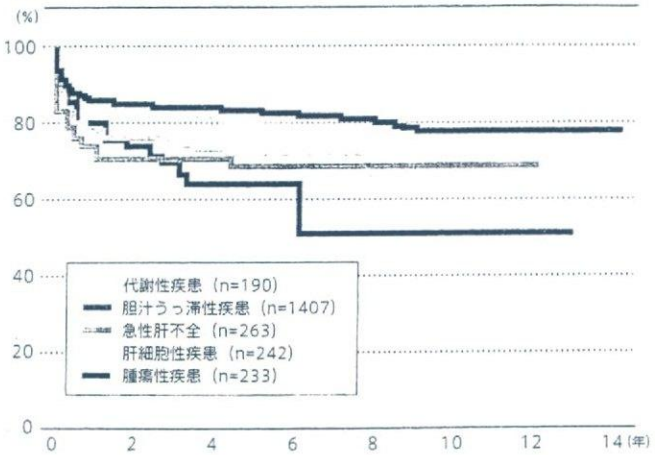
図2 適応疾患別の累積生存率(日本肝移植研究会による)



(非代償期)および劇症肝炎(ウイルス性、自己免疫性、薬剤性、成因不明を含む)である。また、肝硬変に肝細胞癌を合併している場合には、遠隔転移と血管侵襲を認めないもので、肝内に径5cm以下1個、または3cm以下3個以内が存在する場合に限られている。上記以外の場合は、保険が適応されず、原則的に患者の自費負担となる。

生体移植は、健康人である提供者(ドナー)の体にメスを入れるという本質的な問題点を有する医療である。ドナーの手術死亡はあってはならないことであるが、2002年4月New England Journal of Medicine誌は、米国で7人の生体肝ドナーの手術死亡があり、他にも2人が肝提供後に肝不全に陥り肝移植を受けたとの論説を掲載し警鐘を鳴らした(後に死亡者数は3人に訂正)。これを受け、日本肝移植研究会は、わが国の全移植施設を対象とし、生体肝ドナーの術後合併症に関する緊急調査を行った。全症例につき回答を得ることができ、手術関連死亡は1人もなかったが、12.4%のドナーに有意な術後合併症が発生していることが分かった。頻度の高い合併症は、胆汁瘻、胃内容停滞(胃と肝切離面の癒着による)、創感染、腸閉塞など腹部合併症であったが、危険な合併症である肺塞栓が5例あった。ドナーの術後入院日数は15.6±9.6日(mean±SD)であった。提供肝の種類別で比較すると、肝右葉を提供したドナーは合併症の頻度が高く、術後入院日数が長かった。

この調査の後、2003年5月にわが国で初めて生体肝ドナーの手術関連死亡があったが、日本肝移植研究会のドナー安全対策委員会はこの事例につき詳細な検証を行い、誌上報告した。

さらに、日本肝移植研究会は、生体肝ドナー本人を対象として、QOLなどに関するアンケート調査を施行した。61%の方から回答を得ることができ、分析の結果ドナーの健康状態や心理状態などに関して多くの重要な知見を得ることができた。2005年4月に報告書を公開するとともに、日本肝移植研究会のホームページ(http://jlts.umin.ac.jp/)に掲載する予定である。

今後の課題

わが国の脳死肝移植は、症例数が一向に増加しない。生体肝移植はそれを補うべく著明な増加を示しているが、上述したように生体肝ドナーには種々の医学的な合併症が発生しているのみならず、心理的、社会的にも色々な問題があり、決してバラ色の医療ではない。また、海外渡航に道を求める人があるが、C型肝炎の増加に伴い世界的にドナー不足の状態にあり、実際に移植を受けることができる人は限られている。さらに、渡航移植は、海外において、臓器売買に類する行為とのそしりを受ける場合があるとも聞く。以上のような状況を改善するためには、脳死臓器提供の増加が必要であり、いわゆる臓器移植法の改正が望まれる。

繰り返しになるが、脳死肝移植はいまだに保険適応が認められていない。法律で特に規定されている医療が保険適応でないのは異常な状態であり、早急な是正が必要である。

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特集

肝臓の臨床最前線

急性肝不全 劇症肝炎と肝移植

Liver transplantation for fulminant hepatic failure

武田 裕 TAKEDA Yutaka
堂野 恵三 DONO Keizo
梅下 浩司 UMESHITA Koji
永野 浩昭 NAGANO Hiroaki
宮本 敦史 MIYAMOTO Atsushi
丸橋 繁 MARUBASHI Shigeru
門田 守人* MONDEN Morito

Key words 劇症肝炎 肝移植 適応基準

欧米では，1980年代より劇症肝炎の治療として肝移植が施行され，現在では治療法として確立している．一方本邦では，1997年に臓器移植法が施行され，劇症肝炎も脳死肝移植の適応となっているが，日本臓器移植ネットワークのホームページ(http://www.jotnw.or.jp/)によると，2004年12月20日現在で，脳死肝移植はわずかに26例施行されたのみである．したがって，本邦での劇症肝炎に対する肝移植もほとんどが生体肝移植であり，こうした特殊性を含めて，劇症肝炎に対する肝移植について述べたい．

Ⅰ．劇症肝炎とは

急性肝不全(劇症肝炎)はAASLD(American Association for the Study of Liver Disease)のガイドライン[1]では，Acute liver failure(fulminant hepatic failure)として肝移植の適応項目に入っており，先行する肝疾患なく発症から8週間以内に肝性脳症と凝固異常をきたすものと定義され，その原因はアセトアミノフェンとそれ以外の薬剤，A型，B型肝炎，Wilson病と成因不明に分けられている．本邦では，第12回犬山シンポジウムの劇症肝炎の診断基準[2]により，「肝炎のうち初発症状発現後8週間以内に高度の肝機能異常に基づいて昏睡Ⅱ度以上の肝性脳症をきたし，プロトロンビン時間が40%以下を示すものとする．」と定義されている．また，「症状出現後10日以内に脳症が発現する急性型と11日以降に発現する亜急性型がある．」と記載されている(表1)．類縁疾患としては，「プロトロンビン時間が40%以下を示す症例のうち，肝性脳症が認められない，ないしは昏睡Ⅰ度以内の症例は急性肝炎重症型，初発症状出現から8週以降，24週以内に昏睡Ⅱ度以上の脳症を発現する症例は遅発性肝不全に分類する．」とされている．成因に関しては，以前はA型，B型，非A非B型，薬剤性，その他に分類されていたが，現在では，ウイルス性(A型，B型，C型，E型，その他)自己免疫性，薬剤性，成因不明，分類不能の5つに分類されている[3]．

Ⅱ．発症頻度

劇症肝炎の年間推定発生数は1972年の全国集計

大阪大学大学院医学系研究科病態制御外科学教室 *教授

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表1　劇症肝炎の診断基準(難治性の肝疾患に関する研究班，2003年)

劇症肝炎とは，肝炎のうち初発症状出現後8週間以内に高度の肝機能異常に基づいて昏睡Ⅱ度以上の肝性脳症をきたし，プロトロビン時間が40%以下を示すものとする．そのうちには症状出現後10日以内に脳症が発現する急性型と，11日以内に出現する亜急性型がある．

(注1)　先行する慢性肝疾患が存在する場合は劇症肝炎から除外する．ただし，B型肝炎ウイルスの無症候性キャリアからの急性増悪例は劇症肝炎に含めて扱う．

(注2)　薬物中毒，循環不全，妊娠脂肪肝，Reye症候群など肝臓の炎症を伴わない肝不全は劇症肝炎から除外する．

(注3)　肝性脳症の昏睡度分類は犬山分類(1972年)に基づく(表1)．

(注4)　成因分類は「難治性の肝疾患に関する研究班」の指針(2002年)に基づく(表2)．

(注5)　プロトロビン時間が40%以下を示す症例のうち，肝性脳症が認められない，ないしは昏睡Ⅰ度以内の症例は急性肝炎重症型，初発症状出現から8週以降24週以内に昏睡Ⅱ度以上の脳症を発現する症例は遅発性肝不全に分類する．これらは劇症肝炎の類縁疾患であるが，診断に際しては除外として扱う．

特定疾患の申請に際しての臨床調査個人票には(注3)と(注4)のみが記載されています．

では約3,700例[4]であったが，1989年には約1,000例[5]となり1996年の集計においても約1,000例[6]であった．一方，遅発性肝不全(LOHF：late onset hepatic failure)は年間約100例，急性肝炎重症型は約3,000例と推定されている[7]．

Ⅲ．予　　後

劇症肝炎の予後は，1983年から1997年の救命率は急性型で30～40%，亜急性型は10～20%，またLOHFでは約10%と不良であった．しかしながら，近年では救命率の向上が見られ，2002年の全国集計によると，肝移植非実施症例による救命率は，急性型57%，亜急性型23%，LOHF 0%，肝移植実施症例による救命率は，急性型64%，亜急性型76%，LOHF75%であり，肝移植を含めた全症例での救命率は，急性型58%，亜急性型41%，LOHF 30%と治療成績の向上が見られている[3][8]～[10]．

