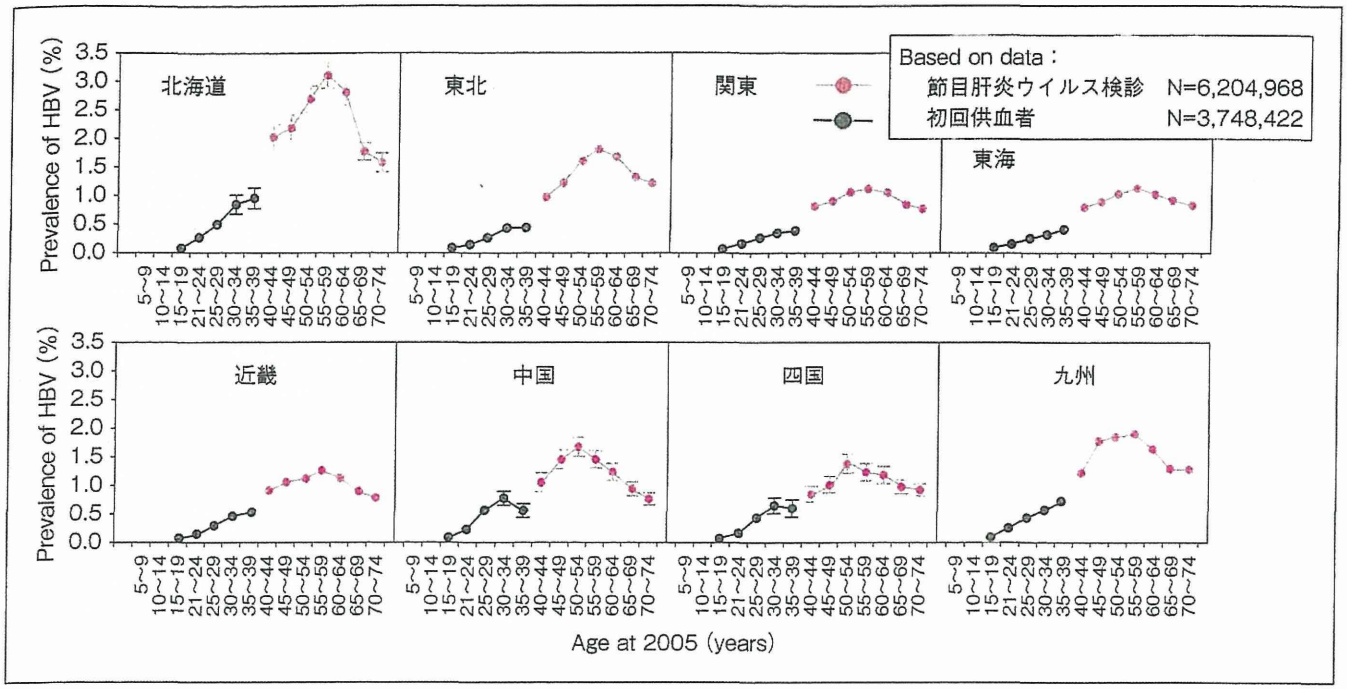

図6 地域別年齢階級別にみたHBVキャリア率 (文献5より引用)

年齢とキャリア率の傾向は地域により高低差が認められるものの，全国で共通に認められている．

V 感染を知らないまま社会に潜在するHBVキャリア数の推計

B型肝炎ウイルスに持続感染している人（キャリア）がどのくらいいるのかを把握することは，社会に対して疾病が与える規模（burden）を測るうえでも重要であり，また，肝癌へ進行する可能性のある人数規模や地域年齢偏在を把握することは，治療戦略や肝癌対策の基礎資料になる．

しかし，肝炎ウイルスに感染している人のほとんどは自覚症状がなく，特にHBVの持続感染者の場合は，肝臓の状態が進行してもなかなか自覚症状が現われないという特性をもっているため，その数を正確に把握することは困難と考えられる．厚労省研究班[7]では，肝炎ウイルスに持続感染している人の社会での存在状態別の人数の把握を，これまでの疫学的調査成績や患者調査，数理疫学手法などを用いて試みている．

まず，前項に示した二つの大規模集団から得られた地域別・年齢階級別HBVキャリア率を用いて，HBVキャリア数の推計を行ったところ，2005年時点では，903,145人（95％ CI：83.7～97.0万人）と算出[5]された（図7）．この値は，初回供血者集団および肝炎ウイルス検診受診者集団を元にした推計値であることから，自身が「感染を知らないまま潜在しているキャリア」の推計数に相当していると考えられる．この集団に対しては，肝炎ウイルス検査受検の機会を設け，自覚症状がなくても一度は肝炎ウイルス検査を受けることを進めることが望ましい．

なお，肝炎ウイルス持続感染者数の全体把握には，「感染を知らないまま潜在しているキャリア」数の他に，「患者としてすでに通院・入院しているキャリア」と「受診しないでいる，あるいは継続受診に至っていないキャリア」，「新規感染によるキャリア」の把握が必要と考えられ，現在，さまざまなアプローチで検討が行われているところである．特に，HBVに関しては，「感染を知ったが医療機関を受診しないでいる，あるいは継続的な医療機関受診には至っていないキャリア」が多いものと推察される．

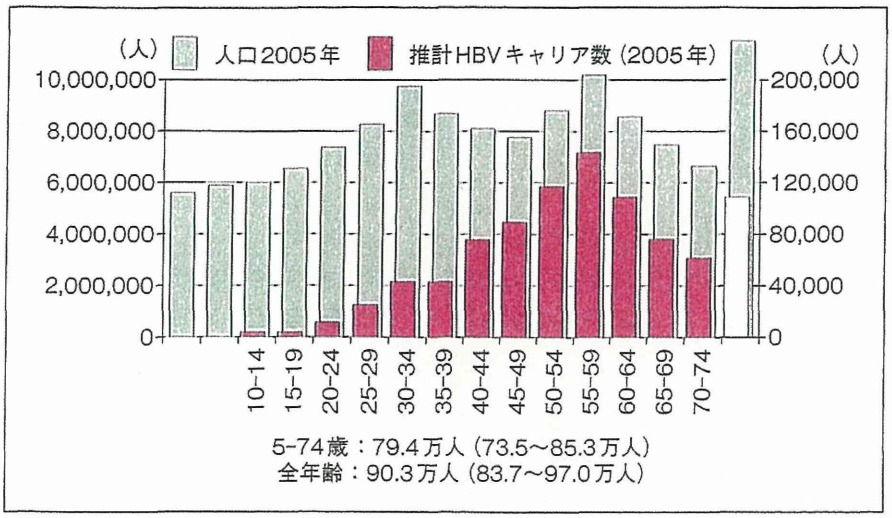

図7 自覚症状がなく社会に潜在するHBVキャリアの推計数
（文献5より引用）

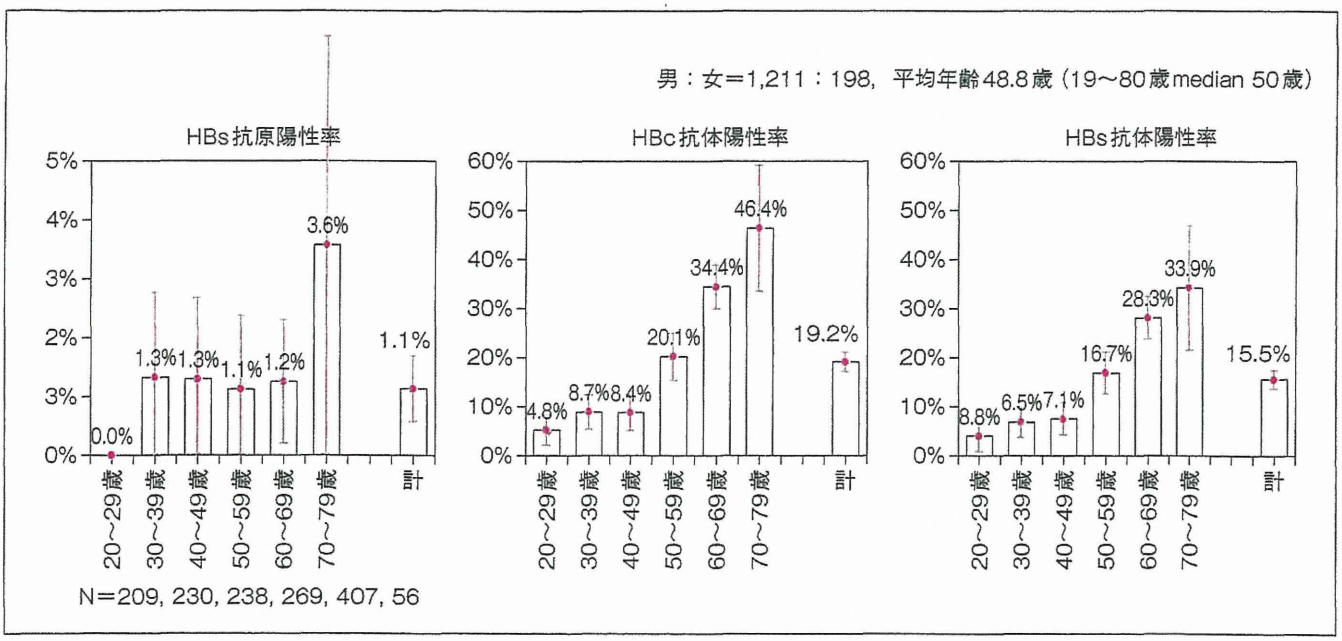

図8 職域集団におけるパイロット調査
年齢別にみたHBs抗原，HBc抗体，HBs抗体陽性率
2011～2012年　広島　N＝1,409人

肝炎ウイルス検査の推進と同時に，感染が判明した場合には必ず肝臓専門医により宿主側とウイルス側の特性を元にした診断を定期的に受け，その後の治療継続と定期的なフォローアップが重要であると考えられる．

Ⅵ 職域集団および妊婦集団のHBV感染状況

出前検査による職域集団および妊婦集団におけるHBV感染状況成績[8]を示す．

職域集団を対象とした「肝炎ウイルス検査」結果を示す（図8）．運輸・サービス業に従事する1,409人（男性1,211人，女性198人；平均年齢：48.8±14.1歳，19歳～80歳）のHBs抗原陽性率は全体で1.1％（95％CI：0.58～1.69％）であり，70歳代では3.6％と高いキャリア率を示した．HBs抗体陽性率とHBc抗体陽性率は全体で15.5％（95％ CI：13.7～17.4％），19.2％（95％CI：17.1～21.2％）であり，両者の組み合わせに

