tance mutation-acquisition pathways, it is possible that resistance mutations had been transmitted.

In contrast to multiple pathways for acquiring d4T resistance in the naïve group, d4T resistance acquisition in the exposed group was much simpler. d4T resistance-related mutations were the most frequently observed mutations, and few K65R or Q151M mutations were detected. As many cases had a history of AZT as mono- or dual therapy, d4T resistance-related mutations appear to have been induced during previous AZT exposure, and these mutations were re-selected by GPOvir treatment. We also observed different mutation patterns in NNRTI resistance. K103N was less prevalent in the exposed group. Some of the differences observed between our two study groups may be attributed to intra- or intermolecular interference, which has been reported to affect drug-resistant mutation-acquisition pathways (Parikh et al., 2006; Quan et al., 1998).

Regarding mutations in the connection and RNase H domains, these two domains are not usually analyzed in clinical samples since most RT inhibitor-resistance mutations map to the DNA polymerase domain of RT (Clavel and Hance, 2004). Thus, less information is available for these domains in CRF01_AE. Therefore, we collected information on these two domains from our cohort. We found that G335C/D and A371V, which have been reported to confer AZT resistance in subtype B (Brehm et al., 2007; Nikolenko et al., 2007), were natural polymorphisms of CRF01_AE, and N348I, E399D, P537S, and I542M appeared to be induced by GPOvir exposure. Among these last 4 mutations, N348I and E399D were reported to affect AZT and NNRTI resistance in subtype B (Hachiya et al., 2008; Poveda et al., 2008); P537S and I542M are two newly discovered mutations in our study.

In conclusion, our study shows the potential of GPOvir for antiretroviral treatment-naïve and -exposed groups and demonstrates differences in drug-resistance acquisition pathways. Selection of pre-existing mutations and different pathways was affected by interference with drug-resistance mutations. Although developing countries currently have no alternative treatment regimen to GPOvir, its usage could be detrimental to salvage regimens because (1) d4T selects the multi-drug-resistance mutations, Q151M and K65R, the latter conferring resistance to tenofovir, and (2) both 3TC and nevirapine have low genetic barriers to acquiring drug resistance. More studies are needed to provide a better basis for selecting second-line treatments after GPOvir failure.

Acknowledgments

We are grateful to all the participants of the Lampang cohort. We appreciate the technical assistance of Mrs. Arunratsamee Katesomboon, Mr. Amornsak Kladnil, Ms. Suthira Kasemsuk, Ms. Sriprai Seneewong-na yuthaya, Ms. Nutira Boonna, and Mr. Prapan Wongnamnong. We are indebted to Dr. Shiino Teiichiro, Dr. Shiro Ibe and Dr. Junko Shibata for guiding the analysis of genetic diversity of the RT gene. We also thank Ms. Claire Baldwin for her assistance in preparing the manuscript. This study was supported by The Ministry of Public Health Thailand, Ministry of Health, Labor and Welfare of Japan, and Human Sciences Grant.

References

Arts, E.J., Quiñones-Mateu, M.E., Albright, J.L., Marois, J.P., Hough, C., Gu, Z., Wainberg, M.A., 1998. 3′-Azido-3′-deoxythymidine (AZT) mediates cross-resistance to nucleoside analogs in the case of AZT-resistant human immunodeficiency virus type 1 variants. J. Virol. 72, 4858–4865.

Boyer, P.L., Sarafianos, S.G., Clark, P.K., Arnold, E., Hughes, S.H., 2006. Why do HIV-1 and HIV-2 use different pathways to develop AZT resistance? PLoS Pathog. 2, e10.

Brehm, J.H., Koontz, D., Meteer, J.D., Pathak, V., Sluis-Cremer, N., Mellors, J.W., 2007. Selection of mutations in the connection and RNase H domains of human immunodeficiency virus type 1 reverse transcriptase that increase resistance to 3′-azido-3′-dideoxythymidine. J. Virol. 81, 7852–7859.

Brenner, B., Turner, D., Oliveira, M., Moisi, D., Detorio, M., Carobene, M., Marlink, R.G., Schapiro, J., Roger, M., Wainberg, M.A., 2003. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. AIDS 17, F1–5.

Chetchotisakd, P., Anunnatsiri, S., Kiertiburanakul, S., Sutthent, R., Anekthananon, T., Bowonwatanuwong, C., Kowadisaiburana, B., Supparatpinyo, K., Horsakulthai, M., Chasombat, S., Ruxrungtham, K., 2006. High rate multiple drug resistances in HIV-infected patients failing nonnucleoside reverse transcriptase inhibitor regimens in Thailand, where subtype A/E is predominant. J. Int. Assoc. Physicians AIDS Care (Chic Ill) 5, 152–156.

Clavel, F., Hance, A.J., 2004. HIV drug resistance. N. Engl. J. Med. 350, 1023–1035.

Delviks-Frankenberry, K.A., Nikolenko, G.N., Barr, R., Pathak, V.K., 2007. Mutations in human immunodeficiency virus type 1 RNase H primer grip enhance 3′-azido-3′-deoxythymidine resistance. J. Virol. 81, 6837–6845.

Deshpande, A., Jauvin, V., Magnin, N., Pinson, P., Faure, M., Masquelier, B., Aurillac-Lavignolle, V., Fleury, H.J., 2007. Resistance mutations in subtype C HIV type 1 isolates from Indian patients of Mumbai receiving NRTIs plus NNRTIs and experiencing a treatment failure: resistance to AR. AIDS Res. Hum. Retroviruses 23, 335–340.

Ehteshami, M., Beilhartz, G.L., Scarth, B.J., Tchesnokov, E.P., McCormick, S., Wynhoven, B., Harrigan, P.R., Götte, M., 2008. Connection domain mutations N348I and A360V in HIV-1 reverse transcriptase enhance resistance to 3′-azido-3′-deoxythymidine through both RNase H-dependent and -independent mechanisms. J. Biol. Chem. 283, 22222–22232.

Gonzales, M.J., Wu, T.D., Taylor, J., Belitskaya, I., Kantor, R., Israelski, D., Chou, S., Zolopa, A.R., Fessel, W.J., Shafer, R.W., 2003. Extended spectrum of HIV-1 reverse transcriptase mutations in patients receiving multiple nucleoside analog inhibitors. AIDS 17, 791–799.

Grossman, Z., Istomin, V., Averbuch, D., Lorber, M., Risenberg, K., Levi, I., Chowers, M., Burke, M., Bar Yaacov, N., Schapiro, J.M., 2004. Genetic variation at NNRTI resistance-associated positions in patients infected with HIV-1 subtype C. AIDS 18, 909–915.

Hachiya, A., Kodama, E.N., Sarafianos, S.G., Schuckmann, M.M., Sakagami, Y., Matsuoka, M., Takiguchi, M., Gatanaga, H., Oka, S., 2008. Amino acid mutation N348I in the connection subdomain of human immunodeficiency virus type 1 reverse transcriptase confers multiclass resistance to nucleoside and nonnucleoside reverse transcriptase inhibitors. J. Virol. 82, 3261–3270.

Hirsch, M.S., Brun-Vézinet, F., Clotet, B., Conway, B., Kuritzkes, D.R., D'Aquila, R.T., Demeter, L.M., Hammer, S.M., Johnson, V.A., Loveday, C., Mellors, J.W., Jacobsen, D.M., Richman, D.D., 2003. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel. Clin. Infect. Dis. 37, 113–128.

Johnson, V.A., Brun-Vezinet, F., Clotet, B., Gunthard, H.F., Kuritzkes, D.R., Pillay, D., Schapiro, J.M., Richman, D.D., 2008. Update of the Drug Resistance Mutations in HIV-1. Top. HIV Med. 16, 138–145.

Julias, J.G., McWilliams, M.J., Sarafianos, S.G., Alvord, W.G., Arnold, E., Hughes, S.H., 2003. Mutation of amino acids in the connection domain of human immunodeficiency virus type 1 reverse transcriptase that contact the template-primer affects RNase H activity. J. Virol. 77, 8548–8554.

Kantor, R., Katzenstein, D., 2003. Polymorphism in HIV-1 non-subtype B protease and reverse transcriptase and its potential impact on drug susceptibility and drug resistance evolution. AIDS Rev. 5, 25–35.

Kimura, M., 1981. Estimation of evolutionary distances between homologous nucleotide sequences. Proc. Natl. Acad. Sci. U.S.A. 78, 454–458.

Kumarasamy, N., Solomon, S., Chaguturu, S.K., Mahajan, A.P., Flanigan, T.P., Balakrishnan, P., Mayer, K.H., 2003. The safety, tolerability and effectiveness of generic antiretroviral drug regimens for HIV-infected patients in south India. AIDS 17, 2267–2269.

Lacey, S.F., Larder, B.A., 1994. Novel mutation (V75T) in human immunodeficiency virus type 1 reverse transcriptase confers resistance to 2′,3′-didehydro-2′,3′-dideoxythymidine in cell culture. Antimicrob. Agents Chemother. 38, 1428–1432.

Lennerstrand, J., Hertogs, K., Stammers, D.K., Larder, B.A., 2001a. Correlation between viral resistance to zidovudine and resistance at the reverse transcriptase level for a panel of human immunodeficiency virus type 1 mutants. J. Virol. 75, 7202–7205.

Lennerstrand, J., Stammers, D.K., Larder, B.A., 2001b. Biochemical mechanism of human immunodeficiency virus type 1 reverse transcriptase resistance to stavudine. Antimicrob. Agents Chemother. 45, 2144–2146.

Liu, L., Lu, H.Z., Henry, M., Tamalet, C., 2007. Polymorphism and drug selected mutations of reverse transcriptase gene in 102 HIV-1 infected patients living in China. J. Med. Virol. 79, 1593–1599.

Loemba, H., Brenner, B., Parniak, M.A., Ma'ayan, S., Spira, B., Moisi, D., Oliveira, M., Detorio, M., Wainberg, M.A., 2002. Genetic divergence of human immunodeficiency virus type 1 Ethiopian clade C reverse transcriptase (RT) and rapid development of resistance against nonnucleoside inhibitors of RT. Antimicrob. Agents Chemother. 46, 2087–2094.

Ministry of Public Health, 2008. The Asian Epidemic Model (AEM) Projections for HIV/AIDS in Thailand: 2005–2025.

Myint, L., Ariyoshi, K., Yan, H., Frater, A.J., Auwanit, W., Pathipvanith, P., Yamada, K., Matsuda, M., Chiba, T., Fujita, K., McClure, M., Weber, J.N., Sugiura, W., 2002. Mutagenically separated PCR assay for rapid detection of M41L and K70R zidovudine resistance mutations in CRF01_AE (Subtype E) human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 46, 3861–3868.

