Table 1 Outcome of liver transplantation for HIV/hepatitis C virus co-infection

Authors	Publication year	Country	*n*	Patient survival (%)		
				1 year	3 years	5 years
de Vera *et al.*[7]	2006	USA	27	67	56	33
Schreibman *et al.*[8]	2007	USA	15	73	73	–
Duclos-Vallee *et al.*[9]	2008	France	35	–	73	51
Terrault *et al.*[10]	2012	USA	89	76	60	–
Miro *et al.*[11]	2012	Spain	84	88	62	54

SPECIAL ISSUES REGARDING LT INDICATIONS FOR HIV/HCV CO-INFECTION

ART-related non-cirrhotic portal hypertension

IN HCV MONO-INFECTED patients, LT should be considered when the patients develop deteriorated liver function as indicated by a Child–Pugh classification of B or C. In HIV/HCV co-infected patients, liver failure due to HCV hepatitis was generally enhanced by ART-related hepatotoxicity, especially non-cirrhotic portal hypertension.[13–15] Accordingly, not only in cases with deteriorated liver function but also in class A cases, the patients can easily develop severe liver dysfunction suddenly,[16,17] so that all HIV/HCV co-infected patients should be carefully followed up so as not to miss the chance for LT. Also, Murillas *et al.* reported that Model for End-Stage Liver Disease (MELD) score is the best prognostic factor in HIV-infected patients,[18] so that HIV/HCV co-infected patients may be considered for LT before MELD score increase to achieve comparable results with HCV mono-infected patients. Several studies showed the aggressive fibrosis in HIV/HCV co-infected patients compared with HCV mono-infected patients,[19,20] but the mechanism of this aggressive fibrosis remains unclear. Recently, transient elastography or acoustic radiation force impulse imaging to check for liver stiffness has been introduced as an effective and non-invasive modality to determine patients' candidacy for LT.[21–23]

Count of CD4$^+$ T lymphocytes

Generally, the count of CD4$^+$ T lymphocytes has been required to be more than 200/μL to perform general elective surgeries in HIV-infected patients,[24] but in HIV/HCV co-infected patients, current studies show that a count of more than 100/μL is acceptable,[25,26] because patients generally have portal hypertension which can cause pancytopenia. In such patients, the ratio of CD4/CD8 is reported to be a feasible marker to predict postoperative complications including opportunistic infections. When the ratio is less than 0.15, the incidence of infectious complications is significantly higher.[27]

Preoperative infections

In regard to latent opportunistic infections that occur before LT, they are not absolute contraindications when they can be expected to be controlled.[28] Infections regarded as contraindications for LT included uncontrollable multidrug resistance HIV infection, chronic *Cryptosporidium enteritis*, progressive multifocal leukoencephalopathy and lymphoma.[29]

MANAGEMENT OF HIV/HCV IN LT

Management of HIV

THE NUMBER OF HIV RNA copies before LT is suggested as an independent risk factor of postoperative mortality, so that HIV should be controlled sufficiently before LT.[30] Accordingly, in the patients who are under consideration to receive LT, ART can be safely stopped before LT because HIV is generally well-controlled for a long period by ART. After LT, ART should be restarted as soon as possible because HIV RNA appears at 3–30 days after ART is stopped,[31] but the timing of restart of ART depends on the patient's condition, including liver function.[32] As long as the liver function has not fully recovered, or partial liver graft such as in LDLT has not sufficiently regenerated yet, ART cannot be started. Castells *et al.* reported in their case–control study that ART was started at a median of 8 days after LT (range, 4–28 days).[33] In principle, the ART administrated after LT should be the same as the pretransplant regimen, but the majority of ART drugs including protease inhibitor (PI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) have interactions with calcineurin inhibitors (CNI) or mammalian target of rapamycin (mTOR),[34] so that the monitoring of blood levels of immunosuppression is extremely important to avoid infectious complications or rejection. Currently, a novel HIV-1 integrase inhibitor, raltegravir (RAL), is expected to be a feasible drug because it has no interactions with CNI, unlike other drugs.[35,36]

Management of HCV

The treatment strategy for HCV in HIV/HCV co-infected patients is the same as in HCV mono-infected patients. Combination therapy of pegylated interferon (PEG IFN) and ribavirin is the standard treatment both before and after LT. The timing of the induction therapy after LT is controversial. A Tokyo group proposed early induction as a preemptive therapy before patients develop hepatitis,[37] while several other reports showed favorable results when the treatment was administrated only after the development of hepatitis was confirmed by liver biopsy.[38,39] Theoretically, the treatment should be started as soon as possible, because in HIV/HCV co-infected patients, HCV recurrence may be accelerated in an immunocompromised state.[30,40] The novel protease inhibitor, telaprevir, is currently introduced as an effective drug to achieve sustained viral response of 70%, even in genotype 1b, with PEG IFN/ribavirin in a non-transplant setting,[41] but this drug is metabolized via cytochrome P450 as a substrate, as are CNI and various protease inhibitors of ART for HIV. Close monitoring of the CNI trough level should be performed, and although triple therapy with telaprevir/PEG IFN/ribavirin is currently reported to be effective to prevent HCV recurrence after LT in HCV mono-infected cases, special attention should be paid when this regimen is adapted in HIV/HCV co-infected patients.

IMMUNOSUPPRESSION

AS PREVIOUSLY MENTIONED, many factors including ART, anti-HCV treatment and an HIV-related immunocompromised state make post-LT immunosuppressive treatment difficult. Many ART drugs, both PI and NNRTI, cause instability in the blood concentration of CNI through the cytochrome P3A4 (CYP3A4)-related metabolism. Most PI cause the overconcentration of CNI by inhibiting CYP3A4, while most NNRTI cause decreased levels of CNI by stimulating CYP3A4.[29,42] As mentioned earlier, RAL is introduced as a key drug in LT in HIV positive patients, because the metabolism of this drug is not related to CYP450, so it does not affect the blood concentration of CNI. Several reports have demonstrated both the *in vitro* and *in vivo* effectiveness of rapamycin in reducing HIV replication,[43–45] and Di Benedetto *et al.* found that rapamycin monotherapy was significantly beneficial in long-term immunosuppression maintenance and HIV control after LT.[46] Mycophenolate mofetil is expected to be an effective immunosuppressive drug because of its efficacy in reducing HIV infection by both virological and immunological mechanisms.[47–49] Using these drugs, a more effective regimen of immunosuppression with ART may be established.

In regard to the steroid, several studies proposed that a steroid-free regimen can be safely applied and effective in LT for HCV cirrhosis. Also, in HIV/HCV co-infected patients, steroid-free protocol may be beneficial to prevent both HIV and HCV recurrence after LT.[50,51]

CONCLUSIONS

LIVER TRANSPLANTATION FOR HIV/HCV co-infected patients remains challenging, but with recent developments in perioperative management and novel drugs for both HIV and HCV, the results are likely to be improved.

REFERENCES

1 Eguchi S, Soyama A, Hidaka M *et al.* Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus coinfection with special reference to hemophiliac recipients in Japan. *Surg Today* 2011; **41:** 1325–31.
2 Weber R, Sabin CA, Friis-Moller N *et al.* Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* 2006; **166:** 1632–41.
3 Rosenthal E, Pialoux G, Bernard N *et al.* Liver-related mortality in human-immunodeficiency-virus-infected patients between 1995 and 2003 in the French GERMIVIC Joint Study Group Network (MORTAVIC 2003 Study). *J Viral Hepat* 2007; **14:** 183–8.
4 Bica I, McGovern B, Dhar R *et al.* Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; **32:** 492–7.
5 Darby SC, Ewart DW, Giangrande PL *et al.* Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. *Lancet* 1997; **350** (9089): 1425–31.
6 Thio CL, Seaberg EC, Skolasky R *et al.* HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *Lancet* 2002; **360** (9349): 1921–6.

7 de Vera ME, Dvorchik I, Tom K *et al.* Survival of liver transplant patients coinfected with HIV and HCV is adversely impacted by recurrent hepatitis C. *Am J Transplant* 2006; **6:** 2983–93.
8 Schreibman I, Gaynor JJ, Jayaweera D *et al.* Outcomes after orthotopic liver transplantation in 15 HIV-infected patients. *Transplantation* 2007; **84:** 697–705.
9 Duclos-Vallee JC, Feray C, Sebagh M *et al.* Survival and recurrence of hepatitis C after liver transplantation in patients coinfected with human immunodeficiency virus and hepatitis C virus. *Hepatology* 2008; **47:** 407–17.
10 Terrault NA, Roland ME, Schiano T *et al.*, Solid Organ Transplantation in HIV: Multi-Site Study Investigators. Outcomes of liver transplant recipients with hepatitis C and human immunodeficiency virus coinfection. *Liver Transpl* 2012; **18:** 716–26.
11 Miro JM, Montejo M, Castells L *et al.*, Spanish OLT in HIV-Infected Patients Working Group Investigators. Outcome of HCV/HIV-coinfected liver transplant recipients: a prospective and multicenter cohort study. *Am J Transplant* 2012; **12:** 1866–76.
12 Tsukada K, Sugawara Y, Kaneko J *et al.* Living donor liver transplantations in HIV- and hepatitis C virus-coinfected hemophiliacs: experience in a single center. *Transplantation* 2011; **91:** 1261–4.
13 Vispo E, Moreno A, Maida I *et al.* Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings. *AIDS* 2010; **24:** 1171–6.
14 Mendizabal M, Craviotto S, Chen T, Silva MO, Reddy KR. Noncirrhotic portal hypertension: another cause of liver disease in HIV patients. *Ann Hepatol* 2009; **8:** 390–5.
15 Kovari H, Ledergerber B, Peter U *et al.*, Swiss HIV Cohort Study. Association of noncirrhotic portal hypertension in HIV-infected persons and antiretroviral therapy with didanosine: a nested case-control study. *Clin Infect Dis* 2009; **49:** 626–35.
16 Merchante N, Girón-González JA, González-Serrano M *et al.* Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. *AIDS* 2006; **20:** 49–57.
17 Ragni MV, Eghtesad B, Schlesinger KW, Dvorchik I, Fung JJ. Pretransplant survival is shorter in HIV-positive than HIV-negative subjects with end-stage liver disease. *Liver Transpl* 2005; **11:** 1425–30.
18 Baccarani U, Adani GL, Bragantini F *et al.* Long-term outcomes of orthotopic liver transplantation in human immunodeficiency virus-infected patients and comparison with human immunodeficiency virus-negative cases. *Transplant Proc* 2011; **43:** 1119–22.
19 Rullier A, Trimoulet P, Neau D *et al.* Fibrosis is worse in HIV-HCV patients with low-level immunodepression referred for HCV treatment than in HCV-matched patients. *Hum Pathol* 2004; **35:** 1088–94.
20 Ragni MV, Moore CG, Soadwa K *et al.*, HHH Study Group. Impact of HIV on liver fibrosis in men with hepatitis C infection and haemophilia. *Haemophilia* 2011; **17:** 103–11.
21 Resino S, Sánchez-Conde M, Berenguer J. Coinfection by human immunodeficiency virus and hepatitis C virus: noninvasive assessment and staging of fibrosis. *Curr Opin Infect Dis* 2012; **25:** 564–9.
22 Merchante N, Rivero-Juárez A, Téllez F *et al.* Liver stiffness predicts clinical outcome in human immunodeficiency virus/hepatitis C virus-coinfected patients with compensated liver cirrhosis. *Hepatology* 2012; **56:** 228–38.
23 Sánchez-Conde M, Miralles P, Bellón JM *et al.* Use of transient elastography (FibroScan®) for the noninvasive assessment of portal hypertension in HIV/HCV-coinfected patients. *J Viral Hepat* 2011; **18:** 685–91.
24 Davison SP, Reisman NR, Pellegrino ED, Larson EE, Dermody M, Hutchison PJ. Perioperative guidelines for elective surgery in the human immunodeficiency virus-positive patient. *Plast Reconstr Surg* 2008; **121:** 1831–40.
25 Miro JM, Torre-Cisnero J, Moreno A *et al.* [GESIDA/GESITRA-SEIMC, PNS and ONT consensus document on solid organ transplant (SOT) in HIV-infected patients in Spain (March, 2005)]. *Enferm Infecc Microbiol Clin* 2005; **23:** 353–62.
26 O'Grady J, Taylor C, Brook G. Guidelines for liver transplantation in patients with HIV infection (2005). *HIV Med* 2005; **6** (Suppl 2)**:** 149–53.
27 Xia XJ, Liu BC, Su JS *et al.* Preoperative CD4 count or CD4/CD8 ratio as a useful indicator for postoperative sepsis in HIV-infected patients undergoing abdominal operations. *J Surg Res* 2012; **174:** e25–30.
28 Grossi PA. Update in HIV infection in organ transplantation. *Curr Opin Organ Transplant* 2012; **17:** 586–93.
29 Joshi D, O'Grady J, Taylor C, Heaton N, Agarwal K. Liver transplantation in human immunodeficiency virus-positive patients. *Liver Transpl* 2011; **17:** 881–90.
30 Ragni MV, Belle SH. Impact of human immunodeficiency virus infection on progression to end-stage liver disease in individuals with hemophilia and hepatitis C virus infection. *J Infect Dis* 2001; **183:** 1112–5.
31 García F, Plana M, Vidal C *et al.* Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. *AIDS* 1999; **13:** F79–86.
32 Neff GW, Bonham A, Tzakis AG *et al.* Orthotopic liver transplantation in patients with human immunodeficiency virus and end-stage liver disease. *Liver Transpl* 2003; **9:** 239–47.
33 Castells L, Escartín A, Bilbao I *et al.* Liver transplantation in HIV-HCV coinfected patients: a case-control study. *Transplantation* 2007; **83:** 354–8.
34 Frassetto LA, Browne M, Cheng A *et al.* Immunosuppressant pharmacokinetics and dosing modifications in HIV-1 infected liver and kidney transplant recipients. *Am J Transplant* 2007; **7:** 2816–20.
35 Armstrong MJ, Corbett C, Rowe IA, Taylor GP, Neuberger JM. HTLV-1 in solid-organ transplantation: current challenges and future management strategies. *Transplantation* 2012; **94:** 1075–84.
36 Tricot L, Teicher E, Peytavin G *et al.* Safety and efficacy of raltegravir in HIV-infected transplant patients cotreated with immunosuppressive drugs. *Am J Transplant* 2009; **9:** 1946–52.
37 Sugawara Y, Tamura S, Yamashiki N *et al.* Preemptive antiviral treatment for hepatitis C virus after living donor liver transplantation. *Transplant Proc* 2012; **44:** 791–3.
38 Tanaka T, Selzner N, Therapondos G, Renner EL, Lilly LB. Virological response for recurrent hepatitis C improves long-term survival in liver transplant recipients. *Transpl Int* 2013; **26:** 42–9.
39 Ueda Y, Takada Y, Marusawa H, Egawa H, Uemoto S, Chiba T. Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. *Transplantation* 2010; **90:** 661–5.
40 Polard E, Camus C, Abault AY *et al.* Retransplantation for acute liver failure due to combined antiviral agents in an HIV-HCV coinfected liver transplant recipient. *Transplantation* 2005; **80:** 1136–8.
41 Barritt AS 4th, Fried MW. Maximizing opportunities and avoiding mistakes in triple therapy for hepatitis C virus. *Gastroenterology* 2012; **142:** 1314–23.
42 Sugawara Y, Tamura S, Kokudo N. Liver transplantation in HCV/HIV positive patients. *World J Gastrointest Surg* 2011; **3:** 21–8.
43 Lin YL, Mettling C, Portales P, Reynes J, Clot J, Corbeau P. Cell surface CCR5 density determines the post-entry efficiency of R5 HIV-1 infection. *Proc Natl Acad Sci U S A* 2002; **99:** 15590–5.
44 Weissman D, Dybul M, Daucher MB, Davey RT Jr, Walker RE, Kovacs JA. Interleukin-2 up-regulates expression of the human immunodeficiency virus fusion coreceptor CCR5 by CD4+ lymphocytes in vivo. *J Infect Dis* 2000; **181:** 933–8.
45 Heredia A, Amoroso A, Davis C *et al.* Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV beta-chemokines: an approach to suppress R5 strains of HIV-1. *Proc Natl Acad Sci U S A* 2003; **100:** 10411–6.
46 Di Benedetto F, Di Sandro S, De Ruvo N *et al.* First report on a series of HIV patients undergoing rapamycin monotherapy after liver transplantation. *Transplantation* 2010; **89:** 733–8.
47 Chapuis AG, Paolo Rizzardi G, D'Agostino C *et al.* Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. *Nat Med* 2000; **6:** 762–8.
48 García F, Plana M, Arnedo M *et al.* Effect of mycophenolate mofetil on immune response and plasma and lymphatic tissue viral load during and after interruption of highly active antiretroviral therapy for patients with chronic HIV infection: a randomized pilot study. *J Acquir Immune Defic Syndr* 2004; **36:** 823–30.
49 Coull JJ, Turner D, Melby T, Betts MR, Lanier R, Margolis DM. A pilot study of the use of mycophenolate mofetil as a component of therapy for multidrug-resistant HIV-1 infection. *J Acquir Immune Defic Syndr* 2001; **26:** 423–34.
50 Marubashi S, Umeshita K, Asahara T *et al.* Steroid-free living donor liver transplantation for HCV – a multicenter prospective cohort study in Japan. *Clin Transplant* 2012; **26:** 857–67.
51 Klintmalm GB, Davis GL, Teperman L *et al.* A randomized, multicenter study comparing steroid-free immunosuppression and standard immunosuppression for liver transplant recipients with chronic hepatitis C. *Liver Transpl* 2011; **17:** 1394–403.

© 2013 The Japan Society of Hepatology

Hepatology Research

Hepatology Research 2013; 43: 502–507 doi: 10.1111/j.1872-034X.2012.01108.x

Original Article

Disease recurrence plays a minor role as a cause for retransplantation after living-donor liver transplantation for primary biliary cirrhosis: A multicenter study in Japan

Hiroto Egawa,[1] Yasuni Nakanuma,[2] Yoshihiko Maehara,[3] Shinji Uemoto,[4] Susumu Eguchi,[5] Yoshinobu Sato,[6] Ken Shirabe,[3] Mitsuhisa Takatsuki,[5] Akira Mori,[4] Masakazu Yamamoto[1] and Hirohito Tsubouchi[7]

[1]*Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo,* [2]*Department of Human Pathology, Graduate School of Medicine, Kanazawa University, Kanazawa,* [3]*Department of Surgery, Graduate School of Medicine, Kyushu University, Fukuoka,* [4]*Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto,* [5]*Department of Surgery, Graduate School of Medicine, Nagasaki University, Nagasaki,* [6]*Department of Surgery, Graduate School of Medicine, Niigata University, Niigata, and* [7]*Digestive Disease and Life-style Related Disease, Health Research Course, Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan*

Aim: To clarify the role of disease recurrence as a cause of graft loss after living-donor liver transplantation (LDLT) for primary biliary cirrhosis (PBC), we investigated explant grafts, as well as the native liver and liver biopsy specimens, of patients who underwent retransplantation.

Methods: Of 516 patients who underwent LDLT for PBC and were registered in the Japanese Liver Transplant Registry, nine patients (1.7%) underwent retransplantation.

Results: Seven patients undergoing retransplantation later than 6 months after primary liver transplantation (LT) were enrolled. All seven patients were female, with ages ranging from 34–57 years, and Model for End-Stage Liver Disease scores ranging 10–28. The right lobe was used as graft in one and the left lobe in six. The initial immunosuppression regimen was tacrolimus in six and cyclosporin in one. The period between the primary LT and retransplantation ranged 11–120 months, with a median of 36 months. Three patients survived and four patients died due to poor graft functions or complications after retransplantation. The primary causes of primary graft loss revealed by histological examination of the explant livers were chronic rejection in three, portal thrombus and/or steatohepatitis in three and outflow block in one. PBC recurrence was observed in 3 and the stage was mild in all.

Conclusion: PBC recurrence has a small impact as a cause of graft loss after LDLT.