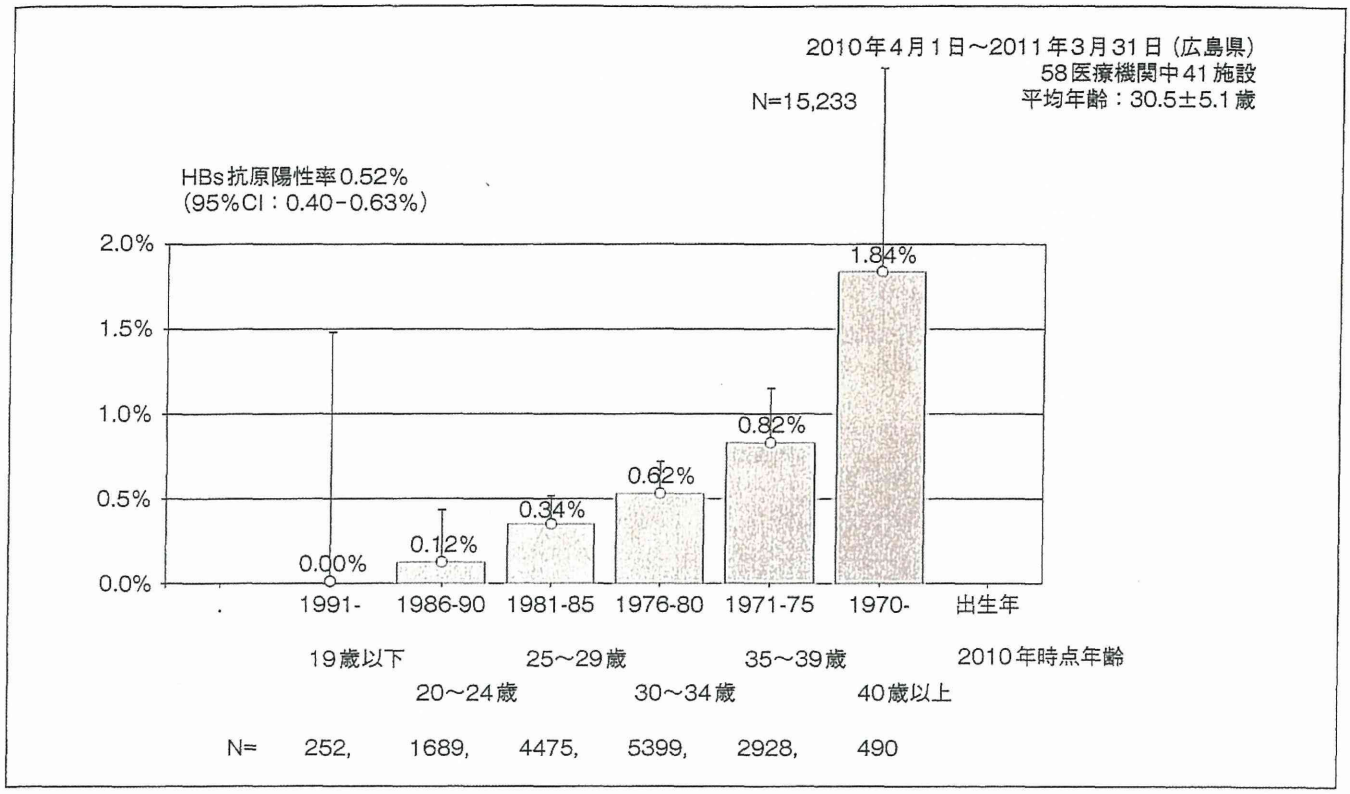

図9 妊婦集団におけるHBs抗原陽性率

（文献7より引用）

よるHBV曝露率は19.5％（95％CI：17.4～21.6％）となった．

次に，広島県の産婦人科を有し分娩を行っている医療機関58施設中協力の得られた41施設を対象に，2010年度に分娩をした妊婦15,233人（平均年齢；30.5±5.1歳，2009年度出生数25,596人の約6割）のHBV感染状況を示す（図9）．HBs抗原陽性率は，全体では0.52％（95％CI：0.40～0.63％）であったが，1986年以降に出生した年齢の若い集団では，HBs抗原陽性例はわずか2例であった．

Ⅶ HBV母子感染の予防対策とその効果

わが国の過去のHBV感染の主な感染経路は母子感染によるものであったことから，HBV母子感染防止事業が1986年出生児を対象として全国規模で実施された．HBVキャリアの母親から出生する児を対象にHBVワクチンとHBIG投与による公費負担による予防対策である．

この事業実施前後に出生した集団のHBs抗原陽性率を岩手県予防協会の成績[9,6]をもとに比較すると，実施前1978～80年に出生した集団では0.75％（対象10,437例），治験開始から事業開始直前1981～85年出生集団では0.22％（対象20,812例），事業開始以後1986～94年出生集団では0.04％（対象56,212例）と激減したこと，また，HBs抗体陽性者に占めるHBc抗体陽性率を同様に比較した成績から事業実施以後のHBs抗体獲得者のほとんどがワクチン接種によるものであることから，わが国のHBV母子感染防止事業は効果的に実施されたことが示されている．

1986年以降の出生集団のHBs抗原陽性率，HBc抗体陽性率は大規模集団の成績からみるときわめて低く，この世代が肝発癌年齢を迎える数十年後にはHBVの持続感染による肝癌は減少していくものと推察される．

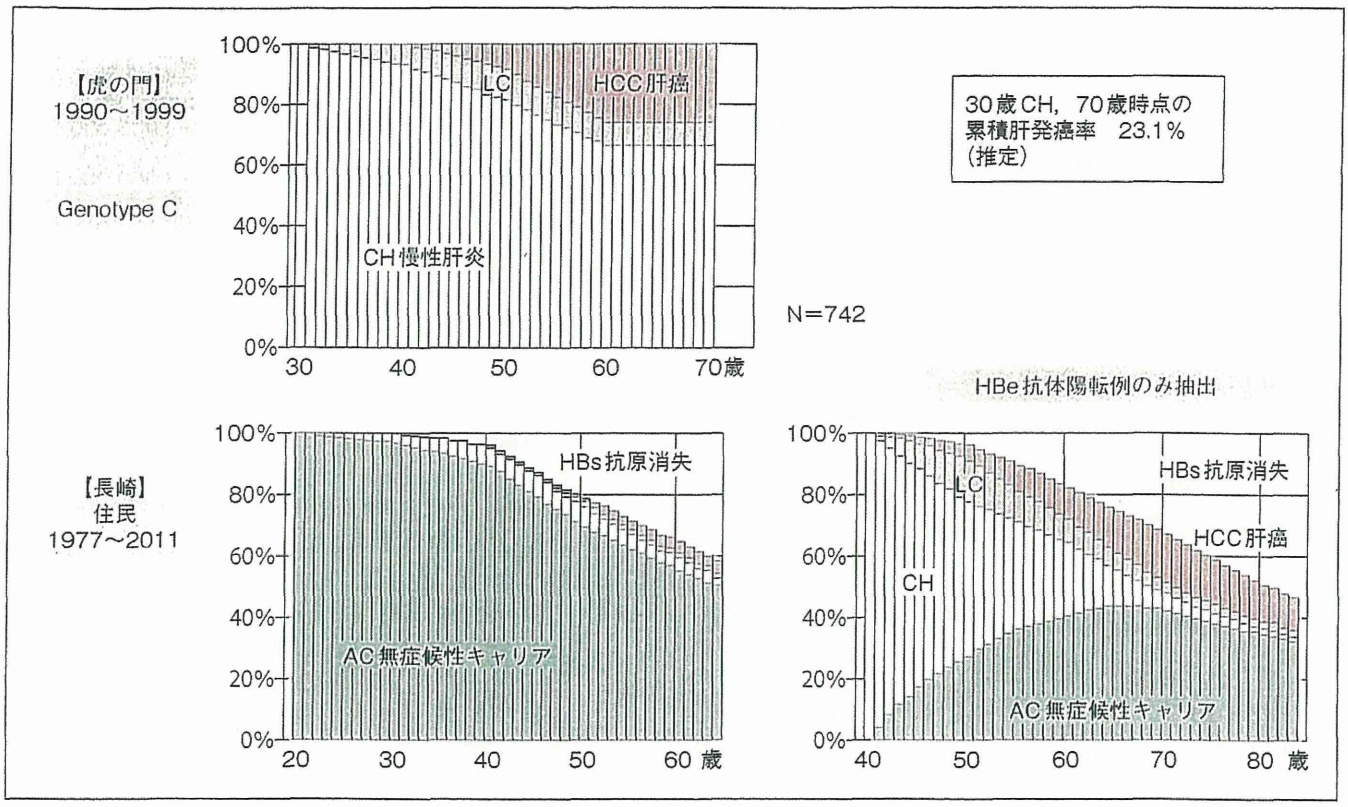

図10 HBV キャリアの自然病態推移
Markov モデルによる数理疫学的推定【抗ウイルス治療介入なしの場合】　（文献 11 より引用）

Ⅷ HBV 持続感染者の自然病態推移 －マルコフモデルによる推定－

HBV 持続感染者の自然病態推移を明らかにすることは，治療介入の必要性や治療効果を評価する際には重要[10,11]である．病院受診群と住民検診群の長期観察資料を元に，数理疫学的手法（Markov 確率モデル）を用いて治療介入のない（抗ウイルス療法をしていない）場合の病態推移を推定[11]し，図 10 に示す．

病院受診した HBV キャリア 742 例（平均観察期間 8.0 年，平均年齢 37.7 歳±12.4，5,632 unit）から genotype C のみ抽出すると，男性 30 歳時点慢性肝炎を起点とした 70 歳時点の累積肝発癌率は男性では推定 23.1％となった．一方，住民検診で発見された HBV キャリアの長期フォロー群 938 例（13,603 unit）では，男性 20 歳時点無症候性キャリアを起点とした 60 歳時点の累積肝発癌率は 3.9％，累積 HBs 抗原消失率は 35.1％と推定された．

さらに，セロコンバージョン例のみを対象として解析を行うと，男性 40 歳時点慢性肝炎を起点とした 70 歳時点の累積慢性肝炎は 5.6％と低いが累積肝発癌率は 16.2％，また，累積 HBs 抗原消失率は 32.6％，無症候性キャリアに落ち着いているのは 42.6％と多様な病態が混在して推定されることが示された．

これらの病態の進展の相違には宿主側あるいはウイルス側の要因が関連していると考えられ，今後さらに，臨床疫学的，ウイルス学的解析が期待されている．

Ⅸ HBV 感染の今後の動向

疫学的視点から，HBV 感染は，感染経路（水平感染，母子感染），感染時期（幼児期，成人期），病態（急性感染，持続感染）に分けて考える必要

がある．

これまでの調査資料からHBV母子感染防止事業開始以降の出生集団（27歳以下，2013年時点）ではきわめて低いHBs抗原陽性率を示していることから，わが国のHBV垂直感染防止は効果をあげたといえる．次世代には母子感染よるHBVキャリアの発生はほぼ消滅することが期待される．

わが国に存在しているHBVキャリアが適切な治療を受けられるよう，治療へ導入するための肝炎ウイルス検査の推進，受検率の低い職域集団への介入，継続受診の必要性強化などはじめとするキャリア対策をさらに進める必要があると考えられる．

また同時に，根本的なHBV撲滅のためには，幼児期および思春期の水平感染防止対策，治療に伴う *de novo*B型肝炎の実態，HBs抗原消失例の臨床病態などをはじめとする，最新のB型肝炎ウイルス感染に関する疫学的・臨床的エビデンスを元に動向をみて行く必要がある．

（田中純子）

文献

1）World Health Organitation Fact Sheet. July 2013
2）Yang Baoping. International Expert Meeting on Hepatitis B Control in the West Pacific,Seoul, 24-25 Nov 2008
3）平成23年（2011）人口動態統計（確定数）の概況．厚生労働省，統計情報・白書 厚生労働省HP：http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei11/
4）日本肝癌研究会：第5回～第18回全国原発性肝癌追跡調査報告，日本肝癌研究会事務局，1982-2009
5）Tanaka J, Koyama T, Mizui M, et al.：Total numbers of undiagnosed carriers of hepatitis C and B viruses in Japan estimated by age-and area-specific prevalence on the national scale. Intervirology 54；185-195, 2011
6）小山 富子　他：岩手県におけるB型肝炎ウイルス・C型肝炎ウイルスの感染状況について．平成24年度厚生労働科学研究費補助金肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究」班　報告書2013
7）大規模集団のキャリア率をもとにしたキャリア数推計の試み　平成21,22年度　肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究」班　報告書，2010, 2011
8）妊婦集団における肝炎ウイルス感染（HBV/HCV）状況調査／職域集団における肝炎ウイルス感染状況に関する研究，平成23年度　厚生労働科学研究費補助金肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究」班　報告書2012
9）Koyama T, Mito H, Takahashi K et al.：Perinatal hepatitis B virus infection in Japan, Congenital and Other Related Infectious Diseases of the Newborn, Elsevier B. V. p141-151, 2006
10）Tanaka J, Kumada H, Ikeda K et al.：Natural histories of hepatitis C virus infection in men and women simulated by the Markov model. J Med Virol, 70；378-386, 2003
11）肝炎ウイルス持続感染者の病態推移に関する数理疫学的研究，平成24年度　厚生労働科学研究費補助金肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究」班　報告書2013