Ⅳ．肝移植の適応

劇症肝炎の肝移植の適応基準は，欧米では，イギリスのKing's College Hospitalの肝移植適応基準や，フランスのPaul Brousse Hospitalの肝移植適応基準が提唱されている(表2)．King's College Hospitalの適応基準は，アセトアミノフェン中毒とそれ以外の成因を分けて適応を決めている．一方，Paul Brousse Hospitalの適応基準は簡便で，肝性脳症(3または4)および年齢30歳以上で，第Ⅴ因子が30%以下，年齢30歳以下で，第Ⅴ因子が20%以下を適応としている．しかしながらこれらの適応基準は，劇症肝炎の成因や治療法の違いなどから，そのまま国内の症例に適応しても正診率は低いことが分かっている[11]．

そこで本邦での予後予測を正確かつ早期に予測するために1996年に日本急性肝不全研究会により劇症肝炎における肝移植適応ガイドライン(表3)が提示されている．ガイドライン作成当時のPPV(positive predictive value：死亡と予測された症例のうち，実際の死亡例の比率)は1.00，NPV(negative predictive value：生存と予測された症例のうち，実際の生存例の比率)は0.82，sensitivity(死亡例のうち，死亡と予測された症例の比率)は0.86，specificity(生存例のうち，生存と予測された症例の比率)は1.00，PA(predictive accuracy 正診率)は0.91であったが，最近の症例による検討では，PPV 0.83，NPV 0.78，sensitivity 0.93，specificity 0.57，PA 0.82と低下している[12]．Specificityの低下は死亡と予測されたが生存した割合が多いことを示しており，内科的治療による救命率の向上とともに，将来見直すべき可能性があると考えられる．

このガイドラインでは，治療開始(脳症発現)から5日後に予後の再予測を行い，内科的治療に反応する症例の見極めを行うことになっている．当初には多くの施設で5日後の再評価後を待つこと

表2 欧州における急性肝不全の移植適応

a：Criteria Adopted in Paul Brousse Hospital for Liver Transplantation in Fulminant Hepatic Failure.
Factor V＜30% if age＞30yr or FactorV＜20% if age＜30yr and Confusion or coma (encephalopathy stage 3 or 4)
b：Criteria Adopted in King's College Hospital for Liver Transplantation in Fulminant Hepatic Failure.
Acetaminophen pH＜7.30(irrespective of grade of encephalopathy) or Prothrombin time＞100s (INR＞7) and serum creatinine＞300μmol/l in patients with grade Ⅲ or Ⅳ encephalopathy. Nonacetaminophen patients Prothrombin time＞100s (INR＞7) (irrespective of grade of encephalopathy) or Any 3 of the following variables (irrespective of grade of encephalopathy)： Age＜10 or＞40yr Etiology—non A, non B hepatitis, halothane hepatitis, idiosyncratic drug reactions Duration of jaundice before onset of encephalopathy＞7days Prothrombin time(INR＞3.5)＞50s Serum bilirubin＞300μmol/l

(文献17より)

表3 劇症肝炎における肝移植適応のガイドライン(1996年日本急性肝不全研究会)

Ⅰ．脳症出現時に次の5項目のうち2項目を満たす場合は死亡と予測して肝移植の登録を行う
(1)年齢：≧45歳
(2)初発症状から脳症発現までの日数：≧11日(すなわち亜急性型)
(3)プロトロンビン時間：＜10%
(4)血清ビリルビン濃度：≧18.0mg/dl
(5)直接/総ビリルビン吐：≦0.67
Ⅱ．治療開始(脳症発現)から5日後における予後の再予測
(1)脳症がⅠ度以内に覚醒あるいは昏睡度でⅡ度以上の改善
(2)プロトロンビン時間が50%以上に改善
以上2項目のうち，認められる項目数が
2 ：生存と再予測して肝移植の登録を取り消す
1 or 0 ：死亡と再予測して肝移植の登録を継続する

が多く，感染症や脳症の進行により，移植不能となったり，術前の病状を悪化させる結果となっていた．しかしながら，脳症出現時に5項目のうちの2項目を満たしていれば脳死肝移植の登録は可能であり，登録のために5日後の再予測を待つ必要はない．特に治療開始後5日以内に肝移植を必要とするhyperacute liver failureのような病態では，5日後の再評価を待つべきではないことを強調したい．

またこのガイドラインは，脳死肝移植の際の基準として，グラフトの公平な分配のための客観的な基準となっている．生体肝移植においては，内科的治療によって改善がみられない場合に，適切なドナーが存在し，十分なインフォームドコンセントがなされて，ドナー候補を含む家族が生体肝移植を希望されれば，脳障害や重症感染症を合併する前に肝移植を施行する方が良いと考えられる[13]．脳死肝移植症例数が少ない本邦では，緊急に対応が必要となる，劇症肝炎に脳死肝移植が対応することは困難であり，劇症肝炎における生体

肝移植適応ガイドラインが必要と考えられる.

V. 内科的治療

内科的治療に関しては詳細を他項に譲るが，原疾患治療としてB型劇症肝炎に対するラミブジン投与やインターフェロンの併用が行われている．また，免疫応答による肝障害を防止する目的にて，サイクロスポリン投与やステロイドパルス療法を，肝再生を目的にPGE1投与やグルカゴンインスリン療法が選択される場合もある．また，劇症肝炎の経過中にはSIRSの状態に陥ることが多く，肝に加えて心肺腎等の多臓器障害をきたし，循環，呼吸，血糖等の全身管理および人工肝補助療法が必要となることから，早期より集中治療の実施が望ましい．人工肝補助療法としては，血漿交換と持続血液濾過透析とを施行する方法の効果が高いと考えられている．劇症肝炎の予後を決定するのみならず，肝移植の禁忌となる感染等の合併症対策も重要である．感染症対策としては，胸部レントゲン，CT等画像検査に加え，各種培養検査を早期に施行し，早期に治療を開始することが重要である．また，選択的消化管内殺菌を施行する施設もある．肝不全に伴う脳浮腫に対しては，脳波，頭部CT，場合によっては頭蓋内圧をモニターする必要があり，アンモニア上昇に対してラルツロース，脳圧降下剤としてマンニトールやグリセオールが投与されている．微小循環障害に対しては，蛋白分解酵素阻害剤が投与される場合もある[14]．

VI. 劇症肝炎に対する肝移植

劇症肝炎に対する肝移植は，先に述べた肝移植の適応にしたがって行うが，もう一つのポイントは移植の禁忌が無いかを検討することである．まずは耐術能があるかで，高齢者(70歳以上)，重篤な心肺合併症，多臓器不全などは適応外となる．脳障害の可逆性に関しては，頭蓋内圧をモニター等から判断されるが，肝性脳症Ⅳ度以上で非可逆性の脳障害をきたしたと考えられる場合も適応外である．また肝移植後は，免疫抑制剤やステロイドが投与されることから感染症の合併は禁忌である．細菌感染症では，明らかな肺炎や敗血症性ショックの状態は移植禁忌である．それ以外の細菌感染症の場合は抗生剤等を投与し適応の検討を続行する．真菌感染症では，アスペルギルス感染症は肝移植の禁忌である[15]．

移植適応のある患者あるいは移植を考慮する患者が現れた場合は，移植施設と綿密な連絡をとり，早期より集中治療を開始し，平行して適応評価と術前検査を行う．移植適応の可能性があれば，早期に患者を移植施設に搬送し，内科的治療を続行するとともに適応判定を行う．画像検査に加えて各種培養検査を早期に施行し，感染症の有無を判定し，感染症が有ればこれに対して，抗生剤投与を行う．予防的抗生剤投与を行う施設もあるが，当施設では予防的抗生剤投与は行っていない．肝性脳症Ⅲ度以上で鎮静剤を使用し，気管内挿管にて人工呼吸管理を行っている場合には，血漿交換後に頭蓋内圧センサーを留置し頭蓋内圧測定を行っている．

また，劇症肝炎の原因検索も平行して行っている．劇症肝炎の成因分類では，ウイルス性，自己免疫性，薬剤性，成因不明，分類不能の5つに分類されるが，例えばB型劇症肝炎であればラミブジン投与やインターフェロンの併用療法の選択や移植後の再感染予防を考慮する．また肝臓の状態や門脈等の血流動態をCT，Echoにて把握している．

これらの情報を踏まえたうえで，当施設では，移植を考慮する患者が現れた場合は，高度救命救急センターのスタッフを中心に，肝臓内科，小児科，消化器外科，小児外科，麻酔科，腎臓内科，神経内科，移植コーディネーターから構成された，劇症肝炎ワーキングを開催し，個別に迅速に検討している．

Ⅶ. 手術方法，術中管理

手術方法に関して，劇症肝移植であるからという理由で特別なことはない．欧米では主に死体肝移植が施行されているが，本邦では他の場合の肝移植と同じく，生体肝移植が選択される場合が多い．通常はレシピエントの肝臓を全摘し同所性にグラフト肝が移植される．しかしながら，劇症肝炎では急性期を乗り切れれば，肝再生の可能性があることから，補助的肝移植が施行される場合がある．補助的肝移植では，レシピエント肝が回復し，グラフト肝による機能補助が不要になった場合に，免疫抑制剤を中止できる可能性がある．APOLT(auxiliary partial orthotopic transplantation)は，レシピエント肝を部分切除または葉切除し，その場所にグラフト肝を移植する術式である．異所性補助的肝移植は，腹腔内スペースの問題と，門脈血流の分配の問題から選択されることは少ない．