Key words: histology, living-donor liver transplantation, primary biliary cirrhosis, recurrence, retransplantation

INTRODUCTION

PRIMARY BILIARY CIRRHOSIS (PBC) is a major indication for liver transplantation (LT). Because autoimmune mechanisms possibly contribute to the etiology of PBC, the possibility of recurrence after transplantation and the impact on the clinical course have been reason for considerable concern. Rates of recurrence have been reported to range 9–35% in deceased-donor LT in Western countries.[1] In living-donor liver transplantation (LDLT) in Japan, the rates have been reported to range 1–40% on the basis of histological evidence.[2–6] However, this range is not reliable because routine liver biopsy is not standard. Furthermore, the impact of recurrence on the clinical course is unclear. The proportion of grafts lost due to disease recurrence was reported to be 2% 10 years after transplantation by Rowe *et al.*[7] On the other hand, Charatcharoenwitthaya *et al.* reported that recurrent PBC was not associated with death or retransplantation.[8] There have been no reports of graft failure secondary to recurrent PBC in Japan, either.[2–6]

The difficulty of performing histological diagnosis of recurrent PBC using needle biopsy specimens is a barrier for studying the impact of recurrent PBC, although histological examination is the gold standard.[6,9,10] Heterogeneity of histological changes is a major hurdle for diagnosis on the basis of needle biopsy specimens. To overcome this problem, we conducted a multicenter study using whole hepatic grafts explanted during retransplantation for PBC.

METHODS

OF 516 PATIENTS who underwent LDLT for PBC and who were registered in the Japanese Liver Transplant Registry, nine patients (1.7%) underwent retransplantation. The demographic data of the recipients and primary donors and information on the clinical courses were obtained.

A current author (Y. N.) performed histological investigation of the native liver, the liver biopsy specimens if present, and the explant grafts. The diagnosis of acute cellular rejection (ACR) and chronic rejection was made according to the Banff criteria.[11,12] Staging of PBC was based on the Nakanuma staging system.[13]

This study was approved by the Ethical Committee of Tokyo Women's Medical University as the central office of the multicenter study, or at each institution if necessary, and it conforms to the provisions of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008).

RESULTS

OF THE NINE patients who underwent retransplantation, two died within 6 months after retransplantation. One died due to graft failure secondary to severe acute rejection and another due to small-for-size syndrome. In both cases, we examined the clinical courses and explanted livers, and confirmed the diagnoses. We enrolled the remaining seven patients in this study.

The demographic and operative data of the recipients and primary donors and the clinical courses are shown in Table 1. All patients were female and had histories of pregnancies. Human leukocyte antigen DR8 was detected in all recipients except no. 5 and in the donors of recipients no. 3, 6 and 7. The donor was the patient's husband in two cases, son in three, sister in one and mother in one.

Primary immunosuppression was performed with a triple regimen consisting of calcineurin inhibitor, steroids and antimetabolites (azathioprine, mizoribine) in three patients, and calcineurin inhibitor and steroids in four patients. The calcineurin inhibitor was tacrolimus in all patients except no. 6 in which cyclosporin was converted to tacrolimus 1 year after transplantation.

All patients were treated with ursodeoxycholic acid (UDCA) and no. 1 and 7 with bezafibrate prior to primary transplantation. All patients were given UDCA after transplantation and only no. 3 was given bezafibrate transiently.

Patients 1, 4, 6 and 7 continued to complain of fatigue even after transplantation. Postoperative complications are shown in Table 1. The period between the primary transplantation and retransplantation ranged 11–120 months, with a median of 36 months. Three patients survived and four patients died due to poor graft functions or complications after retransplantation.

Histological findings of the native liver, the liver biopsy specimens and the explant grafts are summarized in Table 2. The stage of PBC of the native liver was 4 in all patients except no. 7. The primary causes of primary graft loss were chronic rejection in three (no. 2, 3 and 6), portal thrombus in one (no. 7), non-alcoholic steatohepatitis (NASH) in one (no. 4), portal thrombus and NASH in one (no. 5), and outflow block in one (no. 1). Briefly, submassive necrosis from ischemic etiology and liver cirrhosis of chronic congestive etiology were observed in no. 1. Foamy cell arteriopathy, duct loss with degenerative epithelial damage with severe cholestasis, and centrilobular and C-C and P-C bridging fibrosis were observed in no. 2. In both patients 4 and 5 with NASH, the stage had progressed from stage 2 in the biopsy specimens to stage 3 in the explanted livers.[14] Portal vein thromboembolism and altered intrahepatic circulation was also observed in no. 5. Marked centrilobular necrosis and hemorrhage with mild inflammation and fibrosis and portal venopathy with repeated thromboemboli were observed in no. 7.

Recurrence of PBC was observed in no. 2, 6 and 7 in the specimens of on-demand needle or wedge biopsies and confirmed in the explanted livers (Figs 1–3). Histological progression of PBC was very mild or mild and the recurrence was not the main cause of graft failure. We evaluated: (i) mononuclear inflammatory infiltrates; (ii) formation of lymphoid aggregates; (iii)

Correspondence: Professor Hiroto Egawa, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. Email: egawa@ige.twmu.ac.jp

Conflict of interest: none.

Received 6 August 2012; revision 9 September 2012; accepted 12 September 2012.

Table 1 Demographic data, operative data and clinical courses

Patient no.	1	2	3	4	5	6	7
Age (years)	52	40	34	37	47	47	57
Time from diagnosis to LT (months)	22	3	60	55	65	132	99
AMA	>320	80	40	80	NA	Negative	160
Anti-M2 (mg/dL)	1859	1550	NA	NA	NA	NA	152
IgM (mg/dL)	1037.8	172.8	426	115	340	NA	524
IgG (mg/dl)	1945.7	884.2	1774	1373	2921	NA	180
ANA	640	±	Negative	±	Negative	320	NA
Child–Pugh score	7	8	11	12	12	14	10
MELD score	10	11	17	24	22	28	11
Primary donor							
Relation	Husband	Mother	Husband	Sister	Son	Son	Son
Age (years)	50	60	34	47	19	20	23
Sex	Male	Female	Male	Female	Male	Male	Male
Operative variables							
Blood type combination	Compatible	Identical	Identical	Compatible	Compatible	Compatible	Identical
GRWR	1.00	0.95	0.88	0.77	1.07	0.58	0.90
Graft type	Left	Right	Left	Left	Left	Left	Left
Operation time (min)	751	550	665	615	730	680	870
Cold ischemic time (min)	82	38	56	53	111	95	131
Warm ischemic time (min)	53	44	33	40	38	45	41
Blood loss (g)	2400	2470	850	10 320	6190	8005	4500
Postoperative complications	Hemoperitoneum, biliary stenosis, ACR, hepatic vein stenosis	Biliary stenosis, ACR, EBV infection	Chronic rejection	ACR	ACR Artery-portal shunt	Biliary leakage and stenosis	Portal vein thrombosis
Time of retransplantation (months)	39	24	36	88	120	20	11
Outcome of retransplantation	Dead (49 days)	Alive	Dead (59 days)	Alive	Alive	Dead (15 days)	Dead (284 days)
Causes of death	Lung bleeding		Graft failure			Graft failure	Graft failure

ACR, acute cellular rejection; AMA, antimitochondrial antibody; ANA, antinuclear antibody; EBV, Epstein–Barr virus; GRWR, graft recipient weight ratio; Ig, immunoglobulin; LT, liver transplantation; MELD, Model of End-stage Liver Disease; NA, not applicable.

Table 2 Histological findings of the native liver, biopsy specimens and explanted liver

Patient no.	1	2	3	4	5	6	7
PBC staging of native livers							
Stage	4	4	4	4	4	4	2
Bile duct loss	3	3	3	3	3	2	1
Fibrosis	3	2	3	3	3	3	1
Orcein deposition	3	2	3	3	3	2	1
Hepatitis activities	1	1	1	0	0	1	1
Cholangitis activities	0	0	0	0	0	0	0
Needle biopsies							
	Congestion at 6 months	Suspected rPBC (duct loss and hepatitis) at 20 months	No biopsy	rPBC (cholangitis) and NASH at 71 months	rPBC (cholangitis and granuloma) and NASH at 90 months	No biopsy	ACR at 9 months
Main diagnosis	Outflow block	Chronic rejection	Chronic rejection	NASH	PVT and NASH	Chronic rejection	PVT
PBC recurrence	No	Mild (mild chronic cholangitis)	No	Mild (focal duct damage and portal fibrosis)	Mild (focal duct loss and portal inflammation)	No	No

ACR, acute cellular rejection; NASH, non-alcoholic steatohepatitis; PVT, portal vein thrombosis; rPBC, recurrence of PBC.

epithelioid granuloma; and (iv) bile duct damage according to Neuberger's criteria for the diagnosis of recurrent PBC based on liver histology.[15] In patient no. 2, biopsy showed (i) and (iv) (probable recurrence) and the explanted liver showed (i), (ii) and (iv) (definite recurrence); in no. 6, biopsy showed (i), (ii) and (iv) (definite recurrence), and the explanted liver showed (i), (ii) and (iv) (definite recurrence); and in no. 7, biopsy showed (i), (iii) and (iv) (definite recurrence), and the explanted liver showed (i), (ii) and (iv) (definite recurrence).

Case report of three patients with histological diagnoses of recurrent PBC

Patient no. 2 had refractory ACR requiring steroid pulse therapy on postoperative day (POD) 12, 36, 43, 97, 103, 420 and OKT3 monoclonal antibody on POD 434. Liver dysfunction associated with biliary dilatation developed 20 months after LDLT and we performed hepaticojejunostomy and wedge liver biopsy, which revealed suspected recurrence of PBC. Immunosuppression consisted of tacrolimus (3.0 mg/day), steroid (5 mg) and mizoribine (50 mg). Immunoglobulin M was 136, antimitochondrial antibody (AMA) 80 and anti-M2 152 mg/dL. Aggressive liver failure developed despite increased immunosuppression thereafter. She underwent retransplantation 24 months after LDLT.

In patient no. 4, alkaline phosphatase (ALP) began to increase 65 months after LDLT and liver dysfunction developed thereafter. Liver biopsy was performed 71 months after LDLT. Immunosuppression consisted of tacrolimus (2.0 mg/day) and steroid (5 mg). Aspartate aminotransferase (AST) was 44, ALP 432, γ-glutamyltransferase (γ-GT) 17, total bilirubin 1.7 mg/dL, AMA 80 and AMA-M2 155 mg/dL. Tacrolimus was changed to Neoral (Cyclosporine; Novartis, Basel, Switzerland), and mycophenolate mofetil (MMF) (2000 mg/day) was added. Ascites developed 1 year after and liver failure developed. She underwent retransplantation 88 months after LDLT.

In patient no. 5, liver dysfunction developed (AST, 82 IU/L; ALP, 685 IU/L) 50 months after LDLT and was successfully treated with steroid pulse therapy. Liver dysfunction developed and liver biopsy was performed 90 months after LDLT. Total bilirubin was 1.2 mg/dL, AST 57 IU/L, ALP 585 IU/L and γ-GT 48 IU/L. AMA and M2 were not measured. Immunosuppression consisted of tacrolimus only (4.0 mg/day), and MMF (2000 mg) was added thereafter. Portal hypertension started to develop. Radiological examinations yielded a diagnosis of artery-portal shunt of segment 3 of the graft. Shunt

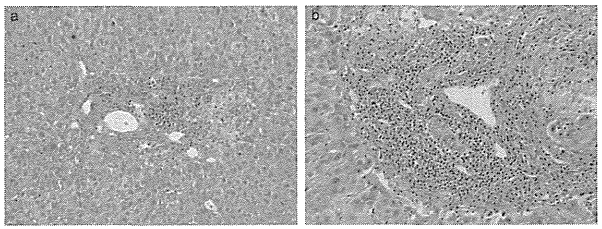


Figure 1 Histological findings of patient no. 2. (a) Wedge liver biopsy at postoperative month 20. Suspected recurrence of primary biliary cirrhosis (PBC) with bile duct loss and mild lobular and portal hepatitis. (b) Second explant liver (allograft). Suspected recurrence of PBC with moderate portal hepatitis and minimal bile duct damage (hematoxylin–eosin, original magnification ×200).

occlusion using metallic coils failed and led to liver failure. She underwent retransplantation 120 months after LDLT.

DISCUSSION

HISTOLOGICAL EXAMINATION IS the gold standard for recurrent PBC. Hubscher *et al.* reported the histological features to be mononuclear portal inflammation, portal lymphoid aggregate, portal granulomas and bile duct damage.[9] These findings are observed also in complications other than recurrent PBC. Lymphoid aggregate can be observed in chronic hepatitis, and bile duct damage and/or vanishing bile duct can be observed in chronic rejection or in the end stage of chronic cholangitis. Foamy cell arteriopathy, which is another specific feature of chronic rejection, is seldom observed on needle biopsy. Duct loss without portal granuloma suggests chronic rejection. The current study focusing on explanted allografts was conducted to avoid these uncertain factors.

Recently, late cellular rejection, chronic hepatitis, and de novo autoimmune hepatitis were discussed as causes of late liver allograft dysfunction.[16] Haga *et al.* reported perivenular lymphoplasmacytic infiltration in a case of their series, which simulated autoimmune hepatitis rather than typical PBC. In our series, ANA was strongly positive prior to primary transplantation in two patients but there were no such findings.

The incidence of recurrent PBC increased along with long-term follow up. Montano-Loza *et al.* studied the cumulative probability of PBC recurrence after LT.[17] Their histological study was not based on protocol biopsy. The overall 5- and 10-year probability of recurrence was 13% and 29%, respectively, in their series. They analyzed risk factors for recurrence and the clinical impacts. Although PBC transplant recipients receiving cyclosporin have a lower risk of disease recurrence, the development of recurrent PBC had no impact on long-term patient survival during 10 years of follow up. The incidence in LDLT based on protocol biopsy was 40% during 10 years of follow up.[3] Besides the increasing incidence, progression of recurrent PBC is still a concern, although progression of recurrent PBC was slow within 10 years of follow up in our series. In Japanese registries of LT, some cases of mortality after 10 years have been reported but information about their causes is not available.[18] A precise study of these cases is required to reveal the risks including recurrence in long-term follow-up.

Protocol biopsies for early diagnosis of recurrent PBC may not be essential to improve clinical courses of

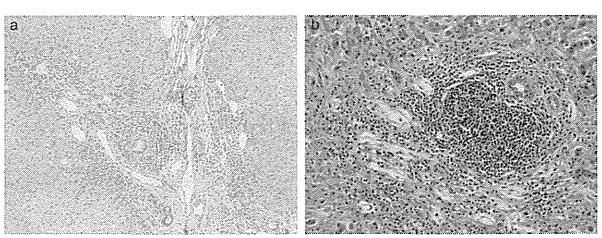


Figure 2 Histological findings of patient no. 4. (a) Needle liver biopsy at postoperative month 71. Recurrence of primary biliary cirrhosis (PBC) with non-suppurative cholangitis and moderate portal hepatitis and fibrosis. (b) Second explant liver (allograft). Suspected recurrence of PBC with focal duct damage and portal inflammation (hematoxylin–eosin, original magnifications: [a] ×150; [b] ×200).

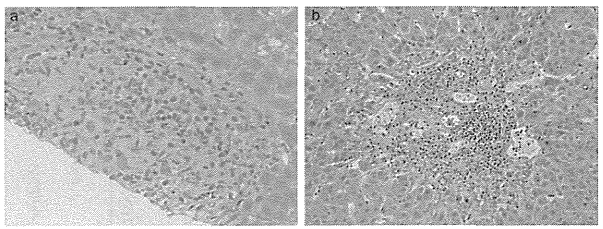


Figure 3 Histological findings of patient no. 5. (a) Needle liver biopsy at postoperative month 90. Recurrence of primary biliary cirrhosis (PBC) with focal cholangitis and epithelioid granuloma. (b) Second explant liver (allograft). Suspected recurrence of PBC with bile duct loss and portal inflammation (hematoxylin–eosin, original magnifications: [a] ×250; [b] ×200).

patients after LT for PBC. Timely biopsies and suitable radiological examinations, when hepatic chemistries deteriorate, are important to improve the clinical course within 10 years after transplantation.

ACKNOWLEDGMENTS

THIS STUDY WAS supported by a Health Labor Sciences Research Grant awarded to The Intractable Hepato-Biliary Disease Study Group in Japan.

REFERENCES

1 Silvera MG, Talwalker JA, Lindor KD, Wiesner RH. Recurrent primary biliary cirrhosis after liver transplantation. *Am J Transplant* 2010; **10:** 720–6.
2 Hashimoto E, Shimada M, Noguchi S *et al.* Disease recurrence after living donor liver transplantation for primary biliary cirrhosis: a clinical and histological follow-up study. *Liver Transpl* 2001; **7:** 588–95.
3 Hashimoto E, Taniai M, Yatsuji S *et al.* Long-term clinical outcome of living-donor liver transplantation for primary biliary cirrhosis. *Hepatol Res* 2007; **37:** S455–61.
4 Takeishi T, Sato Y, Ichida T, Yamamoto S, Kobayashi T, Hatakeyama K. Short-term outcomes of living-related liver transplantation for primary biliary cirrhosis and its recurrence: report of five cases. *Transplant Proc* 2003; **35:** 372–372.
5 Morioka D, Egawa H, Kasahara M *et al.* Impact of leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. *Liver Transpl* 2007; **13:** 80–90.
6 Kaneko J, Sugawara Y, Tamura S *et al.* Long-term outcome of living donor liver transplantation for primary biliary cirrhosis. *Transpl Int* 2012; **25:** 7–12.
7 Rowe IA, Webb K, Gunson BK, Mehta N, Haque S, Neuberger J. The impact of disease recurrence on graft survival following liver transplantation: a single center experience. *Transpl Int* 2008; **21:** 459–65.
8 Charatcharoenwitthaya P, Pimentel S, Talwalkar JA *et al.* Longterm survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. *Liver Transpl* 2007; **13:** 1236–45.
9 Hubscher SG, Elias E, Buckels JA, Mayar AD, McMaster P, Neuberger JM. Primary biliary cirrhosis. Histological eveidence od disease recurrence after liver transplantation. *J Hepatol* 1993; **18:** 173–84.
10 Haga H, Miyagawa-Hayashino Aya TK *et al.* Histological recurrence of autoimmune liver diseases after living-donor liver transplantation. *Hepatol Res* 2007; **37:** S463–S469.
11 International panel. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; **25:** 658–63.
12 Demetris AJ, Adams D, Bellamy C *et al.* Update of the international banff schema for liver allograft rejection: working recommendation for the histopathologic staging and reporting of chronic rejection. An international panel. *Hepatology* 2000; **31:** 792–9.
13 Nakanuma Y, Zen Y, Harada K *et al.* Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. *Pathol Int* 2010; **60:** 167–74.
14 Kleiner DE, Brunt EM, Van Natta M *et al.* Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41:** 1313–21.
15 Neuberger J. Recurrent primary biliary cirrhosis. *Liver Transpl* 2003; **9** (6): 539–46.
16 Banff Working Groups, Demetris AJ, Adeyi O, Bellamy CO *et al.* Liver biopsy interpretation as cause of late liver allograft dysfunction. *Hepatology* 2006; **44:** 489–501.
17 Montano-Loza J, Wasilenko S, Bintner J, Mason AL, Cyclosporine A. Protects against primary biliary cirrhosis recurrence after liver transplantation. *Am J Transplant* 2010; **10:** 852–8.
18 The Japanese Liver Transplant Society. Liver Transplantation in Japan – Registry by the Japanese Liver Transplantation Society. *Ishoku* 2012; **46:** 524–36.