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REVIEW ARTICLE

Prevention of recurrence after curative treatment for hepatocellular carcinoma

Tsuyoshi Kobayashi · Kohei Ishiyama · Hideki Ohdan

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Abstract Hepatocellular carcinoma often recurs even after curative treatment. In addition to its high frequency of metastasis, hepatocellular carcinoma recurrence is characterized by multicentric carcinogenesis arising in the liver damaged by viral infection with the hepatitis B or hepatitis C virus. This is considered to complicate the initial treatment and recurrence prevention strategy for hepatocellular carcinoma, and accordingly, there is no established adjuvant therapy to prevent recurrence. Preventive adjuvant therapy should be administered to high-risk patients, and should be optimized based on individual risk factors. This review will summarize the current status and future prospects of preventive therapy for the recurrence of hepatocellular carcinoma after curative treatment. Although transcatheter arterial embolization/chemoembolization prior to curative treatment can induce tumor necrosis in some patients, several studies have failed to show any improvement in survival. Postoperative interferon therapy may contribute to prolonging the survival in specific groups of patients. No established adjuvant therapy against advanced hepatocellular carcinoma that prevents metastasis has been established so far. Novel treatment strategies incorporating molecular and immunological mechanisms are expected in the future.

Keywords Hepatocellular carcinoma · Adjuvant therapy · Recurrence

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Introduction

Hepatocellular carcinoma (HCC) often recurs even after curative treatment. In addition to its high frequency of metastasis, HCC recurrence is characterized by multicentric carcinogenesis arising in the liver damaged by infections with hepatitis B virus (HBV) and hepatitis C virus (HCV). This is considered to complicate the initial treatment and recurrence prevention strategies for HCC.

According to the Clinical Practice Guidelines for Hepatocellular Carcinoma—The Japan Society of Hepatology 2009 update, the choice of treatment for HCC is based on three factors: the degree of liver damage, the number of tumors and the tumor diameter [1]. Treatments include liver resection, local ablation therapy, transcatheter arterial embolization/chemoembolization (TAE/TACE), hepatic arterial infusion chemotherapy, liver transplantation and palliative care. Among these treatments, liver resection, local ablation therapy and liver transplantation are considered to be curative treatments. However, HCC frequently recurs even after curative treatment, and there is no established adjuvant therapy to prevent recurrence. Such preventive adjuvant therapy should be administered to high-risk patients, and the administration of such treatments should be optimized based on individual risk factors. This review will summarize the current status and future prospects of preventive therapy for HCC recurrence after curative treatment.

Risk factors for the recurrence of hepatocellular carcinoma

The risk factors for HCC recurrence after curative treatment include the tumor stage, vascular invasion, number of tumors, tumor size, capsular formation and liver function [1, 2].

Among these factors, the stage, vascular invasion, number of tumors, tumor size and capsular formation are considered to be related to metastasis. Liver function, however, is likely to be primarily related to the multicentric nature of carcinogenesis. The strategy selected for adjuvant therapy should be based on an individual's risk factors; patients at a high risk of metastasis are good candidates for metastasis-preventing therapies. In high-risk patients with multicentric carcinogenesis, such as those with HCV- or HBV-positive HCC, preventive therapy should include aggressive antiviral therapy.

The vascular invasion and the degree of tumor differentiation play important roles in tumor recurrence after liver transplantation in patients with HCC [2]. Liver transplantation simultaneously treats both the background liver disease and liver tumors; therefore, factors related to the background liver disease are not likely to influence recurrence after transplantation. Cases of early recurrence after transplantation are often caused by the progression of pre-existing micro-metastases, or the implantation of tumor cells circulating in the peripheral blood. Therefore, an effective preventive strategy should focus on providing antitumor effects against these remaining cells. In addition, after liver transplantation, patients must be treated with immunosuppressive agents. Accordingly, it is important to take preventive measures against the reactivation of hepatitis viruses in hepatitis virus-infected patients.

Adjuvant therapy prior to curative treatment

Transcatheter arterial embolization/chemoembolization (TAE/TACE) is a procedure wherein embolic material is introduced into the hepatic artery with or without an antitumor agent. This treatment has been administered to patients with unresectable HCC. Two randomized controlled trials (RCT) demonstrated that TAE/TACE improved the antitumor effect and survival rate compared with conservative treatment in patients with unresectable HCC [3, 4]. TAE/TACE is recommended for patients with advanced hepatocellular carcinoma, which is inoperable, and who are not candidates for local ablation therapy in the Clinical Practice Guidelines for Hepatocellular Carcinoma—The Japan Society of Hepatology 2009 update [1]. TAE/TACE is also administered as a preoperative chemotherapy for resectable HCC prior to hepatic resection or local ablative therapy. More recently, it has been administered prior to liver transplantation.

TAE/TACE prior to hepatic resection

A number of studies have reported inconsistent results on the effect of TAE/TACE prior to hepatic resection. Some reports suggest that TAE/TACE improves the prognosis, while others do not. To date, three RCTs have been reported, and none has shown an improvement in the recurrence-free survival, which was the primary endpoint (Table 1) [5–7]. Although the trials differed in terms of their patient inclusion criteria and embolization methods, the results have been uniformly negative.

TAE/TACE prior to local ablative therapy

The Clinical Practice Guidelines for Hepatocellular Carcinoma—The Japan Society of Hepatology 2009 update recommend that good candidates for local ablation therapy are patients with liver function graded Child–Pugh class A or B, and three or fewer tumors measuring 3 cm or less in diameter [1]. Conventionally, percutaneous ethanol injection (PEI) has been administered; more recently, radiofrequency ablation (RFA) has become popular. However, the

Table 1 Randomized clinical trials about TAE/TACE before curative treatment for hepatocellular carcinoma

References	Cases	Inclusion criteria	Regimen	Treatment	5-year survival (vs. control)	5-year disease-free survival (vs. control)	Result
Wu et al. [5]	52	Larger than 10 cm	TACE (EPI)	RES	40 vs. 50 % (3 years)	32 vs. 60 % (3 years)	Not effective
Yamasaki et al. [6]	97	Solitary, 2 to 5 cm	TAE	RES	62.7 vs. 61.7 %	39.1 vs. 31.1 %	Not effective
Koda et al. [8]	52	Smaller than 3 cm	TACE (EPI)	PEI	40.4 vs. 37.7 %	19.3 vs. 80.1 %[a] (3 years)	Effective (recurrence)
Akamatsu et al. [9]	42	Uninodular	TAE	PEI, RFA	82.4 vs. 82.2 % (3 years)	33.8 vs. 34.3 % (3 years)	Not effective
Zhou et al. [7]	108	Larger than 5 cm	TACE (CDDP, MMC, FU)	RES	30.7 vs. 21.1 %	12.8 vs. 8.9 %	Not effective

TAE transcathether arterial embolization, *TACE* transcathether arterial chemo-embolization, *EPI* epirubicin, *CDDP* cisplatin, *MMC* mitomycin C, *FU* fluorouracil, *RES* resection, *PEI* percutaneous ethanol injection, *RFA* radiofrequency ablation

[a] Recurrence rates

rate of local recurrence after these procedures is high. The use of local ablative therapy preceded by TAE/TACE has been compared to local ablative therapy alone in two RCTs (Table 1) [8, 9]. One RCT compared combination therapy with PEI alone, and the other compared combination therapy with PEI or RFA alone. The results of these studies showed that combination therapy significantly reduced the local recurrence compared with PEI alone, but not with the RFA alone [9]. Neither of the studies found an improvement in the survival rate; therefore, this approach is not recommended in the various treatment guidelines [1, 2, 10].

TAE/TACE prior to liver transplantation

It is not yet clear whether preoperative preventive therapy in patients with HCC improves their prognosis after liver transplantation [11]. Several studies have investigated whether preoperative TACE reduces the recurrence of HCC after liver transplantation, but these were retrospective studies, and no RCT has been reported. Decaens et al. [12] reported no significant difference in the 5-year survival rates (59.4 vs. 59.3 %) and 5-year disease-free survival rates (69.3 vs. 64.1 %) between patients treated with preoperative TACE ($n = 100$) and those without adjuvant therapy ($n = 100$) in a multicenter case–control study. Although other studies have shown a favorable prognosis after transplantation in patients who responded well to TACE, and have demonstrated the efficacy of preoperative TACE in reducing the dropout rate while patients were waiting for liver transplantation, there is no evidence demonstrating that TACE improves the overall or recurrence-free survival [13–15].

Adjuvant therapy after curative treatment

Antiviral therapy in patients with viral hepatitis after curative treatment

Virus eradication by interferon therapy is effective against chronic hepatitis C and compensated cirrhosis type C. Because patients with HCC frequently have viral hepatitis, interferon therapy is administered after curative treatment to eradicate the virus and to repress inflammation. Eight RCTs have investigated the effectiveness of interferon therapy after curative treatment of HCC (Table 2) [16–23]. Shiratori et al. administered PEI to 74 HCV-positive HCC patients with three or fewer tumors; 49 of these patients were subsequently treated with interferon. Interferon therapy did not change the rate of recurrence, but significantly improved the survival [18]. On the other hand, Mazzaferro et al. [21] performed hepatic resection in 150 HCV-positive patients with HCC; 76 of these patients were subsequently treated with interferon. The 5-year recurrence-free survival rate in those treated with interferon (24.3 %) was not significantly different from those not treated with interferon (5.8 %). In summary, one of the RCTs found interferon to be effective in reducing recurrence and improving survival after the curative treatment for HCC, while the other showed a limited effect of interferon in a selected subgroup. However, several meta-analyses of multiple studies have shown that interferon therapy reduced the recurrence and prolonged the survival rate [24–29].