また，劇症肝炎では，門脈や静脈の側副血行路が発達していないため，場合によっては，門脈・下大静脈ー鎖骨下静脈の体外循環バイパスや，一時的な門脈ー下大静脈シャントの作成を行う場合がある．

術中管理に関して通常の生体肝移植または脳死肝移植と大きく変わることはない．ただし，脳浮腫が疑われる場合には，血圧等循環動態の安定に努めるように努力している．

Ⅷ. 術後管理

術後管理に関しても通常の生体肝移植または脳死肝移植と大きく変わることはない．ただし感染症に関しては，重篤なものは禁忌であるが，不顕性に感染が潜んでいる場合が多く，注意を要する．また，術前に肝腎症候群から腎不全を合併している場合も多い．当施設では，無尿の場合は免疫抑制剤をステロイドとMMF(ミコフェノール酸モフェチル)で開始し，腎不全が改善してきた時点でカルシニュリン阻害剤を追加している．

Ⅸ. 当施設での経験

当施設では2001年4月から2004年3月の3年間に17件の劇症肝炎(急性型4例，亜急性型13例)を経験した．ウイルス性(B型)6例，薬剤性3例，成因不明8例であった．脳死肝移植適応ありは13例で，内9例が脳死登録を行い，1例に脳死下の肝移植を施行した．この1例を除く移植適応ありの12例中6例に生体移植ドナーが現れ，生体肝移植を施行した．脳死肝移植を含む劇症肝炎の肝移植症例7例中5例が周術期を乗り越えて生存している(生存率71.4%)．一方，脳死肝移植適応ありとされたが，生体移植ドナー候補がなかった6例中4例が死亡されている(生存率33.3%)．

Ⅹ. 国内および海外の現状

日本肝移植研究会の肝移植症例登録報告[16]によると，2002年末までにわが国での総肝移植数は2,249例であり，ドナー別では，死体肝移植が23例(脳死肝移植21例，心停止肝移植2例)，生体肝移植が2,226例であった．急性肝不全に対して，生体肝移植は266例施行されているが，死体肝移植はわずかに2例施行されたのみである．劇症肝炎の生存率はB型肝炎では3年生存率78.2%，薬剤性では85.7%と成因により若干の差があるが，劇症肝炎全体での1年生存率は72%，3年生存率は71%，5年生存率は68.5%と周術期を乗り越えて生存できれば以後の成績は良好である．一方，欧米では，2003年の米国のUNOS(United Network for Organ Sharing)(OPTN/SRTR)の統計では1993年から2002年までに計3,364例の劇症肝炎に死体肝移植が施行され，1年生存率83.9%，3年生存率75.7%，5年生存率69.4%と報告されている．またヨーロッパのELTR(European Liver Transplant Registry)の統計では1988年から2000

年までに計2,908例の劇症肝炎に肝移植が施行され，1年生存率70%，3年生存率62%，5年生存率59%であった．これらの成績は日本の生体肝移植の成績とほぼ同じである．内科的治療による救命率が，急性型で57%，亜急性型23%であることから考えると，まだまだ限られた状況のなかながら移植医療は標準治療の一選択肢となってきたと考えられる．

XI．ドナーの問題

患者家族にとってみれば，家族の一人が，劇症肝炎により，突然集中治療室に収容されるという緊急な状況下で，短時間の間に治療法の選択を迫られる状況になる．そこで内科的治療が困難であること，肝移植という治療選択があること，脳死肝移植は登録しても現実には可能性が低いこと，生体肝移植にはドナーが必要であることを，切迫した状況下で知らされることになる．日本肝移植研究会の生体肝提供(ドナー)手術に関する指針では，強要のない，自発的意思に基づく提供が明示されている．このことは，緊急性の高い劇症肝炎に対する生体肝移植であっても同じである．限られた時間の中ではあるが，十分なインフォームドコンセントが必要である．当施設でも複数回のインフォームドコンセントを必ず行い，精神科医師による面談も実施している．

おわりに

2004年1月に生体部分肝移植の保険適用疾患が拡大され，劇症肝炎(ウィルス性，自己免疫性，薬剤性，成因不明を含む)も保険適用となった．これまで多額の医療費を患者に負担させてきた現状が改善され，門戸が広げられるとともに，移植医療が内科的治療の困難な劇症肝炎の標準治療となることが期待される．

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Low Incidence of Acute Rejection after Living-Donor Liver Transplantation: Immunologic Analyses by Mixed Lymphocyte Reaction using a Carboxyfluorescein Diacetate Succinimidyl Ester Labeling Technique

Yuka Tanaka, Hideki Ohdan, Takashi Onoe, Hiroshi Mitsuta, Hirotaka Tashiro, Toshiyuki Itamoto, and Toshimasa Asahara

Background. To monitor antidonor alloreactivity for accurate diagnosis of acute rejection after living-donor liver transplantation (LDLT), we used a mixed lymphocyte reaction (MLR) assay using an intracellular fluorescent dye carboxyfluorescein diacetate succimidyl ester (CFSE)-labeling technique (CFSE-MLR) in 29 consecutive patients who underwent adult-to-adult LDLT.

Methods. For patients who developed moderate or severe disorders in liver function, CFSE-MLR was performed together with needle biopsy of the liver allografts immediately after liver dysfunction had occurred. CFSE-labeled peripheral blood mononuclear cells (PBMC) from recipients and irradiated autologous, donor, or third-party PBMC were cultured, and then proliferation and CD25 expression in each of the $CD4^+$ and $CD8^+$ T cell subsets were analyzed by flow cytometry.

Results. Twelve (41.4%) of the 29 patients developed moderate or severe disorders in liver function within 6 months after LDLT. Eight of the 12 patients (overall incidence of 27.6%) suffering from liver function disorder were diagnosed on the basis of liver biopsy results as having mild or moderate acute rejection. However, only 4 of the 12 patients (overall incidence of 13.8%) showed remarkable proliferation of $CD8^+$ T cells in association with CD25 expression on antidonor CFSE-MLR. The other eight patients were eventually diagnosed as having recurrence of original hepatitis, drug-induced hepatotoxicity, or congestion of the anterior segment of the liver allograft by further extensive examinations or in retrospect.

Conclusions. The results of CFSE-MLR assays, which could be used for rigorously monitoring rejection, provided evidence of low incidence of acute rejection after LDLT.

Keywords: Living-donor liver transplantation, Flow cytometry, Mixed lymphocyte reaction, Acute rejection, Immune monitoring.

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The incidence of acute cellular rejection (ACR) after liver transplantation has been reported to be approximately 30% to 60% (*1–3*). Such a wide range might be caused by the difficulty in differential diagnosis from rejection, recurrence of original disease (such as viral hepatitis), and drug-induced hepatotoxicity even by pathologic examinations (*4*). More accurate diagnosis of ACR would reduce risks of morbidity and mortality caused by inappropriate immunosuppressive therapy. To practice a necessary and sufficient immunosuppressive therapy in clinical transplantation, the development of a reliable assay for monitoring immune response is needed. The mixed lymphocyte reaction (MLR) is a widely used method for evaluating immune response to alloantigens in both experimental and clinical transplantation. The MLR assay was initially used to determine the proliferation of host (responder) T cells in response to antigens expressed on leukocytes obtained from the donor (*5, 6*). Later, it was shown that host cytotoxic T cells against antigens of the donor could be generated in MLR (*7*). In addition to constituting the majority of the proliferating cells in allogeneic MLR, the $CD4^+$ T-helper cells secreted cytokines that enabled the killer T cells to undergo functional maturation to possess killer activity (i.e., collaboration between $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells). However, traditional MLR using tritiated thymidine incorporation for quantifying cell division does not enable phenotypic or functional analysis of proliferating cells in such heterogeneous MLR. Flow cytometric (FCM) analysis of lymphocyte division by serial halving of the fluorescence intensity of the intracellular fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE) has recently been used instead of the tritiated thymidine method in the MLR (*8–10*). CFSE stably stains intracellular proteins

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Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan.

Address correspondence to: Hideki Ohdan, Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan.

E-mail: hohdan@hiroshima-u.ac.jp.

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without toxicity, and the fluorescence of each stained cell segregates equally to daughter cells upon cell division, resulting in sequential halving of cellular fluorescence intensity with each successive generation (*11*). When analyzed by FCM, this sequential halving of fluorescence is visualized as distinct peaks or populations of cells and can be used to track cell division in populations of proliferating cells. This, then, allows phenotypic analysis of proliferating cells in addition to determining the number of cells produced in each generation by multicolor FCM analysis. To monitor antidonor alloreactivity for more accurate diagnosis, we have used an MLR assay using intracellular a CFSE-labeling technique (hereafter referred to as CFSE-MLR assay) in patients who underwent adult-to-adult living-donor liver transplantation (LDLT).