© Ann Transplant, 2013: 18: 293-298
DOI: 10.12659/AOT.883953

WWW.annalsoftransplantation.COM
Original Paper

Received: 2012.12.12
Accepted: 2013.04.08
Published: 2013.06.14

Evaluation of immune function under conversion from Prograf to Advagraf in living donor liver transplantation

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Takayuki Tanaka[ABCDEF], Mitsuhisa Takatsuki[ABCDEF], Akihiko Soyama[AD], Yasuhiro Torashima[B], Ayaka Kinoshita[B], Izumi Yamaguchi[B], Tomohiko Adachi[B], Amane Kitasato[B], Tamotsu Kuroki[D], Susumu Eguchi[AEF]

Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Summary

Background: Although some reports have shown the safety and efficacy of conversion from Prograf to Advagraf in liver transplantation, there have been no reports showing the change of immune function after conversion. The aim of this study is not only to analyze the safety and efficacy of conversion from Prograf to Advagraf, but also to evaluate the immune function using the ImmuKnow assay.

Material/Methods: Of the 168 living donor liver transplantation (LDLT) patients, 21 recipients whose liver function was stable after discharge in outpatient clinic and who agreed to conversion from Prograf to Advagraf were enrolled in this study. Liver, renal, and immune functions were retrospectively reviewed.

Results: There were no significant differences in liver and renal function after conversion from Prograf to Advagraf. The intracellular adenosine triphosphate levels before and after conversion were 263±157 and 256±133 ng/ml, respectively, and there was also no significant difference in immune function. None of the recipients showed adverse effects, rejection, or severe infection during the study. It should be further noted that none of the recipients had to increase the dose of Advagraf, while five of 21 recipients (24%) were able to reduce the dose of Advagraf during this study.

Conclusions: Conversion from Prograf to Advagraf in LDLT can be performed safely and effectively without affecting liver, renal, and immune function.

Key words: Advagraf • tacrolimus • ImmuKnow • LDLT

Full-text PDF: http://www.annalsoftransplantation.com/download/index/idArt/883953

Word count: 1455
Tables: 1
Figures: 4
References: 21

Author's address: Susumu Eguchi, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Japan, zip code,852-8501, e-mail: sueguchi@nagasaki-u.ac.jp

Background

Immunosuppressive therapy is essential to preserve graft function in solid organ transplant recipients [1]. Prograf (Astellas Pharma, Inc.), which is a calcineurin inhibitor developed as an oral twice-daily medicine containing tacrolimus, has been the standard therapeutic regimen all over the world [2]. However, the oral twice-daily regimen has led to non-compliance, and non-compliance causes life-threatening rejection and late graft dysfunction [3,4]. To prevent this, Advagraf (Astellas Pharma, Inc.), a modified tacrolimus formation, was developed as an oral once-daily medicine. At present, conversion to Advagraf therapy has been accepted in various stable organ transplant recipients [5–11].

However, there have been no reports that show the actual changes of immune function after conversion. The ImmuKnow assay (Cylex™ ImmuKnow®-the Cylex Immune Cell Function Assay, Cylex, Inc., USA), which was approved by the Food and Drug Administration in 2002, has been shown to be capable of directly measuring the global immune response, especially T-cell-mediated immunity in transplant recipients. This assay has been shown to reliably distinguish between immune profiles of overimmunosuppression and underimmunosuppression and has been reported to be a convenient, non-invasive, *in vitro* assay, and to be effective as an immune monitoring tool for organ transplant recipients [12,13]. The aim of this study is to analyze the safety and efficacy of conversion from Prograf to Advagraf using not only liver and renal function but also immune function using the ImmuKnow assay.

Material and Methods

Patients

A total of 168 recipients underwent living donor liver transplantation (LDLT) from August 1997 to September 2011 at Nagasaki University Hospital. Of these recipients, 21 who underwent conversion from Prograf to Advagraf were enrolled in this study. They included 13 men and 8 women, with a median age at transplantation of 59 (range, 2–73). Original diagnoses included 3 hepatitis C virus (HCV) cirrhosis, 7 hepatitis B virus (HBV) cirrhosis, 5 alcoholic liver cirrhosis, and 6 others. Of these patients, 8 had hepatocellular carcinoma. The characteristics of the patients are shown in Table 1.

Table 1. The characteristic of the recipients.

Variable	Recipients (n=21)
Gender (male: female)	13: 8
Age	59 (2–73)
Original diagnosis*	HBV-LC: 2 HBV-LC/ HCC: 5 HCV-LC/ HCC: 3 Alcoholic LC: 5 BA: 4 FHF: 2
Duration between LDLT and conversion (months)	33 (7–171)
Duration after conversion (months)	8 (3–29)
Dose of Advagraf at conversion (mg/day)	2 (1–4)

* HBV – hepatitis B virus; HCV – hepatitis C virus; LC – liver cirrhosis; HCC – hepatocellular carcinoma; BA – biliary atresia; FHF – fulminant hepatic failure.

Protocol of immunosuppressant

The baseline protocol of immunosuppressants consisted of Prograf and steroids. The steroids were discontinued three to six months after staged reduction, as long as the liver function was stable without rejection. Prograf was initiated at the dose of 1 mg twice a day after transplantation, and regulated to adjust the desired tacrolimus trough level, 10–15 ng/ml within one month after transplantation and 5–10 ng/ml thereafter. In the outpatient clinic, Prograf was gradually reduced as long as the liver function was stable, and maintained at a minimal dose to prevent both adverse effects and rejection. The indications of the conversion were that liver functions had been stable for at least the three previous months in the outpatient clinic before conversion and that the recipient's fully informed consent to conversion was given. The initial dose after conversion to Advagraf started with the dose equivalent to the dose of Prograf at conversion.

Laboratory evaluation

Tacrolimus trough (Tac), total bilirubin (T-Bil), alanine aminotransferase (ALT), estimated Glomerular Filtration Rate (eGFR), serum creatinine (Cr), and fasting blood sugar (FBS) levels were recorded just before conversion and at the last follow-up and evaluated retrospectively.

The ImmuKnow assay

The immune function was evaluated using Cylex™ ImmuKnow®-the Cylex Immune Cell Function Assay (Cylex, Inc. USA). This assay

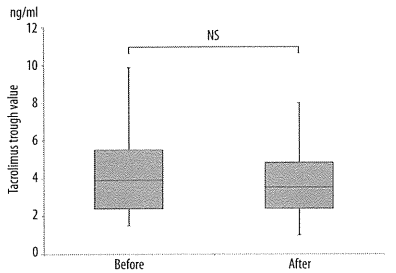


Figure 1. The change of the tacrolimus trough level before and after conversion. Tac levels before and after conversion were 3.9±2.4 and 3.5±2.1 ng/ml, respectively and there was no significant difference in Tac.

was performed according to the manufacturer's protocol [14]. A whole blood sample was collected from each recipient just before conversion and at the last follow-up. The blood sample was collected into an 8-ml sodium heparin vascutainer tube and tested within 10 hours. The whole blood was diluted with a sample diluent, added to a microtiter plate well, and incubated with phytohemagglutinin for 15 to 18 hours in a 37°C, 5% CO_2 incubator. The following day, CD4+ cells were positively selected within the microwells with magnetic particles coated with anti-human CD4 monoclonal antibody (Dynabeads, Dynal, Oslo, Norway) and a strong magnet (model 1050 magnet tray, Cylex, Inc., Columbia, MD) and washed to remove residual cells. A lysing reagent was added to release intracellular adenosine triphosphate (ATP). A luciferin/luciferase mixture was then added to the cell lysate. Within 10 minutes after the addition of the enzyme, released ATP was measured with a GloRunner™ Microplate Luminometer (Turner Biosystems CA).

Statistical analysis

Results for continuous variables were expressed as the median (range). Data for continuous variables were compared using the Mann-Whitney U test. We set statistical significance at $p<.05$.

Results

Change in Tac level and liver functions after conversion.

As shown in Figure 1, the Tac levels before and after conversion were 3.9±2.4 and 3.5±2.1 ng/ml, respectively, and there was no significant difference in Tac. Figure 2 shows liver function. The serum ALT levels before and after conversion were 25±13 and 25±19 IU/l, respectively, and the serum T-Bil levels were 0.9±0.5 and 30.9±0.5 mg/dl, respectively. There was no significant difference in liver function.

Change in renal functions and FBS levels after conversion

Figure 3 shows renal function and FBS level. The serum eGFR levels before and after conversion were 66.8±29.0 and 64.1±27.8 ml/min/1.73 m^2, the serum Cr levels were 0.87±0.23 and 0.82±0.27 mg/dl, and the serum FBS levels were 92±32 and 93±35 mg/dl, respectively. There was no significant difference in renal function or FBS level.

Change in ATP levels after conversion

Figure 4 shows the immune function. The ATP levels before and after conversion were 263±157 and 256±133 ng/ml, respectively. There was also no significant difference in immune function. In addition to these results, none of the recipients showed adverse effects, rejection, or severe

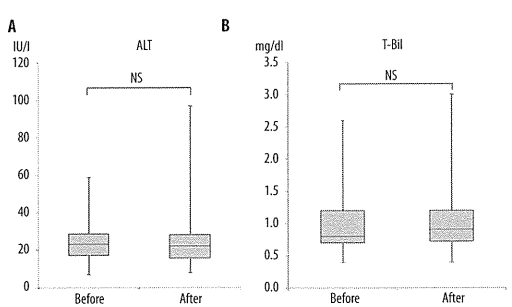


Figure 2. The change of liver functions before and after conversion. (**A**) Serum ALT levels before and after conversion were 25±13 and 25±19 IU/l, respectively. (**B**) Serum T-Bil levels were 0.9±0.5 and 30.9±0.5 mg/dl, respectively. There was no significant difference in liver function.

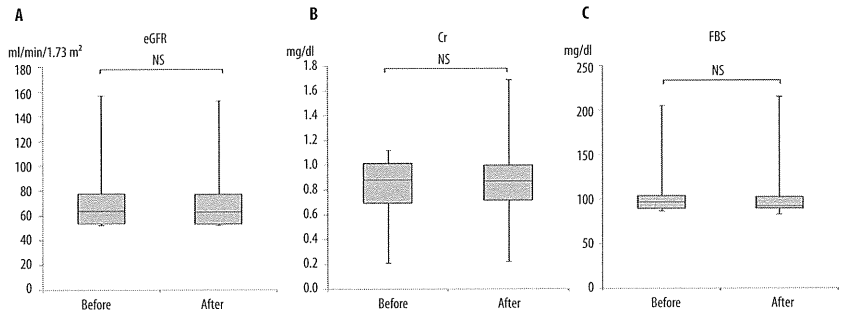


Figure 3. The change of renal functions and FBS before and after conversion. (**A**) Serum eGFR levels before and after conversion were 66.8±29.0 and 64.1±27.8 ml/min/1.73 m^2, respectively. (**B**) Serum Cr levels were 0.87±0.23 and 0.82±0.27 mg/dl, respectively. (**C**) Serum FBS levels were 92±32 and 93±35 mg/dl, respectively. There was no significant difference in renal functions or FBS level.

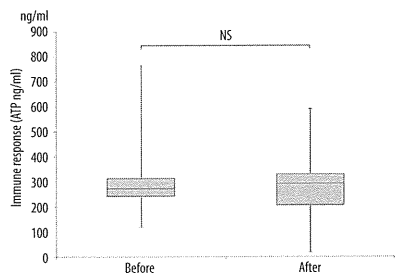


Figure 4. The change of immune function before and after conversion. ATP levels before and after conversion were 263±157 and 256±133 ng/ml, respectively. There was also no significant difference in immune function.

infection during the study. It should also be noted that none of the recipients had to increase the dose of Advagraf, and five of the recipients (24%) could reduce the dose of Advagraf without rejection during this study.

Discussion

Although some reports have shown the safety and efficacy of conversion from Prograf to Advagraf with regard to liver and renal function [8–11], the actual immune function has not yet been clarified. Liver transplantation has been the standard therapeutic option for end-stage liver diseases and reduces the mortality and morbidity of end-stage liver diseases as reflected in the 1- and 5-year survival rates [15–17]. This is mainly the result of improved immunosuppression due to the introduction of a calcineurin inhibitor. Prograf was the immediate-release form of tacrolimus and the oral twice-daily medicine used to prevent various complications in solid organ transplantations and has been accepted as the standard therapeutic regimen all over the world [2,18,19]. However, the estimated rates of nonadherence to immunosuppressive regimens in solid organ transplant recipients range from 15 to 55% [15–17]. Nonadherence has been identified as a leading cause of preventable graft loss [3,4]. It has been proposed that simpler dosing regimens, such as an oral once-daily regimen, may help to improve adherence in transplant recipients [20]. In fact, the prolonged-release form of tacrolimus (Advagraf) was developed as an oral once-daily medicine, and some data have shown that an oral once-daily regimen was associated with an increased likelihood of patient adherence compared with an oral twice-daily regimen [21]. Some reports have evaluated liver and renal function before and after conversion and have shown that the conversion can be applied to liver transplant recipients [8–11]. This study was also able to suggest that conversion does not affect liver and renal function, which is consistent with previous reports.

Additionally, we adapted the ImmuKnow assay to evaluate of the actual immune function. This assay was approved by the US Food and Drug Administration in 2002 for measuring CD4+ T cell immunity [5]. A meta-analysis by Kowaski et al. reported that this assay was useful in monitoring the immune response and assessing the relative risk of infection and rejection [6]. However, no reports have evaluated the safety and efficacy of conversion from Prograf to Advagraf with regard to immune function using this assay. As a result, there

was no significant difference in immune function before and after conversion; this result suggested that conversion also did not affect immune function. In addition, it was important that none of the recipients showed adverse effects, rejection, or severe infection and none had to increase the dose of Advagraf, while five of 21 recipients (24%) were even able to reduce the dose of Advagraf during this study. In our policy of immunosuppression, especially in long-term cases, we reduce and maintain the dose of immunosuppressant as long as possible, keeping the lowest level of tacrolimus needed to prevent rejection. According to the results of this study, Advagraf might be a feasible treatment for avoiding an overdose of tacrolimus.

Conclusions

This study suggested that the conversion of Advagraf can be safely and effectively applied to stable LDLT recipients without affecting liver, renal, and immune function.

Disclosure

The authors have no conflicts of interest or funding to disclose.

References:

1. Chapman JR: The KDIGO clinical practice guideline for the care of kidney transplant recipients. Transplantation, 2010; 89: 644–45
2. Weiler N, Thrun I, Hoppe-Lotichius M et al: Early steroid-free immunosuppression with FK506 after liver transplantation: long-term results of a prospectively randomized double-blinded trial. Transplantation, 2010; 90: 1562–66
3. Gaston RS, Hudson SL, Ward M et al: Late renal allograft loss: noncompliance masquerading as chronic rejection. Transplant Proc, 1999; 31: 21S–23S
4. Denhaerynck K, Dobbles F, Cleemput I et al: Prevalence, consequences, and determinants of nonadherence in adult renal transplant patients: a literature review. Transpl Int, 2005; 18: 1121–33
5. van Hooff JP, Alloway RR, Trunečka P, Mourad M: Four-year experience with tacrolimus once-daily prolonged release in patients from phase II conversion and *de novo* kidney, liver, and heart studies. Clin Transplant, 2010; 25: E1–12
6. Iaria G, Sforza D, Angelico R et al: Switch from twice-daily tacrolimus (Prograf) to once-daily prolonged-release tacrolimus (Advagraf) in kidney transplantation. Transplant Proc, 2011; 43: 1028–29
7. Calia R, Lai C, Aceto P et al: Effects of switching from twice-daily to once-daily tacrolimus formulation on quality of life, anxiety, and transplant benefit perception after kidney transplantation. Transplant Proc, 2011; 43: 1020–23
8. Marin-Gomez LM, Gomez-Bravo MA, Alamo-Martinez JA et al: Evaluation of clinical safety of conversion to Advagraf therapy in liver transplant recipients: observational study. Transplant Proc, 2009; 41: 2184–86
9. Trunečka P, Boillot O, Seehofer D et al: Once-daily prolonged-release tacrolimus (ADVAGRAF) versus twice-dailiy tacrolimus (PROGRAF) in liver transplantation. Am J Transplant, 2010; 10: 2313–23
10. Comuzzi C, Lorenzin D, Rossetto A et al: Safety of conversion from twice-daily tacrolimus (Prograf) to once-daily prolonged-release tacrolimus (Advagraf) in stable liver transplant recipients. Transplant Proc, 2010; 42: 1320–21
11. Perrakis A, Schwarz K, Yedibela S et al: Impact of the conversion of the immunosuppressive regimen from Prograf to Advagraf or to Sirolimus in long-term stable liver transplant recipients: indication, safety, and outcome. Transplant Proc, 2011; 43: 3702–7
12. Kowalski RJ, Post DR, Schneider MC et al: Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. Clin Transplant, 2003; 17: 77–88
13. Kowalski RJ, Post DR, Mannon RB et al: Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. Transplantation. 2006; 82: 663–68
14. Sottong PR, Rosebrock JA, Britz JA, Kramer TR: Measurement of T-lymphocyte responses in whole-blood cultures using newly synthesized DNA and ATP. Clin Diagh Lab Immunol, 2000; 7: 307–11
15. O'Carroll RE, McGregor LM, Swanson V et al: Adherence to medication after liver transplantation in Scotland: a pilot study. Liver Transplant, 2006; 12: 1862–68
16. Butler JA, Roderick P, Mullee M et al: Frequency and impact of nonadherence to immunosuppressants after renal transplantation: a systematic review. Transplantation, 2004; 77: 769–76
17. Vasquez EM, Tanzi M, Benedetti E, Pollak R: Medication noncompliance after kidney transplantation. Am J Health Syst Pharm, 2003; 60: 266–69
18. Kelly PA, Burckart GJ, Venkataramanan R: Tacrolimus: a new immunosuppressive agent. Am J Health Syst Pharm, 1995; 52: 1521–35
19. Spencer CM, Goa KL, Gillis JC: Tacrolimus. An update of its pharmacology and clinical efficacy in the management of organ transplantation. Drugs, 1997; 54: 925–75

20. Morrissey PE, Flynn ML, Lin S: Medication noncompliance and its implications in transplant recipients. Drugs, 2007; 67: 1463–81
21. Weng FL, Israni AK, Joffe MM et al: Race and electronically measured adherence to immunosuppressive medications after decreased donor renal transplantation. J Am Soc Nephrol, 2005; 6: 1839–48

J Gastroenterol (2013) 48:660–670
DOI 10.1007/s00535-012-0667-z

514

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Association of enhanced activity of indoleamine 2,3-dioxygenase in dendritic cells with the induction of regulatory T cells in chronic hepatitis C infection

Koyo Higashitani · Tatsuya Kanto · Shoko Kuroda · Sachiyo Yoshio · Tokuhiro Matsubara · Naruyasu Kakita · Tsugiko Oze · Masanori Miyazaki · Mitsuru Sakakibara · Naoki Hiramatsu · Eiji Mita · Yasuharu Imai · Akinori Kasahara · Alato Okuno · Osamu Takikawa · Norio Hayashi · Tetsuo Takehara

Received: 12 June 2012 / Accepted: 15 August 2012 / Published online: 14 September 2012

Abstract
Background Altered functions of dendritic cells (DCs) and/or increases of regulatory T cells (Tregs) are involved in the pathogenesis of chronic hepatitis C virus (HCV) infection. A tryptophan-catabolizing enzyme, indoleamine 2,3-dioxygenase (IDO), is reported to be an inducer of immune tolerance. Our aim was to clarify whether or not IDO is activated in chronic hepatitis C patients and its role in immune responses.
Methods This study enrolled 176 patients with chronic HCV infection and 37 healthy volunteers. Serum kynurenine concentration was evaluated by high-performance liquid chromatography, and its correlation with clinical parameters was examined. Monocyte-derived DCs were prepared from the subjects and subsequently stimulated with a combination of lipopolysaccharide and interferon-gamma to induce functional IDO (defined as IDO-DCs). The phenotypes, kynurenine or cytokine production, and T-cell responses with IDO-DCs were compared between the patients and healthy volunteers.
Results The serum kynurenine level in the patients was significantly higher than that in the healthy volunteers, and the level of serum kynurenine was positively correlated with the histological activity or fibrosis score. IDO activity in IDO-DCs from the patients was significantly higher than that in IDO-DCs from the volunteers. Furthermore, IDO-DCs from the patients induced more Tregs in vitro compared with those from the volunteers, and the frequency of induced Tregs by IDO-DCs was decreased with an IDO-specific inhibitor.
Conclusions Systemic IDO activity is enhanced in chronic hepatitis C patients in correlation with the degree of liver inflammation and fibrosis. In response to inflammatory stimuli, DCs from the patients tend to induce Tregs, with some of this action being dependent on IDO.