Although interferon was used alone in these studies, the current standard of care for treating chronic hepatitis C is the use of pegylated-interferon in combination with

Table 2 Randomized clinical trials about adjuvant interferon therapy after curative treatment for hepatocellular carcinoma

References	Cases	Inclusion criteria	IFN	Treatment	5-year survival (vs. control)	5-year recurrence rates (vs. control)	Result
Ikeda et al. [16]	20	HCV	IFN beta	RES, PEI	ND	0 vs. 100 % (2 years)	Effective (recurrence)
Kubo et al. [17]	30	HCV	IFN alpha	RES	ND	ND	Effective (survival)
Shiratori et al. [18]	74	HCV	IFN alpha	PEI	68 vs. 48 %	80 vs. 92 %	Effective (survival)
Lin et al. [19]	30	HBV, HCV	IFN alpha	PAI	ND	47 vs. 90 % (4 years)	Effective (recurrence)
Sun et al. [20]	236	HBV	IFN alpha	RES	63.8 vs. 38.8 m[a]	31.2 vs. 17.7 m[b]	Effective (survival)
Mazzaferro et al. [21]	150	HCV	IFN alpha	RES	63.6 vs. 52.4 %	24.3 vs. 5.8 %[c]	Not effective
Lo et al. [22]	80	HBV	IFN alpha	RES	79 vs. 61 %	ND	Not effective
Chen et al. [23]	268	HBV, HCV	IFN alpha	RES	75.4 vs. 72.5 %	42.7 vs. 45.5 %[c]	Not effective

HCV hepatitis C virus, *HBV* hepatitis B virus, *IFN* interferon, *RES* resection, *PEI* percutaneous ethanol injection, *PAI* percutaneous acetic acid injection, *ND* not described

[a] Median survival time

[b] Median disease-free survival time

[c] Recurrence-free survival rates

ribavirin. This combination therapy has demonstrated a higher rate of sustained virological response (SVR) than interferon monotherapy. Some studies have also evaluated its effectiveness as an adjuvant therapy after curative treatment for HCC [30, 31]. Our recent report showed that postoperative administration of pegylated-interferon plus ribavirin in patients with HCV-positive HCC resulted in a 5-year survival rate of 91.7 %, which was significantly higher than that of the historical control group (50.6 %) [31]. In addition, two recent RCTs have investigated telaprevir, an HCV genotype 1 protease inhibitor. The ADVANCE trial investigated the effect of adding telaprevir to the pegylated-interferon plus ribavirin combination therapy (PR group) in 1,088 untreated genotype 1 hepatitis C patients [32]. Telaprevir was used with the combination therapy for either 12 weeks (T12PR) or 8 weeks (T8PR). The SVR was 44 % in the PR group, compared with 75 and 69 % in the T12PR and T8PR groups, respectively. This demonstrates a significant additive effect of the combination therapy. The REALIZE trial investigated the effect of adding telaprevir to pegylated-interferon plus ribavirin combination therapy (PR group) in 663 treated genotype 1 hepatitis C patients [33]. The telaprevir combination therapy was used either for 12 weeks (T12PR) or for 12 weeks after an initial 4 weeks of PR and was followed by 32 weeks of PR (lead-in T12PR48). The SVR was 17 % in the PR group as compared with 64 and 66 % in the T12PR and lead-in T12PR48 groups, respectively, demonstrating a significant improvement. Based on these positive outcomes, telaprevir combination therapy is likely to become a standard therapy used for HCV genotype 1 hepatitis. Although telaprevir is not recommended for elderly patients, patients with thrombocytopenia or with low hemoglobin, this drug is expected to be useful as an adjuvant therapy for selected patients after curative treatment for HCC.

Nucleoside analogues are effective against hepatitis B. The results of an RCT demonstrated that lamivudine suppressed carcinogenesis arising from hepatitis B [34]. Nucleoside analogues suppress the replication of HBV, repress inflammation and reverse liver fibrosis. Although nucleoside analogues are expected to be used an adjuvant therapy in patients with hepatitis B virus, no prospective randomized studies have demonstrated its efficacy. It may be difficult to perform an RCT with or without nucleoside analogues after curative treatment for HCC, because these drugs are already recommended for patients with HBV, especially patients with a high viral load.

Adjuvant chemotherapy after curative treatment

HCC is generally insensitive to anti-cancer drugs, and the response rate to systemic chemotherapy against unresectable advanced HCC is less than 20 %. In many cases, HCC develops on a background of chronic liver disease; accordingly, worsening liver function can lead to insufficient dosing or a deteriorated prognosis. Until the introduction of sorafenib, there was no standard therapy with proven efficacy for unresectable HCC [35]. Hepatic arterial infusion chemotherapy is considered to result in a high local concentration and have fewer adverse systemic effects, because the systemic concentration of the anti-cancer drug is reduced. A number of small RCTs have investigated the use of various adjuvant chemotherapies for reducing the rates of metastasis and recurrence after curative treatment of HCC (Table 3) [36–47]. Oral, intravenous and intrahepatic arterial administration routes have all been investigated individually or in combination. Although two previous RCTs have reported that neither 1-hexylcarbamoyl-5-fluorouracil nor uracil-tegafur could reduce the recurrence of HCC, a recent small RCT reported that oral administration of capecitabine reduced the recurrence rate [36–38]. There is currently no evidence that intravenous chemotherapy is effective. The most frequent route of administration is intrahepatic arterial administration, the efficacy of which was demonstrated in a meta-analysis [48]. However, the included studies were single center experiences, with a small number of patients, and incorporated various treatment regimens. As such, there is no established evidence for a single treatment protocol. Still, some RCTs showed reduced recurrence of HCC with portal vein tumors and improved survival in a population with limited inclusion criteria [39, 45–47]. Because HCC is considered to progress via the portal vein, and patients with complicating portal vein tumors have an increased risk of metastasis and recurrence, hepatic arterial infusion chemotherapy is considered to be important in these patients. Therefore, multicenter studies appropriately designed to target patients with a high risk of metastasis are needed for further investigations.

Sorafenib is a molecule that selectively suppresses receptor tyrosine kinases, including the VEGF receptor and PDGF receptor, as well as the serine/threonine kinase Raf in the MAP kinase cascade [49, 50]. In 2008, the SHARP trial, a large multicenter trial conducted primarily in the US and Europe demonstrated that sorafenib significantly prolonged the survival in patients with advanced hepatocellular carcinoma [35]. Sorafenib is the first agent proven to improve the survival in HCC patients in a large phase III trial. A recent, large clinical trial investigated the efficacy of sorafenib for preventing recurrence after curative therapy for HCC; the results are forthcoming (STORM trial).

Other studies have investigated various adjuvant therapies. In a randomized study, Takayama et al. [51] demonstrated that adoptive immunotherapy after hepatic resection significantly reduced the recurrence rate in 150

Table 3 Randomized clinical trials about postoperative chemotherapy after curative resection for hepatocellular carcinoma

References	Cases	Inclusion criteria	Regimen	5-year survival (vs. control)	5-year disease-free survival (vs. control)	Result
Oral regimens						
Yamamoto et al. [36]	76	Stage II	HCFU vs. observation	ND	ND	Effective (clinical stage I)
Hasegawa et al. [37]	159	Child A/B, without VI	UFT vs. observation	58 vs. 73 %	29 vs. 29 %	Not effective
Xia et al. [38]	60	Child A, within three number of tumors, without VI	Capecitabine vs. observation	62.5 vs. 39.8 %	46.7 vs. 23.3 %	Effective (DFS)
Intravenous or intra-arterial regimens						
Izumi et al. [39]	50	With VI and/or IM	Intra-arterial DXR and MMC vs. observation	50.3 vs. 28.8 %	25.6 vs. 5.9 % (4 years)	Effective (DFS)
Kohno et al. [40]	88	No residual disease	Oral UFT and intra-arterial EPI vs. oral UFT	30 vs. 35 %	17 vs. 14 %	Not effective
Ono et al. [41]	57	Child A or B	Intra-arterial and intravenous EPI and oral HCFU vs. observation	31.5 vs. 57.1 %	32.0 vs. 22.5 %	Not effective
Lai et al. [42]	66	No residual disease	Intra-arterial CDDP and intravenous EPI vs. observation	ND	18 vs. 48 %	Worse outcome
Kwok et al. [43]	40	Child A or B	Intra-arterial CDDP 4 dose vs. 1 dose	40 vs. 55 % (3 years)	40 vs. 44 % (3 years)	Not effective
Shuqun et al. [44]	57	No residual disease	Intra-arterial CBDCA, EPI and MMC with/without thymosin α1 vs. observation	10 vs. 7 vs. 8 m[a]	7 vs. 5 vs. 4 m[a]	Effective
Tanaka et al. [45]	15	With VI and/or IM	Intra-arterial CDDP and FU vs. observation	75 vs. 25 % (3 years)	19 vs. 12.5 % (2 years)	Effective (survival)
Zhong et al. [46]	115	Stage III A, Child A or B	Intra-arterial CBDCA, EPI and MMC vs. observation	22.8 vs. 17.5 %	9.3 vs. 1.7 %	Effective
Peng et al. [47]	126	With VI, within three number of tumors, Child A or B	Intra-arterial FU and DXR vs. observation	21.5 vs. 8.5 %	ND	Effective (survival)

VI vascular invasion, *IM* intrahepatic metastasis, *ND* not described, *MST* median survival time, *HCFU* 1-hexylcarbamoyl-5-fluorouracil, *UFT* uracil-tegafur, *DXR* doxorubicin, *MMC* mitomycin C, *EPI* epirubicin, *CDDP* cisplatin, *FU* fluorouracil, *CBDCA* carboplatin

[a] Median survival time

patients with HCC. Retinoids are reported to suppress secondary carcinogenesis; a small RCT showed the efficacy of a retinoid as a postoperative adjuvant therapy [52]. Lau et al. [53] administered ^{131}I-labeled lipiodol via the hepatic artery, and reported a significant improvement in the progression-free survival and overall survival. However, as these reports were based on single center trials with a small number of patients, no such treatment is currently recommended in the guidelines. Vitamin K was also reported to suppress secondary carcinogenesis; however, an RCT found it to be ineffective against recurrence [54].

Adjuvant therapy after liver transplantation

Liver transplantation is the most effective treatment for early HCC that is unresectable due to deteriorated liver function, with a 5-year survival rate of 60–80 % [11]. Liver transplantation can simultaneously achieve both complete resection of the liver tumor and appropriate treatment of the background liver disease. However, because of complex factors, such as the need for immunosuppressive therapy and the recurrence of viral hepatitis, no standard adjuvant therapy after the liver transplantation in patients with HCC has been established to date. Recurrence after liver transplantation for HCC may theoretically occur due to the growth of occult metastases or to the engraftment of circulating tumor cells. Therefore, Toso et al. have proposed the following five strategies for improving the outcome after transplantation: (1) selecting recipients with low baseline levels of circulating HCC cells before transplantation (further refining the selection criteria), (2) decreasing the peritransplant release of HCC cells (decreasing the handling of the liver), (3) preventing the engraftment of circulating HCC cells in the liver (liver-protecting strategies), (4) using anticancer drugs (killing circulating tumor cells or early metastases), and (5) tuning immunity toward HCC clearance (tumor-customized immunosuppression) [55]. Accordingly, the current studies on adjuvant therapies for the prevention of recurrence have focused on novel antitumor agents including molecular targeting agents and immunotherapy.

Donor liver-derived activated natural killer (NK) cell therapy for the prevention of recurrence after liver transplantation in patients with HCC

As noted above, the recurrence of HCC after liver transplantation is thought to be due to the extrahepatic dissemination of tumor cells that existed preoperatively or were disseminated during the surgical procedure. After liver transplantation, it is necessary to use immunosuppressive drugs, which attenuate the biological defense mechanisms and make it difficult to eliminate residual microscopic cancer cells. These biological defense mechanisms consist of the innate immune and adaptive immune responses. The latter is predominantly associated with rejection and requires immunosuppressive therapy. Therefore, we have investigated the potential of using anticancer immunotherapy that selectively enhances the innate immune response.

NK cells that dominate the innate immune system can distinguish cancer cells from normal cells during the early stages of cancer metastasis, and selectively kill them. We have confirmed that the human liver has abundant NK cells [56]. Unlike NK cells derived from the peripheral blood, stimulating liver NK cells by IL-2 could induce the potent expression of anti-tumor molecules, including tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which selectively targets cancer cells without affecting normal cells. Moreover, we have found that moderately to poorly differentiated hepatocellular carcinomas, which are prone to recur after transplantation, highly express TRAIL receptors, and are thus susceptible to apoptosis resulting from TRAIL-mediated signaling. In a mouse model of liver cancer, the intrahepatic implantation of cancer cells was suppressed by the adoptive transfer of IL-2-stimulated liver NK cells [57]. During the liver transplantation procedure, the liver extracted from the donor is perfused to replace the intra-hepatic blood with tissue preservatives. We have developed a novel system to effectively retrieve NK cells from the perfusate with aseptic manipulation. From January 2006, with the approval of the Ethics Committee of Hiroshima University Hospital, we began clinical studies of this new procedure. Donor liver-derived activated NK cells were introduced with the aim of preventing recurrence after liver transplantation in patients with HCC [58]. To date, 26 patients with cirrhosis complicated with HCC above stage II have been treated with this therapy, and have been observed for safety and clinical outcomes. Although further observation is required, we have observed a significant reduction in recurrence in the treatment group (unpublished data). Furthermore, we are currently working on a cooperative study with researchers at the University of Miami to expand the applications of this treatment to deceased donor liver transplantation. This therapy has gained FDA approval in the United States, and a Phase I clinical study is ongoing (ClinicalTrials.gov identifier: NCT01147380).