Nikolenko, G.N., Delviks-Frankenberry, K.A., Palmer, S., Maldarelli, F., Fivash Jr., M.J., Coffin, J.M., Pathak, V.K., 2007. Mutations in the connection domain of HIV-1 reverse transcriptase increase 3′-azido-3′-deoxythymidine resistance. Proc. Natl. Acad. Sci. U.S.A. 104, 317–322.

Ntemgwa, M., Wainberg, M.A., Oliveira, M., Moisi, D., Lalonde, R., Micheli, V., Brenner, B.G., 2007. Variations in reverse transcriptase and RNase H domain mutations in human immunodeficiency virus type 1 clinical isolates are associated with divergent phenotypic resistance to zidovudine. Antimicrob. Agents Chemother. 51, 3861–3869.

Parikh, U.M., Bacheler, L., Koontz, D., Mellors, J.W., 2006. The K65R mutation in human immunodeficiency virus type 1 reverse transcriptase exhibits bidirectional phenotypic antagonism with thymidine analog mutations. J. Virol. 80, 4971–4977.

Perach, M., Rubinek, T., Hughes, S.H., Hizi, A., 1997. Analysis of HIV-2 RT mutants provides evidence that resistance of HIV-1 RT and HIV-2 RT to nucleoside analogs involves a repositioning of the template-primer. J. Mol. Biol. 268, 648–654.

Poveda, E., de Mendoza, C., Pattery, T., González Mdel, M., Villacian, J., Soriano, V., 2008. Phenotypic impact of resistance mutations on etravirine susceptibility in HIV patients with prior failure to nonnucleoside analogues. AIDS 22, 2395–2398.

Pujari, S.N., Patel, A.K., Naik, E., Patel, K.K., Dravid, A., Patel, J.K., Mane, A.A., Bhagat, S., 2004. Effectiveness of generic fixed-dose combinations of highly active antiretroviral therapy for treatment of HIV infection in India. J. Acquir. Immune Defic. Syndr. 37, 1566–1569.

Quan, Y., Gu, Z., Li, X., Liang, C., Parniak, M.A., Wainberg, M.A., 1998. Endogenous reverse transcriptase assays reveal synergy between combinations of the M184V and other drug resistance-conferring mutations in interactions with nucleoside analog triphosphates. J. Mol. Biol. 277, 237–247.

Saeng-Aroon, S., Yoshida, L.M., Ariyoshi, K., Taguchi, M., Pathipvanich, P., Rojanawiwat, A., Matsuda, M., Kannagi, M., Sawanpanyalert, P., Sugiura, W., Auwanit, W., 2007. An efficient tool for surveying CRF01_AE HIV type 1 resistance in Thailand to combined stavudine-lamivudine-nevirapine treatment: mutagenically separated PCR targeting M184I/V. AIDS Res. Hum. Retroviruses 23, 1461–1468.

Santos, A.F., Lengruber, R.B., Soares, E.A., Jere, A., Sprinz, E., Martinez, A.M., Silveira, J., Sion, F.S., Pathak, V.K., Soares, M.A., 2008. Conservation patterns of HIV-1 RT connection and RNase H domains: identification of new mutations in NRTI-treated patients. PLoS One 3, e1781.

Sungkanuparph, S., Manosuthi, W., Kiertiburanakul, S., Piyavong, B., Chumpathat, N., Chantratita, W., 2007. Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. Clin. Infect. Dis. 44, 447–452.

Sungkanuparph, S., Manosuthi, W., Kiertiburanakul, S., Saekang, N., Pairoj, W., Chantratita, W., 2008. Prevalence and risk factors for developing K65R mutations among HIV-1 infected patients who fail an initial regimen of fixed-dose combination of stavudine, lamivudine, and nevirapine. J. Clin. Virol. 41, 310–313.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tsuchiya, N., Pathipvanich, P., Yasuda, T., Mukoyama, Y., Rojanawiwat, A., Matsubayashi, T., Saeng-aroon, S., Auwanit, W., Matsuyama, A., Sawanpanyalert, P., Ariyoshi, K., 2009. Demographic, socio-economic, behavioral and clinical factors predicting virologic failure with generic fixed-dose combination antiretroviral therapy before universal health insurance coverage in northern Thailand. Southeast Asian J. Trop. Med. Public Health 40, 71–82.

Waters, J.M., O'Neal, W., White, K.L., Wakeford, C., Lansdon, E.B., Harris, J., Svarovskaia, E.S., Miller, M.D., Borroto-Esoda, K., 2009. Mutations in the thumb-connection and RNase H domain of HIV type-1 reverse transcriptase of antiretroviral treatment-experienced patients. Antivir. Ther. 14, 231–239.

WHO, 2003. Scaling up antiretroviral therapy in resource-limited settings: treatment guidelines for a public helath approach.

Yap, S.H., Sheen, C.W., Fahey, J., Zanin, M., Tyssen, D., Lima, V.D., Wynhoven, B., Kuiper, M., Sluis-Cremer, N., Harrigan, P.R., Tachedjian, G., 2007. N348I in the connection domain of HIV-1 reverse transcriptase confers zidovudine and nevirapine resistance. PLoS Med. 4, e335.

Zelina, S., Sheen, C.W., Radzio, J., Mellors, J.W., Sluis-Cremer, N., 2008. Mechanisms by which the G333D mutation in human immunodeficiency virus type 1 Reverse transcriptase facilitates dual resistance to zidovudine and lamivudine. Antimicrob. Agents Chemother. 52, 157–163.

Zhou, J., Paton, N.I., Ditangco, R., Chen, Y.M., Kamarulzaman, A., Kumarasamy, N., Lee, C.K., Li, P.C., Merati, T.P., Phanuphak, P., Pujari, S., Vibhagool, A., Zhang, F., Chuah, J., Frost, K.R., Cooper, D.A., Law, M.G., 2007. Experience with the use of a first-line regimen of stavudine, lamivudine and nevirapine in patients in the TREAT Asia HIV Observational Database. HIV Med. 8, 8–16.

Dig Dis Sci (2010) 55:820–825
DOI 10.1007/s10620-009-0777-5

ORIGINAL ARTICLE

Regeneration of Graft Livers and Limited Contribution of Extrahepatic Cells After Partial Liver Transplantation in Humans

Susumu Eguchi · Mitsuhisa Takatsuki · Kosho Yamanouchi · Yukio Kamohara · Yoshitsugu Tajima · Takashi Kanematsu

Received: 4 January 2009 / Accepted: 23 February 2009 / Published online: 19 March 2009

Abstract *Background* Liver regeneration is still not fully understood. Partial liver transplantation (LT) can provide the opportunity to investigate the mechanisms of liver regeneration, including the contribution of extrahepatic cells to liver regeneration. *Methods* Of 61 patients transplanted with partial liver graft between August 1997 and October 2006, 56 patients were studied, including 49 adults and 7 children. Sequential computed tomography volumetric analysis was performed for volume measurement, while proliferating cell nuclear antigen (PCNA) labeling index was investigated for liver cell proliferation in nonprotocol liver biopsy specimens. In addition, 15 male recipients who had female liver grafts were investigated in order to detect Y chromosomes as extrahepatic cells in nonprotocol liver biopsy specimens. *Results* Graft volume per standard liver volume was markedly increased after adult-to-adult living-donor (LD) LT. In pediatric transplants, there was no volume increase over time. PCNA labeling index was vigorous in adult-to-adult LDLT in the early period after LDLT. No Y chromosome was evident in hepatocytes from female-donor male-recipient grafts during or after liver regeneration. However, in the cases of failing grafts of this type, many Y-chromosome-positive cells were observed in the graft liver. The character of those cells was CD34(−), CK9(−), hepatocyte-specific antigen(−), and CD68(+/−). *Conclusion* In adult-to-adult LDLT, vigorous liver regeneration occurs in the graft liver, demonstrated by not only volumetric but cell kinetic analysis. Involvement of extrahepatic cells in normal liver regeneration seems limited.

S. Eguchi (✉) · M. Takatsuki · K. Yamanouchi · Y. Kamohara · Y. Tajima · T. Kanematsu
Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
e-mail: sueguchi@net.nagasaki-u.ac.jp

Keywords Living-donor liver transplantation · Liver regeneration · Extrahepatic cells

Introduction

The mechanism of liver regeneration is still not fully understood. Although vigorous liver regeneration after living-donor liver transplantation (LDLT) has been reported by us and others [1–3], it has been assessed by imaging studies such as computed axial tomography (CAT) scan, not hepatocyte cell division. In the present study, we took the opportunity to use liver biopsy specimens to verify liver regeneration in partial liver recipients during various periods after LDLT.

In addition, during liver regeneration it has been reported that extrahepatic cells, especially bone marrow (BM)-derived cells, are mobilized and involved [4–6]. However, details regarding how extrahepatic cells are involved and how much they contribute to normal liver regeneration have not been fully elucidated [7–10]. Therefore, we investigated liver biopsy specimens from female-donor male-recipient grafts, in which only XX cells should be present in the graft liver. We used fluorescent in situ hybridization (FISH) to detect Y chromosomes in the liver to identify extrahepatic cells in the liver upon liver regeneration.

Materials and Methods

Patients

Of 61 patients who underwent LDLT between August 1997 and October 2006 at Nagasaki University Hospital, 56 Japanese patients with survival times of more than 3 months

were included for volumetric analysis. For adult recipients, right lobe grafts were transplanted in 40 recipients, while left-side grafts (8 extended left lobe graft, 1 left lobe graft) were performed in 9 recipients. Seven pediatric cases with left lateral lobe graft also underwent volumetric study. Adult patients were defined as those over 16 years old. When liver function test was deranged, total 93 liver biopsies were carried out, consisting of 83 in adult cases and 10 in pediatric cases, and were prepared for proliferative cell nuclear antigen (PCNA) staining. Within these, a total of 24 liver biopsies were performed in 15 recipients on indication from a pool of 19 male recipients (XY) who were transplanted with female livers (XX).

Methods of LDLT

All partial liver grafts were preserved in University of Wisconsin solution and implanted using a piggyback technique. In general, graft selection was based on the results of volumetric studies using CAT scans to obtain ratios of graft volume to standard liver volume of more than 35% in the recipients.

A dual or triple immunosuppressive regimen was used, which included tacrolimus or cyclosporine A, steroid, and mycophenolate mofetil. Patients with compromised renal function were given induction therapy with interleukin-2 antibodies. Biopsy-proven rejections were treated if clinical and laboratory signs mandated steroid bolus treatment. Steroid-resistant rejections were treated with OKT3.