Keywords Hepatitis C virus · Dendritic cell · Regulatory T cell · Indoleamine 2,3-dioxygenase

Electronic supplementary material The online version of this article (doi:10.1007/s00535-012-0667-z) contains supplementary material, which is available to authorized users.

K. Higashitani · T. Kanto (✉) · S. Kuroda · S. Yoshio · T. Matsubara · N. Kakita · T. Oze · M. Miyazaki · N. Hiramatsu · T. Takehara
Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka, Japan
e-mail: kantot@gh.med.osaka-u.ac.jp

M. Sakakibara
Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari, Osaka, Japan

E. Mita
National Hospital Organization Osaka National Hospital, 2-1-14 Hoenzaka, Chuo, Osaka, Japan

Y. Imai
Ikeda Municipal Hospital, 3-1-18 Jonan, Ikeda, Osaka, Japan

A. Kasahara
Department of General Medicine, Osaka University Hospital, 2-15 Yamadaoka, Suita, Osaka, Japan

A. Okuno · O. Takikawa
Laboratory of Radiation Safety, National Center for Geriatrics and Gerontology, 35 Gengo, Morioka, Obu, Aichi, Japan

N. Hayashi
Kansai Rosai Hospital, 3-1-69 Amagasaki, Hyogo, Japan

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. It is estimated that 170 million people are chronically infected with HCV and are at risk of developing liver cirrhosis and/or hepatocellular carcinoma [1]. Approximately 70 % of those exposed to HCV progress to a chronically infected state [2]. The mechanisms of HCV leading to persistent infection have been ascribed to escape mutations of the HCV genome and insufficient immune responses to HCV in hosts, but the precise mechanisms are still largely unknown.

Dendritic cells (DCs) are key regulators of the immune system and are capable of promoting or suppressing T-cell responses depending on their environment [3, 4]. One of the crucial machineries of HCV-induced immune dysfunction is impaired abilities of DCs. Several research groups, including ours [5, 6] have demonstrated that DCs from chronically HCV-infected patients have lower ability to stimulate T cells and to drive T-helper 1 (Th1) polarization than those from healthy controls [7, 8]. Regulatory T cells (Tregs) are specialized suppressor cells that maintain immune tolerance against auto-reactive T cells or against pathogens [9]. In patients with chronic HCV infection, the frequency of Tregs in peripheral blood mononuclear cells (PBMCs) is higher than that in healthy individuals, suggesting the active roles of Tregs in immune alteration or alleviation of inflammation [10, 11]. However, the mechanisms of DC dysfunction or Treg expansion in chronic HCV infection have not been completely elucidated.

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyzes the initial and rate-limiting steps in the catabolism of the essential amino acid tryptophan (Trp), resulting in the generation of kynurenine (Kyn). IDO is widely expressed in human tissues [12] and cell subsets [13] and is induced during inflammation by interferon-gamma (IFN-γ) and/or other inflammatory cytokines [14–16]. Recent studies have demonstrated a crucial role of IDO in the induction of immune tolerance during infection, pregnancy, transplantation, autoimmunity, and cancers [17–21]. IDO expressed by DCs promotes immune tolerance by inhibiting T-cell activation and proliferation or by inducing Tregs through Trp starvation and/or the accumulation of Trp catabolites, such as Kyn, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid [22–25]. With respect to chronic HCV infection, a small-sized study showed that IDO expression was up-regulated in the liver and was associated with increased serum IDO activity [26]. However, the functions of IDO in immune cells in HCV infection still remain obscure.

In this study, we aimed to clarify whether or not IDO in DCs has a role in chronic HCV infection. We found that systemic IDO activity was enhanced in chronic hepatitis C patients. By comprehensively comparing the function of IDO-expressing DCs between the patients and healthy volunteers, we showed that IDO in DCs may be related to the induction of Tregs.

Subjects, materials, and methods

Subjects

This study enrolled 176 patients chronically infected with HCV serotype 1 (CHC group) who had been followed at Osaka University Hospital (Suita, Japan), National Hospital Organization Osaka National Hospital (Osaka, Japan), or Ikeda Municipal Hospital (Ikeda, Japan). All of them were confirmed to be positive for both serum anti-HCV antibody and HCV-RNA but were negative for other viral infections, including hepatitis B virus (HBV) and human immunodeficiency virus. The presence of other liver diseases, such as alcoholic, metabolic, or autoimmune hepatitis was ruled out, and the presence of liver cirrhosis and hepatocellular carcinoma was excluded by the use of laboratory and imaging analyses. As controls, we examined 37 healthy volunteers (HV group), working as medical staff at Osaka University Hospital, who were negative for HCV and HBV markers. As disease controls, 13 patients with chronic HBV infection followed at National Hospital Organization Osaka National Hospital were also enrolled. They were positive for hepatitis B surface (HBs) antigen and had abnormal levels of alanine aminotransferase (ALT). The characteristics of the group were: male/female 10/3, hepatitis B envelope (HBe) antigen-positive/HBe antigen-negative 6/7, mean age 43.9 ± 15.0 years, mean serum ALT level 218.7 ± 282.5 IU/L, and mean HBV-DNA level [assayed by the COBAS AmpliPrep™/COBAS TaqMan™ HBV test (Roche, Branchburg, NJ, USA)] 6.1 ± 2.3 Log copies/mL. At enrollment, written informed consent was obtained from each subject. The study protocol was approved by the ethics committee of each institution.

In this study, because of the limitations of sampling from multiple centers, the conditions for blood collection and preservation differed among the facilities. Thus, for the precise comparison of IDO activity between the patients and healthy volunteers, firstly, we examined the samples collected and preserved under the same conditions at Osaka University Hospital (Cohort I, Table 1). Secondly, because liver biopsy was not carried out in Cohort I patients, we used another cohort (Cohort II, Table 1) for our analysis of the correlation between IDO activity and clinical parameters. Cohort II consisted of the remaining 127 patients, whose samples were collected at National Hospital Organization Osaka National Hospital or Ikeda Municipal Hospital. Histological examination was performed according to the METAVIR scoring system. The clinical backgrounds of the patients in Cohorts I and II, except for HCV-RNA quantity, were not different.

Table 1 Clinical backgrounds of subjects

	HV (Cohort I)	CHC (Cohort I)	CHC (Cohort II)
N	37	49	127
Male/female	20/17	24/25	58/69
Age (years)[a]	44.3 ± 14.6[b]	57.8 ± 12.6	56.5 ± 10.9
ALT (IU/L)[a]	ND	55.8 ± 39.9	64.6 ± 47.9
Plts ($\times 10^4/\mu L$)[a]	ND	16.8 ± 6.4	17.3 ± 6.1
HCV-RNA[c] (Log copies/mL)[a]	ND	6.1 ± 1.0	6.6 ± 0.6[b]
METAVIR activity (A0/1/2/3)	ND	ND	10/78/35/4
METAVIR fibrosis (F0/1/2/3/4)	ND	ND	0/70/29/21/7

CHC chronic hepatitis C patients, *HV* healthy volunteers, *ALT* alanine aminotransferase, *Plts* platelets, *ND* not determined

[a] Values are expressed as means ± SD

[b] Statistical significance was analyzed by the Mann–Whitney *U*-test ($P < 0.05$), compared with CHC group (Cohort I)

[c] Serum HCV-RNA titer was quantitated using the COBAS AmpliPrep™/COBAS TaqMan™ HCV test (Roche)

Reagents and antibodies

Recombinant human interleukin-4 (IL-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF) were purchased from PeproTech (Rocky Hill, NJ, USA). Recombinant human IFN-γ was purchased from R&D Systems (Minneapolis, MN, USA). Lipopolysaccharide (LPS) from *Escherichia coli*, L-tryptophan, L-kynurenine, and 1-methyl-L-tryptophan (1-MT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein monoclonal antibodies (mAbs) against human CD4 (clone, SK3), CD11c (B-ly6), CD25 (M-A251), CD40 (5C3), CD80 (L307.4), CD86 (IT2.2), CD127 (HIL-7R-M21), CD274/PD-L1 (MIH1), HLA-DR (L243), Foxp3 (259D/C7), and isotype control Abs were purchased from BD Biosciences (San Jose, CA, USA).

Generation of CD14+ monocyte-derived dendritic cells

Monocyte-derived DCs (MoDCs) were generated from CD14+ cells as reported previously [27]. In brief, CD14+ cells were cultured for 7 days at 37 °C and 5 % CO_2 in DC culture medium [Iscove's modified Dulbecco's medium (IMDM; Gibco Laboratories, Grand Island, NY, USA) supplemented with 10 % fetal calf serum, 50 IU/mL of penicillin, 50 mg/mL of streptomycin, 2 mM of L-glutamine, 10 mM of Hepes buffer, and 10 mM of nonessential amino acids] in the presence of 20 ng/mL of IL-4 and 50 ng/mL of GM-CSF. On day 5 of the culture, cells were stimulated with 50 ng/mL of LPS and/or 50 ng/mL of IFN-γ to induce functional IDO, and cultured for 48 h. On day 7, cells were harvested and subjected to phenotypic and functional analysis. At the same time, the supernatant of the culture was also collected and subjected to cytokine assays. As controls, unstimulated MoDCs were also prepared.

Flow cytometric analysis

For the analysis of cell surface markers, cells were stained as reported previously [27]. In this study, Tregs were defined as CD4+CD25+CD127-Foxp3+ cells, the frequency of which in PBMCs was analyzed as reported previously [11]. Flow cytometric analyses were performed with the use of a FACSCantoII flow cytometer (BD Biosciences). Analyses of data were done with FACSDiva 6.1 software (BD Biosciences).

Analysis of IDO activity by high-performance liquid chromatography (HPLC)

For the measurement of Kyn and Trp, the HPLC analysis was performed according to the procedure developed by Takikawa et al. [28]. As an index of IDO activity in vivo, the serum kynurenine-to-tryptophan ratio (KTR) was determined by HPLC [26, 29], after deproteinization by the addition of one-tenth volume 2.4 M perchloric acid and centrifugation at 20000×*g* for 10 min. To assay the functional IDO in MoDCs in vitro, the cells were harvested on day 7 of the culture, washed, and resuspended in Hanks' balanced salt solution (HBSS; Gibco Laboratories) containing 100 μM L-Trp. The cells were incubated for an additional 24 h, and Kyn in the culture supernatants was determined by HPLC. IDO activity in vitro was expressed as the concentration of Kyn (μM) in the supernatant, converted from 100 μM L-Trp by IDO.

T-cell stimulation and cytokine analyses

Naive CD4+ T cells were isolated from the allogeneic healthy volunteer using a Naive CD4+ T Cell Isolation Kit II (Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions. After 7 days of the culture, the graded numbers of IDO-DCs (MoDCs stimulated with LPS and IFN-γ for 48 h) were co-cultured with 1×10^5 naive CD4+ T cells in DC culture medium for 4 days. An IDO-specific inhibitor, 1-MT, was used to confirm the specificity of the IDO activity in the T-cell responses. On day 0 of the co-culture, 1-MT was added to IDO-DCs and T-cell cultures at a final concentration of 1 mM. On day 4, half of the supernatants were collected to assess the Th1/Th2 polarization, which was done by measuring the various cytokines. Next, WST-8 reagent in the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) was added to the cultures, followed by incubation for 4 h. The T-cell proliferation index was measured at the absorbance 450 nm of reduced WST-8 using the plate reader. Assays were performed in triplicate wells.

Cytokine bead assay

To analyze the cytokine secretion of IDO-DCs and of naive CD4+ T cells primed with IDO-DCs, the concentrations of IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13, IFN-γ, or tumor necrosis factor-alpha (TNF-α) in the supernatants were assayed using the Cytometric Bead Array System (BD Biosciences) according to the manufacturer's instructions.

Treg induction

To assess the potential effects of IDO on Treg induction from naive CD4+ T cells, the cells were primed with allogeneic IDO-DCs at a 10:1 ratio in HBSS containing 100 μM L-Trp. After 7 days, the primed T cells were harvested and assessed for their surface phenotype and intracellular Foxp3 expression. Phenotyping of the cells after the co-culture was performed using anti-CD4-PerCP, anti-CD25-APC, and anti-CD127-PE. To exclude dead lymphocytes after the co-culture, Near-IR LIVE/DEAD Fixable Dead Cell Stain (Invitrogen, Carlsbad, CA, USA) was used, according to the manufacturer's instructions. Next, the cells were fixed, permeabilized, and stained with anti-Foxp3-Alexa Fluor 488, using the Human FoxP3 Buffer Set (BD Biosciences) according to the manufacturer's instructions. The frequency of CD4+CD25+CD127-Foxp3+ Tregs generated from each priming culture condition was determined by flow cytometry. As described above, 1 mM of 1-MT was added on day 0 to test for IDO-dependent effects.

Statistical analysis

The values were analyzed by nonparametric tests–the Mann–Whitney *U*-test, the Wilcoxon signed rank test, or Spearman's rank correlation test–or by linear regression analysis, using GraphPad Prism software, version 5.04 (Graph Pad Software, San Diego, CA, USA). A *P* value of <0.05 was considered to be statistically significant.

Results

Systemic IDO activity is enhanced in chronic hepatitis C patients

To examine whether or not IDO activity is up-regulated in chronically HCV-infected patients, we compared the serum Kyn and Trp levels between the groups in Cohort I. The serum KTR was significantly higher in the CHC group than that in the HV group (Fig. 1a). Furthermore, we found that the concentration of Kyn in the CHC group was significantly higher than that in the HV group, whereas the levels of Trp were comparable in the two groups (Fig. 1a). These results show that the KTR level in serum, as a surrogate for systemic IDO activity, was higher in chronic hepatitis C patients than in uninfected controls. Furthermore, as the KTR and Kyn levels were correlated (data not shown), the serum Kyn level can be regarded as a surrogate marker for systemic IDO activity.

Next, in order to examine whether or not the enhanced systemic IDO activity was specific for chronically HCV-infected patients, we compared serum Kyn concentrations among chronic hepatitis B patients, chronic hepatitis C patients (Cohort II), and healthy subjects. The serum Kyn concentration in chronic hepatitis B patients was significantly higher than those in the healthy subjects and the patients with chronic hepatitis C (chronic hepatitis B patients: 2.42 ± 0.11 μM, healthy subjects, 1.12 ± 0.09 μM, chronic hepatitis C patients in Cohort II: 2.04 ± 0.06 μM), suggesting that systemic IDO activity is enhanced in chronic HBV infection as well.

Systemic IDO activity correlates with activity grade and fibrosis stage in the liver

Next, to investigate the underlying mechanisms of enhanced IDO activity in chronically HCV-infected patients, we assessed whether or not serum Kyn levels in Cohort II were correlated with various clinical parameters and the METAVIR scores. A significant positive correlation was observed between serum Kyn levels and the histological activity or fibrosis scores (Fig. 1b). However, there was no correlation between the Kyn level and age, ALT level, or HCV-RNA quantity (Fig. 1b). These results show that the more advanced the inflammation and fibrosis of the liver, the higher the serum Kyn, and vice versa. The inverse correlation between serum Kyn and platelet counts was consistent with the correlation between Kyn and the fibrosis score (Fig. 1b).

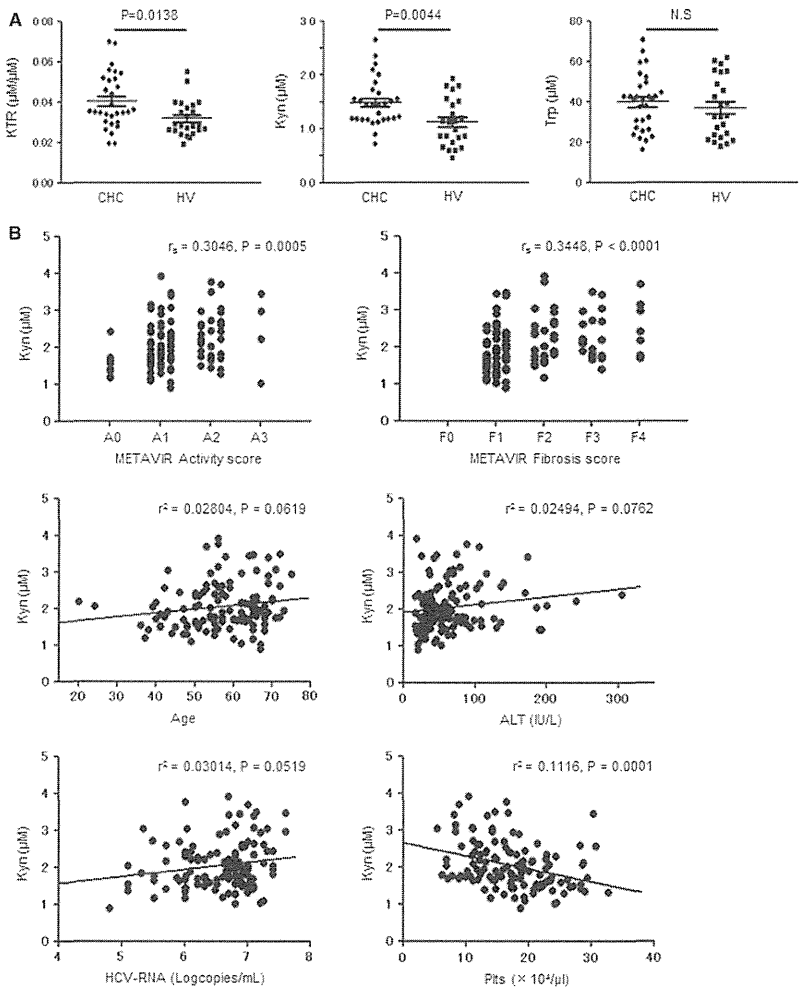


Fig. 1 Systemic indoleamine 2,3-dioxygenase (IDO) activity is enhanced in chronic hepatitis C patients. **a** Serum kynurenine (*Kyn*) and tryptophan (*Trp*) were assayed by HPLC as described in "Subjects, materials, and methods", and the kynurenine-to-tryptophan ratio (*KTR*) was calculated from their concentrations. Scatter plots of 30 chronic hepatitis C patients (*CHC*) and 24 healthy volunteers (*HV*) are shown. *Horizontal bars* depict mean ± SEM. Statistical analyses were performed using the nonparametric Mann–Whitney *U*-test. **b** Correlation analyses were performed between the serum Kyn concentration and histological scores in the liver, and clinical parameters (age, alanine aminotransferase [*ALT*], hepatitis C virus [*HCV*]-RNA titers, and platelet counts [*Plts*]) in 127 chronic hepatitis C patients. Spearman's correlation or simple linear regression analyses were performed. r_s Spearman's correlation coefficient, r^2 linear regression coefficient. *N.S* not significant

Lipopolysaccharide and IFN-γ induce functional IDO in DCs

DCs have been reported to be the most prominent IDO inducer in blood cells in response to inflammatory stimuli [13]. We first assayed the IDO activity (i.e., production of Kyn) of unstimulated MoDCs from chronic hepatitis C patients and found that they did not induce functional IDO (Fig. 2a). In order to simulate the inflammatory condition of DCs in vivo, we examined whether or not IDO was inducible in MoDCs with different combinations of cytokines for various incubation times. In this context, we examined the IDO activity of MoDCs stimulated with LPS alone, IFN-γ alone, or LPS plus IFN-γ for 48 h. The Kyn concentration in media from MoDCs stimulated with LPS alone did not differ from that in unstimulated MoDCs, whereas Kyn concentrations in media from MoDCs stimulated with IFN-γ alone or LPS plus IFN-γ were elevated (Fig. 2a). These results show that the combination of LPS and IFN-γ for 48 h significantly induces functional IDO in MoDCs. Therefore, in the following experiments, we used a combination of LPS and IFN-γ to induce functional IDO.