Conclusion

HCC recurrence is characterized by metastasis, as well as multicentric carcinogenesis. Postoperative antiviral adjuvant therapy, especially interferon therapy, may contribute

to prolonging the survival in specific groups of patients. However, no effective therapy against advanced HCC that prevents metastasis has been established. Novel treatment strategies incorporating molecular and immunological mechanisms are expected in the future.

References

1. The Japan Society of Hepatology. Clinical Practice Guidelines for Hepatocellular Carcinoma—The Japan Society of Hepatology 2009 update. Hepatol Res. 2010;40(Suppl 1):2–144.
2. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020–2.
3. Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet. 2002;359:1734–9.
4. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology. 2002;35:1164–71.
5. Wu CC, Ho YZ, Ho WL, Wu TC, Liu TJ. K. Preoperative transcatheter arterial chemoembolization for resectable large hepatocellular carcinoma: a reappraisal. Br J Surg. 1995;82:122–6.
6. Yamasaki S, Hasegawa H, Kinoshita H, Furukawa M, Imaoka S, Takasaki K, et al. A prospective randomized trial of the preventive effect of pre-operative transcatheter arterial embolization against recurrence of hepatocellular carcinoma. Jpn J Cancer Res. 1996;87:206–11.
7. Zhou WP, Lai EC, Li AJ, Fu SY, Zhou JP, Pan ZY, et al. A prospective, randomized, controlled trial of preoperative transarterial chemoembolization for resectable large hepatocellular carcinoma. Ann Surg. 2009;249:195–202.
8. Koda M, Murawaki Y, Mitsuda A, Oyama K, Okamoto K, Idobe Y, et al. Combination therapy with transcatheter arterial chemoembolization and percutaneous ethanol injection compared with percutaneous ethanol injection alone for patients with small hepatocellular carcinoma: a randomized control study. Cancer. 2001;92:1516–24.
9. Akamatsu M, Yoshida H, Obi S, Sato S, Koike Y, Fujishima T, et al. Evaluation of transcatheter arterial embolization prior to percutaneous tumor ablation in patients with hepatocellular carcinoma: a randomized controlled trial. Liver Int. 2004;24:625–9.
10. European Association For The Study Of The Liver. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56:908–43.
11. Belghiti J, Carr BI, Greig PD, Lencioni R, Poon RT. Treatment before liver transplantation for HCC. Ann Surg Oncol. 2008; 15:993–1000.
12. Decaens T, Roudot-Thoraval F, Bresson-Hadni S, Meyer C, Gugenheim J, Durand F, et al. Impact of pretransplantation transarterial chemoembolization on survival and recurrence after liver transplantation for hepatocellular carcinoma. Liver Transpl. 2005;11:767–75.
13. Millonig G, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, Ladurner R, et al. Response to preoperative chemoembolization correlates with outcome after liver transplantation in patients with hepatocellular carcinoma. Liver Transpl. 2007;13:272–9.
14. Porrett PM, Peterman H, Rosen M, Sonnad S, Soulen M, Markmann JF, et al. Lack of benefit of pre-transplant locoregional hepatic therapy for hepatocellular cancer in the current MELD era. Liver Transpl. 2006;12:665–73.
15. Majno PE, Adam R, Bismuth H, Castaing D, Ariche A, Krissat J, et al. Influence of preoperative transarterial lipiodol chemoembolization on resection and transplantation for hepatocellular carcinoma in patients with cirrhosis. Ann Surg. 1997;226:688–701.
16. Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, et al. Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. Hepatology. 2000;32:228–32.
17. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. Br J Surg. 2002;89:418–22.
18. Shiratori Y, Shiina S, Teratani T, Imamura M, Obi S, Sato S, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. Ann Intern Med. 2003;138:299–306.
19. Lin SM, Lin CJ, Hsu CW, Tai DI, Sheen IS, Lin DY, et al. Prospective randomized controlled study of interferon-alpha in preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. Cancer. 2004;100:376–82.
20. Sun HC, Tang ZY, Wang L, Qin LX, Ma ZC, Ye QH, et al. Postoperative interferon alpha treatment postponed recurrence and improved overall survival in patients after curative resection of HBV-related hepatocellular carcinoma: a randomized clinical trial. J Cancer Res Clin Oncol. 2006;132:458–65.
21. Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, et al. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. Hepatology. 2006;44:1543–54.
22. Lo CM, Liu CL, Chan SC, Lam CM, Poon RT, Ng IO, et al. A randomized, controlled trial of postoperative adjuvant interferon therapy after resection of hepatocellular carcinoma. Ann Surg. 2007;245:831–42.
23. Chen LT, Chen MF, Li LA, Lee PH, Jeng LB, Lin DY, et al. Long-term results of a randomized, observation-controlled, phase III trial of adjuvant interferon Alfa-2b in hepatocellular carcinoma after curative resection. Ann Surg. 2012;255:8–17.
24. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. J Hepatol. 2010;52:889–94.
25. Breitenstein S, Dimitroulis D, Petrowsky H, Puhan MA, Mullhaupt B, Clavien PA. Systematic review and meta-analysis of interferon after curative treatment of hepatocellular carcinoma in patients with viral hepatitis. Br J Surg. 2009;96:975–81.
26. Zhang CH, Xu GL, Jia WD, Ge YS. Effects of interferon alpha treatment on recurrence and survival after complete resection or ablation of hepatocellular carcinoma: a meta-analysis of randomized controlled trials. Int J Cancer. 2009;124:2982–8.
27. Singal AK, Freeman DH Jr, Anand BS. Meta-analysis: interferon improves outcomes following ablation or resection of hepatocellular carcinoma. Aliment Pharmacol Ther. 2010;32:851–8.
28. Miao RY, Zhao HT, Yang HY, Mao YL, Lu X, Zhao Y, et al. Postoperative adjuvant antiviral therapy for hepatitis B/C virus-related hepatocellular carcinoma: a meta-analysis. World J Gastroenterol. 2010;16:2931–42.
29. Miyake Y, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. J Viral Hepat. 2010;17:287–92.
30. Hagihara H, Nouso K, Kobayashi Y, Iwasaki Y, Nakamura S, Kuwaki K, et al. Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. Int J Clin Oncol. 2011;16: 210–20.

31. Tanimoto Y, Tashiro H, Aikata H, Amano H, Oshita A, Kobayashi T, et al. Impact of pegylated interferon therapy on outcomes of patients with hepatitis C virus-related hepatocellular carcinoma after curative hepatic resection. Ann Surg Oncol. 2012;19:418–25.
32. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011;364: 2405–16.
33. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection. N Engl J Med. 2011;364:2417–28.
34. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351:1521–31.
35. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359:378–90.
36. Yamamoto M, Arii S, Sugahara K, Tobe T. Adjuvant oral chemotherapy to prevent recurrence after curative resection for hepatocellular carcinoma. Br J Surg. 1996;83:336–40.
37. Hasegawa K, Takayama T, Ijichi M, Matsuyama Y, Imamura H, Sano K, et al. Uracil-tegafur as an adjuvant for hepatocellular carcinoma: a randomized trial. Hepatology. 2006;44:891–5.
38. Xia Y, Qiu Y, Li J, Shi L, Wang K, Xi T, et al. Adjuvant therapy with capecitabine postpones recurrence of hepatocellular carcinoma after curative resection: a randomized controlled trial. Ann Surg Oncol. 2010;17:3137–44.
39. Izumi R, Shimizu K, Iyobe T, Ii T, Yagi M, Matsui O, et al. Postoperative adjuvant hepatic arterial infusion of Lipiodol containing anticancer drugs in patients with hepatocellular carcinoma. Hepatology. 1994;20:295–301.
40. Kohno H, Nagasue N, Hayashi T, Yamanoi A, Uchida M, Ono T, et al. Postoperative adjuvant chemotherapy after radical hepatic resection for hepatocellular carcinoma (HCC). Hepatogastroenterology. 1996;43:1405–9.
41. Ono T, Nagasue N, Kohno H, Hayashi T, Uchida M, Yukaya H, et al. Adjuvant chemotherapy with epirubicin and carmofur after radical resection of hepatocellular carcinoma: a prospective randomized study. Semin Oncol. 1997;24:S6–18–S6–25.
42. Lai EC, Lo CM, Fan ST, Liu CL, Wong J. Postoperative adjuvant chemotherapy after curative resection of hepatocellular carcinoma: a randomized controlled trial. Arch Surg. 1998;133:183–8.
43. Kwok PC, Lam TW, Lam PW, Tang KW, Chan SC, Hwang JS, et al. Randomized controlled trial to compare the dose of adjuvant chemotherapy after curative resection of hepatocellular carcinoma. J Gastroenterol Hepatol. 2003;18:450–5.
44. Shuqun C, Mengchao W, Han C, Feng S, Jiahe Y, Wenming C, et al. Combination transcatheter hepatic arterial chemoembolization with thymosin alpha1 on recurrence prevention of hepatocellular carcinoma. Hepatogastroenterology. 2004;51:1445–7.
45. Tanaka S, Shimada M, Shirabe K, Maehara S, Harimoto N, Tsujita E, et al. A novel intrahepatic arterial chemotherapy after radical resection for advanced hepatocellular carcinoma. Hepatogastroenterology. 2005;52:862–5.
46. Zhong C, Guo RP, Li JQ, Shi M, Wei W, Chen MS, et al. A randomized controlled trial of hepatectomy with adjuvant transcatheter arterial chemoembolization versus hepatectomy alone for Stage III A hepatocellular carcinoma. J Cancer Res Clin Oncol. 2009;135:1437–45.
47. Peng BG, He Q, Li JP, Zhou F. Adjuvant transcatheter arterial chemoembolization improves efficacy of hepatectomy for patients with hepatocellular carcinoma and portal vein tumor thrombus. Am J Surg. 2009;198:313–8.
48. Mathurin P, Raynard B, Dharancy S, Kirzin S, Fallik D, Pruvot FR, et al. Meta-analysis: evaluation of adjuvant therapy after curative liver resection for hepatocellular carcinoma. Aliment Pharmacol Ther. 2003;17:1247–61.
49. Zhu AX. Development of sorafenib and other molecularly targeted agents in hepatocellular carcinoma. Cancer. 2008;112: 250–9.
50. Kudo M. Signaling pathway and molecular-targeted therapy for hepatocellular carcinoma. Dig Dis. 2011;29:289–302.
51. Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet. 2000;356:802–7.
52. Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. N Engl J Med. 1996;334:1561–7.
53. Lau WY, Lai EC, Leung TW, Yu SC. Adjuvant intra-arterial iodine-131-labeled lipiodol for resectable hepatocellular carcinoma: a prospective randomized trial-update on 5-year and 10-year survival. Ann Surg. 2008;247:43–8.
54. Yoshida H, Shiratori Y, Kudo M, Shiina S, Mizuta T, Kojiro M, et al. Effect of vitamin K2 on the recurrence of hepatocellular carcinoma. Hepatology. 2011;54:532–40.
55. Toso C, Mentha G, Majno P. Liver transplantation for hepatocellular carcinoma: five steps to prevent recurrence. Am J Transplant. 2011;11:2031–5.
56. Ishiyama K, Ohdan H, Ohira M, Mitsuta H, Arihiro K, Asahara T. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. Hepatology. 2006;43:362–72.
57. Ohira M, Ohdan H, Mitsuta H, Ishiyama K, Tanaka Y, Igarashi Y, et al. Adoptive transfer of TRAIL-expressing natural killer cells prevents recurrence of hepatocellular carcinoma after partial hepatectomy. Transplantation. 2006;82:1712–9.
58. Ohira M, Ishiyama K, Tanaka Y, Doskali M, Igarashi Y, Tashiro H, et al. Adoptive immunotherapy with liver allograft-derived lymphocytes induces anti-HCV activity after liver transplantation in humans and humanized mice. J Clin Invest. 2009;119:3226–35.