Investigation for Liver Regeneration

Incremental growth of the liver in volume was measured by serial CAT scans using Flexi Trace software (Tree Star, Inc., U.S.A.) at 0, 1–2 weeks, and 3 months after LDLT [1]. In liver biopsy specimens, expression of PCNA (DACO, Carpinteria, CA) was analyzed for intrahepatic proliferation [11].

Four-micrometer liver sections were deparaffinized in xylene and hydrated in graded ethanol. After deparaffinization, rehydration, and heating in 95°C buffer, sections were incubated with each antibody and subsequently with Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Japan). Incubation was performed overnight at 4°C and followed by a wash in three changes of phosphate buffered saline (PBS) for 5 min. For all stainings, the reaction product was developed with the use of 3-diaminobenzidine tetrahydrochloride and H_2O_2. The sections were counterstained with Meyer hematoxylin–eosin.

For hepatocyte staining, the goat anti-human hepatocyte-specific antigen Ab (R&D system, Minneapolis, MN), and 2nd Ab biotinylated rabbit anti-goat Ig (DAKO, Carpinteria, CA) were used. For the staining of CK7 (bile duct marker), CD68 (macrophage marker) and CD34 (hematopoietic cells) were used, respectively, according to the manufacturer's protocol.

Fluorescent In Situ Hybridization (FISH)

FISH was performed in our reference laboratory (SRL, Nagasaki, Japan). Sections from paraffin-embedded biopsied liver tissues were placed on silane-coated glass slides. The slides were deparaffinized immediately in two rinses of 1,000 g/l xylene for 10 min each. Each slide was rehydrated in an ethanol series for 5 min. The slides were then treated with 0.2 mol/l HCl for 20 min, followed by 2 × SSC (0.3 mol/l sodium chloride and 0.03 mol/l sodium citrate) for 20 min at 80°C, treated with 0.05 mg/ml proteinase K in TEN [0.05 mol/l Tris–HCl, pH 7.8, 0.01 mol/l ethylenediamine tetraacetic acid (EDTA), and 0.01 mol/l sodium chloride] for 10 min at 37°C, and placed in 40 g/l formaldehyde in PBS for 10 min. Both FISH probes and target DNA were denatured simultaneously for 10 min at 90°C, and the slides were incubated overnight at 42°C, placed in 2 × SSC for 10 min at 42°C, washed twice in 2 × SSC/500 g/l formaldehyde formamide for 5 min each at 42°C, washed 2 × SSC for 5 min at 42°C, and counterstained in 2 × SSC/0.03 μg/ml 4',6-diamidino-2-phenylindole (DAPI).

Statistical Analysis

For the data, Mann–Whitney *U* test was used. Differences were considered statistically significant for *P*-value less than 0.05.

Results

Liver Volume

Graft volume per standard liver volume at 0, 1, 3, and 6 months after adult-to-adult LDLT was 53.2%, 95.9%, 98.5%, and 101.2% in right lobe grafts and 41.1%, 81.9%, 92.7%, and 102.4% in left-sided grafts, respectively (Fig. 1). Since volume changes in pediatric LDLT were not evident, they are not included in the figure.

DNA Synthesis in the Liver

PCNA labeling index was vigorous in adult-to-adult LDLT in the early period after LDLT, while it was not evident in pediatric LDLT (Fig. 2).

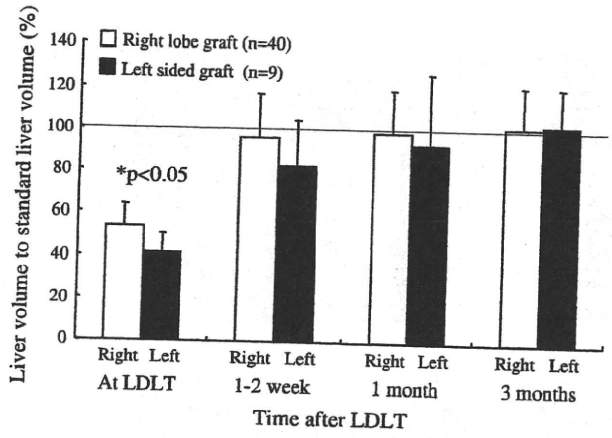

Fig. 1 Liver regeneration of right lobe or left lobe graft liver after adult-to-adult LDLT using volumetric analysis using CAT scan. *LDLT* living-donor liver transplantation, *CAT* computed axial tomography

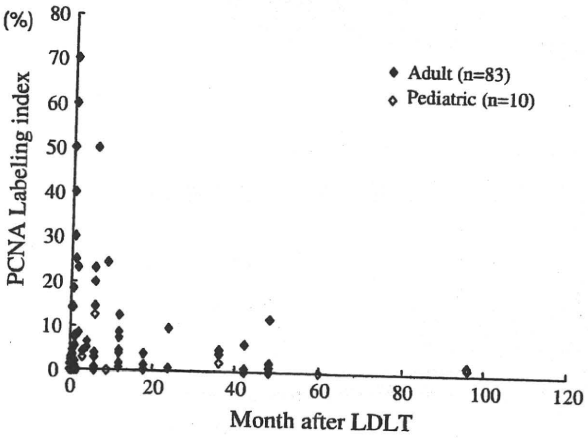

Fig. 2 PCNA labeling index after LDLT using immunohistochemical staining. *PCNA* proliferating cell nuclear antigen, *LDLT* living-donor liver transplantation

FISH and Immunohistochemical Staining for Y-Positive Cells

Y chromosome was not evident in hepatocytes of female-donor male-recipient grafts after normal liver regeneration in adult-to-adult LDLT recipients (Fig. 3, case 1). As seen in this case, when graft livers did not receive any damage and underwent normal liver regeneration, existence of Y-chromosome-positive cells was limited with FISH examination. However, in the case of failing graft, such as in cases 11–13, many Y-chromosome-positive cells were observed in zone 1 of the graft liver (Fig. 3, case 11).

For these cases, immunohistochemical staining was performed in the area with Y chromosomes. CD34(−), CK9(−), hepatocyte Ag(−), and CD68(+/−) were observed using immunohistochemical staining (Fig. 4, case 11). In the case of chronic liver damage (Fig. 5, case 15) after LDLT due to biliary complication, a few Y-positive cells were also detected with nonspecific staining for CD34, CK9, hepatocyte Ag, and CD68. Results of immunohistochemical staining are summarized in Tables 1 and 2.

Discussion

In this report, we showed liver regenerative response after partial LT using not only volumetric CAT scan study but also PCNA labeling of biopsy specimens. Previously, we reported vigorous liver regenerative response after partial liver regeneration and investigated liver regenerative growth factors after liver regeneration [11]. Herein, we showed a clear difference in proliferation of graft liver according to recipient body size and blood flow due to the difference in responses when transplanted in adults and children with different standard liver volumes. We did not carry out statistical analysis on PCNA index since it exhibited wide deviation. Liver regeneration remains an unsolved phenomenon, but our results show that it could be related to factors in recipients, as we reported previously [1]. Since protocol biopsy tends to be avoided because of risk of hemorrhage etc., further investigation is needed to assess cell proliferation noninvasively aside from CAT scan. Also since liver biopsy was not done on protocol, rejection or inflammation could have affected the data of PCNA staining. Although it would be interesting to investigate the difference in liver regeneration between patients after liver resection and those after partial liver transplantation, biopsy specimen from patients after liver resection cannot be obtained because of risk of complications. Therefore this also remains for further investigation. Our liver specimens from liver transplant recipients were obtained because of on-demand liver biopsy.

In addition, for combinations of female donor (XX) and male recipient (XY), the Y chromosome was investigated in the biopsy specimen of the female liver (XX) in order to investigate the contribution of extrahepatic cells to liver regeneration. Previously, in an in vivo experiment conducted in 2000, it was reported that hepatocytes could be derived from BM cells [12]. Subsequently, in 2001, Baccarani et al. [13] reported that, in human recipients, replacement of a female liver venous endothelium with male BM showed the possibility of involvement of BM cells in liver rearrangement. Fujii et al. [4] reported that BM cells participated in liver regeneration after hepatectomy, whereas the majority of cells were committed to sinusoidal endothelial cells. Very recently, Conzelmann et al. [5], using their reduced-size LT model, reported that recipient-derived progenitor cells were present and might contribute to liver regeneration in mice. However, in 2005 Di Campli et al. [7] reported no evidence of hematopoietic

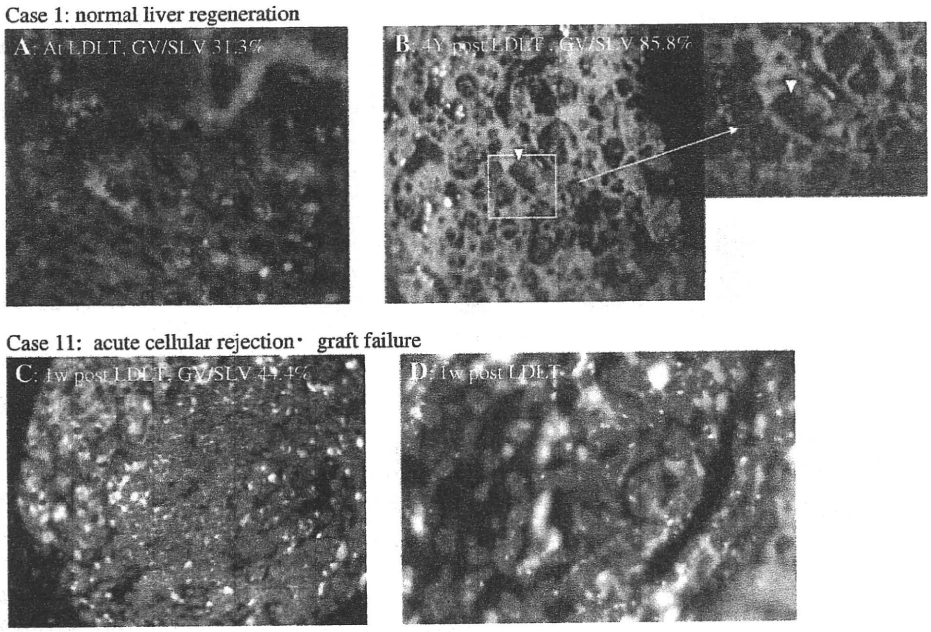

Fig. 3 FISH for Y chromosome in liver biopsy specimens. Case 1 showed normal liver regeneration after LDLT. **a** At the time of LDLT, few Y-chromosome-positive cells were seen. **b** With time, although GV/SLV increased, a few Y-chromosome-positive cells were seen only in the sinusoid. **c** Case 11 had severe acute rejection at 1 week after LDLT. **d** In the biopsy specimen, massive accumulation of Y-chromosome-positive cells was seen, mimicking hepatic structure. *FISH* fluorescent in situ hybridization, *GV/SLV* graft volume versus standard liver volume ratio