DCs from chronic hepatitis C patients induce more IDO in response to LPS and IFN-γ than those from healthy volunteers

First, we compared the phenotype of IDO-DCs and unstimulated MoDCs from each group. The expressions of CD40, CD80, CD86, HLA-DR, and CD274/PD-L1 on IDO-DCs were significantly up-regulated compared with those on unstimulated MoDCs, and their expression levels were not different between the CHC and HV groups (Fig. 2b).

Next, we examined the concentration of Kyn in the culture supernatants. In the CHC group, Kyn levels from MoDC culture were significantly enhanced by the stimulation with LPS and IFN-γ (Fig. 2c). Moreover, the Kyn levels in the IDO-DC culture from the CHC group were significantly higher than those in the HV group, whereas those in unstimulated MoDCs did not differ between the groups (Fig. 2c). This increase of Kyn was blocked by the addition of 1-MT, showing that the production of Kyn is specifically dependent on IDO activity (Fig. 2c). These results show that IDO activity is enhanced more in DCs from chronic hepatitis C patients than in DCs from healthy subjects.

Finally, we compared the ability of IDO-DCs to produce various cytokines. The levels of IL-6, IL-10, IL-12p70, and TNF-α from IDO-DCs were not different between the hepatitis C patients and healthy controls (Fig. 2d).

Fig. 2 Enhanced induction of IDO in dendritic cells (DCs) from chronic hepatitis C patients in response to a combination of lipopolysaccharide (*LPS*) and interferon-γ (*IFN-γ*). **a** The levels of Kyn in the culture supernatants of monocyte-derived DCs (MoDCs) in the presence of LPS (50 ng/mL) and/or IFN-γ (50 ng/mL) were determined by HPLC, as described in "Subjects, materials, and methods". The results are expressed as the mean ± SEM from 4 chronic hepatitis C patients. $*P < 0.05$ by nonparametric Wilcoxon signed rank test. *Controls*, unstimulated MoDCs. **b** Phenotype analysis of IDO-DCs was performed as described in "Subjects, materials, and methods". The values are expressed as mean fluorescence intensity (*MFI*). The MFI of each marker is represented as the mean ± SEM from 9 patients and 7 healthy volunteers. $*P < 0.05$ by nonparametric Wilcoxon signed rank test. *IDO-DCs*, MoDCs stimulated with LPS and IFN-γ for 48 h. **c** The levels of Kyn in the culture supernatants were assayed by HPLC as described in "Subjects, materials, and methods". The samples were obtained from MoDCs in the presence (IDO-DCs) or absence (controls) of a combination of LPS and IFN-γ. In parallel, the same experiments were performed in the presence or the absence of 1 mM of 1-methyl-L-tryptophan (*1-MT*). The results are expressed as the mean ± SEM from 12 chronic hepatitis C patients and 10 healthy controls. $*P < 0.05$ by Wilcoxon signed rank test, $**P < 0.05$ by Mann–Whitney *U*-test. **d** The levels of cytokines in the culture supernatants from IDO-DCs were assayed with the Cytometric Bead Array System, as described in "Subjects, materials, and methods". *Bars* depict the mean concentration of each cytokine ± SEM from 10 healthy volunteers and 10 chronic hepatitis C patients. *IL* interleukin, *TNF-α* tumor necrosis factor-α

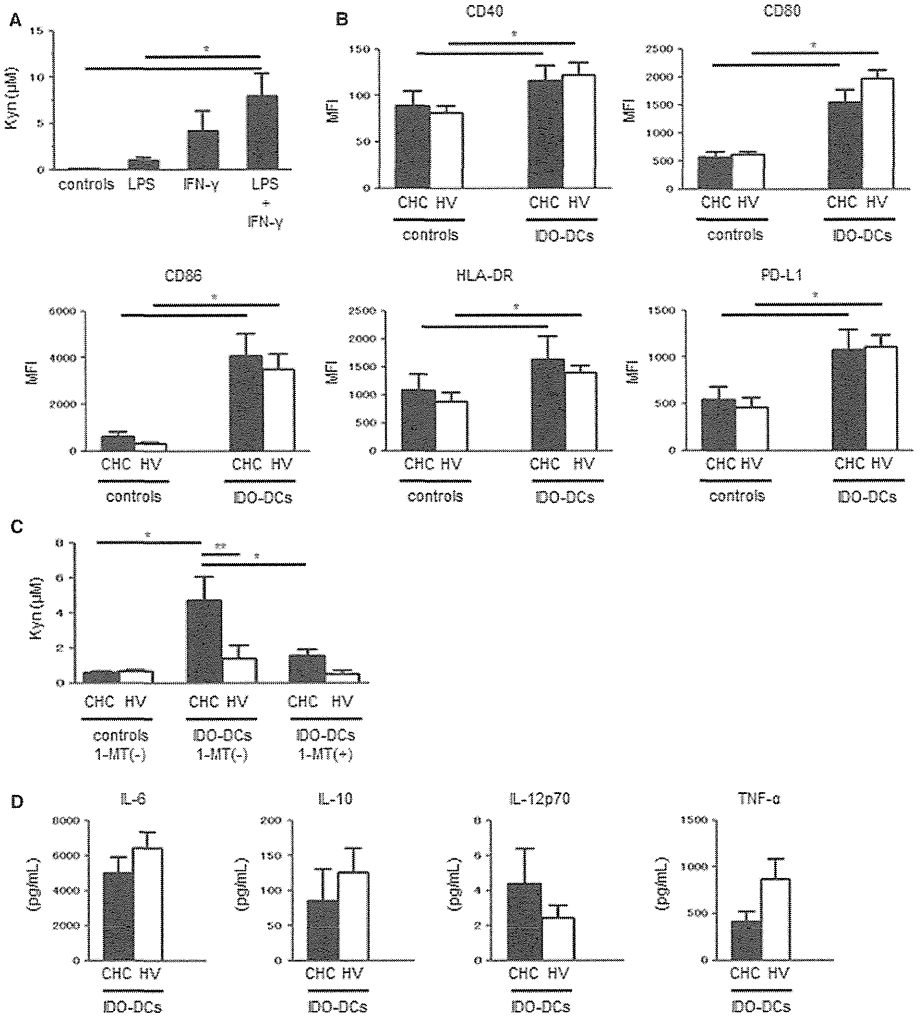
IDO is not involved in allogeneic T-cell proliferation and Th1/Th2 differentiation with DCs from chronic hepatitis C patients

With regard to the allogeneic CD4+ T-cell response, IDO-DCs from the CHC group tended to have a lower stimulatory capacity than those from the HV group (Fig. 3a). To examine whether this phenomenon was dependent on IDO activity, we compared T-cell proliferation with IDO-DCs in the presence and absence of 1-MT. The CD4+ T-cell responses with IDO-DCs were not restored by the addition of 1-MT, regardless of HCV infection (Fig. 3a).

In order to examine whether functional IDO in DCs is involved in Th1/Th2 differentiation, we quantified cytokines in the supernatants obtained from the co-culture of IDO-DCs and CD4+ T cells. In samples from chronic hepatitis C patients, the levels of Th1 cytokines (IL-2, IFN-γ) and Th2 cytokines (IL-4, IL-10, IL-13) tended to be higher than the levels in samples from healthy volunteers, though the difference was not significant. The levels of all cytokines, except for IL-4, tended to decrease with the addition of 1-MT (Fig. 3b). Thus, IDO in DCs is not actively involved in Th1/Th2 differentiation.

IDO is involved in the induction of regulatory T cells

We examined whether or not IDO in DCs was involved in the generation of Tregs. With IDO-DCs from the CHC



group, the frequency of Tregs after the co-culture was significantly higher than that with IDO-DCs from the HV group (Fig. 4a). Such Treg frequency from the culture of the CHC group was significantly reduced in the presence of 1-MT (Fig. 4a). These results show that functional IDO in DCs is partially involved in the generation of Tregs in vitro.

A significant correlation exists between peripheral Treg frequency and serum IDO activity

Finally, we examined whether or not the frequency of Tregs in PBMCs and serum Kyn levels were correlated in our subjects. In the chronic hepatitis C patients, a positive correlation was observed between these parameters (Fig. 4b).

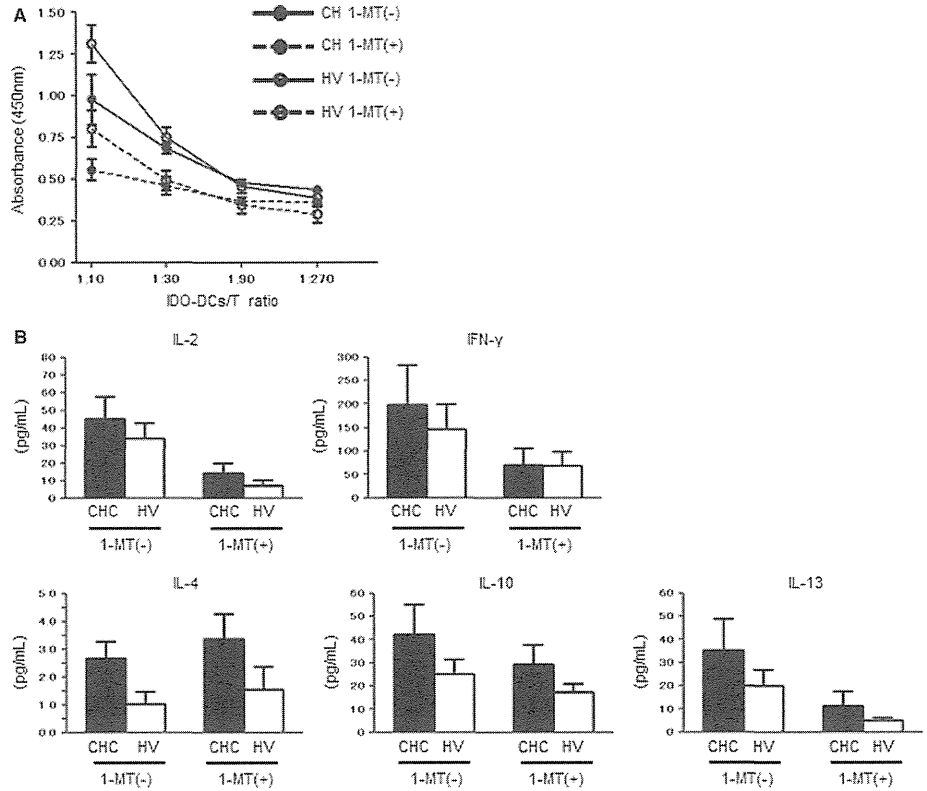


Fig. 3 IDO is not involved in lower allogeneic T-cell response and Th1/Th2 differentiation with DCs from chronic hepatitis C patients. **a** Allogeneic mixed lymphocyte reaction (MLR) with IDO-DCs was performed as described in "Subjects, materials, and methods". *Closed circles* are the 450-nm absorbance obtained with IDO-DCs from the CHC group, and *open circles* are that obtained with IDO-DCs from the HV group. *Dotted lines* are the 450-nm absorbance obtained with IDO-DCs from both groups with the addition of 1-MT. *Vertical bars* indicate the mean ± SEM from 5 chronic hepatitis C patients and 5 healthy volunteers. **b** The levels of cytokines in the supernatants of co-culture of IDO-DCs and naive CD4+ T cells in the presence or absence of 1-MT were assayed with the Cytometric Bead Array System. Results are expressed as the mean ± SEM from 5 patients and 5 healthy controls. *IDO-DCs*; see Fig. 2 legend

However, no significant correlation was observed between peripheral Treg frequency and clinical parameters (i.e., age, ALT, HCV-RNA titers, or platelet counts) (data not shown). These results suggest that an increase in serum Kyn, or enhanced IDO activity, is involved in the increased frequency of Tregs in the PBMCs of HCV-infected patients.

Discussion

In comparison with healthy subjects, we have shown that in chronic hepatitis C patients: (1) systemic IDO activity is enhanced; (2) DCs from these patients exhibit enhanced IDO activity in response to LPS and IFN-γ; (3) IDO-DCs from these patients are more capable than IDO-DCs from healthy volunteers of inducing Tregs in vitro; and (4) the frequency of Tregs in PBMCs is positively correlated with the serum Kyn concentration. Based on these data, it seems that enhanced IDO activity in chronic HCV infection may be one of the mechanisms of Treg induction.

Mammals have two enzymes that catabolize the first and rate-limiting step in the degradation of Trp, resulting in the production of downstream metabolites collectively known as Kyn. The first enzyme is tryptophan 2,3-dioxygenase

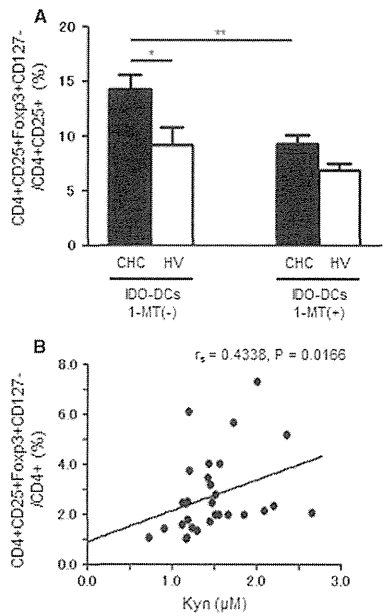


Fig. 4 IDO is involved in the induction of regulatory T cells. **a** After IDO-DCs were generated from the CHC or HV group, naive CD4+ T cells were co-cultured for 7 days with IDO-DCs in the presence or absence of 1-MT. The cultured T cells were stained with relevant antibodies (*Abs*) and analyzed with a FACSCantoII flow cytometer. The percentage of regulatory T cells was determined by the positive ratio of CD4+CD25+CD127-Foxp3+ cells to CD4+CD25+ T cells, as described in "Subjects, materials, and methods". Results are expressed as the mean ± SEM from 9 chronic hepatitis C patients and 5 healthy controls. $^{*}P < 0.05$ by Mann–Whitney *U*-test, $^{**}P < 0.05$ by the Wilcoxon signed rank test. *IDO-DCs*; see Fig. 2 legend. **b** The correlation between the serum Kyn level and the frequency of regulatory T cells was analyzed in 30 chronic hepatitis C patients. The frequency of regulatory T cells was expressed as the percentage of CD4+CD25+CD127-Foxp3+ T cells in CD4+ T cells assessed by FACS. r_s Spearman's correlation coefficient

(TDO), which is expressed primarily in the liver and catabolizes excess dietary Trp to maintain its serum concentration. The second one is IDO, which is expressed in a wider range of tissues, but by a limited range of cell types. In general, TDO is constitutively expressed and is not regulated by inflammatory mediators, while IDO expression is inducible by antigen-presenting cells and is subject to complex regulation by various immunological signals. For the analysis of IDO activity, several modalities have been used, including HPLC and colorimetric and mass spectrometric assays [29, 30]. In the present study, to measure Trp and Kyn, we utilized HPLC owing to its reproducibility, as well as its high-throughput feature. By measuring large numbers of samples, we demonstrated that systemic IDO activity (as expressed by serum KTR) in chronic hepatitis C patients was enhanced compared with that in healthy controls. In addition, we found that increases in KTR were dependent on increased serum Kyn, but not on Trp. Thus, we used Kyn levels as a surrogate for IDO activity.

It is yet to be clarified which type of cell is the source of Kyn in chronic hepatitis C patients. Two possibilities exist for its origin; one is the liver and the other is DCs. We observed positive correlations between serum Kyn levels and the degree of liver inflammation or fibrosis in the present study, suggesting that IDO in the liver may play some role in Kyn production. In support of this possibility, up-regulation of IDO in the liver and increased serum KTR have been reported in patients with chronic HCV infection [26]. It is well known that the inflamed liver is infiltrated by numerous activated immune cells, such as T cells, natural killer (NK) cells, macrophages, and DCs. Thus, it is likely that activated T cells or NK cells release IFN-γ or other cytokines and subsequently induce IDO in hepatocytes or co-existing DCs.

Several investigators have reported that some of the critical stimuli for inducing IDO are inflammatory cytokines or Toll-like receptor (TLR) agonists [14–16, 30–34]. Among them, IFN-γ is reported to play a prominent role in inducing IDO in cancer cells, and the origin of the IFN-γ is presumed to be infiltrated lymphocytes [31]. Furthermore, LPS is regarded as a potent stimulant that induces and sustains IDO in DCs. Therefore, we hypothesized that DCs exposed to some inflammation or fibrosis-related factors express IDO, thereby regulating the immune response in chronic hepatitis C patients. In this study, we used MoDCs for functional assays of IDO in DCs. In order to simulate the inflammatory condition in vivo, we stimulated MoDCs with various combinations of factors, as described above. We found that a combination of IFN-γ and LPS strongly enhanced IDO activity in MoDCs, with this activity being more significantly enhanced in the MoDCs from chronically HCV-infected patients than in those from the healthy controls (Fig. 2a, c). However, the other cytokines failed to enhance IDO activity in MoDCs. Moreover, we confirmed that IDO activity was also enhanced in myeloid dendritic cells (MDCs), stimulated with a combination of IFN-γ and LPS, from the healthy volunteers (Supplementary Figure 1). Because blood MDCs and plasmacytoid DCs (PDCs) are scarce in PBMCs, we used MoDCs as representative cells for the functional analysis of IDO. Thus, in this study, we used a combination of LPS and IFN-γ for MoDCs to induce functional IDO and termed these cells 'IDO-DCs'.

It is intriguing that MoDCs from chronic hepatitis C patients expressed more functional IDO in response to IFN-γ and LPS than the MoDCs from the healthy controls. The simplest reason for this finding would be that such a difference occurs owing to a difference in receptor expressions on DCs. However, this is unlikely, because our previous work showed that TLR4 transcripts in immature MoDCs did not differ between patients with chronic hepatitis C and healthy controls [27]. In addition, in the present study, flow cytometric analysis revealed that the expression of CD119 (IFN-γ receptor α chain) on MoDCs did not differ between the two groups (data not shown). The next possible explanation of the finding that MoDCs from chronic hepatitis C patients expressed more functional IDO in response to IFN-γ and LPS than those from the healthy controls is that there was an influence of other cytokines produced from the stimulated MoDCs in an autocrine fashion. It has been reported that a balance between Th1 and Th2 cytokines has some impact on IDO expression [31]. Finally, the signaling pathways downstream of IFN-γ and LPS may differ between the groups. Jung et al. [32] reported that LPS-induced IDO expression was mediated by IFN-γ-independent mechanisms, including phosphatidylinositol-3-kinase (PI3K) and Jun-N-terminal kinase (JNK) pathways, in murine bone marrow-derived DCs, while IFN-γ-induced IDO expression was regulated by the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathways. As shown in the present study (Fig. 2a), the levels of IDO activity in MoDCs were additively enhanced with LPS and IFN-γ, suggesting the presence of some cross-talk between these signals. Further investigation focusing on the signaling pathway of functional IDO induction is needed to clarify this issue.

Numerous reports have shown that IDO is involved in immune tolerance. As for the mechanisms underlying its involvement, the starvation of Trp could inhibit T-cell proliferation by way of the general control nonrepressed 2 (GCN2) kinase and eukaryotic initiation factor 2α (eIF2α) pathway [35] or the mammalian target of rapamycin (mTOR) and PI3K pathway [36]. Accumulation of Kyn and its metabolites could exert an immune-modulating effect. In the present study, serum Kyn levels were higher in HCV-infected patients than in the healthy controls, whereas Trp levels were comparable in the two groups, suggesting that an increase of Kyn derivatives contributes to immune modulation.