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INVITED COMMENTARY

Is living donor liver transplantation really equivalent to deceased donor liver transplantation?*

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*Invited commentary on "Living donor versus deceased donor liver transplantation: A surgeon-matched comparison of recipient morbidity and outcomes", by Reichman *et al.*

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Living donor liver transplantation (LDLT) has developed as an alternative to deceased donor liver transplantation (DDLT) in order to overcome the critical shortage of deceased organ donations. Particularly in regions with low deceased donation rates, like Asian, LDLT for end stage liver disease significantly reduces the risk of death or drop off the wait list without compromising post-transplant survival. A preference for LDLT to DDLT may depend on the original disease representing the indication for liver transplantation (LT). LDLT offers a timely alternative to DDLT for patients with hepatocellular carcinoma (HCC). However, the higher recurrence rate of HCC after LDLT and the indication criteria remain controversial. One of the recent quantitative meta-analyses revealed the comparable patient survival rates and no significant differences in the recurrence rates between LDLT and DDLT recipients [1]. Another meta-analysis provided evidence of lower disease-free survival (DFS) after LDLT compared with DDLT for HCC [2]. Hence, LDLT likely represents an acceptable option that does not compromise patient survival or increase HCC recurrence in comparison with DDLT at this moment.

Early data suggested that patients with Hepatitis C virus (HCV) that received a LDLT had worse outcomes, including increased rates of cholestatic HCV than did recipients of DDLT [3,4]. This is currently thought to be because of an increased rate of biliary complications or other problems seen during the learning curve of early LDLT experience. More recent data demonstrated that there is no difference in recurrent HCV between recipients of DDLT and LDLT [5,6]. The latest meta-analysis demonstrated that LDLT was equivalent to DDLT in terms of long-term patient or graft survival, HCV recurrence, and acute rejection with a potential lower short-term graft survival [7].

There are limited convincing data comparing outcomes of LDLT and DDLT for autoimmune hepatitis (AIH) and cholestatic liver diseases. It has been previously reported that the overall survival outcomes of LDLT were similar to DDLT in patients with AIH and primary biliary cirrhosis

[8]. In contrast, patients with primary sclerosing cholangitis undergoing LDLT, especially with biologically related donors, are thought to have a higher risk to develop recurrent disease compared with the DDLT setting, probably because of sharing antigens targeted by autoimmunity between recipients and the related donors [9]. Further prospective studies at transplant centers performing both LDLT and DDLT might be needed to confirm these issues.

Regardless of such original disease, LDLT offers several advantages over DDLT, which include the reduction in waiting time mortality, the reduction in cold ischemic time (CIT) and the feasibility of various preoperative interventions, such as nutritional treatment for both the donor and recipient [10]. However, it remains unclear whether those advantages offset disadvantages peculiar to LDLT, such as the smaller graft volume than DDLT and the highly technical procedure, which may be associated with higher complication rates. This seems to be caused by a fact that direct comparison of the results between LDLT and DDLT inevitably involves various biases in nature.

Reichman *et al.* [11] have performed a retrospective matched-cohort study to compare postoperative complication rate and patient survival in the two groups of patients submitted to LDLT and to DDLT. Six clinical variables for recipients: age, Meld, date of transplant, gender, primary diagnosis, and recipient surgeon were matched in each group (n = 145 in each group). They found that the overall complication rate was similar between two groups. In further detail, biliary complications were higher in LDLT although the complications that occurred in the DDLT were strongly associated with graft loss. Graft and patient survival outcomes for LDLT versus DDLT were similar. From those findings, they concluded that LDLT offers an excellent alternative to DDLT in areas of deceased donor organ shortages. This study defined surgical complications that are more frequent in LDLT, i.e., biliary complications (34% and 17% in LDLT and DDLT cohorts, respectively). Despite a higher rate of complications among LDLT recipients, complications leading to death were not significantly higher in LDLT in the experienced center. These findings, in concert with the current common consent that the incidence of complications, even biliary complications, can decline with center experience to levels comparable with DDLT [12], underscore the impact of the learning curve on this highly technical procedure. Potential recipients need to hear about both the rates of complications after LDLT and DDLT, and this study with control for recipient variables will help to define those rates. As pointed out by the authors, this study left control for donor variables out of consideration, despite a well known fact that donor age/gender and donor-recipient human leukocyte antigen matching correlate with either the incidence of certain complications or the severity of original disease recurrence. Nevertheless, this case control comparison of the outcome of LDLT and DDLT convincingly reported that these procedures had different complication profiles but the overall outcomes were similar with expert management, suggesting that the biological advantage in LDLT could compensate for a higher rate of surgical complications caused by greater technical complexity.

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References

1. Liang W, Wu L, Ling X, *et al.* Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl* 2012; **18**: 1226.
2. Grant RC, Sandhu L, Dixon PR, *et al.* Living vs. deceased donor liver transplantation for hepatocellular carcinoma: a systematic review and meta-analysis. *Clin Transplant* 2013; **27**: 140.
3. Garcia-Retortillo M, Forns X, Llovet JM, *et al.* Hepatitis C recurrence is more severe after living donor compared to cadaveric liver transplantation. *Hepatology* 2004; **40**: 699.
4. Gaglio PJ, Malireddy S, Levitt BS, *et al.* Increased risk of cholestatic hepatitis C in recipients of grafts from living versus cadaveric liver donors. *Liver Transpl* 2003; **9**: 1028.
5. Shiffman ML, Stravitz RT, Contos MJ, *et al.* Histologic recurrence of chronic hepatitis C virus in patients after living donor and deceased donor liver transplantation. *Liver Transpl* 2004; **10**: 1248.
6. Terrault NA, Shiffman ML, Lok AS, *et al.* Outcomes in hepatitis C virus-infected recipients of living donor vs. deceased donor liver transplantation. *Liver Transpl* 2007; **13**: 122.
7. Hu A, Liang W, Zheng Z, Guo Z, He X. Living donor vs. deceased donor liver transplantation for patients with hepatitis C virus-related diseases. *J Hepatol* 2012; **57**: 1228.
8. Kashyap R, Safadjou S, Chen R, *et al.* Living donor and deceased donor liver transplantation for autoimmune and cholestatic liver diseases–an analysis of the UNOS database. *J Gastrointest Surg* 2010; **14**: 1362.
9. Graziadei IW. Live donor liver transplantation for primary sclerosing cholangitis: is disease recurrence increased? *Curr Opin Gastroenterol* 2011; **27**: 301.
10. Kaido T, Uemoto S. Does living donation have advantages over deceased donation in liver transplantation? *J Gastroenterol Hepatol* 2010; **25**: 1598.
11. Reichman TW, Katchman H, Tanaka T, *et al.* Living donor vs. deceased donor liver transplantation: a surgeon-matched comparison of recipient morbidity and outcomes. *Transpl Int* 2013; **26**: 780.
12. Freise CE, Gillespie BW, Koffron AJ, *et al.* A2ALL Study Group. Recipient morbidity after living and deceased donor liver transplantation: findings from the A2ALL Retrospective Cohort Study. *Am J Transplant* 2008; **8**: 2569.

ELSEVIER

Comparative Analysis of T-Cell Depletion Method for Clinical Immunotherapy—Anti–Hepatitis C Effects of Natural Killer Cells Via Interferon-γ Production

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ABSTRACT

Liver transplantation (LT) is a life-saving treatment for liver cirrhosis patients with hepatocellular carcinoma (HCC). However, 10%–20% HCC recurrence rate after LT is due to the immunosuppression inducing tumor growth. We recently reported a novel immunotherapy with donor liver natural killer (NK) cells to prevent HCC and hepatitis C virus (HCV) recurrence after LT. In this cell processing procedure, Muromonab-CD3 (Orthoclone OKT3, an anti-CD3 antibody) was added to the culture medium to deplete $CD3^+$ T cells to prevent graft-versus-host disease. However, the manufacture of OKT3 was discontinued in 2010, when other treatments with similar efficacy and fewer side effects became available. In this study, we examined alternative reagents for T-cell depletion-MACS GMP CD3 pure (GMP CD3), antithymocyte globulin, and alemtuzumab-for NK cell immunotherapy in the allogeneic setting. We observed that GMP CD3 showed exactly the same effects on liver mononuclear cells as OKT3, including activation of NK cells and depletion of T cells. Interestingly, binding of T-cell depletion antibodies to NK cells led to an anti-HCV effect via interferon-γ production. These results with the use of in vitro culture systems suggested that antibodies which produce T-cell depletion affected NK cell function.

Liver failure and hepatocellular carcinoma (HCC) caused by chronic hepatitis C virus (HCV) infection are the most common indications for liver transplantation (LT). The incidences of both conditions have been projected to increase further. On the one hand, the rate of HCC recurrence after LT is 10%–20%.[1,2] On the other hand, recurrent HCV infection in the allograft, which is universal, occurs immediately after LT and is associated with accelerated progression to liver cirrhosis, graft loss, and death.[3,4] These recurrences remains the most serious issue with LT. The use of postoperative immunosuppressants poses an additional risk for recurrences and hinders the use of chemotherapeutic or interferon (IFN) agents.[5,6] However, no definitive treatment or prevention for HCC recurrence after LT is known.

Natural killer (NK) cells are innate immune lymphocytes that are identified by their expression of the CD56 surface antigen and the absence of CD3 markers.[7,8] NK cells can directly kill targets through the release of granzymes, which are granules containing perforin and serine proteases, and/or by surface-expressed ligands that engage and activate death receptors expressed on target cells. Unlike T cells, NK cells do not require the presence of a specific antigen to kill cancer cells, modified cells, or invading infectious microbes. NK cells are abundant in the liver, in

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contrast to their relatively small distribution in peripheral lymph and lymphatic organs in rodents[9–11] and humans.[12,13] In addition, hepatic NK cells in humans have been shown to mediate cytotoxic activity against HCC[12] and to display anti-HCV effects[14] compared with their peripheral blood counterparts. We have successfully applied adoptive immunotherapy with liver NK cells to LT recipients with HCC in Japan and the United States.[14–16] In this regimen, LT recipients are injected intravenously with interleukin (IL) 2–activated NK cells derived from the donor liver allograft. After treatment with IL-2 and OKT3 (Orthoclone OKT3, an anti-CD3 monoclonal antibody [mAb]; Ortho Biotech, Raritan, NJ), liver NK cells expressed significantly elevated levels of the tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), a crucial molecule for killing of tumor cells. Furthermore, these cells showed great cytotoxicity against HCC without any effect on normal cells.[12]

OKT3, a potent immunosuppressant, has been shown to reverse renal allograft rejection episodes.[17,18] It has also been widely used for immunotherapy, as well as to expand cytotoxic T cells[19] and enhance the activity of lymphokine-activated killer (LAK) cells,[20–25] and prevent graft-versus-host disease (GVHD).[26–29] In the latter setting, administration of OKT3-coated T cells in vivo opsonizes for the reticuloendothelial system to subsequently trap or lyses cells.[30–32] This method has been used for clinical NK therapy in Japan, achieving protection against GVHD.[14] However, because of its numerous side effects, the availability of better-tolerated alternatives, and its declining use, OKT3 has been recently removed from the market. Therefore, alternative reagents need to be evaluated for this immunotherapy. In the present study, we evaluated the effect of alternative reagents-GMP CD3 (MACS GMP CD3 pure; Miltenyi Biotec, Bergisch Gladbach, Germany), antithymocyte globulin (Thymoglobulin; Genzyme, Cambridge, MA), and alemtuzumab (Campath; Genzyme) using culture systems with NK and T cells for subsequent application in clinical trials.