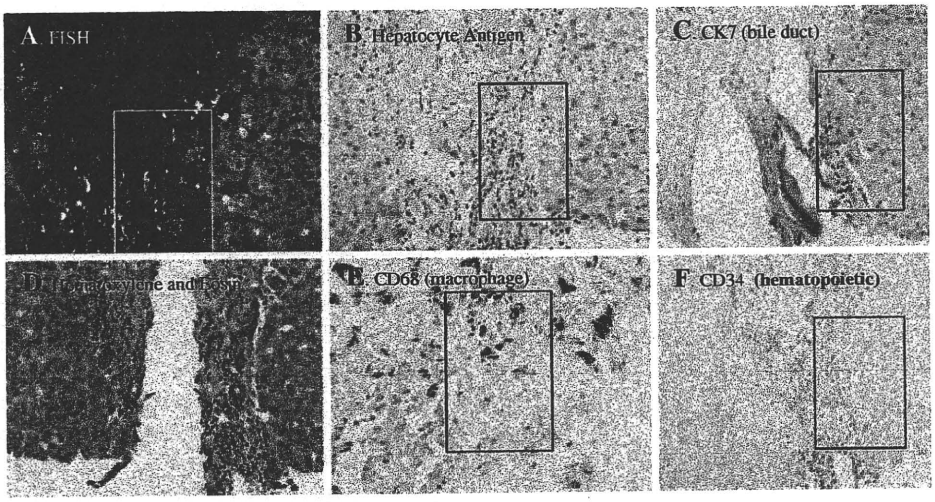

Fig. 4 Immunohistochemical stainings in case 11. Characterization of Y-chromosome-positive cells was attempted in corresponding area. **a** FISH showing Y-chromosome-positive cells (white square), **b** hepatocyte antigen was not positive in the black square, **c** CK7 (cytokeratin 7, bile duct) was not positive in the black square, **d** hematoxylin and eosin staining, **e** CD68 (macrophage) was partially positive in the black square, **f** CD34 (hematopoietic cell) was not positive in the black square

stem cell mobilization in patients who underwent hepatectomy or in patients with acute liver failure. Similarly, in 2006, Moritoki et al. [8], using green fluorescent protein transgenic mice, demonstrated that BM cell transfer seemed not to contribute to the differentiation of cholangiocytes in a chronic cholestasis model. In 2007, Tomiyama [6] reported the limited contribution of cells originating from intact extrahepatic tissue in hepatocyte regeneration in transplanted rat livers. Thus, it is still unknown whether extrahepatic cells such as BM cells could contribute to liver regeneration or liver repair, especially in humans.

In our study, we did not find many Y-chromosome-positive cells after liver transplantation with normal liver regeneration. If extrahepatic cells had been involved and integrated into normal liver regeneration, they should have stayed and been found in the liver biopsied a long time after LDLT. This is indirect evidence that would seem to rule out extrahepatic cell contribution to normal liver regeneration in humans, in contrast to previous reports [12, 13]. On the other hand, when failing livers were biopsied, many Y-chromosome-positive cells were present. Although we could not clearly show the origin of those Y-positive cells, circulating macrophages were candidate sources

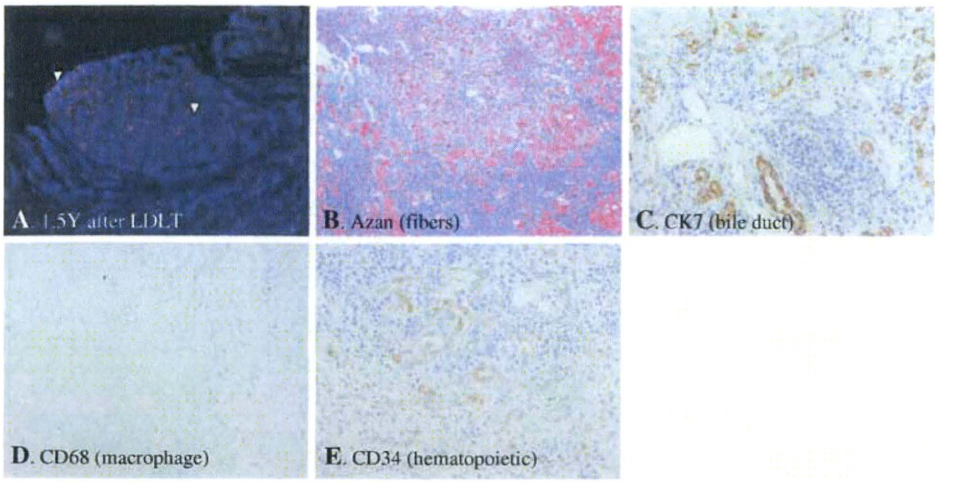

Fig. 5 Immunohistochemical stainings in case 15, secondary biliary cirrhosis after LDLT. **a** FISH showing Y-chromosome-positive cells (white square), **b** Azan staining was positive, showing the presence of liver fibrosis, **c** CK7 (cytokeratin 7, bile duct) was not positive in the black square, **d** CD68 (macrophage) was partially positive in the black square, **e** CD34 (hematopoietic cell) was not positive in the black square. *LDLT* living-donor liver transplantation

Table 1 Demographics of male recipients with female donors

Case no.	Age	Gender	Etiology	Donor	Blood type match	Graph type	Biopsy period after LDLT	Comments	Outcome
1	16	M	FHF	Mother	Identical	L	3d, 4Y	None	Survived
2	5	M	BA	Mother	Identical	LL	2M, 8Y	Cholestasis	Survived
3	56	M	LC-B/HCC	Sister	Identical	R	1.5M, 1.8Y, 2Y mild ACR	None	Survived
4	20	M	FHF	Aunt	Identical	R	1M, 5M, 2Y	Cholestasis	Survived
5	58	M	LC-C	Sister	Identical	R	2M	Hepatitis	Survived
6	56	M	LC-B/HCC	Daughter	Identical	R	8M	Vanishing BD	Survived
7	56	M	LC-B/HCC	Daughter	Identical	R	9M (Re-LDLT)	Poor quality	Survived
8	56	M	LC-B/HCC	Wife	Identical	R	6M	Mild ACR	Survived
9	58	M	LC-C/HCC	Daughter	Incompatible	R	3W	Hepatitis	Survived
10	62	M	LC-C	Sister	Compatible	L	1.5M	Moderate ACR	Survived
11	41	M	PBC	Wife	Identical	R	1W, 1M (autopsy)	Severe ACR	Died (2M)
12	50	M	LC-B	Wife	Identical	R	10d (graft failure)	Malcirculation	Died (1M)
13	57	M	LC-C/HCC	Wife	Identical	R	10d, 2M (graft failure)	Moderate ACR	Died (2M)
14	47	M	LC-Al	Sister	Identical	R	3.8Y (liver cirrhosis)	Poor quality	Died (3.8Y)
15	51	M	LC-C	Sister	Identical	R	2.5Y (chronic liver failure)	Biliary cirrhosis	Died (2.5Y)

FHF fulminant hepatic failure, *BA* biliary atrasia, *ACR* acute cellular rejection, *LC-B* liver cirrhosis due to hepatitis B, *LC-C* liver cirrhosis due to hepatitis C, *LC-Al* liver cirrhosis due to alcohol hepatitis, *HCC* hepatocellular carcinoma, *PBC* primary biliary cirrhosis, *d* days, *M* months, *Y* years, *LDLT* living-donor liver transplantation

Table 2 Summary of results

	Normal regeneration	Acute graft failure	Chronic graft failure
Y chromosome	−	++	+
Hepatocyte antigen	−	−	−
CK7 (bile duct)	−	−	−
CD68 (macrophage)	−	Partial +	−
CD34 (hematopoietic)	−	+	+

because some cells were positive for CD68, which we used to identify macrophages. However, CD34, used for hematopoietic cells, was negative, which indicated that those Y-positive cells did not have hematopoietic origins. In addition, there may be significant sampling variability in liver biopsy specimens from a single liver biopsy, which may not necessarily be representative of the entire liver. In liver chronically damaged by biliary complication, Y-chromosome-positive cells were not as numerous as seen in the case of acute graft failure. In addition, despite the information about expression of progenitor cell markers such as c-kit and Thy-1, we did not investigate this in this study; this awaits further investigation. With regard to CD68(+) Y chromosome(+) cells, we presume that they are regular macrophages from recipient side to dispose of damaged cells in failing liver, not special multipotent stem cells expressing CD68.

In conclusion, in adult-to-adult LDLT, vigorous liver regeneration occurs in graft livers. Involvement of extrahepatic cells in normal adult-to-adult liver regeneration seems limited.

References

1. Eguchi S, Yanaga K, Sugiyama N, Okudaira S, Furui J, Kanematsu T. Relationship between portal venous flow and liver regeneration in patients after living donor right-lobe liver transplantation. *Liver Transpl.* 2003;9:547–551. doi:10.1053/jlts.2003.50128.
2. Akamatsu N, Sugawara Y, Kaneko J, et al. Effects of middle hepatic vein reconstruction on right liver graft regeneration. *Transplantation.* 2003;76:832–837. doi:10.1097/01.TP.0000085080.37235.81.
3. Marcos A, Fisher RA, Ham JM, et al. Liver regeneration and function in donor and recipient after right lobe adult to adult living donor liver transplantation. *Transplantation.* 2000;69:1375–1379. doi:10.1097/00007890-200004150-00028.
4. Fujii H, Hirose T, Oe S, et al. Contribution of bone marrow cells to liver regeneration after partial hepatectomy in mice. *J Hepatol.* 2002;36:653–659. doi:10.1016/S0168-8278(02)00043-0.
5. Conzelmann LO, Hines IN, Kremer M, Perry AW, Lemasters JJ, Wheeler MD. Extrahepatic cells contribute to the progenitor/stem cell response following reduced-size liver transplantation in mice. *Exp Biol Med (Maywood).* 2007;232:571–580.
6. Tomiyama K, Miyazaki M, Nukui M, et al. Contribution of cells of intact extrahepatic tissue origin to hepatocyte regeneration in transplanted rat liver. *Transplantation.* 2007;83:624–630. doi:10.1097/01.tp.0000253942.16061.d9.
7. Di Campli C, Piscaglia AC, Giuliante F, et al. No evidence of hematopoietic stem cell mobilization in patients submitted to hepatectomy or in patients with acute on chronic liver failure. *Transplant Proc.* 2005;37:2563–2566. doi:10.1016/j.transproceed.2005.06.072.
8. Moritoki Y, Ueno Y, Kanno N, et al. Lack of evidence that before marrow cells contribute to cholangiocyte repopulation during experimental cholestatic ductal hyperplasia. *Liver Int.* 2006;26:457–466. doi:10.1111/j.1478-3231.2006.01250.x.
9. am Esch JS 2nd, Knoefel WT, Klein M, et al. Portal application of autologous CD133+ bone marrow cells to the liver: a novel concept to support hepatic regeneration. *Stem Cells.* 2005;23:463–470.
10. Ferry N, Hadchouel M. Liver regeneration: with a little help from marrow. *J Hepatol.* 2002;36:695–697. doi:10.1016/S0168-8278(02)00054-5.
11. Eguchi S, Okudaira S, Azuma T, et al. Changes in liver regenerative factors in a case of living-related liver transplantation. *Clin Transplant.* 1999;13:536–544. doi:10.1034/j.1399-0012.1999.130616.x.
12. Theise ND, Badve S, Saxena R, et al. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology.* 2000;31:235–240. doi:10.1002/hep.510310135.
13. Baccarani U, Donini A, Risaliti A, Bresadola F. Replacement of liver venous endothelium. *Lancet.* 2001;357:2137. doi:10.1016/S0140-6736(00)05209-0.