In chronic HCV infection, the mechanisms of IDO-mediated immune tolerance remain unclear. In the present study, we have shown that IDO-DCs are involved in the generation of Tregs in vitro, and the specificity of this involvement was confirmed by the effect of 1-MT. In order to exclude the possibility that 1-MT is cytotoxic to DCs and naive CD4+ T cells, we performed a dye exclusion test or WST-8 assay. Even at the highest concentration of 1-MT, the percentages of viable DCs and the proliferation of T cells were not decreased compared with the findings at the lower concentrations, suggesting that 1-MT was not cytotoxic to cells (Supplementary Figure 2A,B). A possible link between enhanced IDO activity and an increase in Treg frequency was observed in the chronic hepatitis C patients in this study. Thus, it is possible that IDO activity may be partially involved in Treg induction.

Several research groups, including ours, have reported that the frequency and the suppressor function of Tregs are higher in chronic hepatitis C patients than in controls [10, 11]. However, the mechanisms of Treg induction or activation are still largely unknown. Various molecules in DCs, including IL-10, transforming growth factor-beta (TGF-β), programmed cell death 1 ligand 1 (PD-L1), and IDO, are key differentiation molecules for Tregs in various clinical settings. Although the level of TGF-β from DCs was not evaluated in the present study, the levels of IL-10 production and PD-L1 expression did not differ between the HCV-infected patients and the healthy controls (Fig. 2b, d). In this study, the addition of 1-MT did not completely suppress Treg induction by IDO-DCs in vitro. Thus, it is suggested that other factors, such as IL-10, TGF-β, and PD-L1, are also involved in Treg induction. Cytotoxic T-lymphocyte antigen 4 (CTLA-4), which is capable of inducing functional IDO in DCs, has been reported as one of the key molecules for Treg induction [37]. In the present study, the induction of Tregs with IDO-DCs was not altered in the presence of masking anti-CTLA-4 antibody (data not shown), suggesting that CTLA-4 is not involved in this setting.

In conclusion, we have demonstrated that systemic IDO activity is enhanced in chronic hepatitis C patients, and this activity is influenced by histological activity and fibrosis. DCs express functional IDO in response to inflammatory stimuli and, presumably, induce Tregs. Targeting IDO with its specific inhibitor 1-MT could serve as a potential modality to improve the immune response to HCV.

Acknowledgments This study was funded in part by Grants-in-Aid from the Ministry of Health, Labor and Welfare of Japan and the Ministry of Education, Science and Culture of Japan (ID 22590729 and 22590730).

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41–52.
2. Seeff LB. Natural history of chronic hepatitis C. Hepatology. 2002;36:S35–46.

3. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392:245–52.
4. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol. 2003;21:685–711.
5. Kanto T, Hayashi N, Takehara T, Tatsumi T, Kuzushita N, Ito A, et al. Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. J Immunol. 1999;162:5584–91.
6. Kanto T, Inoue M, Miyatake H, Sato A, Sakakibara M, Yakushijin T, et al. Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. J Infect Dis. 2004;190:1919–26.
7. Bain C, Fatmi A, Zoulim F, Zarski JP, Trepo C, Inchauspe G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. Gastroenterology. 2001;120:512–24.
8. Murakami H, Akbar SM, Matsui H, Horiike N, Onji M. Decreased interferon-alpha production and impaired T helper 1 polarization by dendritic cells from patients with chronic hepatitis C. Clin Exp Immunol. 2004;137:559–65.
9. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol. 2004;22:531–62.
10. Cabrera R, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, et al. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology. 2004;40:1062–71.
11. Itose I, Kanto T, Kakita N, Takebe S, Inoue M, Higashitani K, et al. Enhanced ability of regulatory T cells in chronic hepatitis C patients with persistently normal alanine aminotransferase levels than those with active hepatitis. J Viral Hepat. 2009;16:844–52.
12. Yamazaki F, Kuroiwa T, Takikawa O, Kido R. Human indolylamine 2,3-dioxygenase. Its tissue distribution, and characterization of the placental enzyme. Biochem J. 1985;230:635–8.
13. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol. 2004;4:762–74.
14. Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J. 1991;5:2516–22.
15. Puccetti P. On watching the watchers: IDO and type I/II IFN. Eur J Immunol. 2007;37:876–9.
16. Braun D, Longman RS, Albert ML. A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation. Blood. 2005;106:2375–81.
17. Boasso A, Herbeuval JP, Hardy AW, Anderson SA, Dolan MJ, Fuchs D, et al. HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells. Blood. 2007;109:3351–9.
18. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998;281:1191–3.
19. Hainz U, Jurgens B, Heitger A. The role of indoleamine 2,3-dioxygenase in transplantation. Transpl Int. 2007;20:118–27.
20. Platten M, Ho PP, Youssef S, Fontoura P, Garren H, Hur EM, et al. Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. Science. 2005;310:850–5.
21. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9:1269–74.
22. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science. 2002;297:1867–70.
23. Terness P, Bauer TM, Rose L, Dufter C, Watzlik A, Simon H, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med. 2002;196:447–57.
24. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. J Immunol. 2008;181:5396–404.
25. Jurgens B, Hainz U, Fuchs D, Felzmann T, Heitger A. Interferon-gamma-triggered indoleamine 2,3-dioxygenase competence in human monocyte-derived dendritic cells induces regulatory activity in allogeneic T cells. Blood. 2009;114:3235–43.
26. Larrea E, Riezu-Boj JI, Gil-Guerrero L, Casares N, Aldabe R, Sarobe P, et al. Upregulation of indoleamine 2,3-dioxygenase in hepatitis C virus infection. J Virol. 2007;81:3662–6.
27. Yakushijin T, Kanto T, Inoue M, Oze T, Miyazaki M, Itose I, et al. Reduced expression and functional impairment of Toll-like receptor 2 on dendritic cells in chronic hepatitis C virus infection. Hepatol Res. 2006;34:156–62.
28. Takikawa O, Yoshida R, Kido R, Hayaishi O. Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. J Biol Chem. 1986;261:3648–53.
29. Suzuki Y, Suda T, Furuhashi K, Suzuki M, Fujie M, Hahimoto D, et al. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. Lung Cancer. 2010;67:361–5.
30. Suh HS, Zhao ML, Rivieccio M, Choi S, Connolly E, Zhao Y, et al. Astrocyte indoleamine 2,3-dioxygenase is induced by the TLR3 ligand poly(I:C): mechanism of induction and role in antiviral response. J Virol. 2007;81:9838–50.
31. Godin-Ethier J, Pelletier S, Hanafi LA, Gannon PO, Forget MA, Routy JP, et al. Human activated T lymphocytes modulate IDO expression in tumors through Th1/Th2 balance. J Immunol. 2009;183:7752–60.
32. Jung ID, Lee CM, Jeong YI, Lee JS, Park WS, Han J, et al. Differential regulation of indoleamine 2,3-dioxygenase by lipopolysaccharide and interferon gamma in murine bone marrow derived dendritic cells. FEBS Lett. 2007;581:1449–56.
33. Fujigaki H, Saito K, Fujigaki S, Takemura M, Sudo K, Ishiguro H, et al. The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. J Biochem. 2006;139:655–62.
34. Fujigaki S, Saito K, Sekikawa K, Tone S, Takikawa O, Fujii H, et al. Lipopolysaccharide induction of indoleamine 2,3-dioxygenase is mediated dominantly by an IFN-gamma-independent mechanism. Eur J Immunol. 2001;31:2313–8.
35. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity. 2005;22:633–42.
36. Cobbold SP, Adams E, Farquhar CA, Nolan KF, Howie D, Lui KO, et al. Infectious tolerance via the consumption of essential amino acids and mTOR signaling. Proc Natl Acad Sci USA. 2009;106:12055–60.
37. Li R, Perez N, Karumuthil-Melethil S, Prabhakar BS, Holterman MJ, Vasu C. Enhanced engagement of CTLA-4 induces antigen-specific CD4+CD25+Foxp3+ and CD4+CD25− TGF-beta 1+ adaptive regulatory T cells. J Immunol. 2007;179:5191–203.

J Gastroenterol (2013) 48:535–543
DOI 10.1007/s00535-012-0657-1

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Incidence of hepatocellular carcinoma in HCV-infected patients with normal alanine aminotransferase levels categorized by Japanese treatment guidelines

Naoki Harada · Naoki Hiramatsu · Tsugiko Oze · Ryoko Yamada · Mika Kurokawa · Masanori Miyazaki · Takayuki Yakushijin · Takuya Miyagi · Tomohide Tatsumi · Shinichi Kiso · Tatsuya Kanto · Akinori Kasahara · Masahide Oshita · Eiji Mita · Hideki Hagiwara · Yoshiaki Inui · Kazuhiro Katayama · Shinji Tamura · Harumasa Yoshihara · Yasuharu Imai · Atsuo Inoue · Norio Hayashi · Tetsuo Takehara

Received: 18 May 2012 / Accepted: 25 July 2012 / Published online: 14 September 2012

Abstract

Background This study was conducted to evaluate Japanese treatment guidelines for patients with chronic hepatitis C virus (HCV) infection and normal alanine aminotransferase (N-ALT) levels from the viewpoint of the incidence of hepatocellular carcinoma (HCC).

Methods Four groups of patients with chronic HCV infection treated with pegylated interferon (Peg-IFN) plus ribavirin, and classified according to the N-ALT guidelines, were examined for HCC incidence: group A (n = 353), ALT ≤30 IU/L and platelet (PLT) ≥15×10^4/mm^3; group B (n = 123), ALT ≤30 IU/L and PLT <15×10^4/mm^3; group C (n = 233), 30 < ALT ≤ 40 IU/L and PLT ≥15×10^4/mm^3; and group D (n = 100), 30 < ALT ≤ 40 IU/L and PLT <15×10^4/mm^3. The mean observation period was 36.2 ± 16.5 months

Results In groups A and C, the HCC incidence was low even in patients with non-response (NR) (cumulative rates at 3 years, 0.0 and 2.9 %, respectively). In groups B and D, 14.5 and 5.3 % of NR patients had developed HCC at 3 years, but none of the patients with sustained virologic response (SVR) or relapse had developed HCC. In group B, no patients with mild fibrosis developed HCC irrespective of the antiviral effect of the treatment. Among patients with PLT <15×10^4/mm^3 (group B plus group D), the HCC incidence was significantly lower in patients with SVR and relapse than in NR patients ($p < 0.001$, $p = 0.021$, respectively).

Conclusion These results suggest that N-ALT patients with PLT <15×10^4/mm^3 could be candidates for early antiviral therapy.

N. Harada · N. Hiramatsu (✉) · T. Oze · R. Yamada · M. Kurokawa · M. Miyazaki · T. Yakushijin · T. Miyagi · T. Tatsumi · S. Kiso · T. Kanto · A. Kasahara · T. Takehara
Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, 565-0871 Suita, Osaka, Japan
e-mail: hiramatsu@gh.med.osaka-u.ac.jp

M. Oshita
Osaka Police Hospital, Osaka, Japan

E. Mita
National Hospital Organization Osaka National Hospital, Osaka, Japan

H. Hagiwara · N. Hayashi
Kansai Rosai Hospital, Amagasaki, Japan

Y. Inui
Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Japan

K. Katayama
Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan

S. Tamura
Minoh City Hospital, Minoh, Japan

H. Yoshihara
Osaka Rosai Hospital, Sakai, Japan

Y. Imai
Ikeda Municipal Hospital, Ikeda, Japan

A. Inoue
Osaka General Medical Center, Osaka, Japan

Keywords Hepatitis C virus · Normal alanine aminotransferase · Pegylated interferon plus ribavirin combination therapy · Cumulative carcinogenesis rate · Treatment guidelines

Introduction

Continuous hepatitis C virus (HCV) infection causes liver inflammation and can lead to liver fibrosis, which may progress to cirrhosis and hepatocellular carcinoma (HCC) [1–4]. Because HCV carriers with persistent normal alanine aminotransferase (PNALT) levels have minimal liver inflammation and the progression of liver fibrosis in such patients is slow, they are generally considered to be at low risk for carcinogenesis [5–7]. Moreover, patients with PNALT had not been considered as candidates for antiviral therapy in the era of interferon (IFN) monotherapy because of reports of ALT flare-up owing to antiviral therapy in some cases (47–67 %) [8–10].

However, in recent years, the antiviral efficacy of pegylated IFN (Peg-IFN) plus ribavirin combination therapy for patients with chronic HCV infection has been reported to be equivalent for patients with normal alanine aminotransferase (N-ALT) levels and those with elevated ALT levels [11–15]. In addition, for patients with PNALT, there have been fewer cases of ALT flare-up caused by Peg-IFN plus ribavirin combination therapy than with IFN monotherapy [12, 15]. Thus, patients with chronic HCV infection and N-ALT have come to be treated with Peg-IFN plus ribavirin combination therapy.

Treatment guidelines for patients with chronic HCV infection and N-ALT levels have been prepared by a Japanese group conducting "Research on Hepatitis" supported by Health and Labour Sciences Research Grants from the Japanese Government. In these guidelines, HCV carriers with N-ALT (≤40 IU/L) are categorized into four groups according to their ALT levels (≤30 or ≥31 IU/L) and platelet (PLT) counts (≥15 or $<15 \times 10^4/mm^3$). Briefly, the therapeutic strategies are as follows: patients with ALT levels of more than 31 IU/L are candidates for antiviral treatment, but observation is recommended for patients with ALT levels of <30 IU/L. However, the goal of antiviral treatment is to improve the long-term prognosis, including inhibition of HCC. Therefore, the indication of antiviral therapy for patients with chronic HCV infection and N-ALT should be decided based on whether or not Peg-IFN plus ribavirin combination therapy can suppress the cumulative rate of HCC incidence and improve prognosis. It is thus very important to examine the effect of inhibition of HCC induced by antiviral therapy in patients with chronic HCV infection and N-ALT.

In the present study, we evaluated the treatment guidelines for patients with chronic HCV infection and N-ALT from the viewpoint of HCC inhibition by analyzing the differences in the cumulative rates of HCC incidence among the above four groups. The treatment guidelines also recommend that if patients with ALT ≤30 IU/L and PLT $<15 \times 10^4/mm^3$ have moderate to severe liver fibrosis (F2–4), they should receive antiviral therapy. We also evaluated the effect of Peg-IFN plus ribavirin on HCC incidence according to the degree of fibrosis in this group.

Patients and methods

This retrospective study was conducted by Osaka University and institutions participating in the Osaka Liver Forum. Among patients with chronic HCV infection who had received Peg-IFN plus ribavirin combination therapy from December 2004 to December 2009, four groups of patients, classified according to the N-ALT guidelines, who had not suffered from HCC, were examined for their HCC incidence: group A ($n = 353$), ALT ≤30 IU/L and PLT $\geq 15 \times 10^4/mm^3$; group B ($n = 123$), ALT ≤30 IU/L and PLT $<15 \times 10^4/mm^3$; group C ($n = 233$), $30 <$ ALT ≤ 40 IU/L and PLT $\geq 15 \times 10^4/mm^3$; and group D ($n = 100$), $30 <$ ALT ≤ 40 IU/L and PLT $<15 \times 10^4/mm^3$. The Kaplan–Meier method was used to examine the cumulative rates of HCC incidence in the four groups. Excluded from this study were patients who developed HCC within 12 months from the start of Peg-IFN plus ribavirin combination therapy, patients with co-infection with hepatitis B or human immunodeficiency virus, patients with drug-induced or alcoholic liver disorders, and patients with autoimmune hepatitis. The protocol was performed after obtaining informed consent from each patient before treatment in accordance with the ethical guidelines of the Declaration of Helsinki amended in 2008. This study was approved by the Institutional Review Board and registered in the Universal Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN unique trial number, C000000197).

Treatment protocol

All patients received Peg-IFN alpha-2b (PEGINTRON; Merck & Co., Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; MSD) for the duration of the study. Peg-IFN alpha-2b was given subcutaneously once weekly at a dosage of 60–150 μg/kg based on body weight (body weight 35–45 kg, 60 μg; 46–60 kg, 80 μg; 61–75 kg, 100 μg; 76–90 kg, 120 μg; 91–120 kg, 150 μg) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight (body weight ≤60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg), according to the standard treatment protocol for Japanese patients. Dose modification according to the intensity of the hematological adverse effects followed, as a rule, the manufacturer's drug information. The dose of Peg-IFN alpha-2b was reduced to 50 % of the assigned dose if the white blood cell (WBC) count declined to $<1500/mm^3$, the neutrophil count declined to $<750/mm^3$, or the PLT count declined to $<8 \times 10^4/mm^3$, and was discontinued if the WBC count declined to $<1000/mm^3$, the neutrophil count declined to $<500/mm^3$, or the PLT count declined to $<5 \times 10^4/mm^3$. Ribavirin was also reduced, from 1000 to 600 mg, or 800 to 600 mg, or 600 to 400 mg, if the hemoglobin (Hb) level decreased to <10 g/dL, and was discontinued if the Hb level decreased to <8.5 g/dL. Both Peg-IFN alpha-2b and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. During this therapy, no medicine containing iron or hematopoetic growth factors, such as erythropoietin alpha, or granulocyte–macrophage colony-stimulating factor, was administered. The serum HCV RNA levels were qualitatively analyzed using the COBAS AMPLICOR HCV Test, version 2.0 (lower limit of detection 50 IU/mL; Roche Diagnostics, Branchburg, NJ, USA), and the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml). In the patients with HCV genotype 1, as a rule, treatment duration was 48 weeks, but the patients with detectable HCV RNA (≥50 IU/mL) at week 12 and undetectable HCV RNA (<50 IU/mL) at week 24 were treated for 72 weeks. Patients with HCV genotype 2 were treated for 24 weeks.

Definition of virologic response

A sustained virologic response (SVR) was defined as undetectable HCV RNA at the end of treatment and at 24 weeks after completion of treatment. A relapse was defined as undetectable HCV RNA at the end of treatment but detectable HCV RNA at 24 weeks after completion of treatment. A non-response (NR) was defined as detectable HCV RNA at the end of treatment.

Histological evaluation

Liver biopsy was performed immediately before initiation of the Peg-IFN plus ribavirin combination therapy. Liver biopsy specimens were scored using the METAVIR system, and the grade of activity and stage of fibrosis were evaluated [16].

HCC surveillance

Ultrasonography or computed tomography (CT) was carried out before the initiation of the Peg-IFN plus ribavirin combination therapy and every 3–6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by CT or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings from CT. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out, with the patient's consent, or the patient was carefully followed until a diagnosis was possible with a definite observation by CT or angiography.

End point

The observation period was defined as the period from the start of Peg-IFN plus ribavirin combination therapy. Patients who developed HCC and patients whose treatments were switched to other types of IFN therapy were defined as censored cases at that point in time.

Statistical analysis

Baseline data for various demographic, biochemical, and virologic characteristics of the patients were expressed as means ± SD. To analyze differences between baseline data among the four groups, analysis of variance or the χ^2 test was performed. The Kaplan–Meier method was used to calculate the cumulative incidence of HCC. The prognostic relevance of clinical variables and HCC incidence was evaluated by univariate analysis with the log-rank test. A value of $p < 0.05$ (two-tailed) was considered to indicate significance. The statistical software used for this analysis was IBM SPSS for Windows v. 19.0.0 (SPSS, Armonk, NY, USA).