MATERIALS AND METHODS

Isolation of Liver Mononuclear Cells

Liver mononuclear cells (LMNCs) from liver perfusates were isolated by gradient centrifugation with Ficoll-Hypaque (GE Healthcare, Pittsburgh, PA) before suspension in X-Vivo 15 medium (Lonza, Walkersville, MD) supplemented with 100 μg/mL gentamicin (APP Pharmaceuticals, Schaumburg, IL), 10% human AB serum (Valley Biomedical, Winchester, VA), and 10 U/mL sodium heparin (APP Pharmaceuticals), as previously described.[16] Our Institutional Review Board (IRB) approved this study.

Cell Culture

LMNCs were cultured with 1,000 U/mL human recombinant IL-2 (Proleukin; Novartis, Emeryville, CA) in culture medium at 37°C in an atmosphere supplemented with 5% CO_2. LMNCs were exposed to a OKT3 (1 μg/mL), GMP CD3 (1 μg/mL), antithymocyte globulin (100 μg/mL), or alemtuzumab (100 μg/mL) at 1 day before cell harvest. After 4 days of culture, cells were subjected to further analyses.

Flow Cytometry

All flow cytometry (FCM) analyses were performed on an LSR II Flow Cytometer (BD Biosciences, San Jose, CA). The following mAbs were used for surface staining of the lymphocytes: fluorescein isothiocyanate–conjugated anti-CD3 (HIT3a; BD Pharmingen, San Diego, CA) or anti-CD56 (B159; BD Pharmingen); phycoerythrin (PE)-conjugated anti-TRAIL (RIK-2; BD Pharmingen), anti-NKp44 (P44-8.1; BD Pharmingen), or anti-CD158b (CH-L; BD Pharmingen); allophycocyanin (APC)–conjugated anti-CD56 (B159; BD Pharmingen), anti-CD25 (M-A251; BD Pharmingen), or anti-NKG2A (Z199; Beckman Coulter, Fullerton, CA); APC-eFluor780–conjugated anti-CD3 (UCHT1; eBioscience, San Diego, CA); PE-Cy7–conjugated anti-CD69 (FN50; Biolegend, San Diego, CA), or anti-NKG2D (1D11; Biolegend); eFluor 605NC–conjugated anti-CD16 (eBioCB16; eBioscience); Alexa Fluor 647–conjugated anti-NKp30 (P30-15; Biolegend); peridinin chlorophyll protein complex (PerCP)-Cy5.5–conjugated anti-CD158a (HP-MA4; eBioscience); and biotin-conjugated anti-CD122 (Mik-b3; BD Pharmingen), anti-NKp46 (9E2; Biolegend), or CD132 (TuGh4; BD Pharmingen). The biotinylated mAbs were visualized with the use of PerCP-Cy5.5-streptavidin (eBioscience) or PE-Cy7-streptavidin (Biolegend). Dead cells were excluded by light scatter and 4′,6-diamidino-2-phenylindole staining (DAPI; Invitrogen, Carlsbad, CA). FCM analyses were performed with Flowjo software (Tree Star, Ashland, OR).

Cytotoxic Assay

The cytotoxicity assay was performed by FCM as previously described.[16] Briefly, target cells labeled with 0.1 μmol/L carboxyfluorescein diacetate succinimidyl ester Cell Tracer Kit (Invitrogen) for 5 minutes at 37°C in 5% CO_2 were washed twice in phosphate-buffered saline solution, resuspended in complete medium, and counted with the use of trypan blue staining. The effector and target cells were coincubated at various ratios for 1 hour at 37°C in 5% CO_2. As a control, target cells or effector cells were incubated alone in complete medium to measure spontaneous cell death after DAPI was added to each tube. The data were analyzed with the use of Flowjo software. Cytotoxic activity was calculated as a percentage with the following formula: % cytotoxicity = [(% experimental $DAPI^+$ dead targets) − (% spontaneous $DAPI^+$ dead targets)]/[(100 − (% spontaneous $DAPI^+$ dead targets)] × 100.

ELISA

IFN-γ production of LMNCs during the culture was measured by enzyme-linked immunosorbent assay (ELISA) (Biolegend). Supernates collected after the incubation were stored at −80°C until further use. IFN-γ ELISA was performed according to the manufacturer's instructions.

Coculture with HCV Replicon Cells

The Huh7/Rep-Feo cell line (HCV replicon cells) was kindly provided by Dr N Sakamoto (Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan). The HCV subgenomic replicon plasmid, pRep-Feo, was derived from pRep-Neo (originally pHCVIbneo-delS).[33] pRep-Feo carries a fusion gene comprising firefly luciferase and neomycin phosphotransferase, as described elsewhere.[34,35] After culture in the pres-

ence of G418 (Invitrogen), Huh7/Rep-Feo cell lines showed stable expression of the replicons. We used transwell tissue culture plates (pore size 1 μm; Costar, Cambridge, MA) for coculture experiments. HCV replicon cells (10^5 cells) were incubated in the lower compartment with various numbers of lymphocytes in the upper compartment. The HCV replicon cells in the lower compartments were collected at 48 hours after the coculture for luciferase assays in duplicate with the use of a luminometer (TriStar LB 941; Berthold Technologies, Oak Ridge, TN) with the Bright-Glo Luciferase Assay System (Promega, Madison, WI).

Statistical Analysis

Data are presented as mean ± SEM. The statistical difference between results were analyzed by Student *t* test (2 tailed), using the Statistical Package for the Social Sciences (SPSS) software version 19 for Windows (IBM Corp, Armonk, NY). *P* values of ≤.05 were considered to be statistically significant.

RESULTS

Effect on the Surface Phenotype of LMNCs

In 5 LMNC preparations, the addition of OKT3 GMP CD3 to IL-2–stimulated LMNCs decreased $CD3^+CD56^-$ T cells to 0.2% ± 0.1% and 0.2% ± 0.1%, respectively, from the IL-2–only control value of 28.1% ± 12.3%. In contrast, $CD3^+CD56^-$ T cells were retained among LMNCs with the addition of antithymocyte globulin or alemtuzumab: 3.3% ± 2.0% and 17.2% ± 7.3%, respectively. The proportion of $CD3^-CD56^+$ NK cells increased by ~10% in all groups (Fig 1A).

Addition of OKT3 or GMP CD3 to IL-2–stimulated LMNCs maintained both activation and inhibitory markers on NK cells. Interestingly, the expressions of TRAIL, CD25 (IL-2αR), and CD132 (IL-2γR) were increased in the antithymocyte globulin group. Furthermore, both antithymocyte globulin and alemtuzumab completely blocked the expression of CD16 on NK cells (Fig 1B).

Cytotoxic Capacity

Cytotoxicity assays were performed with the use of freshly isolated cultured LMNCs as effectors and K562 cells as targets. Fig 2 shows freshly isolated LMNCs barely mediated cell death, whereas IL-2–stimulated LMNCs produced significant cytotoxicity. Although the ratios of $CD3^-CD56^+$ to $CD3^+CD56^+$ cells varied after treatment with various

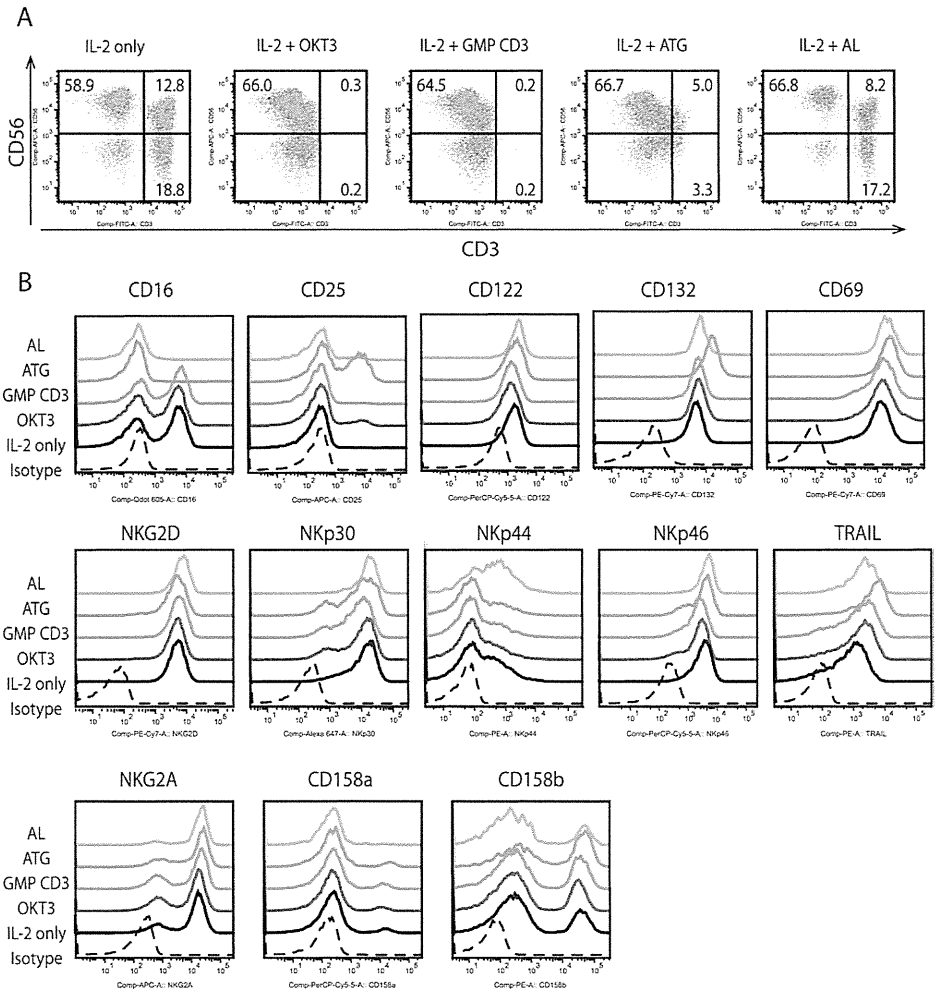

Fig 1. Effect of the T-cell depletion antibodies on the phenotypic characteristics of liver mononuclear cells (LMNCs). LMNCs obtained from cadaveric donors were stimulated with IL-2 (1000 U/mL) for 4 days. Anti-CD3 mAb (OKT3; 1 μg/mL), MACS GMP CD3 pure (GMP CD3; 1 μg/mL), antithymocyte globulin (ATG; 100 μg/mL), or alemtuzumab (AL; 100 μg/mL) was added to the culture medium 1 day before cell harvesting. (A) The LMNCs were stained with monoclonal antibodies against CD3 and CD56. The numbers indicate the mean percentages of the population. (B) Histograms show the logarithmic fluorescence intensities obtained on staining for each surface marker after gating on the $CD3^-CD56^+$ NK cells. Dotted lines indicate negative control samples with isotype-matched mAbs. The flow cytometry dot plot and histogram profiles represent 5 independent experiments. TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

