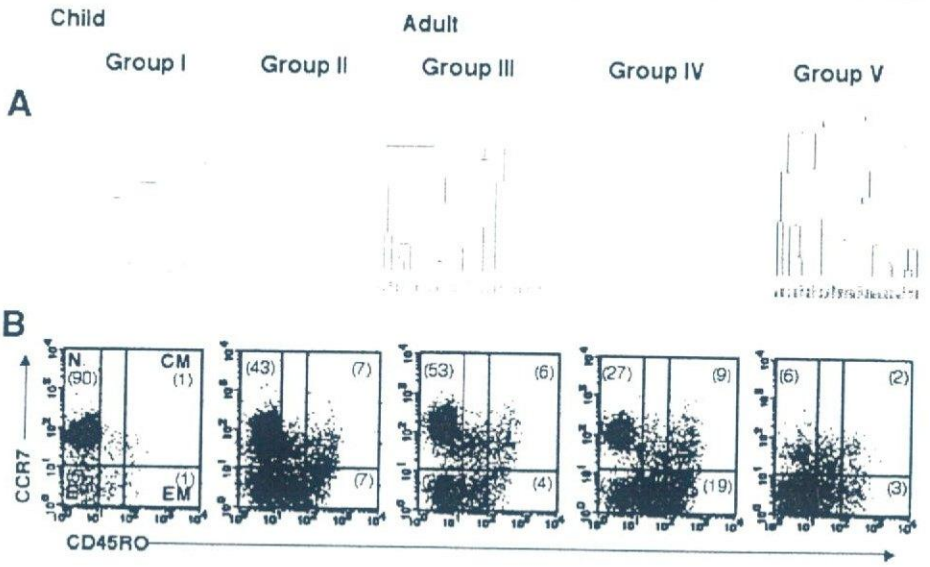

Figure 1. (A) Dendrogram of hierarchical clustering. (B) CD45RO and CCR7 are coexpressed on the subsets of peripheral blood $CD8^+$ T cells. Lymphocytes were stained with monoclonal antibodies to CD45RO and CCR7, which identified 4 subsets of $CD8^+$: 1 naive (N) ($CD45RO^-CCR7^+$); and 3 memory subsets, CM ($CD45RO^+CCR7^+$), EM ($CD45RO^+CCR7^-$), and effector T cells (E) ($CD45RO^-CCR7^-$). Percentages of cells in each subset are shown.

surface phenotype and functions of antigen-specific $CD8^+$ T cells. However, our study aims to clarify the phenotypic and functional changes of the $CD8^+$ subpopulation in many recipients (with or without viral infection) classified according to $CD8^+CD45$ isoform profiles prior to LDLT. Analysis of global nonspecific $CD8^+$ T cells was therefore used to follow up the relation of $CD8^+$ T cell function to the clinical outcome.

Heparinized venous blood samples were obtained 1 hour prior to surgery and then at 0, 1, 3, 6, 12, 36, and 120 hours, and every week following graft reperfusion for 4 months. As control samples, venous peripheral blood was collected from 54 healthy laboratory personal and medical students (32 men, 22 women; mean age 30 ± 3 yr standard error, range 4-69 yr). Since CD45RA and CD45RO expression are mutually exclusive we measured only the CD45RO isoform, but we used CD45RA for measuring cytokine. Naive T cells were defined as $CD45RO^-CCR7^+$; central/memory (CM) T cells as $CD45RO^+CCR7^+$; effector/memory (EM) T cells as $CD45RO^+CCR7^-$; and effector T cells as $CD45RO^-CCR7^-$.[6]