PATIENTS AND METHODS

Patient Population

Twenty-nine consecutive patients who underwent adult-to-adult LDLT at Hiroshima University Hospital were enrolled in this study. The 29 patients included 17 males and 12 females, ranging in age from 28 to 68 (mean±SD 52.3±7.2) years. Original diseases of the patients are shown in Table 1. The graft donors were 18 children, 4 siblings, 4 spouses, 2 parents, and 1 other relative, with ages ranging from 18 to 61 (mean 36.3±6.0) years.

Immunosuppressive Protocol

The basic immunosuppressive regimen after LDLT consisted of tacrolimus/cyclosporine and methylprednisolone, with doses gradually being tapered off. In patients with hepatitis B or C virus (HCV), the dose of methylprednisolone was rapidly tapered off, and administration was stopped within 1 month after LDLT, which would be beneficial for preventing enhanced viral replication. Instead, basiliximab was usually administered (20 mg on days 0 and 4 after LDLT) to those patients. When patients developed significant disorders in liver function as determined by laboratory tests after LDLT, CFSE-MLR was performed together with needle biopsy of the liver allografts immediately after liver dysfunction had occurred. Unless graft liver dysfunction was progressive, immunosuppressive treatment usually was not intensified. However, some patients in whom liver function

TABLE 1. Patient characteristics and compatibility

Patient no.	Age at LTx (years)	Sex	Original diagnosis	Viral hepatitis	Donor	HLA mismatches, A-B-C-DR	Liver allograft dysfunction
1	63	M	Liver cirrhosis with HCC	HCV	Offspring	0-1-0-1	−
2	50	M	Liver cirrhosis with HCC	HCV	Spouse	1-1-1-1	−
3	66	F	Liver cirrhosis with HCC	HCV	Offspring	1-1-0-2	−
4	62	M	Liver cirrhosis with HCC	HBV	Offspring	1-1-0-1	−
5	40	M	Fluminant hepatitis	—	Sibling	0-1-0-0	−
6	48	F	Autoimmune hepatitis	—	Sibling	2-2-1-1	+
7	52	M	Liver cirrhosis	HCV	Offspring	0-1-0-1	−
8	57	M	Liver cirrhosis with HCC	HBV	Offspring	1-1-0-1	+
9	58	M	Liver cirrhosis (Alcoholic)	—	Offspring	1-1-1-0	+
10	46	F	Liver cirrhosis (Alcoholic)	—	Spouse	2-1-0-2	−
11	66	F	Liver cirrhosis with HCC	HCV	Offspring	1-0-0-1	−
12	56	M	Fluminant hepatitis	HBV	Offspring	1-1-0-1	−
13	59	F	Liver cirrhosis	HCV	Offspring	1-1-0-1	+
14	56	F	Liver cirrhosis with HCC	HCV	Offspring	1-1-1-1	−
15	49	M	Liver cirrhosis with HCC	HCV	Offspring	0-1-1-1	+
16	60	M	Liver cirrhosis with HCC	HCV	Offspring	1-1-0-ND	−
17	54	M	Liver cirrhosis with HCC	HBV	Offspring	1-1-1-ND	−
18	55	F	Liver cirrhosis with HCC	HCV	Sibling	0-0-0-0	−
19	49	M	Liver cirrhosis with HCC	HBV	Offspring	0-1-0-2	−
20	28	M	Liver cirrhosis	HCV	Parent	1-0-0-ND	−
21	47	M	Liver cirrhosis with HCC	HCV	Offspring	1-1-1-1	+
22	51	F	Secondary biliary chirosis	—	Other relative	0-0-0-0	+
23	43	M	Liver cirrhosis with HCC	HBV	Spouse	1-1-1-2	+
24	28	M	Insulinoma (Liver metastasis)	—	Parent	0-0-0-1	+
25	57	F	Liver cirrhosis	HCV	Spouse	2-1-1-1	+
26	58	M	Liver cirrhosis	HBV	Offspring	1-1-1-1	−
27	44	F	Autoimmune hepatitis	—	Sibling	0-0-0-1	+
28	46	F	Liver cirrhosis with HCC	HBV	Offspring	1-1-0-1	−
29	68	F	Liver cirrhosis with HCC	HCV	Offspring	1-1-0-1	+

LTx, liver transplantation; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; ND, not done; HLA, human leukocyte antigen.

progressively worsened, received low-dose steroid-pulse therapy (125–250 mg/day of methylprednisolone for 2–3 days). Patients who were eventually diagnosed as having acute rejection by liver-allograft biopsy and CFSE-MLR assay received additional steroid-pulse or OKT 3 therapy if necessary. However, when antidonor MLR revealed a hypo-response, the patient did not receive further antirejection therapy.

CFSE Labeling

Peripheral blood mononuclear cells (PBMC, 1×10^7 cells/mL) were resuspended in phosphate-buffered saline (PBS). 5-(and 6)-CFSE (Molecular Probes, Inc., Eugene, OR) was added to make a final concentration of 5 μM, and the cells were gently mixed and incubated for 15 minutes at 37 °C in a CO_2 incubator protected from light. Labeling of cells was stopped by adding cold PBS with 2% fetal bovine serum (Sanko, Tokyo, Japan), and the cells were then washed and resuspended in MLR medium, that is, RPMI culture medium containing 15% controlled process serum replacement-type 3 (Sigma, St. Louis, MO), 50 μM 2-mercaptoethanol (Katayama, Osaka, Japan), 1% HEPES buffer (Gibco, NY, NY), and 100 IU/mL penicillin-100 μg/mL streptomycin (Gibco, NY, NY).

MLR Assay

The PBMC prepared from recipients (autologous control), donors, and healthy volunteers (third-party control) as stimulator cells were irradiated with 30 Gy, and those as responder cells from recipients were labeled with CFSE, as described above. Both the stimulator and responder cells in the MLR medium were adjusted to 2×10^6 cells/mL of medium and cocultured in a total volume of 2 mL of medium in 24-well flat-bottom plates (BD Labware, Franklin Lakes, NJ) at 37°C in a 5% CO_2 incubator in the dark for 5 days. After MLR culture, nonadherent cells were harvested and stained with either phycoerythrin-conjugated CD4 or CD8 monoclonal antibodies (mAbs; BD Farmingen, San Diego, CA) together with allophycocyanin-conjugated CD25 mAb (BD Farmingen). Four-color FCM was performed on a FACSCalibur dual-laser cytometer (Becton Dickinson, Mountain View, CA) using standard Cell Quest acquisition/analysis, and fluorescence compensation was achieved using an appropriate single fluorochrome-labeled sample. Dead cells were excluded from the analysis by light-scatter or propidium iodide.

Quantifying Proliferation of $CD4^+$ and $CD8^+$ T Cells

Precursor frequency (PF), proliferation index (PI), and stimulation index (SI) were quantitatively estimated using a method described previously (*10, 12*). The CFSE fluorescence intensity of the peak of cell division, which was divided once, shows a half value of CFSE-fluorescence intensity of the peak of nonreactive cell division. Divisions of reactive cells, which were identified and determined by their CFSE intensities, were labeled from 0 to n as dividing time. A single cell dividing n times will generate 2^n daughter cells. With use of this mathematical relationship, the number of division precursors was extrapolated from the number of daughter cells of each division and from proliferation events and PF in $CD4^+$ and $CD8^+$ T-cell subsets. With use of these values, proliferation events and PI were calculated. SI was calculated by dividing PI of allogeneic combinations by those of self-control.

RESULTS

Clinical Characteristics

The target blood levels of calcineurin inhibitors were achieved in all 29 recipients in this series (i.e., trough whole-blood levels of tacrolimus were maintained between 8 and 15 ng/mL in the first few postoperative weeks and thereafter between 5 and 10 ng/mL, and those of cyclosporine were maintained between 100 and 200 ng/mL in the first few postoperative weeks and thereafter between 100 and 150 ng/mL). Twelve (41.4%) of the 29 patients developed significant disorders in liver function (levels of serum bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were routinely measured as indexes of liver function) within 6 months after LDLT (Table 2). In 6 of those 12 patients, immunosuppressive treatment was not intensified because liver dysfunction was not progressive. However, the other six patients (including 4 patients who were eventually diagnosed by CFSE-MLR as having ACR) in whom liver function progressively worsened received low-dose steroid-pulse therapy (125–250 mg/day of methylprednisolone for 2–3 days) after CFSE-MLR, and needle biopsy had been performed but before their results had been obtained. Eight of the 12 patients suffering from liver function disorder were diagnosed as having mild or moderate ACR by liver-allograft biopsy (overall incidence of 27.6%). The other four patients in whom ACR was ruled out by results of liver biopsy were retrospectively diagnosed as having either drug-induced hepatotoxicity (in 2 patients) or congestion of the anterior segment of the liver allograft (in 2 patients). In contrast, only 4 of the 12 patients suffering from liver-function disorder showed remarkable proliferation of $CD8^+$ T cells in association with CD25 expression on anti-donor CFSE-MLR (overall incidence of 13.8%). In those four patients, antirejection therapy consisting of steroid pulse (250 mg/day for 3–5 days) with OKT 3 therapy (in 2 patients) or without OKT3 therapy (in 2 patients) resulted in remarkable improvement. Four patients who were diagnosed as having ACR but did not show significant antidonor responses of CD8 T cells in the CFSE-MLR were eventually diagnosed as having recurrence of original hepatitis (i.e., HCV in 2 patients and autoimmune hepatitis [AIH] in 1 patient) or drug-induced hepatotoxicity (in 1 patient) by results of further extensive examinations (i.e., detection of elevation of HCV RNA levels or autoantibodies/globulins in peripheral blood). In the patients diagnosed as having HCV recurrence, immunosuppressive treatment was not intensified. In contrast, in the patients diagnosed as having AIH recurrence, mycophenolate mofetil was administered (750–1,500 mg/day) together with tacrolimus and methylprednisolone.