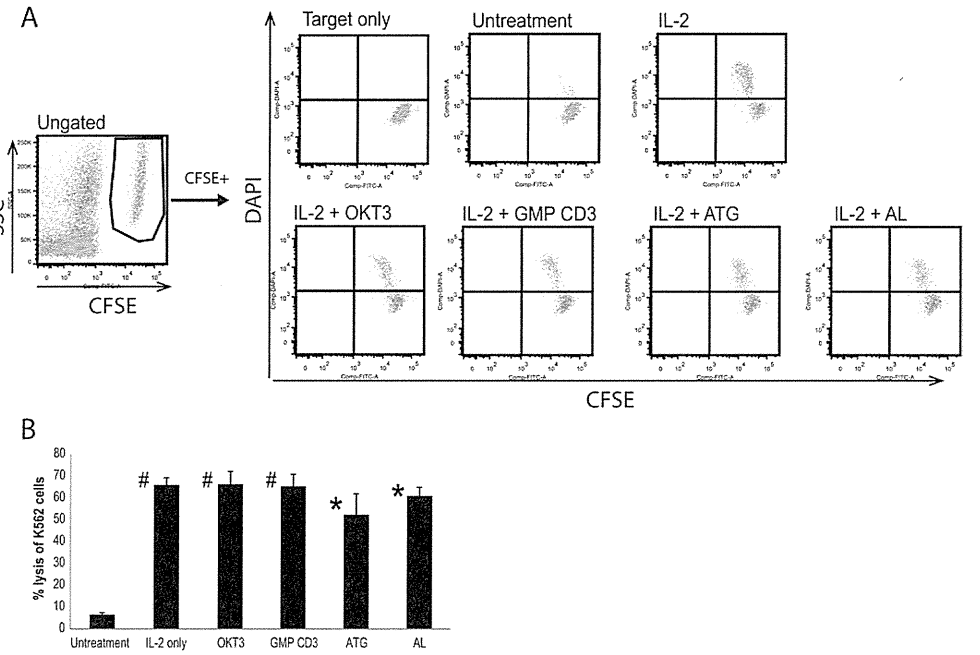

Fig 2. Antitumor effect of the T-cell depletion antibodies on IL-2–stimulated liver mononuclear cells (LMNCs). The NK cell cytotoxic activities of untreated cells and IL-2–stimulated LMNCs treated with various reagents were analyzed by a flow cytometry (FCM)–based cytotoxic assay. (A) Gate is set on cells to discriminate $CFSE^+$ targets from LMNCs. Gate is set on target to obtain the number of live and dead K562 cells. The FCM dot plot profiles represent 5 independent experiments. (B) The data represent the mean ± SEM of the percentage of target lysis at effector-to-target (E:T) ratios of 10:1 (5 LMNCs; $^{\#}P < .01$; $^{*}P < .05$ vs untreated group, *t* test).

T-cell depletion reagents for 4 days in culture, all cultured LMNCs exhibited vigorous cytotoxicity against K562. LMNCs treated with antithymocyte globulin showed slightly decreased cytotoxicity compared with the other groups, but the difference was not significant. This tendency was similar to that reported in an earlier study.[36] The cultured LMNCs did not show cytotoxicity against self-lymphoblasts (data not shown).

Anti-HCV Activity

IL-2–cultured LMNCs inhibited 40% luciferase reporter activity compared with freshly isolated LMNCs (Fig 3A). As we have reported before, the anti-HCV effect of IL-2–activated LMNCs was strongly enhanced by OKT3 treatment.[14] GMP CD3 treatment showed ~80% decreased HCV replication, which was almost the same effect as that caused by OKT3. Surprisingly, antithymocyte globulin and alemtuzumab treatment also elicited robust anti-HCV effects on LMNCs. We previously reported that IFN-γ secreted from LMNCs activated by IL-2 and OKT3 was responsible for the anti-HCV activity of these cells.[14] Cultured LMNCs also actively produced large amounts of IFN-γ (Fig 3B), which probably played a pivotal role in their anti-HCV activity.

DISCUSSION

In this study, we discovered GMP CD3 to be an alternative reagent to OKT3 for immunotherapy using liver NK cells.

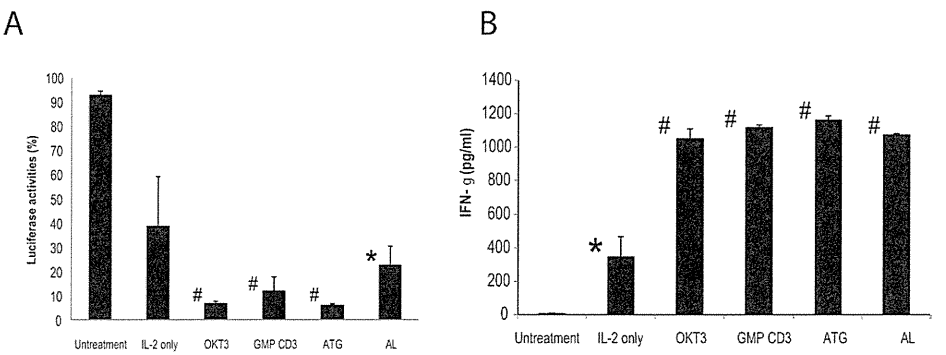

Fig 3. Anti–hepatitis C virus (HCV) effect of the T-cell depletion antibodies on IL-2–stimulated liver mononuclear cells (LMNCs). The LMNCs cultured for 4 days in the presence of IL-2 and various reagents were incubated with HCV replicon–containing cells for 48 hours in transwell tissue culture plates (effector-to-target ratio, 10:1). (A) Luciferase activity of HCV replicon–containing cells in the presence of effectors, normalized to luciferase activity in the absence of effectors. The difference in anti-HCV effect between the reagent-treated LMNCs and the freshly isolated LMNCs was statistically significant (5 LMNCs; $^{\#}P < .01$; $^{*}P < .05$ vs untreated group, *t* test). (B) IFN-γ production during the culture, as measured by ELISA [mean ± SEM (5 samples; $^{\#}P < .01$; $^{*}P < .05$ vs untreated group, *t* test)].

We compared the phenotypes and functions of LMNCs after treatment with various T-cell depletion regents, showing that GMP CD3 displayed same results as OKT3. Treatment with other T-cell depletion reagents, such as antithymocyte globulin and alemtuzumab, revealed unexpectedly strong cytotoxicity and anti-HCV effects on liver NK cells. Although antithymocyte globulin and alemtuzumab are difficult to use in immunotherapy because they completely bind the CD16 ligand on NK cells, these antibodies might affect NK cell function in in vitro culture systems.

This in vitro study showed that after treatment with GMP CD3 the degree of T-cell contamination and the NK cell phenotype and function, were similar to those after OKT3 treatment. T-Cell contamination was significantly decreased by either GMP CD3 or OKT3 treatment (Fig 1A). The 0.2% $CD3^+$ T-cell persistence in the final product represents an acceptable level for allogeneic transplantation.[16] Residual OKT3-coated T cells were dysfunctional. The NK cell percentage was the same in both groups. GMP CD3 treatment did not affect NK cell phenotype, including activation receptors, inhibitory receptors, and TRAIL. $CD3^-CD56^+$ NK cells expressed CD16, CD69, NKG2D, NKp30, NKp40, NKp46, TRAIL, and killer cell immunoglobulin–like receptors (KIRs), such as CD158a and CD158b (Fig 1B). Functional assays revealed that cytotoxicity and anti-HCV activity were maintained after GMP CD3 treatment. These results were reasonable, because both OKT3 and GMP CD3 are mouse IgG2as, whose Fc R receptor binds poorly to CD16. No animal- or human-derived components were used for the manufacture of this antibody. GMP CD3 is a reagent for research use and ex vivo cell culture processing only. It is not intended for in vivo human applications. GMP CD3 is manufactured and tested under a certificated ISO 9001 quality system in compliance with relevant GMP guidelines. It was designed following the recommendations of USP 1043 on ancillary materials.[36] GMP CD3 has been applied to expand cytokine-induced killer cells.[37]

In this study, we chose to examine the effects of other T-cell depletion antibodies. Currently, a wide variety of both polyclonal antibodies (antithymocyte globulin) and mAbs (alemtuzumab) are routinely used to deplete T cells in organ transplantation. Antithymocyte globulin contains a wide variety of antibody specificities directed toward immune response antigens, adhesion and cell trafficking molecules, and markers of heterogeneous pathways, including CD2, CD3, CD4, CD8, CD11a, CD16, CD25, CD44, CD45, HLA-DR, and HLA class I.[38] Alemtuzumab is the humanized form of a murine anti-CD52 mAb, a membrane glycoprotein with unknown function that is expressed on lymphocytes, macrophages, monocytes, and eosinophils. It is especially highly expressed on lymphocytes (up to 5% of surface antigens), explaining its powerful immunodepletion. Interestingly, antithymocyte globulin enhances the expression of IL-2 receptors (CD25 and CD132) and alemtuzumab of the activation receptor (NKp44) on NK cells (Fig 1B). Under IL-2 stimulation, either antithymocyte globulin– or alemtuzumab-treated liver NK cells showed strong cytotoxicity and anti-HCV activity (Fig 2 and 3). Our results clearly support the conclusion of other authors that binding of antithymocyte globulin to NK cells leads to cell activation and IFN-γ production.[36,39] The possible mechanism is that the binding of antithymocyte globulin or alemtuzumab to CD16 produces NK cell activation and degranulation.[40] However, antithymocyte globulin and alemtuzumab have also been reported to be potent to induce NK cell death and impair cytotoxicity.[41,42] When used for immunotherapy, antithymocyte globulin– or alemtuzumab-binding NK cells are destroyed through immunologic mechanisms such as complement-mediated and/or antibody-dependent cytotoxicity.[43]

In summary, we have shown the effects of GMP CD3 antibody to be similar to those of OKT3, namely, depletion of T cells and induction of NK cell phenotype and function. We have already applied this method to clinical immunotherapy using liver NK cells for liver transplant patients with HCC (ClinicalTrial.gov identifier: NCT01147380) after IRB and Food and Drug Administration approval in the United States. Our findings also support the hypothesis that T-cell depletion antibodies affect NK cell function with the use of in vitro culture systems.

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REFERENCES

1. Levi DM, Tzakis AG, Martin P, et al. Liver transplantation for hepatocellular carcinoma in the model for end-stage liver disease era. *J Am Coll Surg*. 2010;210:727–736.
2. Saab S, Yeganeh M, Nguyen K, et al. Recurrence of hepatocellular carcinoma and hepatitis B reinfection in hepatitis B surface antigen–positive patients after liver transplantation. *Liver Transpl*. 2009;15:1525–1534.
3. Petrovic LM. Early recurrence of hepatitis C virus infection after liver transplantation. *Liver Transpl*. 2006;12:S32–S37.
4. Brown RS. Hepatitis C and liver transplantation. *Nature*. 2005;436:973–978.
5. Vivarelli M, Cucchetti A, la Barba G, et al. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. *Ann Surg*. 2008;248:857–862.
6. Chinnadurai R, Velazquez V, Grakoui A. Hepatic transplant and HCV: a new playground for an old virus. *Am J Transplant*. 2012;12:298–305.
7. Trinchieri G. Biology of natural killer cells. *Adv Immunol*. 1989;47:187–376.
8. Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. *Blood*. 1990;76:2421–2438.
9. Takahashi M, Ogasawara K, Takeda K, et al. LPS induces NK1.1+ alpha beta T cells with potent cytotoxicity in the liver of mice via production of IL-12 from Kupffer cells. *J Immunol*. 1996;156:2436–2442.
10. Seki S, Habu Y, Kawamura T, et al. The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells,