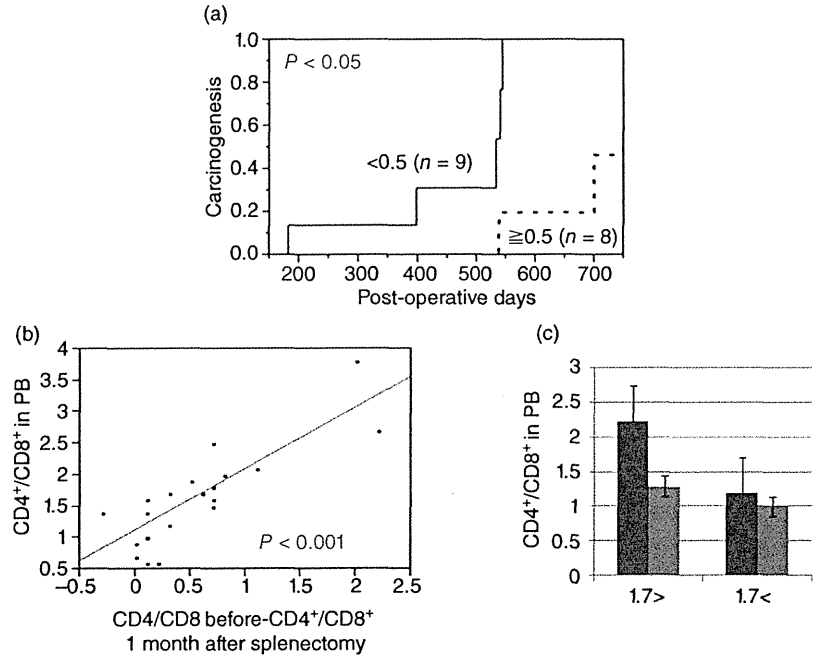

Figure 6 (a) Correlation between carcinogenesis, the perioperative period and 1 month later. The occurrence of hepatocellular carcinoma was significantly lower in cases in which the difference in the $CD4^+/CD8^+$ ratio between the perioperative period and 1 month later was over 0.5. (b,c) Correlation in peripheral blood (PB) between the $CD4^+/CD8^+$ ratio before surgery and differences in the $CD4^+/CD8^+$ ratios before splenectomy and 1 month after splenectomy. (b) A positive correlation in PB was observed between the $CD4^+/CD8^+$ ratio before splenectomy and differences in the $CD4^+/CD8^+$ ratio between pre-splenectomy and 1 month after splenectomy. (c) The postoperative (1 month after splenectomy) $CD4^+/CD8^+$ ratio significantly decreased in groups in which the preoperative value was larger than 1.7. ■, pre; ■, post.