Histology

Discrepancy between liver-biopsy and MLR results is likely to be caused by the difficulty of differential diagnosis between ACR and recurrence of original hepatitis (HCV and AIH) (*4*). In general, histologic characteristics of ACR are portal inflammatory infiltrates and nonsuppurative cholangitis with or without endotheliitis (Fig. 1, A and B). In con-

TABLE 2. Results of liver allograft biopsy and CFSE-MLR

Patient no.	Timing of biopsy and MLR (postLTx days)	Histopathologic diagnosis	CFSE-MLR stimulation index				$CD25^+$ cells among proliferating $CD8^+$ cells (%)	
			CD4		CD8			
			Donor	Third	Donor	Third	Donor	Third
6	12	Acute rejection (mild)	1.4	2.0	1.6	2.4	41.7	69.1
8	170	Acute rejection (mild)	0.4	0.4	1.1	0.9	19.9	17.1
9	68	Focal necrosis and bile stasis	0.6	2.6	0.3	0.4	ND	ND
13	59	Acute rejection (mild)	1.2	2.4	3.5[a]	1.4	69.7	30.1
15	29	Acute rejection (mild)	0.7	1.5	1.2	1.3	27.5	20.2
21	58	Acute rejection (mild)	0.4	1.1	0.9	1.3	9.0	43.6
22	26	Bile stasis	1.8	1.8	1.0	3.8	33.3	85.5
23	15	Acute rejection (mild)	14.5	5.2	68.3[a]	14.7	76.6	82.8
24	21	Centilobular hepatocellular degeneration	4.4	4.8	1.5	4.0	27.0	64.6
25	14	Acute rejection (mild)	3.3	1.5	3.6[a]	1.2	67.0	7.6
27	21	Acute rejection (moderate)	5.1	12.8	16.3[a]	9.3	80.8	82.3
29	30	Focal necrosis and bile stasis	4.5	2.5	2.4	2.1	1.4	5.7

[a] Data showing significant proliferation of CD8+ T cells in anti-donor MLR.
ND, not done; MLR, mixed lymphocyte reaction; CFSE, carboxyfluorescein diacetate succinimidyl ester; LTx, liver transplantation.

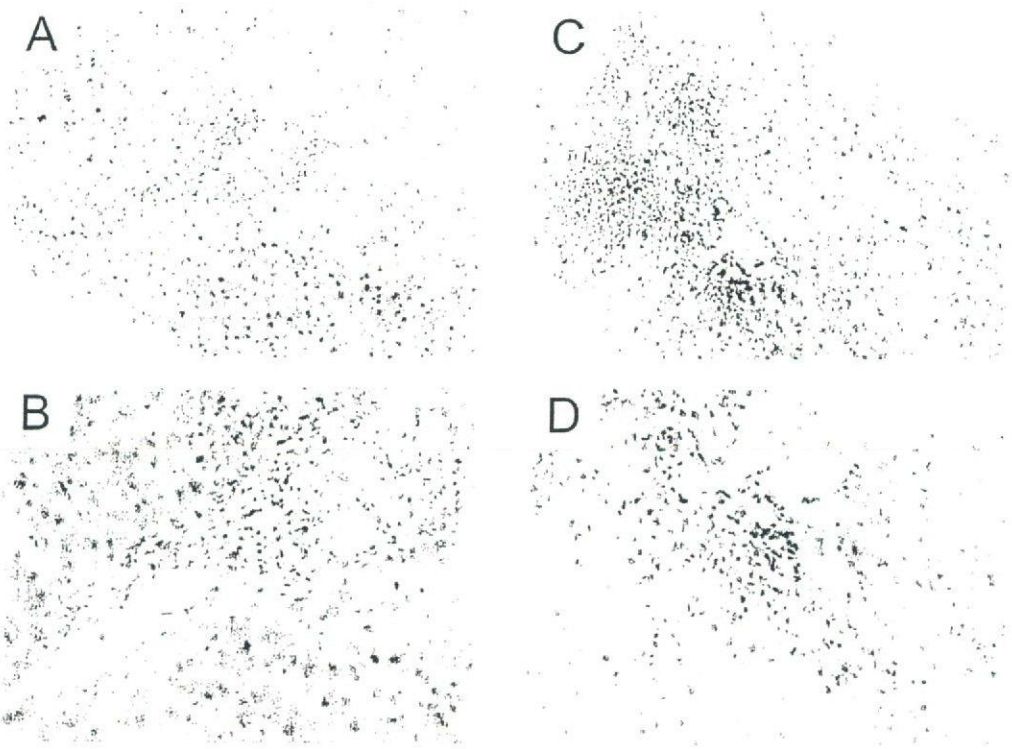

FIGURE 1. Representative histopathologic findings of liver allograft biopsies. (A) (magnification, ×100) and (B) (×200): portal inflammatory infiltrates and nonsuppurative cholangitis with endotheliitis were observed, leading to the histologic diagnosis of acute rejection (patient 23). (C) (×100) and (D) (×200): mild periportal hepatitis with lymphoid aggregates, the most common biopsy presentation of recurrent hepatitis C virus (HCV), was observed (patient 21). Because lymphoid cholangitis and endotheliitis were also found, recurrent HCV was difficult to distinguish from acute rejection. This patient was eventually diagnosed as having HCV recurrence.

trast, mild periportal hepatitis with lymphoid aggregates, with or without fatty change, is the most common biopsy presentation of recurrent HCV. It has been well noticed that lymphoid cholangitis and endotheliitis also are found, and in these instances, recurrent HCV is difficult to distinguish from acute rejection (Fig. 1, C and D). This was true of the two patients in this series who were diagnosed as having ACR by liver biopsy but were eventually diagnosed as having HCV recurrence. Recurrence of AIH is usually defined by the presence of autoantibodies and elevated globulins in association with periportal hepatitis in the absence of viral infection or rejection. The presence of periportal hepatitis with lymphoplasmacytic infiltrates, plasma cells, piecemeal necrosis, and bridging fibrosis in liver-biopsy samples is consistent with recurrent disease of AIH. However, the features of histopathology at the early phase of AIH recurrence were similar to

those for ACR in this series, making it difficult to distinguish between them.

Immune Monitoring by CFSE-MLR Assay

A representative FCM profile in a patient showing hyper-response in antidonor MLR is shown in Figure 2A. When compared with anti–third-party MLR, higher levels of CD4 and CD8 T-cell proliferation were observed. A comparable or even higher level of CD25 expression on the proliferating CD8 T cells in antidonor MLR suggested cytotoxicity activity against donor cells in this patient. A representative FCM profile in a patient showing hypo-response in antidonor MLR is shown in Figure 2B. When compared with anti–third-party MLR, limited levels of CD4 and CD8 T-cell proliferation were observed. Absence of CD25 expression on the proliferating CD8 T cells in antidonor MLR but the presence of that in anti–third-party MLR suggested a lack of cytotoxicity against donor cells in this patient (we have confirmed that only $CD25^+$ proliferating $CD8^+$ T cells have cytotoxic activity against donor cells in our preliminary studies). The SIs in each alloreactive $CD4^+$ and $CD8^+$ T cells in response to antidonor and anti–third-party MLR are shown in Table 2. CFSE-MLR could be a useful tool for precise diagnosis even when differential diagnosis between rejection and recurrence of viral hepatitis is difficult by pathologic examinations. The higher SI in both CD4 and CD8 T cells in antidonor MLR than those in anti–third-party MLR reflects strong antidonor reactivity, confirming the accuracy of diagnosis of ACR.