Case Report

Two–Staged Living Donor Liver Transplantation for Fulminant Hepatic Failure

Katsuhiko Yanaga[1,3], Susumu Eguchi[1,3], Mitsuhisa Takatsuki[1], Sadayuki Okudaira[1], Yoshitsugu Tajima[1], Takashi Kanematsu[1]

[1]Department of Surgery, Nagasaki University Graduate School of Medical Sciences, Nagasaki, Japan
[2]Department of Surgery, Jikei University School of Medicine, Tokyo, Japan
[3]Co-first-authors: Katsuhiko Yanaga and Susumu Eguchi
Corresponding Author: Susumu Eguchi, MD, FACS
Department of Surgery, Nagasaki University Graduate School of Medical Sciences
Nagasaki, 852-8501, Japan
Tel: +81958197316, Fax: +81958197319, E-mail: sueguchi@nagasaki-u.ac.jp

KEY WORDS: Two–staged; Liver transplantation; Living–related; Hepatectomy

ABBREVIATIONS: Fulminant Hepatic Failure (FHF); Living Donor Liver Transplantation (LDLT); Continuous Hemodiafiltration (CHDF)

SUMMARY

We reported a first successful and life-saving two-staged living-related liver transplantation for a patient with imminent brain death due to fulminant hepatic failure that otherwise had to be performed after a pre-treated and scheduled blood-type incompatible liver transplantation. The patient was anhepatic for 6 hr 34 min, and continuous hemodiafiltration was given throughout the operation. The patient recovered quickly and was extubated within 24 hr after transplant. This two-staged procedure is useful for emergency living-related liver transplantation that needs to be performed when the operating room is busy with other emergency or scheduled surgical procedures, and may allow clearance of toxic metabolites during the anhepatic period.

INTRODUCTION

For fulminant hepatic failure (FHF), liver transplantation is an established and effective therapy (1). Nevertheless, the therapeutic window is narrow, and the procedure needs to be performed as an emergency operation. If the allograft is to be obtained from a live donor, two emergency operations need to be performed simultaneously and in a coordinated fashion at a single institution (2). Such a circumstance is demanding not only for the surgeons but also for the anesthesiologists, operating room personnel and facility as well as for the blood bank. We were confronted with an instance in which we had to perform two living-related liver transplants at the same time, i.e., an emergency living donor liver transplantation (LDLT) for a patient with imminent brain-death due to FHF and a scheduled blood-type incompatible LDLT after full preparatory treatment with repeated plasma exchange to reduce serum isoagglutinin titer. We report our experience with a two-staged LDLT, i.e., total hepatectomy and temporary portocaval shunting which was followed by allograft implantation to prevent brain stem herniation for a patient with FHF in such a difficult situation.

CASE REPORT

A 34-year-old woman suffered from fulminant hepatic failure due to hepatitis B and was transferred to our hospital on May 7, 2001. In spite of plasma exchange and continuous hemodiafiltration (CHDF), she developed grade IV coma (responsive only to painful stimuli) with the prothrombin time below 12% and serum ammonia of 176 microgm/ml. She was judged to require emergency liver transplantation in the evening of May 10, 2001, when her father, 67-years-old and blood type identical, volunteered to be a donor. His preoperative work-up and informed consent was completed by the end of the same day.

In the mean time, an 11 month-old-girl with biliary atresia, status-post Kasai's operation was scheduled and being prepared for blood-type incompatible (A to O) LDLT from her mother the next morning. She had undergone two courses of preoperative plasma exchange under general anesthesia to reduce the serum anti-A isoagglutinin titer. The family of the infant was extremely reluctant to postpone their transplant and became nervous about any negative influence on their transplant from the emergency liver transplantation for FHF. We therefore considered performing emergency LDLT immediately after the elective LDLT. Nevertheless, the woman with FHF started to exhibit decerebrate posture in spite of CHDF and plasma exchange, and computed tomography revealed brain edema, which suggested imminent brain death. We confronted with the need to perform two LDLT at the same time.

For this difficult clinical situation, we decided to perform after donor operation for the blood-type incompatible transplant a total hepatectomy and end-to-side portocaval shunting for the patient with FHF, and then to implant the allograft after the first elective blood-type incompatible LRLT was

Hepato-Gastroenterology 2010; 57:146-148

finished (Figure 1). This modification would prevent the development of brain stem herniation or hemodynmic instability, while allowing the already set LDLT as scheduled.

After the native total hepatectomy with preservation of the inferior vena cava, the patient was anhepatic for 6 hr 34 min, during which she was placed on an end-to-side portocaval shunt. The shunt flow was 462ml/min by Doppler ultrasound, and splanchnic decompression was adequate with no signs of mesenteric petechiae or intestinal edema. CHDF was given throughout the operation, and her blood ammonia and lactate levels were lowered even during the anhepatic period (Figure 1). The donor right lobe weighed 750gram, with the graft weight/estimated liver volume of 66.3%. The explant liver weighed 520gram and exhibited massive necrosis. The operation for the recipient took 19hr 8min, and the estimated blood loss was 2,300gram.

For postoperative immunosuppression, tacrolimus and steroids were given. The patient's postoperative course was uneventful, and she woke up on the first postoperative day, when she was extubated. She recovered without neurological deficits and was transferred to the medical service on postoperative day 30 with normal liver function and stable blood tacrolimus level. At 12 months posttransplant, she is on tacrolimus 1mg b.i.d. only and remains well with normal liver function and without any restriction as a housewife. The child who received a blood-type incompatible liver from her mother was discharged 39 days after transplant and remains well with normal liver function without immunosuppression.

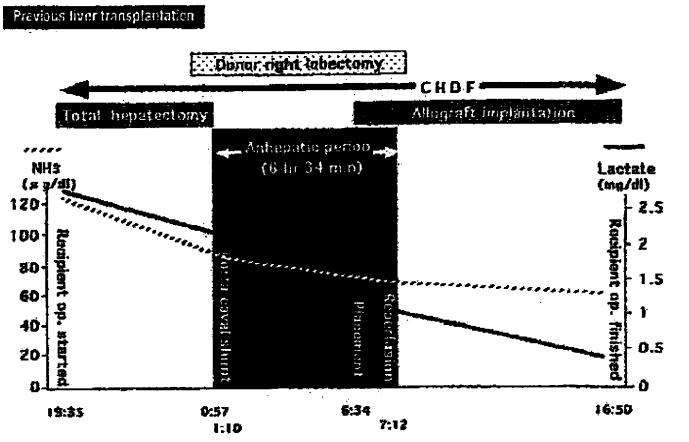

FIGURE 1 Operative course of the two-staged living-related liver transplantation for a patient with fulminant hepatic failure.

DISCUSSION

Keeping a patient with FHF alive and as a liver transplant candidate can be a challenging problem because of the narrow therapeutic window. The concept of two-staged liver transplantation was first reported as a desperate attempt by Ringe *et al.* (3) in 1988 for patients with primary graft non-function of the liver allograft or for intractable hemorrhage during hepatic resection. They later added severe hepatic trauma and FHF as indications for such a procedure (2, 3). The rationale for such a procedure for FHF is based on a clinical observation that the presence of a necrotic liver causes cardiovascular instability and renal as well as respiratory insufficiency which is described as 'toxic liver syndrome'. Husberg *et al.* (6) in 1991 described hepatic devascularization rather than total hepatectomy for three patients with FHF and noted improvement in the acidosis with diuresis after isolation of the failing liver. Rozga *et al.* (7) in 1993 reported combination of hypothermia, plasma exchange, and extracorporeal liver support with total hepatectomy. Their patient was anhepatic for 14 hr but recovered completely after two liver transplants. In our patient, we used CHDF throughout the anhepatic period. As compared to other 5 patients with FHF in our institution, our patient woke up much faster after LDLT. This may be in part due to the clearance of toxic metabolite during the anhepatic period by CHDF. In this regard, intentional anhepatic preconditioning by early total hepatectomy of the failing liver in combination with CHDF or artificial liver support before allograft implantation may facilitate recovery from acute liver failure.

A controversial issue in performing the two-staged liver transplantation for FHF has been the uncertainty with the availability of liver allografts and rather poor outcome (8). In LDLT, however, a viable graft can certainly be obtained at any given time, provided that the donor is willing, medically suitable for donation, and mentally supported.

Furthermore, although liver transplantation for FHF itself is a life-saving procedure, the influence of this procedure on the surgical practice at a hospital level is significant, especially if donor operation is also performed at the same time. We believe that for low-volume transplant centers, the two-stage LDLT for FHF can be performed in combination with emergency surgical procedures.

REFERENCES

1. **Williams R, Wendon J:** Indications for orthotopic liver transplantation in fulminant hepatic failure. Hepatology 1994; 20: 5S.
2. **Matsunami H, Makuuchi M, Kawasaki S, et al:** Living-related liver transplantation in fulminant hepatic failure. Lancet 1992; 340: 1411.
3. **Ringe B, Pichlmayr R, Lubbe N, Bornscheuer A, Kuse E:** Total hepatectomy as temporary approach to acute hepatic or primary graft failure. Transplant Proc 1988; 20: 552.
4. **Ringe B, Pichlmayr R, Ziegler H, et al:** Management of severe hepatic trauma by two-stage total hepatectomy

and subsequent liver transplantation. Surgery 1991; 109: 792.