The monoclonal antibodies used to stain cell surface antigens were as follows: allophycocyanin (Coulter Immunotech, Miami, FL) or phycoerythrin-cyanin-5-conjugated (Coulter Immunotech, Marseilles, France) anti-CD4 or CD8, fluorescein isothiocyanate (FITC)-conjugated anti-CD45RO (Nichirei, Tokyo, Japan), TRI-COLOR-conjugated anti-CD45RA (Caltag Laboratories, Burlingame, CA), phycoerythrin (PE)-conjugated anti-CD3 (Coulter Immunotech, Miami, FL), FITC-conjugated anti-CD19 (Coulter Immunotech, Marseilles, France), PE-conjugated anti-human CCR7 (DakoCytomation, Kyoto, Tokyo, Japan), PE-conjugated anti-CD27 (Coulter Immunotech, Marseilles, France), and FITC-conjugated anti-CD28 (Nichirei, Tokyo, Japan). We used isotype-matched controls for intracellular staining. Cells were exposed to the antibodies for 30 minutes at 4°C and were washed twice with phosphate buffered saline; 5,000 cells were analyzed. FITC- and PE-labeled mouse immunoglobulin G were used as isotype-matched background controls. We analyzed the stained cells on a fluorescence-activated cell sorter Calibur flow cytometer by 3- and 4-color analysis, using CELL Quest software version 3.3 (BD Biosciences, San Jose, CA).

Flow Cytometric Detection of Cytokine Production and Intracellular Staining for Perforin

Flow cytometric measurement of cytokine production was performed as described previously.[22] In summary, 10^6 cells/mL were stimulated for 4 hours (interferon-gamma, IFN-γ, FITC-conjugated anti-IFN-γ, Becton Dickinson, San Jose, CA; tumor necrosis factor-alpha, tumor necrosis factor-alpha, FITC-conjugated anti-tumor necrosis factor-alpha, BD Bioscience, San Diego, CA) with a mixture of phorbol 12-myristate 13-acetate (25 ng/mL; Sigma-Aldrich Chemical, St. Louis, MO) and ionomycin (1 μg/mL; Sigma-Aldrich). The Golgi inhibitor brefeldin A (10 μg/mL; Sigma-Aldrich) was added for retention of intracellular cytokines. The cells were then stained for surface markers with PE, phycoerythrin-cyanin-5 (or TRI-COLOR), and allophycocyanin-conjugated antibodies, permeabilized with fluorescence-activated cell sorter lysing solution and fluorescence-activated cell sorter permeabilizing solution (BD Biosciences, San Diego, CA), and then stained for the indicated intracellular cytokines with FITC or PE-conjugated antibodies.

We measured intracellular perforin in $CD8^+$ cells without previous stimulation, and used the permeabilization and staining protocol described above for cytokine analysis. For the perforin analysis, the cells were treated with fixing buffer (Caltag Laboratories, Austria) for 20 minutes at room temperature, washed with phosphate buffered saline-0.1% fetal calf serum, and permeabilized with a permeabilization buffer (Caltag Laboratories, Austria) for 20 minutes at room temper-

TABLE 1. Hierarchical Clustering into 5 Groups

Group	n	Age (yr)	% Naive T cells	% CM T cells	% EM T cells	% Effector T cells	% CD27$^+$CD28$^+$ subsets	% IFN-γ	% TNF-α	% Perforin
Child										
I	24	3 ± 3	84.91 ± 7.54	0.85 ± 0.85	1.65 ± 2.10	7.98 ± 4.36	83.19 ± 7.26	0.84 ± 0.71	0.25 ± 0.37	3.61 ± 4.32
II	9	7 ± 5	44.95 ± 11.70	1.52 ± 3.53	8.93 ± 7.50	32.03 ± 8.44	59.97 ± 15.82	8.82 ± 3.99	4.28 ± 1.17	19.65 ± 7.29
Adult										
III	26	46 ± 11	53.66 ± 8.14	6.31 ± 3.47	7.70 ± 6.60	18.60 ± 7.93	70.62 ± 8.76	9.74 ± 5.38	9.70 ± 6.10	15.68 ± 6.68
IV	30	55 ± 9	19.85 ± 11.14	12.33 ± 5.55	18.10 ± 10.31	27.83 ± 12.82	44.23 ± 17.37	13.43 ± 8.57	13.32 ± 7.23	24.33 ± 11.46
V	23	49 ± 13	23.43 ± 10.75	3.73 ± 1.89	8.57 ± 5.40	46.71 ± 14.22	46.48 ± 17.10	27.58 ± 13.71	24.92 ± 16.25	32.11 ± 14.06

NOTE: Values are expressed as mean ± SD.

ature. They were then stained with antiperforin (δG9) (BD PharMingen, Crowley, UK) followed by addition of R-PE-CY5-conjugated F(ab')$_2$ fragment of rabbit anti-mouse immunoglobulin (DakoCytomation) secondary antibody.