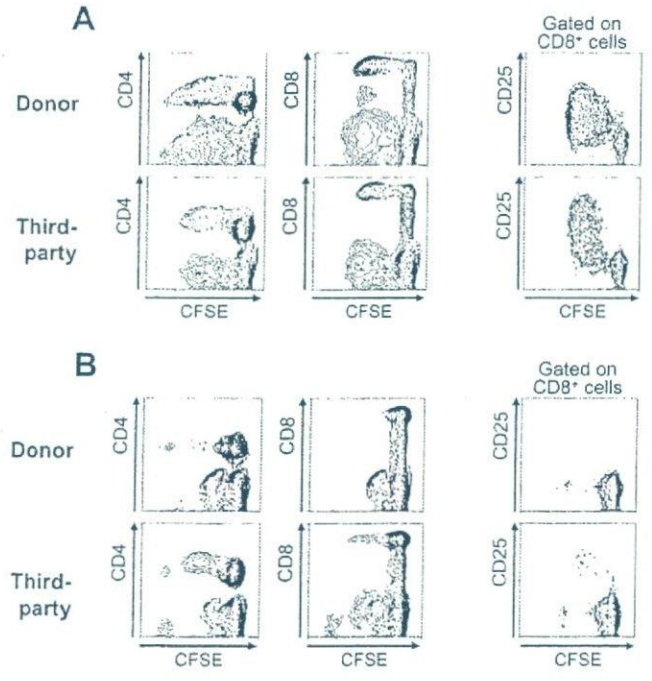

FIGURE 2. (A) Flow cytometry (FCM) profiles in the patient whose histologic appearance of liver allograft biopsy is shown in Figure 1, A and B (patient 23). When compared with anti–third-party mixed lymphocyte reaction (MLR), high levels of CD4 and CD8 T-cell proliferation were observed. A comparable or even higher level of CD25 expression on early proliferating CD8 T cells in both antidonor MLRs was observed. (B) FCM profiles in the patient whose histologic appearance of liver allograft biopsy is shown in Figure 1, C and D (patient 21). When compared with anti–third-party MLR, limited levels of CD4 and CD8 T-cell proliferation were observed. CD25 expression on proliferating CD8 T cells was undetectable in the anti-donor MLR. CFSE, carboxyfluorescein diacetate succinimidyl ester.

DISCUSSION

Antidonor alloreactivity, defined as the number and phenotype of alloreactive precursors in the recipient, can be used to monitor rejection or reduction/withdrawal of immunosuppression. Monitoring such alloreactivity using PBMCs in recipients of transplants does not necessarily mirror what will occur in the allograft tissue because allografts are regulated by infiltrating lymphocytes. However, it has been reported that the frequencies of donor-reactive T cells in PBMCs are closely linked with those in lymphocytes infiltrating allografts (*13*), indicating the validity of evaluation of T-cell responses to allogeneic stimulation using PBMCs for monitoring alloreactivity in transplant recipients. MLR using PBMCs is a widely used method for evaluating T-cell responses to allogeneic stimulation in both experimental and clinical transplantation. However, in conventional forms using tritiated thymidine incorporation, proliferative MLR bulk cultures have very little predictive value because of its low level of reproducibility in the context of transplantation (*14*). The low level of reproducibility of conventional MLR might be caused at least in part by the presence of nonviable cells (which might include unexpectedly surviving stimulator cells) that still have the ability to incorporate tritiated thymidine. By applying a CFSE-based method, the proliferation of viable $CD4^+$ and $CD8^+$ responder T cells in response to allostimulation could be separately quantified using multiparameter FCM. The lack of proliferation of both $CD4^+$ and $CD8^+$ T cells in antidonor MLR would reflect suppression of antidonor response. When remarkable proliferation was observed in $CD4^+$ T cells but not in $CD8^+$ T cells, we did not observe cytotoxic activity against donor cells in the subsequent CML assay in our preliminary studies (data not shown). In contrast, remarkable proliferation of $CD8^+$ T cells would reflect strong antidonor response. We further examined CD25 expression on the proliferating $CD8^+$ T cells by multicolor FCM. The remarkable elevation of CD25 expression on proliferating $CD8^+$ T cells might reflect their cytotoxic activity toward donor cells. In our preliminary studies, the proliferative activity levels of $CD4^+$ and $CD8^+$ T cells were generally higher in MLRs using PBMCs from the spouse than in MLRs using PBMCs from offspring or parents, even before transplantation. This may reflect the greater susceptibility to ACR in recipients of liver allografts from unrelated donors than in recipients of liver allografts from related donors. Consistent with this speculations, MLR-proven ACR occurred in 2 (50%) of the 4 patients receiving LDLT from their spouse but in only 2 (4%) of the 25 patients receiving LDLT from their offspring or parents in the present study. Although the usefulness of CFSE-MLR for the prediction of ACR remains to be elucidated, the results obtained by using this method provided evidence of low incidence of ACR after LDLT compared with previously reported results.

Possible alternative methodologies to diagnose ACR using laboratory-based immunologic modalities might include limiting dilution assay (LDA) and enzyme-linked immunospot (ELISPOT). LDAs provide precise quantification of immunity to a given stimulus and allow the estimation of frequencies of antigen-specific cells participating in an immune response (*15*). Although LDAs have been shown to be specific and reproducible as a measurement of alloreactivity (*16*), conflicting data regarding the usefulness of measurement of cytotoxic T-cell precursors for diagnosis/prediction of rejection in the context of solid-organ transplantation have been reported (*17, 18*). The ELISPOT assay is based on the detection of a cytokine produced by single cells after stimulation with mitogens or antigens (*19*). It has been used to identify the presence of donor-specific T cells in patients before surgery (*20*). However, data indicating the usefulness of this method for diagnosing ACR are less abundant at present.

One of the most difficult challenges in the care of HCV-positive liver-transplant recipients is the differentiation between ACR and HCV recurrence, which can have considerable histologic overlap (*4*). Although polymerase chain reaction allows identification of HCV RNA in biopsy tissue in such difficult cases (*21*), this method could not distinguish between HCV infection alone versus HCV infection complicated by ACR. Attempts to distinguish between these at the intrahepatic gene response level have been made in two studies. The first study analyzed ACR and HCV infection versus HCV infection alone by using gene array analysis (*22*). It has been found that ACR and recurrence of HCV are associated with distinct mRNA expression pattern (i.e., ACR is most notably associated with the relative over-expression of immune activation genes such as major histocompatibility complex classes I and II, tumor necrosis factor [TNF]-α, granzyme B, and complement components). Zekry et al. (*23*) concentrated on Th1 versus Th2-like gene expression and found that ACR in the setting of HCV infection was more like ACR in non–HCV-infected patients and was associated with increase in interleukin (IL)-10 and IL-4 gene expression rather than the IL-2/interferon-γ/TNF-α response seen more in chronic HCV alone. Higher average daily steroid dose and use of OKT 3 have both been associated with more severe recurrence of HCV, presumably through enhancing viral replication or attenuating viral clearance (*24–26*). Minimizing exposure to immunosuppressants for HCV-infected liver-transplant recipients thus requires accurate distinction of recurrence of HCV from ACR. Diagnosis of ACR is based on the detection of biochemical evidence of graft dysfunction and the presence of suggestive allograft histology, including distinct lymphocytic infiltrate patterns. The presence of a modest cellular infiltrate and biochemical abnormalities, however, are not specific to ACR. In addition to the above-described methods, CFSE-MLR also has potential as a tool for diagnosing ACR in HCV-reinfected patients. Additional studies with larger sample sizes are required to confirm this possibility.

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Scientific paper

Indications of partial hepatectomy for transplantable hepatocellular carcinoma with compensated cirrhosis

Toshiyuki Itamoto, M.D.*, Hideki Nakahara, M.D., Hirotaka Tashiro, M.D., Hideki Ohdan, M.D., Hiroshi Hino, M.D., Makoto Ochi, M.D., Toshimasa Asahara, M.D.

Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, 1-2-3, Kasumi, Minami-Ku, Hiroshima, 734-8551, Japan

Abstract

Background: The appropriate treatment strategy for transplantable hepatocellular carcinoma (HCC) patients with compensated cirrhosis remains controversial.

Methods: Surgical outcomes were reviewed in 136 cirrhotic patients with transplantable HCC who had undergone partial hepatectomy. Transplantable HCC was defined as that corresponding to Milan's criteria.

Results: The adverse prognostic factors for both survival and disease-free survival were histologic surgical margin of 5 mm or less, Child-Pugh B, and the presence of hepatitis C virus infection. The overall 5-year survival and disease-free survival rates of patients with 1 or none of the adverse prognostic factors were 73% and 33%, respectively, whereas those of patients with 2 or 3 adverse prognostic factors were 36% and 17%, respectively.

Conclusions: Transplantable HCC patients with 2 or 3 adverse prognostic factors should be considered candidates for liver transplantation, whereas patients with only 1 or none of the adverse prognostic factors are good candidates for partial hepatectomy.

Keywords: Hepatocellular carcinoma; Hepatectomy; Cirrhosis; Liver transplantation; Milan's criteria

Hepatocellular carcinoma (HCC) in the early stage is curable by various surgical approaches including partial hepatectomy and orthotopic liver transplantation (OLT). Partial hepatectomy is the obviously preferred treatment for HCC patients without cirrhosis and can cure the tumor immediately with low mortality and transfusion rates [1]. However, partial hepatectomy is more technically challenging for cirrhotic patients. The underlying cirrhosis is responsible for a high recurrence rate, and patients are exposed to long-term complications of cirrhosis. On the other hand, OLT, by treating both the tumor and the cirrhosis, offers better long-term survival for selected HCC patients with a cancer recurrence rate of less than 15% [2–6]. OLT is the treatment of choice for unresectable small HCC (≤5 cm) in patients with decompensated cirrhosis [2,7,8]. Some recent studies have suggested that OLT is applicable to selected HCC patients with compensated cirrhosis [9]. However, OLT is limited by the insufficient availability of donor organs and long waiting times. Thus, the optimal surgical approach for transplantable and resectable HCC patients with compensated cirrhosis is less clearly defined [10].