5. **Ringe B, Lubbe N, Kuse E, Frei U, Pichlmayr R:** Management of emergencies before and after liver transplantation by early total hepatectomy. Transplant Proc 1993; 25: 1090.
6. **Husberg BS, Goldstein RM, Klintmalm GB, et al:** A totally failing liver may be more harmful than no liver at all: three cases of total hepatic devascularization in preparation for emergency liver transplantation. Transplant Proc 1991; 23: 1533.
7. **Rozga J, Podesta L, LePage E, et al:** Control of cerebral oedema by total hepatectomy and extracorporeal liver support in fulminant hepatic failure. Lancet 1993; 342: 898.
8. **Lee WM:** Total hepatectomy for acute liver failure: don't take out my liver! Gastroenterology 1994; 107: 894.

World J Surg (2010) 34:1034–1038
DOI 10.1007/s00268-010-0424-5

World Journal of Surgery

Predictor for Histological Microvascular Invasion of Hepatocellular Carcinoma: A Lesson from 229 Consecutive Cases of Curative Liver Resection

Susumu Eguchi · Mitsuhisa Takatsuki · Masaaki Hidaka · Akihiko Soyama · Tetsuo Tomonaga · Izumi Muraoka · Takashi Kanematsu

Published online: 2 February 2010

Abstract
Background Microscopic vascular invasion is an important risk factor for recurrent hepatocellular carcinoma (HCC), even after curative liver resection or orthotopic liver transplantation. To predict microscopic portal venous invasion, the following two questions were examined retrospectively: Is it possible to detect microvascular invasion preoperatively? What are the characteristics of a group of early HCC recurrences even with no microvascular invasion?
Methods Study 1 included 229 patients with HCC who underwent curative liver resection between 1991 and 2008; 127 had HCC without microscopic portal venous invasion, and 52 had HCC with microscopic portal venous invasion (MPVI). These two distinct groups were analyzed with regard to various clinicopathologic factors. Subsequently, we specifically investigated if HCCs <5 cm with vascular invasion ($n = 32$) have some characteristics that would allow detection of latent microvascular invasion. Study 2 included 127 HCC patients without MVPI; 42 had a recurrence within 2 years, and 85 patients were recurrence-free for at least 2 years. These two distinct groups were analyzed with regard to various clinicopathologic factors.
Results HCC diameter of >5 cm, the macroscopic appearance of HCC, and high levels of preoperative des-γ-carboxyprothrombin are significant prognostic factors in identifying microvascular invasion of HCC. The strongest predictor of early recurrence (within 2 years) was the serum α-fetoprotein level in patients without clear microvascular invasion.
Conclusions Tumor size, macroscopic appearance, and high tumor marker levels are important elements in identifying the group of patients with a low HCC recurrence rate after curative liver resection.

S. Eguchi (✉) · M. Takatsuki · M. Hidaka · A. Soyama · T. Tomonaga · I. Muraoka · T. Kanematsu
Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
e-mail: sueguchi@nagasaki-u.ac.jp; sueguchi@net.nagasaki-u.ac.jp

Introduction

Microvascular invasion is a strong prognostic factor for hepatocellular carcinoma (HCC), even after curative liver resection. Moreover, after orthotopic liver transplantation (OLT), which is the ultimate removal of a malignant tumor, microvascular invasion remains a significant prognostic factor as HCC becomes systemic through invasion of peripheral portal or hepatic veins and subsequent spread [1–3]. Therefore, vascular invasion has always been included as an indication for OLT, including in the Milan criteria [4]. Macrovascular invasion to the first and second ramifications of the portal vein can be diagnosed by computed tomography (CT) or other imaging techniques; however, microvascular invasion to minute peripheral areas, such as the third branch of the portal vein, is difficult to detect with current imaging modalities. Therefore, to predict the outcome of liver transplantation or curative liver resection more accurately, it is necessary to identify factors that indicate or predict the microvascular invasion of HCC.

We performed 229 curative liver resections for HCC during a period of 17 years. Using the data from these cases, we carried out a retrospective analysis to identify factors that can predict the recurrence of HCC based on the histological findings of HCC in the resected liver.

Patients and methods

Between 1991 and 2008, a total of 229 curative liver resections were performed in the Department of Surgery of Nagasaki University Hospital, Nagasaki, Japan. Of 229 HCCs, 50 had vascular invasion up to the second branch of the portal vein. The remaining 179 patients were analyzed in the study. The average age of the patients was 65 years (range 20–85 years). There were 143 men and 36 women, and the median follow-up period was 45.5 months.

The macroscopic appearance of HCC was classified into four types: type 1, single nodular type; type 2, single nodular type with extranodular growth; type 3, contiguous multinodular type formed by a cluster of small, contiguous nodules; type 4, infiltrative type [5]. As tumor markers, serum levels of α-fetoprotein (AFP) and des-γ-carboxy-prothrombin (DCP) were measured. We conducted two distinct retrospective studies.

Study 1

To identify factors for detecting microscopic portal venous invasion preoperatively, we examined 179 of our 229 patients (excluding those with vascular invasion to the first and/or second branches of the portal vein). Of these 179 patients, 127 had no histologically proven microscopic portal venous invasion, hepatic venous invasion, or intrahepatic metastasis. The remaining 52 had microscopic vascular invasion regardless of the presence or absence of intrahepatic metastasis of HCC. Subsequently, after we learned that more microvascular invasion occurred in large tumors (≥5 cm), we were interested in whether HCCs <5 cm with microvascular invasion have some characteristics that would allow detection of latent microvascularity. When we limited our examination to patients with a single HCC lesion of <5 cm in diameter, we had 102 patients without and 31 patients with microvascular invasion.

Study 2

The second study was performed to identify the group of patients who suffered early recurrence—defined as recurrence within 2 years—even though they showed no microscopic portal venous invasion. Of the 127 patients without proven microvascular invasion, 42 suffered early recurrence and 85 experienced recurrence after 2 years. These two distinct groups were analyzed with regard to various clinicopathologic factors. For this study, necroinflammatory activity (grade) and the degree of fibrosis (stage) as determined by Knodell et al. were calculated by routine histologic examination [6].

Statistical analysis

All analyses were conducted with Stat-View. Univariate analysis was performed using the Pearson chi-squared test for categorical factors and the Mann–Whitney test for numerical values. Multivariate analysis was conducted with a logistic regression model. Odds ratios (ORs) and the corresponding 95% confidence interval (CI) were computed to assess the strength of association. Any P values of <0.05 were considered statistically significant.

Results

Study 1

Univariate analysis revealed that the size of the HCC, the number of HCC lesions, the macroscopic appearance of HCC, and tumor markers (AFP, DCP) had a significant predictive value (Table 1). Multivariate logistic regression analysis revealed that the size of the HCC and its macroscopic appearance were significant independent risk factors for microvascular invasion by HCC (Table 2).

When we limited our examination to patients with a single HCC lesion of < 5 cm diameter, we had 102 patients without and 31 patients with microvascular invasion. Significant predictive factors for microvascular invasion were the macroscopic appearance of the HCC and a high DCP level (Table 3). With respect to the macroscopic appearance of the HCC, types 2 and 3 were significantly predictive of microvascular invasion of HCC.

Study 2

Only AFP had a significant predictive value for identifying patients likely to experience recurrence within 2 years even if there was no histologic evidence of microvascular invasion (Table 4). Moreover, a positive rate of hepatitis C antibody in the early recurrence group was higher than in the group with recurrence after 2 years. Grading (necroinflammatory response) and staging (fibrosis) were not statistically different between the two groups.

Discussion

In the present study, a tumor diameter of ≥5 cm, its macroscopic appearance, and the DCP level were significant predictive factors for microvascular invasion, which cannot be detected by current imaging techniques; this is consistent with the findings of a previous report by Shirabe et al. [7] The macroscopic appearance of type 2 or type 3 HCC, which can be evaluated in imaging studies, also

Table 1 Association of microvascular invasion of HCC: all HCCs

Parameter	Microvascular invasion		
	Positive ($n = 52$)	Negative ($n = 127$)	P
Age (years), median and range	64 (20–85)	65 (44–81)	NS
Sex (M:F)	41:11	102:25	NS
HBsAg-positive	20 (38.5%)	36 (28.3%)	NS
HCV Ab-positive	20 (38.5%)	63 (50.8%)	NS
Liver damage (A:B)	48:4	114:13	NS
HCC size (cm), median (range)	5.2 (0.5–17)	3 (0.8–11.5)	<0.001
No. of HCCs, median and range	1 (1–5)	1 (1–6)	<0.01
Macroscopic appearance of HCC (types 1/2/3/4)	8/17/19/1 (7 unclassified)	65/26/20/2 (14 unclassified)	<0.001
Tumor markers (median and range)			
AFP	95 (1.6–454,300)	12.7 (1.2–13,840)	<0.001
DCP	845 (6–76,600)	24 (0–69,150)	<0.05

HCC hepatocellular carcinoma, *HBsAg* hepatitis B virus surface antigen, *HCV Ab* hepatitis C virus antibody, *AFP* α-fetoprotein, *DCP* des-γ-carboxyprothrombin

Table 2 Logistic regression of factors associated with microvascular invasion of HCC: all HCCs

Parameter	Coefficient	Odds ratio (95% CI)
Size of HCC	0.517	1.678* (1.275–2.208)
No. of HCC	−0.42	0.657 (0.181–2.384)
Macroscopic appearance of HCC		
Type 1		Reference
Type 2	2.569	13.047** (1.514–112.439)
Type 3	3.229	25.253* (3.289–193.913)
Type 4	4.098	60.205** (2.574–1408.257)

CI confidence interval

* $P < 0.01$

** $P < 0.05$

predicts microvascular invasion. Therefore, in cases of type 2 or 3 HCC, early recurrence can be carefully monitored even after OLT.

With respect to tumor markers, the DCP level was an important factor in estimating the malignant potential of HCC without microscopic vascular invasion even after curative liver resection. Furthermore, even when the HCC is limited to a single lesion <5 cm in diameter (as described in the Milan criteria), an elevated DCP level implies a poor prognosis after curative resection. For these small, single HCCs, the DCP level was found to be a better predictor of vascular invasion than the macrovascular appearance of the HCC.