Results

Baseline characteristics of patients categorized by the treatment guidelines

The baseline clinical features of the patients are shown in Table 1. There were significant differences in age; sex; body mass index (BMI); HCV genotype; past history of IFN therapy; grade and stage of liver histology; WBC, neutrophil, and PLT counts; Hb levels; and virologic response among the four groups. The mean ages of the patients in groups B and D were significantly higher than those of the patients in groups A and C. The proportion of males was lowest in group A (26 %) and highest in group C (41 %). The proportion of patients with progression of liver fibrosis (F3–4) diagnosed by the METAVIR score was 7.8 % among all patients tested and highest in group D (22.5 %). In groups B and D, peripheral blood cell counts (WBC, neutrophils, Hb, PLT) were significantly lower and the

Table 1 Baseline characteristics of the patients with chronic HCV infection and normal ALT levels

	Group A ALT ≤30 IU/L PLT count ≥15 × 10^4/mm^3	Group B ALT ≤30 IU/L PLT count <15 × 10^4/mm^3	Group C 30 < ALT ≤ 40 IU/L PLT count ≥15 × 10^4/mm^3	Group D 30 < ALT ≤ 40 IU/L PLT count <15 × 10^4/mm^3	*p* value
Number of patients	353	123	233	100	
Age (years)	55.6 ± 11.3	60.3 ± 8.4	54.6 ± 11.8	60.7 ± 8.6	<0.001
Sex: male/female	95/258	44/79	95/138	35/65	0.005
BMI (kg/m^2)	22.6 ± 3.3	22.1 ± 3.0	23.2 ± 3.4	22.3 ± 2.6	0.029
HCV genotype: 1/2	203/144	86/35	180/52	81/16	<0.001
HCV RNA (KIU/mL), mean ± SD	2333 ± 1664	2276 ± 1478	2261 ± 1599	2354 ± 1644	0.998
Past IFN therapy: naïve/experienced[a]	266/81	79/41	173/52	63/33	0.018
Histology[b]: activity: A0/A1/A2/A3	32/179/48/1	6/64/23/0	20/105/36/1	0/46/24/1	0.026
Fibrosis: F0/F1/F2/F3/F4	41/169/40/9/1	4/49/29/7/5	16/107/31/7/1	0/34/21/13/3	<0.001
White blood cell count (/mm^3)	5543 ± 1606	4405 ± 1211	5601 ± 1638	4677 ± 1337	<0.001
Neutrophil count (/mm^3)	3008 ± 1213	2332 ± 948	2999 ± 1243	2578 ± 1026	<0.001
Hemoglobin (g/dL)	13.3 ± 1.3	13.3 ± 1.4	13.9 ± 1.4	13.3 ± 1.3	<0.001
Platelet count (×10^4/mm^3)	21.1 ± 4.7	12.2 ± 2.1	21.3 ± 4.8	12.1 ± 2.2	<0.001
ALT (IU/L)	22.8 ± 5.2	23.5 ± 5.4	35.4 ± 2.9	35.8 ± 2.9	<0.001
Virologic response: SVR/relapse/NR	218/82/53	59/32/32	133/51/49	44/26/30	0.005

BMI body mass index, *ALT* alanine aminotransferase, *HCV* hepatitis C virus, *IFN* interferon, *SVR* sustained virologic response, *NR* non-response, PLT platelet

[a] Virologic response to previous treatment was unknown for 22 patients

[b] Fibrosis stages are evaluated on a scale of 0–4 and activity grades are evaluated on a scale of 0–3 according to the METAVIR histological score. Fibrosis data were not available for 222 patients. Activity data were not available for 223 patients

numbers of patients with progression of liver fibrosis were significantly higher than in groups A and C. The mean duration of the observation period was 36.2 ± 16.5 months.

Antiviral efficacy of Peg-IFN plus ribavirin combination therapy

In genotype 1 patients, the rates of SVR, relapse, and NR were 50.7, 25.1, and 24.1 %, respectively, in group A; 39.5, 24.4, and 36.0 % in group B; 52.2, 23.9, and 23.9 % in group C; and 39.5, 25.1, and 35.2 % in group D. Although there was no significant difference in the treatment effect among the four groups, the SVR rate was significantly higher in groups A and C than that in groups B and D (groups A and C: SVR 51.4 %, relapse 24.5 %, NR 24.0 %; groups B and D: SVR 39.5 %, relapse 25.1 %, NR 35.2 %, $p = 0.012$). In genotype 2 patients, the rates of SVR, relapse, and NR were 77.8, 20.1, and 2.1 %, respectively, in group A; 65.7, 31.4, and 2.9 % in group B; 75.0, 15.4, and 9.6 % in group C; and 62.5, 31.3, and 6.3 % in group D. Although there was no significant difference in the treatment effect among the four groups, the SVR rate tended to be higher in groups A and C than that in groups B and D (groups A and C: SVR 77.0 %, relapse 18.9 %, NR 4.1 %; groups B and D: SVR 64.7 %, relapse 31.4 %, NR 8.9 %, $p = 0.152$).

Cumulative rate of HCC incidence according to the treatment effect of Peg-IFN plus ribavirin combination therapy

Eleven patients developed HCC during the observation period, and all were infected with HCV genotype 1. Figure 1 shows the cumulative rates of HCC incidence according to the treatment effect in the four groups.

In group A, no patients developed HCC during the 3 years of observation, regardless of the effect of Peg-IFN plus ribavirin combination therapy. Moreover, among those with SVR and relapse, no patients developed HCC during the 3-year observation period, while in NR patients the cumulative rate of HCC incidence at 5 years was 4.0 %. No significant difference in HCC incidence was found among the patients with SVR, relapse, and NR ($p = 0.071$) (Fig. 1a). In group C, no significant difference in HCC incidence was found among the patients with SVR, relapse, and NR (cumulative rates of HCC at 3 years, 2.2, 0.0, and 2.9 %, respectively; at 5 years, 3.7, 0.0, and 2.9 %, respectively, $p = 0.631$) (Fig. 1c). In group B, a marginally significant difference was found in HCC incidence among patients with SVR, relapse, and NR ($p = 0.054$), and patients with SVR had a significantly lower rate of HCC incidence than that of patients with NR (SVR vs. relapse, $p = 0.346$, SVR vs. NR, $p = 0.013$, relapse vs. NR, $p = 0.250$). Of the NR patients, 14.5 % had developed HCC at 3 years, while none of the SVR or relapse patients had developed HCC at 3 years (Fig. 1b). In group D, there was a significant difference in HCC incidence among patients with SVR, relapse, and NR ($p = 0.006$), and patients with SVR or relapse had a significantly lower rate of HCC incidence than patients with NR (SVR vs. NR, $p = 0.012$, relapse vs. NR, $p = 0.047$). In the NR patients, 5.3 % had developed HCC at 3 years and 25.0 % had developed HCC at 5 years, but none of the SVR or relapse patients had developed HCC at 3 years (Fig. 1d).

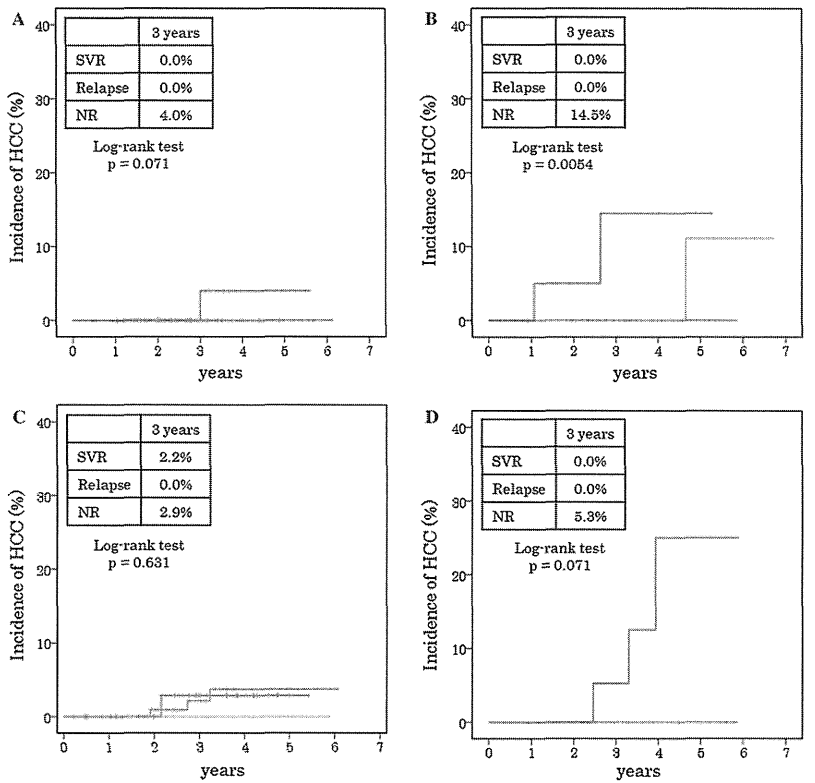


Fig. 1 Cumulative rates of hepatocellular carcinoma (*HCC*) incidence in groups A, B, C, and D, categorized according to the treatment effect of pegylated interferon (Peg-IFN) plus ribavirin combination therapy. **a** Group A (patients with alanine aminotransferase [ALT] level ≤30 IU/L and platelet [PLT] count ≥15 × 10^4/mm^3), **b** group B (patients with ALT ≤30 IU/L and PLT <15 × 10^4/mm^3), **c** group C (patients with 30 < ALT ≤ 40 IU/L and PLT ≥15 × 10^4/mm^3), **d** group D (patients with 30 < ALT ≤ 40 IU/L and PLT < 15 × 10^4/mm^3). *Blue line* patients with sustained virologic response (*SVR*), *green line* patients with relapse, *red line* patients with non-response (*NR*)

In the analysis of the differences in the cumulative rates of HCC incidence in the patients with 30 < ALT ≤ 40 IU/L (group C plus group D), the *p* value for a significant difference was 0.059 among the patients with SVR, relapse, and NR (Fig. 2). In the analysis of the differences in the cumulative rates of HCC incidence among the patients with PLT counts of less than 15 × 10^4/mm^3 (group B plus group D), there was a significant difference in HCC incidence among patients with SVR, relapse, and NR ($p < 0.001$), and patients with SVR or relapse had a significantly lower rate of HCC incidence than patients with NR (cumulative rates of HCC incidence at

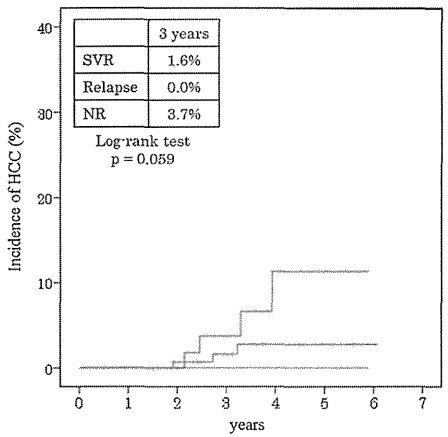


Fig. 2 Cumulative rates of HCC incidence according to ALT levels. Cumulative rates of HCC incidence in patients with ALT levels of $30 < \text{ALT} \leq 40$ IU/L (group C plus group D). *Blue line* patients with sustained virologic response, *green line* patients with relapse, *red line* patients with non-response

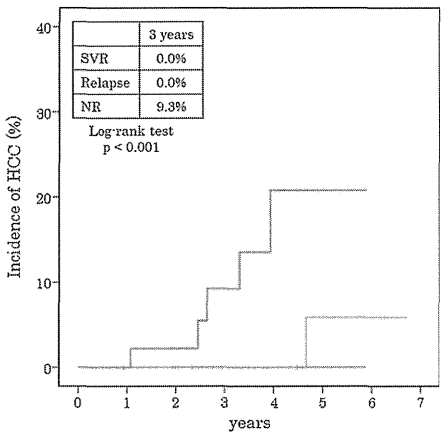


Fig. 3 Cumulative rates of HCC incidence according to PLT counts. Cumulative rates of HCC incidence in patients with PLT counts of $<15 \times 10^4/\text{mm}^3$ (group B plus group D). *Blue line* patients with sustained virologic response, *green line* patients with relapse, *red line* patients with non-response

3 years, 0.0, 0.0, and 9.3 %, respectively; at 5 years, 0.0, 11.1, and 20.8 %, respectively; SVR vs. NR, $p < 0.001$, relapse vs. NR, $p = 0.021$) (Fig. 3).

Cumulative rate of HCC incidence in group B according to the stage of liver fibrosis

Based on the pattern of the Japanese treatment guidelines, we categorized the patients in group B into two groups according to the stage of liver fibrosis (F0–1 or F2–4) and compared the cumulative rates of HCC incidence. Patients with no fibrosis or mild fibrosis (F0–1) showed no HCC development regardless of the virologic response (SVR, relapse, or NR). Of note, in those with moderate to severe fibrosis (F2–4) in group B, there was no significant difference in HCC incidence among patients with SVR, relapse, and NR ($p = 0.174$), although SVR patients tended have a lower rate of HCC incidence than NR patients (SVR vs. relapse, $p = 0.414$, SVR vs. NR, $p = 0.071$, relapse vs. NR, $p = 0.383$). No patient in the SVR or relapse groups developed HCC, while the cumulative rate of HCC incidence at 3 years for the NR group was 25.0 % (Fig. 4).

Discussion

Patients with chronic HCV infection and N-ALT have been reported to show the possibility of ALT flare-up during the natural course of the disease (22–27 %) [17, 18] and to develop moderate to severe progression of liver fibrosis (5–30 %) [18–21]. However, very low cumulative incidences of HCC have been reported among patients with average ALT integration values less than or equal to 20 IU/L (5-year, 0.0 %, 10-year, 3.6 %) [22]. Therefore, it remains controversial whether HCV eradication by antiviral therapy can reduce the incidence of HCC in patients with chronic HCV infection and N-ALT [23–26].

The definition of N-ALT remains unclear because its cutoff value is still under consideration [22, 27, 28]. In Japan, treatment guidelines for patients with chronic HCV infection and N-ALT define N-ALT as serum ALT levels of ≤40 U/L, and the therapeutic strategy is decided after categorizing patients into four groups according to ALT levels and PLT counts. However, the indication of antiviral therapy should be based on whether or not HCC incidence can be suppressed by the antiviral therapy. Therefore, we examined the treatment guidelines from the viewpoint of inhibiting HCC in patients with chronic HCV infection and N-ALT.

In the present study, the antiviral efficacy of Peg-IFN plus ribavirin combination therapy for patients with chronic HCV infection and N-ALT was almost equivalent to the efficacy in those with elevated ALT levels, as previously reported [11–15]. The SVR rate was significantly higher in groups A and C than in groups B and D for patients with genotype 1, and the same tendency was found

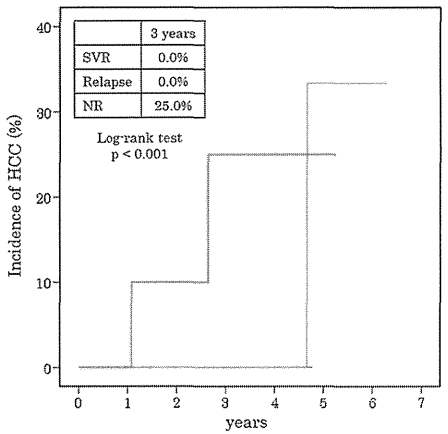


Fig. 4 Cumulative rates of HCC incidence in group B patients ($\text{ALT} \leq 30$ IU/L and $\text{PLT} < 15 \times 10^4/\text{mm}^3$) with moderate to severe liver fibrosis (F2–4), according to the treatment effect of Peg-IFN plus ribavirin combination therapy. *Blue line* patients with sustained virologic response, *green line* patients with relapse, *red line* patients with non-response

for those with genotype 2. The reason for this was considered to be that groups B and D included many patients with moderate to severe liver fibrosis (F3–4, 17.0 %), which can lead to a lower SVR rate [23, 29, 30].

The present study revealed the cumulative rates of HCC incidence according to the treatment effect in the four groups. In group D, the cumulative rate of HCC incidence in the SVR and relapse patients was significantly lower than that for the NR patients. This result supports the recommendation by the treatment guidelines that patients in group D be managed in the same way as patients with chronic hepatitis C (CH-C) and elevated ALT levels.

In group B patients, the treatment guidelines recommend antiviral therapy for those who have moderate to severe liver fibrosis (F2–4). In our present study, patients with no fibrosis to mild fibrosis (F0–1) did not develop HCC, and in the patients with moderate to severe fibrosis (F2–4), the cumulative rate of HCC incidence tended to be lower in the SVR group than that in the NR group ($p = 0.071$). These results also indicate the appropriateness of the Japanese treatment guidelines. However, further study is needed because of the small number of cases studied here.

It appears that group A patients have time to wait for therapy with the next generation of direct antiviral agents (DAAs), such as Peg-IFN plus ribavirin plus a second-generation protease inhibitor, because none of the patients had developed HCC at 3 years. Even in group C, for which the treatment guidelines recommend antiviral therapy, there was no significant difference in the cumulative rate of HCC incidence among the SVR, relapse, and NR patients, with the incidence being below 5 % at 3 years. Accordingly, patients with PLT counts of more than $15 \times 10^4/\text{mm}^3$ (groups A or C) have time to wait until the next generation of DAAs becomes available, because patients with PLT counts of more than $15 \times 10^4/\text{mm}^3$ have a low 3-year carcinogenesis rate.

The Japanese treatment guidelines recommend antiviral therapy for patients with $30 < \text{ALT} \leq 40$ IU/L levels. However, in the present study, in the patients with $30 < \text{ALT} \leq 40$ IU/L levels, the p value for a significant difference in the cumulative rate of HCC incidence among the patients with SVR, relapse, and NR was 0.059. This result indicates that the patients with $30 < \text{ALT} \leq 40$ IU/L levels have the potential to be candidates for antiviral therapy, and further study is needed to clarify this. However, these patients may not be candidates for immediate antiviral therapy because the cumulative rates of HCC incidence at 3 years in the patients with SVR, relapse, and NR were low (cumulative rates of HCC at 3 years: 1.6, 0.0, and 3.7 %). On the other hand, as mentioned above, in the patients with PLT counts of $<15 \times 10^4/\text{mm}^3$, the cumulative rate of HCC incidence was significantly lower in the SVR and relapse patients than that in the NR patients (cumulative rates of HCC at 3 years: 0.0, 0.0, and 9.3 %; at 5 years: 0.0, 11.1, and 20.8 %; $p < 0.001$). This result suggests that patients with PLT counts of $<15 \times 10^4/\text{mm}^3$ may be candidates for antiviral therapy.

A limitation of this study was that the incidence of HCC was not compared between a treatment group and a non-treatment group. This study showed the suppressive effect of antiviral therapy on HCC incidence by comparing patients according to the treatment's antiviral effect. Peg-IFN plus ribavirin combination therapy has become acceptable for patients with chronic HCV infection and N-ALT levels. However, if there were no difference in HCC incidence between patients with SVR and non-SVR in the group receiving Peg-IFN plus ribavirin combination therapy, it would not be necessary for patients with chronic HCV infection and N-ALT to receive this therapy. In this study, we compared the incidence of HCC according to the treatment effect in HCV-infected patients with N-ALT levels categorized by the Japanese treatment guidelines. Indeed, although our results did not demonstrate that N-ALT patients should be treated, they indicated that it could be appropriate to treat N-ALT patients, because the incidence of HCC in these patients with SVR was suppressed compared with that in the NR patients.

In conclusion, in patients with N-ALT and PLT counts of $<15 \times 10^4/\text{mm}^3$ who received Peg-IFN plus ribavirin

combination therapy, the cumulative rate of HCC incidence was significantly lower in those with SVR or relapse than in those with NR. Therefore, HCV-infected patients with N-ALT and PLT counts of $<15 \times 10^4/mm^3$ could be candidates for early antiviral therapy for the purpose of reducing the risk of developing HCC.

Acknowledgments Other institutions and participants in the Osaka Liver Forum are: Higashi Osaka General Hospital, S Iio; Sumitomo Hospital, A Yamada; Toyonaka Municipal Hospital, M Inada; National Hospital Organization Osaka Minami Medical Center, T Hijioka; Yao Municipal Hospital, H Fukui; Kinki Central Hospital of Mutual Aid Association of Public School Teachers, E Hayashi; Osaka Koseinenkin Hospital, T Ito; Itami City Hospital, Y Saji; Suita Municipal Hospital, T Nagase; Ashiya Municipal Hospital, A Takeda; Saiseikai Senri Hospital, K Suzuki; NTT West Osaka Hospital, A Kaneko; National Organization Minami Wakayama Medical Center, M Kato; Otemae Hospital, Y Doi; Kano General Hospital, S Kubota; Nishinomiya Municipal Central Hospital, H Ogawa; Osaka Kaisei Hospital, N Imaizumi; Saso Hospital, M Nishiuchi; and Meiwa Hospital, Y Hayakawa. This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from the Ministry of Health, Labor and Welfare of Japan, and by a Grant-in Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan. None of the authors has any financial relationship relevant to this study to disclose.

Conflict of interest Dr Kanto belongs to an endowed department sponsored by MSD. Dr Takehara received donations from MSD and Chugai Pharmaceutical CO., LTD.

References

1. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology. 1997;112:463–72.
2. Serfaty L, Aumaitre H, Chazouilleres O, Bonnand AM, Rosmorduc O, Poupon RE, Poupon R. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. Hepatology. 1998;27:1435–40.
3. Hu KQ, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. Hepatology. 1999;29:1311–6.
4. National Institutes of Health Consensus Development Conference Statement. Management of hepatitis C 2002 (June 10–12, 2002). Gastroenterology. 2002;123:2082–99.
5. Nutt AK, Hassan HA, Lindsey J, Lamps LW, Raufman JP. Liver biopsy in the evaluation of patients with chronic hepatitis C who have repeatedly normal or near-normal serum alanine aminotransferase levels. Am J Med. 2000;109:62–4.
6. Marcellin P. Hepatitis C: the clinical spectrum of the disease. J Hepatol. 1999;31(Suppl 1):9–16.
7. Martinot-Peignoux M, Boyer N, Cazals-Hatem D, Pham BN, Gervais A, Le Breton V, Levy S, et al. Prospective study on antihepatitis C virus-positive patients with persistently normal serum alanine transaminase with or without detectable serum hepatitis C virus RNA. Hepatology. 2001;34:1000–5.
8. Sangiovanni A, Morales R, Spinzi G, Rumi M, Casiraghi A, Ceriani R, Colombo E, et al. Interferon alfa treatment of HCV RNA carriers with persistently normal transaminase levels: a pilot randomized controlled study. Hepatology. 1998;27:853–6.
9. Pradat P, Alberti A, Poynard T, Esteban JI, Weiland O, Marcellin P, Badalamenti S, et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. Hepatology. 2002;36:973–7.
10. Tassopoulos NC. Treatment of patients with chronic hepatitis C and normal ALT levels. J Hepatol. 1999;31(Suppl 1):193–6.
11. Arora S, O'Brien C, Zeuzem S, Shiffman ML, Diago M, Tran A, Pockros PJ, et al. Treatment of chronic hepatitis C patients with persistently normal alanine aminotransferase levels with the combination of peginterferon alpha-2a (40 kDa) plus ribavirin: impact on health-related quality of life. J Gastroenterol Hepatol. 2006;21:406–12.
12. Bini EJ, Mehandru S. Sustained virological response rates and health-related quality of life after interferon and ribavirin therapy in patients with chronic hepatitis C virus infection and persistently normal alanine aminotransferase levels. Aliment Pharmacol Ther. 2006;23:777–85.
13. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology. 2002;36:S47–56.
14. Hiramatsu N, Inoue Y, Oze T, Kurashige N, Yakushijin T, Mochizuki K, Miyagi T, et al. Efficacy of pegylated interferon plus ribavirin combination therapy for hepatitis C patients with normal ALT levels: a matched case–control study. J Gastroenterol. 2011;46(11):1335–43.
15. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, Shiffman M, et al. Peginterferon alfa-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology. 2004;127:1724–32.
16. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 1996;24:289–93.
17. Tsuji K, Yamasaki K, Yamanishi M, Kawakami M, Shirahama S. Risk of alanine aminotransferase flare-up among asymptomatic hepatitis C virus RNA carriers: a 10-year follow-up study. J Gastroenterol Hepatol. 2001;16:536–40.
18. Puoti C, Castellacci R, Montagnese F. Hepatitis C virus carriers with persistently normal aminotransferase levels: healthy people or true patients? Dig Liver Dis. 2000;32:634–43.
19. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009;49:1335–74.
20. Puoti C, Guido M, Mangia A, Persico M, Prati D. Clinical management of HCV carriers with normal aminotransferase levels. Dig Liver Dis. 2003;35:362–9.
21. Puoti C, Magrini A, Stati T, Rigato P, Montagnese F, Rossi P, Aldegheri L, et al. Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal or abnormal alanine transaminase levels. Hepatology. 1997;26:1393–8.
22. Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, et al. Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. J Hepatol. 2009;50:729–35.
23. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347:975–82.
24. Puoti C, Bellis L, Martellino F, Guarisco R, Dell' Unto O, Durola L, Galossi A. Chronic hepatitis C and 'normal' ALT levels: treat the disease not the test. J Hepatol. 2005;43:534–5.
25. Puoti C, Bellis L, Galossi A, Guarisco R, Nicodemo S, Spilabotti L, Unto OD. Antiviral treatment of HCV carriers with persistently normal ALT levels. Mini Rev Med Chem. 2008;8:150–2.
26. Okanoue T, Makiyama A, Nakayama M, Sumida Y, Mitsuyoshi H, Nakajima T, Yasui K, et al. A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. J Hepatol. 2005;43:599–605.
27. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002;137:1–10.
28. Ahmed A, Keeffe EB. Chronic hepatitis C with normal aminotransferase levels. Gastroenterology. 2004;126:1409–15.
29. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358:958–65.
30. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140:346–55.

＜短　報＞

C型慢性肝炎SVR例におけるHCV-RNAの一過性陽性例の検討

福田　安伸[1)]　長瀬　良彦[1)]　北川紗里香[1)]　路川　陽介[1)]　平石　哲也[1)]
公文　大輔[1)]　黄　世揚[1)]　馬場　哲[1)]　山田　典栄[2)]　小林　稔[2)]
池田　裕喜[2)]　高橋　秀明[2)]　松永光太郎[2)]　松本　伸行[2)]　奥瀬　千晃[2)]
四柳　宏[3)]　鈴木　通博[1)2)*]

緒言：C型慢性肝炎（CH-C）に対する抗ウイルス療法の重要な効果予測となる血中HCV-RNA陰性化時期の判断には，リアルタイムPCR法を測定原理とするTaqMan®法が広く用いられている．陰性化は，1.2 Log IU/mL未満，シグナル検出なし（＜1.2－）により判断される．しかし，一部症例において血中HCV-RNA陰性化後，陽性（＜1.2＋），陰性を繰り返す例が認められ，これら症例の多くは血中にHCV-RNAが低レベルで残存し，治療終了後に再燃を来す．一方，治療中や終了後治療効果判定前に一過性に陽性を示すもSVRに至る例が認められる．さらに，SVR判定後の長期経過観察例においても一過性陽性例が散見される．今回，これらSVR例における一過性陽性例の背景，経過につき検討を行った．

対象および方法：1）治療中～SVR判定前一過性陽性例の検討：2007年7月のリアルタイムPCR法（TaqMan® HCV Test，Roche Diagnostics東京）導入から2011年12月末までに，当院および関連施設で抗ウイルス療法（IFN単独またはPeg IFN＋Rib併用）を施行し，SVR（治療6カ月後の時点での血中HCV-RNA陰性）を確認したCH-Cは157例である．内訳は，セログループ1型の高ウイルス量（5.0 Log IU/L≦）：G1-HIGHが75例，G1-HIGH以外（others）は82例であり，othersはG1-LOW 13例，セログループ2型（SG2）が69例であった．これら症例よりSVR判定前にHCV-RNAが陰性化後一過性に陽性を示した8例を対象として患者背景，ウイルス量の推移，一過性陽性時期の検討に加え，SVR判定後の観察にてHCV-RNAの再出現があるか否かを検討した．2）SVR後一過性陽性例の検討：SVR判定後，3～6カ月毎を原則とした定期採血によりHCV-RNAの経過観察を行っているCH-C 235例（G1-HIGH 158例）から，HCV-RNA一過性陽性が確認された19例の一過性陽性時期，患者背景，その後のHCV-RNAの再出現の有無を検討した．

結果：1）治療中～SVR判定前：一過性陽性例は8例に認められ，背景および全経過をFig. 1に示す．治療時平均年齢60.6±9.1歳，男性3，女性5例，全例初回治療，G2が6例，内低ウイルス量（G2-LOW）は5例，G1が2例，内G1-LOWは1例である．治療法は，β-IFN1例，Peg-IFNα2a 3例，Peg-IFNα2b＋Rib 4例であり，治療期間は8週の短期投与が1例，24週が6例，48週が1例である．HCV-RNA一過性陽性時期は治療中が4例であり，内3例は治療開始後1週の時点での陰性化例である．治療終了後SVR評価前は4例であり，内2例は治療終了後4週の直後の評価にて検出された．Case 7の1.7 Log IU/mlを除き，いずれも＜1.2＋で検出された．なお4例の一過性陽性時点の同時採血による保存血清を用いた再検ではHCV-RNAはいずれも陰性を示した．さらにCase 5はSVR後の組織内HCV-RNAの検討においても陰性を示した．Case 6は，SVR判定後24週後に＜1.2＋の再度一過性陽性を示した．8例は，現時点において，SVR後3.5年を最長に，全例ALTの上昇は認めずHCV-RNA陰性を示している．

2）SVR後：一過性陽性例は19例に認められ，背景およびSVR後の経過をFig. 2に示す．治療時平均年齢51.1±12.3歳，男性11，女性8例であり，初回治療が15例，G1-HIGHは7例，G2は12例，内低ウイルス量2例である．治療法は，G1-HIGHはPeg-IFNα2b＋Rib 48週～72週，G2に対しては，低ウイルス量はβ-IFN 8週，Consensus IFN12週，高ウイルス量はPeg-IFNα

1）川崎市立多摩病院消化器・肝臓内科
2）聖マリアンナ医大消化器・肝臓内科
3）東京大学感染症内科
*Corresponding author: michstmu@marianna-u.ac.jp
＜受付日2012年11月5日＞＜採択日2013年2月21日＞

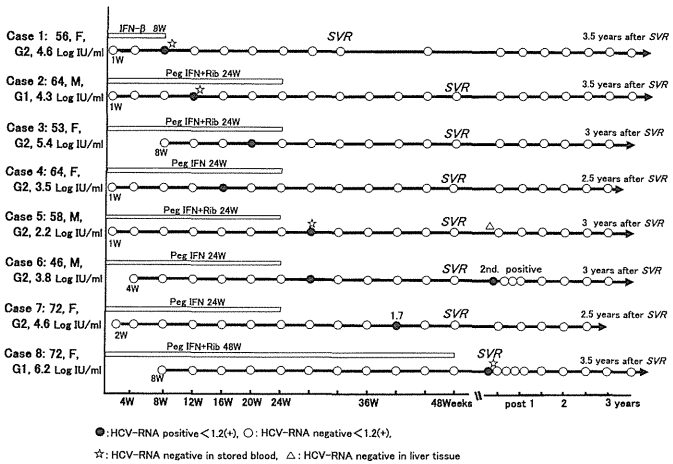


Fig. 1　Clinical course of the transient sero-positive cases in antiviral treatment.

2a 24週が1例，残る9例はPeg-IFNα2b＋Rib 24週～48週である．HCV-RNA一過性陽性時期は，SVR判定後3カ月から8.5年で認められ，Case2で2.8，Case10で1.9 LogIU/mlで定量可能であった．なおCase 3, 10, 15の3例に再度検出が認められた．定量可能で検出され陰性化し，2カ月後に再度検出が認められたCase10では，ALTの変動（71～175 IU/L）を伴っていた．Case 17では組織内HCV-RNAが検索され，結果は陰性を示した．現時点において19例に，SVR後最長11.5年，平均5年の観察期間にて肝細胞癌の発生は認めていない．

考案：TaqMan PCR法によるHCV-RNAの測定は，広範な測定範囲をカバーし，かつ高感度の測定法であり，CH-Cの診断および治療に広く使用されている．TaqMan PCR法は，その原理よりHCV-RNAの存在なしにシグナルが検出されることは考えにくい測定法である．さらに本法の測定過程は，専用スピッツを用い，サンプルチューブの取り扱いに際しても他検体と重複する事の無い全自動測定であるため，コンタミネーションによる偽陽性の可能性も極めて少ない測定法である[1)2)]．

今回の治療中～SVR判定前の検討においては，8例中7例（88%）はothers例であり，治療開始後4週以内のHCV-RNA陰性（RVR）が確認され，SVRが期待できる例であった．治療中に確認された4例中3例（75%）は，治療開始後1週の超早期の陰性化例であり，血中のHCV-RNAの急速な低下との関係が示唆された．治療終了後SVR判定までに一過性陽性を示した4例については，通常は陽性を確認した時点で再燃（relapse）と判断される例である．4例中2例は治療終了4週後の最初の測定にて一過性陽性を示し，これらは体内のHCV-RNAのごく少量が治療終了により，血中で一時的に検出されるも，増殖に至らなかった可能性も考えられた．なお全8例中SVR判定後に再度一過性の陽性を示す例が1例（13%）認められたこと，肝組織内HCV-RNA検索された例では陰性であったことなどから，SVR判定例における肝臓以外のPBMC等に残存するHCVが[3)4)]，一過性に血中へ流出した可能性も考えられた．今回の治療中～SVR判定前の検討におけるothers例にSVR例は82例認められ，内7例（8.7%）が一過性陽性例であった．治療反応も良く，治療効果が期待されるothers例に，一過性陽性例が比較的多く存在することを念頭に置き，一過性陽性に対する治療期間の延長の判断，および治療後の再燃の判断は慎重に行う必要があると思われた．しかし，一方では，一過性陽性時点

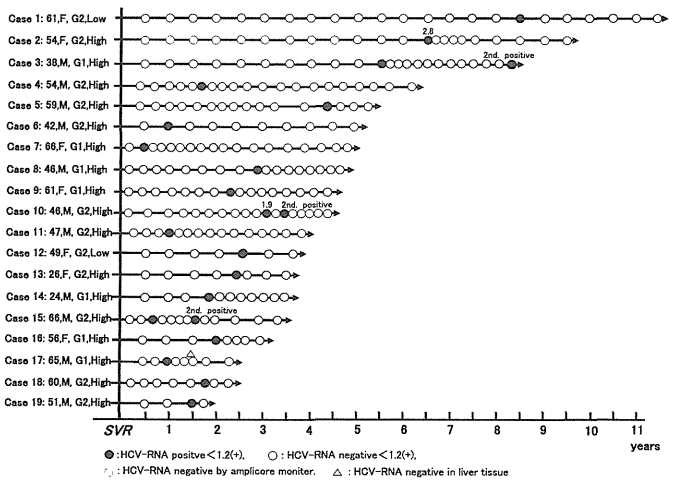


Fig. 2 Clinical course of the transient sero-positive cases in long-term follow up after SVR diagnosis.
A review of the cases with transient sero-positive HCV-RNA during antiviral treatment and after sustained virological response in chronic hepatitis C.

における同時採血の保存血清を用いたHCV-RNAの測定ではいずれも陰性を示し，一部の血液中のみにHCV-RNAが検出されたことになる．

この乖離については，一過性陽性を示した検体においてHCV-RNA量が極めて少ない状態を想定すると，再検した検体が凍結保存検体であり凍結溶解により少数存在したHCV-RNAが分解した可能性，またHCV-RNA量が少ないためTaqManPCR法におけるプライマーが結合できる確率が低く，保存血清では陽性を確認することが困難である可能性が考えられた[5]．しかし，いずれの保存血清においても再現性が得られない事実は，精度の高いTaqManPCR法における偽陽性の可能性も完全には否定できず検討する必要があると思われた．

SVR後の検討においては，SVR後の経過観察例235例中19例(8.1%)に一過性陽性が認められた．現在SVR後のHCV-RNA測定間隔にガイドラインはなく，その測定はSVR後の時期にもよるが各施設で必ずしも一定ではないと考えられる．今回の陽性率は，SVR後HCV-RNAを3～6カ月間隔でTaqManPCR法によるHCV-RNA測定を行い，平均観察期間が5年で得られた結果である．一過性陽性が検出された19例では，検出後1～3カ月間隔の短期間でのHCV-RNAの経過観察が行われ，多くの例ではHCV-RNAの持続陰性が確認されている．しかし，19例のうち3例（16%）に再陽性が認められた．これら経過から，SVR判定例においても一部症例は，肝臓以外も含め体内にHCV-RNAが残存する可能性があり，定期的に血中HCV-RNAの測定を続けることにより一過性陽性として検出されることは，決してまれな現象ではないと思われた．さらにその陽性率は，より短期間での経過観察が行われた際には増加する可能性が考えられた．今回の19例は現時点では，HCV-RNAの持続陽性に伴う明らかな肝炎の再燃を認めないことから，SVR後のHCV-RNA一過性陽性の多くは臨床上SVRと扱って問題ない例と考えられる．しかし，少数ではあるが，ALT変動例，定量可能例，再度検出例が認められ，これら症例を中心に慎重な経過観察を行うことが，一過性陽性の臨床的意義を明らかにするものと思われた．

結論：C型慢性肝炎SVR判定例において，判定前後の経過観察中にHCV-RNAが一過性に陽性となる例が存在する．

索引用語：C型慢性肝炎，HCV-RNA，
一過性陽性

文献：1）狩野吉康，赤池　淳，山崎　克，他．医学と薬学　2007；58：137—149　2）田原和子，出口松夫，中野　卓，他．JJCLA　2007；32：245—252　3）Mohamad HI, El-Bab HK, Kamal AM. Hepatogastroenterology 2011；58：107—108　4）Cavalherio NP, Filgueiras TH, Melo CE, et al. BJID 2007; 11: 471—474　5）Vermehren J, Kau A, Gartner BC, et al. J Clin Microbiol 2008; 46: 3880—3891

本論文内容に関連する著者の利益相反：なし

英文要旨

A review of the cases with transient sero-positive HCV-RNA during antiviral treatment and after susutained virological response in chronic hepatitis C

Yasunobu Fukuda[1], Yoshihiko Nagase[1], Sarika Kitagawa[1], Yosuke Michikawa[1], Tetsuya Hiraishi[1], Daisuke Kumon[1], Seiyo Ko[1], Satoshi Baba[1], Norie Yamada[2], Minoru Kobayashi[2], Hiroki Ikeda[2], Hideaki Takahashi[2], Kotaro Matsunaga[2], Nobuyuki Matsumoto[2], Chiaki Okuse[2], Hiroshi Yotsuyanagi[3], Michihiro Suzuki[1,2]*

The real-time polymerase chain reaction (PCR) techniques are widely used to diagnose sustained virological response (SVR). And we occasionally come across transient sero-positive HCV-RNA cases during antiviral therapy and after diagnosis of SVR in chronic hepatitis C.

Eight SVR cases showed transient sero-positivity of HCV-RNA during and within 6 months after IFN treatment (Study 1). And 19 SVR cases showed it in long-term follow up after the diagnosis of SVR (Study 2). Seven out of 8 Study 1 patients were cases with other than sero group 1 with high viral load. Four patients showed sero-positivity during IFN treatment, and rest of patients showed it within 6 months after the IFN treatment, when we diagnose SVR. The viral load of one of them was even quantifiable, and another one of them showed re-positivity after SVR diagnosis.

In Study 2, 12 out of 19 patients were cases with other than sero group 1 with high viral load. Three patients showed transient positivity of HCV-RNA with quantifiable viral load. And 3 patients showed repeated transient positivities in a long-term follow up. Transient sero-positive HCV-RNA was occasional incidence in SVR patients after IFN treatment for CH-C especially in cases with other than serogroup 1 with high viral load. Although its clinical importance is unclear, HCV is supposed to present in most of those patients. We would be better to beware of the presence of such cases in the follow up of SVR patients.

Key words: chronic hepatitis C, HCV-RNA, transient sero-positive HCV-RNA
Kanzo 2013; 54: 294—297

1) Department of Gastroenterology and Hepatology, Kawasaki City Tama Hospital
2) Department of Gastroenterology and Hepatology, St Marianna University School of Medicine
3) Department of Infectious Disease, The University of Tokyo
*Corresponding author: michstmu@marianna-u.ac.jp