We clarify the indication of partial hepatectomy or OLT for cirrhotic patients with transplantable HCC, evaluating multiple factors affecting the prognosis of transplantable HCC patients who have undergone partial hepatectomy.

Methods

Between January 1990 and December 2000, 401 consecutive HCC patients underwent partial hepatectomy in the Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science at Hiroshima University. Among them, 136 cirrhotic patients with transplantable HCC who had undergone curative partial hepatectomy were enrolled in this study. Transplantable HCC corresponding to Milan's criteria [2] was defined as single tumors less than 5 cm in diameter or with 3 nodules, each

* Corresponding author. Tel.: +81-82-257-5222; fax: +81-82-257-5224.

E-mail address: titamoto@hiroshima-u.ac.jp

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Table 1
Overall and disease-free survival rates according to clinicopathologic factors

	Overall survival (%)				Disease-free survival (%)			
	3-year	5-year	10-year	*P* value	3-year	5-year	10-year	*P* value
All cases (n = 136)	76	55	22	—	44	25	9	—
Age (y)								
≤60 (n = 56)	71	45	28	.5124	44	20	4	.6953
>60 (n = 80)	79	62	13		44	28	—	
Sex								
Male (n = 102)	77	55	24	.6752	42	26	7	.5979
Female (n = 34)	73	57	19		51	20	—	
Type of hepatitis virus*								
Non-HCV (n = 37)	88	75	48	.0086	70	37	19	.0055
HCV (n = 98)	72	49	14		35	21	—	
Child-Pugh grade								
A (n = 105)	79	62	25	.0031	51	29	12	.0058
B (n = 31)	62	25	—		20	10	0	
Preoperative AFP level								
≤400 (n = 114)	77	56	22	.6450	42	26	11	.9957
>400 (n = 22)	71	56	22		55	21	0	
Microscopic vascular invasion								
No (n = 108)	77	55	24	.4483	46	26	—	.3907
Yes (n = 28)	69	59	14		37	21	21	
Number of tumors								
Single (n = 111)	75	57	21	.8873	42	26	—	.2790
2 or 3 (n = 25)	76	46	25		52	18	18	
Tumor distribution								
Unilobar (n = 124)	74	54	22	.4653	44	25	9	.3477
Bilobar (n = 12)	88	66	—		48	—	—	
Microscopic capsular invasion								
No (n = 63)	74	50	16	.3297	51	27	—	.3771
Yes (n = 73)	77	60	29		39	23	12	
Histologic grading†								
Well (n = 26)	76	64	—	.5105	44	39	—	.6726
Moderate (n = 95)	77	53	21		43	23	—	
Poor (n = 6)	22	22	22		25	25	25	
Tumor stage‡								
I (n = 50)	77	61	27	.1268	55	32	—	.2989
II (n = 74)	78	54	20		40	22	8	
III (n = 12)	56	37	—		27	—	—	
CLIP score								
0 or 1 (n = 108)	77	58	22	.6317	44	26	—	.6545
2 or 3 (n = 28)	68	41	22		46	16	16	
Preoperative TAE								
No (n = 44)	72	49	—	.2809	49	27	—	.6038
Yes (n = 92)	77	58	23		42	24	9	
Type of hepatectomy								
Limited resection (n = 114)	72	53	24	.7406	43	26	11	.7680
Segmentectomy or more (n = 22)	90	67	—		49	24	0	
Histologic surgical margin (mm)								
≤5 (n = 67)	66	45	12	.0029	34	21	—	.0092
>5 (n = 69)	85	65	30		55	28	11	

AFP = α fetoprotein; CLIP = Cancer of the Liver Italian Program; TAE = transcatheter arterial embolization.
* Hepatitis C antibody was not measured in 1 patient.
† Histologic grading of the tumors in 9 patients could not be evaluated owing to 100% necrosis by TAE.
‡ The tumor stage was assessed according to the Liver Cancer Study Group of Japan.

less than 3 cm in diameter, without macroscopic vascular invasion or extrahepatic spread of the tumor. Curative partial hepatectomy was defined as removal of all recognizable tumors. Cirrhosis was confirmed by histologic examination of a resected specimen.

The 136 patients included 102 men and 34 women with a mean age of 61 years (range, 36–79 y). Mean and median sizes in diameter of the resected tumors were 2.4 cm and 2.2 cm, respectively. Data on age, sex, type of hepatitis virus, Child-Pugh class [11], preoperative α-fetoprotein level, mi-

Table 2
Results of Cox's proportional hazards analysis for survival after partial hepatectomy

Variables	Beta value	SE	*P* value	Relative risk	95% confidence interval
Histologic surgical margin (≤5 mm)	.666	.259	.0100	1.947	1.173–3.232
Child-Pugh grade (B)	.647	.295	.0282	1.909	1.072–3.401
Hepatitis virus (C)	.734	.335	.0286	2.084	1.080–4.020

croscopic vascular invasion, number of tumors, distribution of tumors in the liver, microscopic capsular invasion, histologic grading of the tumor, tumor stage, Cancer of the Liver Italian Program score [12], preoperative transarterial embolization, type of partial hepatectomy, and histologic surgical margin were collected and analyzed. Histologic grading of the tumor and tumor stage were examined according to the classification of the Liver Cancer Study Group of Japan [13].

The indication of hepatectomy was determined according to Child-Pugh classification. Child-Pugh class C was regarded as a contraindication for hepatectomy. The selection of type of hepatectomy was made on the basis of liver function and tumor location. Liver function was assessed by Child-Pugh classification and the indocyanine green retention rate at 15 minutes. Hepatectomy of less than one segment according to Couinaud's [14] segmentation was defined as limited resection. There was a tendency to select limited resection in cases of severe cirrhosis or tumors located on the surface of the liver.

Follow-up evaluation after the surgery consisted of clinical physical examinations, blood chemistry tests, and measurements of levels of tumor markers, including α-fetoprotein and des-gamma-carboxy prothrombin, every month for 2 years. After 2 years, the patients were assessed every 3 months. Patients were examined by ultrasound every 3 months and by computed tomographic scan every 6 months. When recurrence was indicated by any of these examinations, patients underwent hepatic angiography. The median follow-up period for survivors was 52 months (range, 15–139 mo).

Overall survival rates and disease-free survival rates were calculated using the Kaplan-Meier method and were compared using the log-rank test. Disease-free survival was calculated by considering any death or recurrence as an event. Independent and significant prognostic factors were assessed using multivariate Cox's proportional hazards model among the variables found to be significant by univariate analysis. Statistical significance was defined as P less than .05. All analyses were performed using SAS statistical software (SAS Institute Inc, Cary, NC).

Results

One patient died within 1 month after partial hepatectomy (mortality rate, .7%), and another 3 patients died during the initial hospital stay (in-hospital mortality rate, 2.9%). The causes of death of those 4 patients were liver failure (2 patients), intra-abdominal sepsis (1 patient), and severe enteritis caused by methicillin-resistant *Staphylococcus aureus* (1 patient).

The overall survival rates at 3, 5, and 10 years were 76%, 55%, and 22%, respectively, and the corresponding disease-free survival rates were 44%, 25%, and 9%, respectively.

Table 1 shows a summary of results of univariate analyses according to the clinicopathologic factors. Histologic surgical margin of 5 mm or less ($P = .0029$), Child-Pugh class B ($P = .031$), and hepatitis C virus (HCV) positivity ($P = .0055$) were significant adverse prognostic factors for survival (Table 1). Similarly, HCV positivity ($P = .0055$), Child-Pugh class B ($P = .0058$), and histologic surgical margin of 5 mm or less ($P = .0092$) were significant adverse prognostic factors for disease-free survival.

In the multivariate analyses, histologic surgical margin of 5 mm or less ($P = .01$ and $P = .0191$), Child-Pugh class B ($P = .0282$ and $P = .0407$), HCV positivity ($P = .0286$ and $P = .0191$) also were independent variables related to adverse survival and adverse disease-free survival, respectively (Tables 2 and 3).

The overall survival rates at 3, 5, and 10 years of 68 patients with none or only 1 of the 3 adverse prognostic factors were 90%, 73%, and 30%, respectively. The corresponding survival rates of 67 patients with 2 or 3 adverse prognostic factors were 61%, 36%, and 12%, respectively (Fig. 1). Disease-free survival at 3, 5, and 10 years of 68 patients with none or only 1 of the 3 adverse prognostic factors were 64%, 33%, and 15%, respectively. The corre-

Table 3
Results of Cox's proportional hazards analysis for disease-free survival after partial hepatectomy

Variables	Beta value	SE	*P* value	Relative risk	95% confidence interval
Hepatitis virus (C)	.611	.261	.0191	1.842	1.105–3.071
Histologic surgical margin (≤5 mm)	.506	.216	.0191	1.659	1.086–2.535
Child-Pugh class (B)	.503	.246	.0407	1.654	1.021–2.677

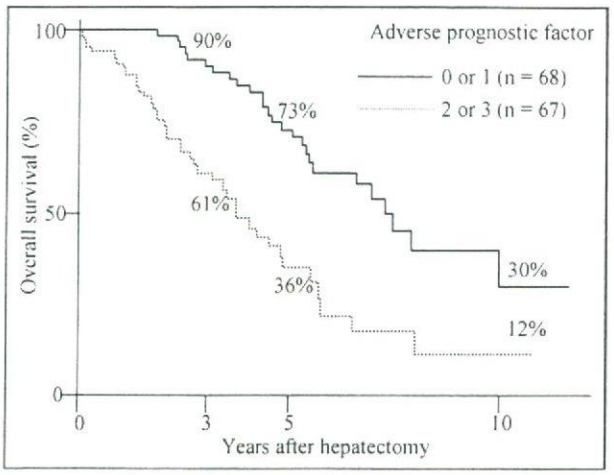

Fig. 1. Cumulative survival curves for patients with none or only 1 of the 3 adverse prognostic factors (n = 68) and patients with 2 or 3 adverse prognostic factors (n = 67). There was a significant difference between these 2 groups (P <.0001).

sponding disease-free survival rates of 67 patients with 2 or 3 adverse prognostic factors were 23%, 17%, and 0%, respectively (Fig. 2). There were significant differences between these 2 groups (P <.0001).

Table 4 shows the patterns of recurrence, disease-free intervals, and treatments for recurrence. Cancer recurrence occurred in 83 (61.0%) of the 136 patients, with a median time to recurrence of 22.0 months (range, 1–83 mo). Patterns of recurrence were divided into 2 groups. One group was transplantable recurrence corresponding to Milan's criteria [2]. The other group was more advanced recurrence. At the time of diagnosis of HCC recurrence, the patterns of recurrence were transplantable recurrence in 57 (67%) patients and advanced recurrence in 26 (23%) patients. Advanced recurrence included recurrences in the liver remnant without distant metastasis in 22 patients and recurrences with distant metastasis in 4 patients (Table 4). There was no significant difference in disease-free interval between these 2 groups. Two patients who underwent living-donor liver transplantation for recurrences, 1 patient at 1 year and 7 months and 1 patient at 6 years and 4 months after initial hepatectomies, are alive without cancer recurrence. The overall survival rates after the diagnosis of HCC recurrence at 3 and 5 years were 64% and 31% in patients with transplantable recurrence, respectively, and 10% and 5% in patients with advanced recurrence, respectively (Table 4). There was a significant difference between these 2 groups (P <.0001).

Discussion

Although numerous previous studies have reported the prognostic factors in resectable HCC patients [15–20], there were few reports [9] of cirrhotic patients with transplantable HCC corresponding to Milan's criteria [2]. It is very important to clarify the prognostic factors in cirrhotic patients with transplantable and resectable HCC for deciding the indications of partial hepatectomy or OLT. The present study clarified that a histologic surgical margin of 5 mm or less, HCV positivity, and Child-Pugh class B were significant and independent adverse prognostic factors for both overall survival and disease-free survival in patients with transplantable HCC. No tumor factors such as vascular invasion or the number of tumors were included in the adverse prognostic factors. The results were different from those of other investigations that were designed for resectable HCC patients [15–20]. The reason was that we designed this study for cirrhotic patients with resectable and transplantable HCC who met Milan's criteria, excluding HCC patients with macroscopic tumor thrombus, large tumors, or 4 or more nodules.

In patients with HCC, securing a wide surgical margin at surgery may prevent microscopic metastases and/or microscopic tumor thrombus being left around the resected tumor and may prevent subsequent recurrences [21]. We treated HCC patients by partial hepatectomy while keeping a sufficient margin of more than 1 cm as far as possible. Consequently, we failed to secure a 5-mm margin histologically in half of our patients owing to unexpected tumor extension microscopically or undesirable tumor location [22–24]. When a tumor is located near the large glissons or hepatic veins, major hepatectomy must be performed to secure a sufficient surgical margin. However, major hepatectomy cannot be used in many cirrhotic patients. Although the surgical margin is determined definitively after pathologic examination of the resected specimen, we could predict preoperatively with various imaging modalities whether we would keep a sufficient margin or not at surgery. The majority of Child-Pugh class B patients undergo partial hepatectomy with insufficient surgical margin because of their poor hepatic functional reserve and cannot be treated inten-

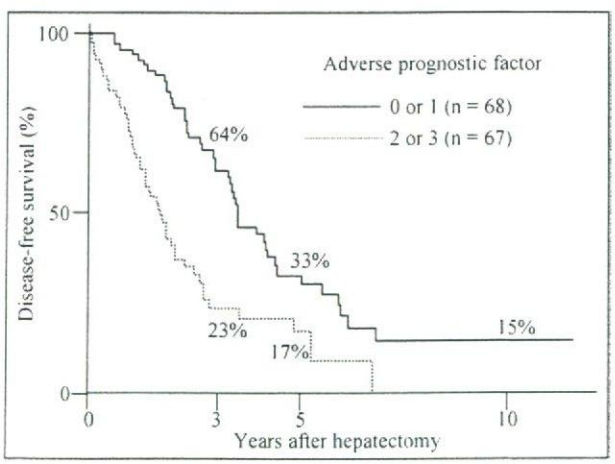

Fig. 2. Disease-free survival curves for patients with none or only 1 of the 3 adverse prognostic factors (n = 68) and patients with 2 or 3 adverse prognostic factors (n = 67). There was a significant difference between these 2 groups (P <.0001).

Table 4
Patterns of recurrence, disease-free interval, treatments for recurrence, and survival after recurrence

Pattern of recurrence	N (%)	Disease-free interval (mo)			Treatments for recurrence	N	Survival after recurrence (%)	
		Mean	Median	Range			3-year	5-year
Transplantable	57 (69)	29	24	3–83	Partial hepatectomy	19	64	31
					Living donor liver transplantation	2		
					Open MCT or RFA	5		
					Percutaneous ablation therapy*	30		
					TAE	37		
Advanced	26 (31)	23	12	1–74	Partial hepatectomy	3	10	5
					Open MCT or RFA	1		
					Resection of distant metastasis	5		
					Percutaneous ablation therapy*	3		
					TAE	22		

MCT = microwave coagulation therapy; RFA = radiofrequency ablation; TAE = transcatheter arterial embolization.
* Percutaneous ablation therapies included percutaneous ethanol injection, MCT, and RFA.

sively when they have recurrences in the remnant liver. Moreover, it has been reported that multicentric HCC occurred more frequently in patients with poor hepatic functional reserve than in those with good hepatic functional reserve [25]. HCV-positive patients have multicentric recurrences more often than do hepatitis B virus–positive patients [26,27]. Therefore, the results regarding adverse prognostic factors obtained in the present study are reasonable considering the behavior of HCC arising from a cirrhotic liver and the hepatic functional reserve of cirrhotic patients.

Partial hepatectomy could have a good outcome (5-year survival rate of 73%) for selected cirrhotic patients with transplantable HCC who have none or only 1 of the 3 adverse prognostic factors described earlier. However, the results of partial hepatectomy in patients with 2 or 3 adverse prognostic factors were not satisfactory (5-year survival rate of 36%), and those patients, therefore, might not have been appropriate candidates for partial hepatectomy.

OLT results in the widest possible resection margin for the cancer, removes the remaining liver tissue that is at risk for the development of HCC, and restores hepatic function. It is a logical treatment for HCC and should be the first choice of treatment for patients in whom the outcome of partial hepatectomy was not good. Patients with 2 or 3 adverse prognostic factors might have been candidates for OLT, if possible, not partial hepatectomy, as an initial treatment of HCC. Unfortunately, the limited availability of donor organs with the resulting delay in transplantation makes OLT less effective and less available to individual patients. Living-donor liver transplantation eliminates many of these obstacles if the patient has a suitable living donor.

About 70% of the HCC patients with recurrence after partial hepatectomy in this study had a transplantable HCC when diagnosed as having HCC recurrence. Although patients with transplantable recurrence had a better prognosis than that of patients with advanced recurrence, the 5-year survival rate after diagnosis of recurrence was only 31% despite intensive treatment. Accordingly, salvage transplantation [28,29] may be applicable to such patients and improve the prognosis of HCC patients with recurrence in the remnant liver. Results of long-term follow-up studies using larger numbers of patients are required to establish the role of OLT for recurrent HCC.

In conclusion, the significant and independent adverse prognostic factors in transplantable HCC patients with compensated cirrhosis who have undergone partial hepatectomy include the impossibility of securing a sufficient margin, Child-Pugh class B, and HCV positivity. Patients with none or only 1 of the 3 adverse prognostic factors have a good indication of partial hepatectomy as an initial treatment for HCC. However, patients with 2 or 3 adverse prognostic factors should be considered as candidates for OLT. Salvage liver transplantation as a novel strategy may improve the prognosis of selected patients with recurrence in the remnant liver after partial hepatectomy.

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