According to the results of the present study 2, AFP predicted the early recurrence of HCC even without proven microvascular invasion in the resected specimen. As documentation of microvascular invasion may be difficult because of the width of the slice in the tumor, the possibility of microvascular invasion in patients with early recurrence cannot be ruled out. Our findings indicate that even when an HCC that meets the Milan criteria (a single lesion <5 cm diameter) is removed by curative liver

Table 3 Association of microvascular invasion of HCC: single HCCs <5 cm diameter

Parameter	Microvascular invasion		
	Positive ($n = 31$)	Negative ($n = 102$)	P
Age (years), median and range	61 (37–85)	65 (44–81)	NS
Sex (M:F)	2:4	81:21	NS
HBsAg-positive	12 (38.7%)	28 (27.4%)	<0.05
HCV Ab-positive	12 (38.7%)	52 (50.9%)	NS
Child-Pugh (A:B)	30:1	94:8	NS
Macroscopic appearance (types 1/2/3/4)	6/11/10/1 (3 data sets missing)	55/23/13/1 (10 data sets missing)	<0.001
Tumor markers (median and range)			
AFP	97.9 (1.6–454,300)	13 (1.2–81)	NS
DCP	1307 (1.8–76,600)	71 (0–8520)	<0.001

Table 4 Factors for early recurrence within 2 years without proven microvascular invasion

Parameter	Early recurrence		*P*
	Yes ($n = 42$)	No ($n = 85$)	
Age, median (range)	65 (45–77)	66 (44–81)	NS
Sex (M:F)	35:7	67:18	NS
HBsAs-positive	12 (28.6%)	27 (31.7%)	NS
HCV Ab-positive	23 (54.8%)	39 (45.9%)	<0.05
Child Pugh (A:B)	36:6	80:5	NS
HCC size (cm), median and range	3 (1–11.5)	3 (0.8–11.4)	NS
No. of HCCs, median and range	1 (1–6)	1 (1–2)	NS
Macroscopic appearance (types 1/2/3/4)	21/10/5/1 (5 unclassified)	45/14/16/2 (8 unclassified)	NS
Tumor markers (median and range)			
AFP	41.4 (2–1714)	8.3 (1.2–13,840)	<0.05
DCP	61 (0–69,150)	24 (6–3,999)	NS
HAI			
Grading	5 (0–13)	5 (0–13)	NS
Staging	3 (0–4)	2 (0–4)	NS

HAI hepatitis activity index

resection and OLT, patients should be carefully monitored for early recurrence when the AFP level is elevated. Usually, after curative liver resection, recurrence within 2 years appears mostly as an intrahepatic metastasis through vascular invasion, whereas recurrences occurring 2 years after R0 are regarded as multicentric HCCs, which are a different clone from the first resected HCC. In other words, it is not usual that recurrence occurs after 2 years through microvascular invasion.

When considering expansion of the indication criteria for OLT for HCC, the prediction of vascular invasion should be a key point because a previous report showed its importance even after total eradication of the diseased liver [8]. The present study found that the macroscopic appearance of the HCC and tumor markers are important as predictors of microvascular invasion and that DCP in particular can be used to detect latent microvascular invasion of HCC even in patients with a single lesion of <5 cm diameter. Furthermore, the AFP level can be used to predict early recurrence after curative removal of HCC, which implies latent microvascular invasion because early recurrence is generally thought to indicate intrahepatic metastasis of a primary HCC through the portal vein [9]. In contrast, recurrence after 2 years is usually regarded as a second occurrence of HCC in the diseased liver (multicentric occurrence) [10]. Because the diseased liver is removed during OLT, intrahepatic metastasis through microvascular invasion is more important than the multicentric occurrence of HCC after the procedure.

Recently, using thin-sliced explant liver we showed that preoperatively undetectable HCC does not have a prognostic impact on outcome or recurrence of HCC after liver transplantation [11]. The characteristics of undetectable HCCs included a minute (median size 6 mm), well-differentiated appearance (80%), with indistinct margins (85.3%) and without vascular invasion (94%). There was no recurrence in any patients at the time of follow-up (median follow-up period was 30.1 months). In fact, tumor markers in almost all patients were within normal limits. Together with these results, it was found that in small HCCs with low tumor marker levels there was an absence of microvascular invasion of the HCC.

As a subgroup analysis, we investigated the group of patients with HCCs <5 cm in diameter to determine predictors for microscopic vascular invasion. As it has already been widely reported that HCCs ≥5 cm in diameter have a greater chance of spreading through microvascular invasion, the prediction of microvascular invasion is not important for those patients. Even when the patient meets other criteria for liver transplantation, if the HCC is ≥5 cm OLT is contraindicated because of the high risk of recurrence. In the studies described herein, therefore, we tried to find potential microscopic vascular invasion using criteria other than the size of the HCC.

In conclusion, tumor size, the macroscopic appearance of the HCC, and the DCP level are important factors that can be used to identify the group of patients with a low probability of recurrence of HCC after curative liver resection. The AFP level can also be used as a predictor of latent microscopic vascular invasion and early recurrence.

References

1. Iwatsuki S, Starzl TE, Sheahan DG (1991) Hepatic resection versus transplantation for hepatocellular carcinoma. Ann Surg 214:221–228

2. Bismuth H, Chiche L, Adam R et al (1993) Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. Ann Surg 218:145–151
3. Roayaie S, Frischer JS, Emre SH et al (2002) Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. Ann Surg 235:533–539
4. Mazzaferro V, Regalia E, Doci R et al (1996) Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 334:693–699
5. Shimada M, Rikimaru T, Hamatsu T et al (2001) The role of macroscopic classification in nodular-type hepatocellular carcinoma. Am J Surg 182:177–182
6. Knodell RG, Ishak KG, Black WC et al (1981) Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1:431–435
7. Shirabe K, Itoh S, Yoshizumi T et al (2007) The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma: with special reference to the serum levels of des-gamma-carboxy prothrombin. J Surg Oncol 95:235–240
8. Yao FY, Ferrell L, Bass NM et al (2002) Liver transplantation for hepatocellular carcinoma: comparison of the proposed UCSF criteria with the Milan criteria and the Pittsburgh modified TNM criteria. Liver Transpl 8:765–774
9. Vivarelli M, Cucchetti A, La Barba G et al (2008) Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. Ann Surg 248:857–862
10. Kim BW, Kim YB, Wang HJ et al (2006) Risk factors for immediate post-operative fatal recurrence after curative resection of hepatocellular carcinoma. World J Gastroenterol 12:99–104
11. Hidaka M, Eguchi S, Okudaira S et al (2009) Multicentric occurrence and spread of hepatocellular carcinoma in whole explanted end-stage liver. Hepatol Res 39:143–148

ELSEVIER

Macrophage-Dominant Sialadenitis in Human T-Cell Leukemia Virus Type I–Associated Myelopathy After Living-Donor Liver Transplantation

H. Nakamura, T. Ichikawa, T. Nakamura, A. Kawakami, N. Iwamoto, T. Matsuzaki, H. Miyaaki, S. Yamasaki, H. Ida, S. Eguchi, T. Hayashi, K. Nakao, T. Kanematsu, and K. Eguchi

ABSTRACT

A 64-year-old man who suffered from human T-cell leukemia virus type I (HTLV-I)–associated myelopathy (HAM) after living-donor liver transplantation (LDLT) for liver cirrhosis due to hepatitis C virus infection complained of xerostomia. Although exocrine function test results were positive, autoantibodies including anti-SS-A/SS-B antibodies and sialography showed negative findings. Labial salivary gland biopsy revealing infiltration of 60 counts of mononuclear cells (MNCs) in minor salivary glands led to a diagnosis of Sjögren's syndrome-like sialadenitis. Immunohistochemistry demonstrated dominant CD68 staining and major histocompatibility complex class II on the surface of infiltrating MNCs. Herein we have reported a rare condition of macrophage-dominant sialadenitis in a patient with HAM after LDLT.

BOTH hepatitis C virus (HCV) and human T-cell leukemia virus type I (HTLV-I) have been reported to be associated with the onset of Sjögren's syndrome (SS).[1,2] Because HCV infection demonstrates exocrine dysfunction along with sialadenitis, the American-European Consensus Group for SS excluded HCV infection in the diagnosis of SS.[3] We have previously reported that an epidemiological study showed a high prevalence of SS in anti–HTLV-I antibody–positive subjects.[4] In this case, a complication of HTLV-I–associated myelopathy (HAM) after living-donor liver transplantation (LDLT) has recently been reported.[5] Herein, we have additionally reported the emergence of unusual sialadenitis in this patient.

CASE REPORT

The complication of HAM in this patient was already reported by Soyama et al.[5] Briefly, LDLT was performed for a patient who had decompensated liver cirrhosis due to HCV infection in August 2002. Both the patient and his younger sister donor were seropositive for anti–HTLV-I antibody. Immediately after LDLT in October 2002, interferon (IFN) α-2b and ribavirin were administered after we confirmed recurrence of the HCV infection, but HAM appeared 18 months after LDLT. Although pegylated IFN α-2b and ribavirin were administered for 48 weeks against the HCV infection, no response was observed to the recurrent active hepatitis.

When the patient was admitted in September 2008, xerostomia was newly detected. The new clinical manifestations of HAM included spastic gait and bladder symptoms. Elevation of aspartate aminotransferase (53 IU/L), alanine aminotransferase (47 IU/L), and immunoglobulin (Ig)G (2630 mg/dL) were observed with normal total bilirubin (0.7 mg/dL). Type IV collagen and quantitative HCV ribonucleoprotein were elevated at 290 ng/mL (normal, ≤140) and 7.1 log IU/mL (normal, undetected) with reduced total branched chain amino acids (285 μmol/L; normal, 379–688). A liver biopsy, which had resulted in a hospital admission in September 2008, showed chronic hepatitis with fibrous enlargement of the portal area, inflammatory cell infiltration, and piecemeal necrosis. The relative copy number of HTLV-I against

From the Unit of Translational Medicine (H.N., A.K., N.I., S.Y., H.I., K.E.) Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Department of Gastroenterology and Hepatology (T.I., T.M., H.M., K.N.), Department of Molecular Microbiology and Immunology (T.N.), Nagasaki University Graduate School of Biomedical Sciences, Department of Surgery (S.E., T.K.), Nagasaki University Graduate School of Biomedical Sciences, and Department of Pathology (T.H.), Nagasaki University Hospital, Nagasaki City, Japan.

All authors declare no conflicts of interest in this article.

Address reprint requests to Hideki Nakamura, Department of Immunology and Rheumatology, Unit of Translational Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki City, Nagasaki 852-8501, Japan. E-mail: nakamura_hideki911@yahoo.co.jp, nhideki@nagasaki-u.ac.jp

0041-1345/–see front matter
doi:10.1016/j.transproceed.2010.04.050

β-globin in the peripheral blood sample was $2.56 \times 10^2/10^4$ cells by real-time polymerase chain reaction. Along with xerostomia, both the Saxon test (1.1 g/2 minutes; <2 g, positive) and Schirmer test (3 mm/5 minutes; <5 mm, positive) were positive with negative results for anti-SS-A/SS-B antibodies and sialography. However, minor salivary gland biopsy (Fig 1A) demonstrated more than 60 counts of mononuclear cell (MNC) infiltration, which were confirmed as dominantly macrophages. Although the patient showed signs of xerostomia, positive exocrine dysfunction, and MNC infiltration into the minor salivary gland (MSG), SS was excluded according to the criteria determined by the American-European Consensus Group.[3] Immunohistochemistry using monoclonal antibodies for MSG demonstrated positive staining of CD68 on the infiltrating MNCs (Fig 1B). Compared with the prevalence of CD68, the prevalence of CD4 (Fig 1C) or CD8 (Fig 1D) was less than that of CD68, macrophage. Major histocompatibility (MHC) class II was found in human tonsil as a positive control (Fig 1E) and MNCs in the MSG of this patient (Fig 1F). Written informed consent for the use of the biopsy specimen was obtained from the patient.

DISCUSSION

HCV-related SS has been reported to be characterized by a high prevalence of cryoglobulinemia with a low frequency of anti-SS-A/SS-B antibodies.[6] In our case, sialadenitis without SS-related autoantibodies was compatible with the characteristics of HCV-related SS. However, HCV infection usually shows infiltration of CD4+ T lymphocytes into the MSG, which is incompatible with the present macrophagic infiltration. Furthermore, it has previously been reported that IFNs have the potential to cause autoimmune diseases, such as autoimmune thyroid diseases, systemic lupus erythematosus, rheumatoid arthritis, or SS.[7,8] Unoki et al reported that administration of IFN-alpha-2b for a patient with type C chronic active hepatitis induced SS with sicca symptoms and elevation of autoantibodies, suggesting that IFN per se has a potential to form autoimmune disorders in patients with viral hepatitis.[9]

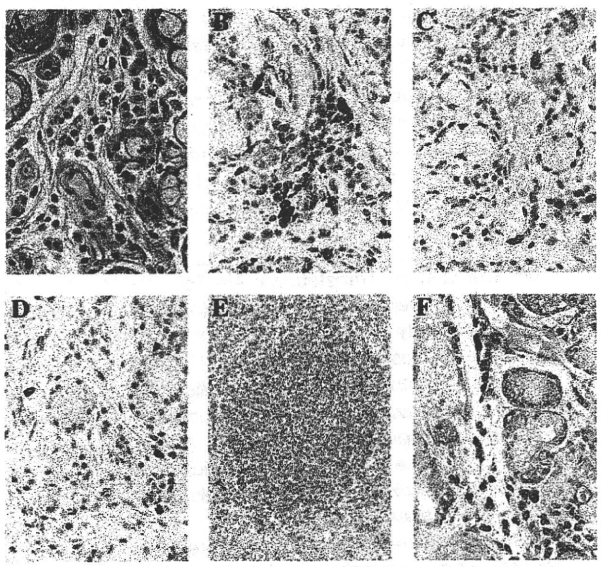

Fig 1. Phenotypic markers expressed in the minor salivary gland (MSG). Immunohistochemistry was performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the MSG using the streptavidin-biotin method. Primary antibodies were used as follows: **(A)** hematoxylin-eosin staining, **(B)** CD68, **(C)** CD4, **(D)** CD8, **(E)** MHC class II staining in human tonsil (positive control), and **(F)** MHC class II staining of the MSG of the patient. (Original magnification for A–D and F, ×200; E ×100.) Hematoxylin was used as a counterstain.

With regard to HTLV-I infection, prognosis of HTLV-I–positive renal transplant recipients has been previously reported,[10] observing that both living-related and cadaveric kidneys from HTLV-I carriers may be used for HTLV-I–seropositive recipients because of the low occurrence of adult T-cell leukemia. HTLV-I is also one of the candidates to trigger sialadenistis. We have previously reported a high prevalence of SS among patients with HAM.[2] However, the predominant phenotype of MNCs in HAM-SS patients was CD4+ T lymphocytes, which was similar to the type of MNCs in HTLV-I–seronegative SS patients.

Previously Ishiguro et al[11] established a rat model of HTLV-I infection in which massive foamy macrophages infiltrated the spinal cord and clinical manifestations of the rat resembled those of HAM patients. Meanwhile, our patient showed macrophage-dominant MNC infiltration into the MSG. Although the pathogenesis of the rat model might be different from that of human HAM, because lymphocytic infiltration is an apparent characteristic of HAM patients, an unrecognized trigger might have induced infiltration of macrophages into the MSG in our patient.

Graft-versus-host disease (GVHD) is considered to be a candidate cause of sialadenitis. Fujiwara et al[12] have previously reported sialadenitis in experimental GVHD in an animal model, in which nonirradiated mice were injected with spleen cells developing chronic GVHD. In their report, sialadenitis was observed predominantly with CD4+ T lymphocytes, although with a low frequency of macrophages, B cells, or plasma cells. However, chronic GVHD has rarely been reported after LDLT. Sun et al[13] reported a case of GVHD at 4 months after cadaveric liver transplantation. In their report, the patient showed gastrointestinal symptoms, which were determined to be T-lymphocyte infiltration based on a colonic biopsy.

In summary, the mechanism by which sialadenitis is induced remains to be clarified. However, both active hepatitis and HAM have the potential for viral-induced recruitment of mononuclear infiltration. Furthermore, double viral infection may provoke a strong elimination reaction compared with a single viral infection. Although an antiviral reaction is considered to be increased by innate immunity through Toll-like receptors,[14] intense antigen-presentation capacity might be yielded by induction of macrophages.

REFERENCES

1. Ramos-Casals M, Loustaud-Ratti V, De Vita S, et al: Sjögren syndrome associated with hepatitis C virus: a multicenter analysis of 137 cases. Medicine (Baltimore) 84:81, 2005

2. Nakamura H, Kawakami A, Tominaga M, et al: Relationship between Sjögren's syndrome and human T-lymphotropic virus type I infection: follow-up study of 83 patients. J Lab Clin Med 135:139, 2000

3. Vitali C, Bombardieri S, Jonsson R, et al: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 61:554, 2002

4. Terada K, Katamine S, Eguchi K, et al: Prevalence of serum and salivary antibodies to HTLV-1 in Sjögren's syndrome. Lancet 344:1116, 1994

5. Soyama A, Eguchi S, Takatsuki M, et al: Human T-cell leukemia virus type I-associated myelopathy following living-donor liver transplantation. Liver Transpl 14:647, 2008

6. Ramos-Casals M, De Vita S, Tzioufas AG: Hepatitis C virus, Sjögren's syndrome and B-cell lymphoma: linking infection, autoimmunity and cancer. Autoimmun Rev 4:8, 2005

7. Wilson LE, Widman D, Dikman SH, et al: Autoimmune disease complicating antiviral therapy for hepatitis C virus infection. Semin Arthritis Rheum 32:163, 2002

8. Bhakri H, Sriskandan K, Davis T, et al: Recombinant gamma interferon and autoimmune thyroid disease. Lancet 2:457, 1985

9. Unoki H, Moriyama A, Tabaru A, et al: Development of Sjögren's syndrome during treatment with recombinant human interferon-alpha-2b for chronic hepatitis C. J Gastroenterol 31:723, 1996

10. Nakamura N, Tamaru S, Ohshima K, et al: Prognosis of HTLV-I-positive renal transplant recipients. Transplant Proc 37: 1779, 2005

11. Ishiguro N, Abe M, Seto K, et al: A rat model of human T lymphocyte virus type I (HTLV-I) infection. 1. Humoral antibody response, provirus integration, and HTLV-I-associated myelopathy/tropical spastic paraparesis-like myelopathy in seronegative HTLV-I carrier rats. J Exp Med 176:981, 1992

12. Fujiwara K, Sakaguchi N, Watanabe T: Sialoadenitis in experimental graft-versus-host disease. An animal model of Sjögren's syndrome. Lab Invest 65:710, 1991

13. Sun B, Zhao C, Xia Y, et al: Late onset of severe graft-versus-host disease following liver transplantation. Transpl Immunol 16:250, 2006

14. Kawai T, Akira S: Antiviral signaling through pattern recognition receptors. J Biochem 141:137, 2007

Int Surg 2010;95:197–204

Significance of PET/CT in Determining Actual TNM Staging for Patients With Various Lung Cancers

Kenya Chiba[1], Midori Isoda[2], Masako Chiba[3], Takashi Kanematsu[4], Susumu Eguchi[4]

[1]*Department of Surgery, and* [2]*Dermatology,* [3]*Internal Medicine, Nisi-Isahaya Hospital, Nagasaki, Japan, and* [4]*Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*

We investigated the difference in TNM stage of lung cancer provided by PET/CT (combining positron emission tomography and computed tomography) as compared with TNM stage obtained with conventional imaging studies (CI) with contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) with iron contrast media. Sixty-seven cases of lung cancer were included in this study. Overall, the rate of correction of TNM staging was 70.1% after PET/CT. The correction rate for each factor was 32.8% in T, 37.3% in N, and 37.3% in M. High rates of correction were observed in small cell lung cancer (SCLC), with 75% (6/8 cases) obtained by PET/CT. When SCLCs were divided into limited disease (n = 6) involving 1 hemithorax, including mediastinal and contralateral hilar lymph nodes, and others (extensive disease, n = 2), the correction rate was as high as 80% for limited disease. In conclusion, PET/CT can provide actual TNM staging and recognition for oncologists in staging, which would not mislead to selection of inadequate subsequent treatment.

Key words: PET/CT – TNM stage – Lung cancer

With the development of fluoro-deoxy-glucose (FDG) in the late 1900s, positron emission tomography (PET) emerged as a new technology for cancer diagnosis.[1] However, it was difficult to learn the precise anatomic locations of lesions because images obtained by PET were as vague as those obtained by scintigraphic images.

After the appearance of PET combined with computed tomography (CT) devices (hereinafter abbreviated as "combined PET/CT") in 2000, CT images of cancer lesions and the sites thereof were clearly enhanced by FDG, thereby further increasing the precision of images of the range and metastasis of a lesion, resulting in the provision of very

Reprint requests: Susumu Eguchi, MD, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
Tel.: +81 95 819 7316; Fax: +81 95 819 7319; E-mail: sueguchi@nagasaki-u.ac.jp