Statistical Analysis

We classified recipients into 2 groups: pediatric (under 18 yr of age) and adult. We performed hierarchical cluster analysis in both groups, using JMP 5 (SAS Institute, Cary, NC)[23] to obtain clusters of recipients having similar proportions of naive, CM, EM, and effector T cells.

We determined bivariate correlations by Spearman rank correlation. Comparisons for continuous variables between groups were performed using Student's *t*-test and analysis of variance. Comparisons for proportions between groups were performed using Fisher's exact test. Survival curves were estimated using the Kaplan-Meier method, and log-rank tests were applied to test associations between group and survival time. All statistical tests were 2-sided, with significance defined as $P < 0.05$. Statistical analyses were performed using the statistical software package StatView 5 (Abacus Concepts, Berkeley, CA).

RESULTS

Hierarchical Clustering by Preoperative CD8$^+$CD45 Isoform Profiles

The existence of 5 groups, classified according to hierarchical clustering of our 112 recipients, was clear, as seen in the dendrogram (Fig. 1A). CD45RO and CCR7 were coexpressed on a subset of peripheral blood CD8$^+$ T cells in a typical recipient of each group (Fig. 1B). The proportion of cells in the different compartments was reasonably stable in the same group, but more variable across the 5 groups. In pediatric recipients the pretransplantation mean proportion of naive T cells was 85% in Group I and 45% in Group II; the effector T cell population was only marginal in Group I, but was high in Group II (Table 1). In adults, the naive T cell population was considerably lower in Groups IV and V than in Group III. The CD8$^+$ T cells in Group IV included the greatest number of EM T cells, and in Group V included the greatest number of effector T cells. In Groups IV and V the proportion of IFN-γ, tumor necrosis factor-alpha and perforin expression were markedly higher than in Groups I, II, and III. Table 2 shows statistical differences between the 5 groups in their proportions of the CD8$^+$ T cell subpopulation and their function. There were significantly large differences in CD45 isoforms between the 5 groups. In particular, the effector T cell proportion in Group V was significantly higher than in Groups III and IV. The proportion of IFN-γ differed significantly between Group IV and V recipients; tumor necrosis factor-alpha and perforin expression did not differ.

Table 3 profiles the recipients and donors. The study group included 53 recipients who underwent LDLT for

TABLE 2. Results of *P*-values Presenting Differences Among 5 Groups in the Relative Proportion of $CD8^+$ T Cell Subpopulation

Variable	Child	Adult			
	Group I vs. II *P**	*P*†	Group III vs. IV *P**	Group III vs. V *P**	Group IV vs. V *P**
Age (yr)	0.0577	0.0148	0.0021	0.2857	0.1035
% Naive T cells	<0.0001	<0.0001	<0.0001	<0.0001	0.2449
% CM T cells	0.0070	<0.0001	<0.0001	0.0027	<0.0001
% EM T cells	0.0005	<0.0001	<0.0001	0.6171	0.0002
% Effector T cells	<0.0001	<0.0001	0.0025	<0.0001	<0.0001
% $CD27^+CD28^+$ subsets	<0.0001	<0.0001	<0.0001	<0.0001	0.6819
% IFN-γ	0.0061	0.0003	0.1847	0.0002	0.0163
% TNF-α	<0.0001	0.0058	0.1571	0.0043	0.0748
% Perforin	<0.0001	<0.0001	0.0092	<0.0001	0.0552

Abbreviations: ANOVA, analysis of variance; CM, central/memory T cell subsets, EM, effector/memory T cell subsets.
**P*-values are based on Student's *t*-test.
†*P*-values are based on ANOVA.

chronic HCV or HBV infection. One Group IV recipient was coinfected simultaneously with HCV and HBV, and was involved in the HCV and HBV groups. The majority of Group III (53.8%), Group IV (76.7%), and Group V (69.6%) recipients suffered from chronic HCV- and/or HBV-infection. HCC was more prevalent in Groups IV and V (approximately 70%) than in Group III (36%). The 3 adult groups did not differ significantly in clinical status according to the Model for End-Stage Liver Disease score.[24] ABO blood group-incompatible LDLT was carried out in 3 children and 15 adults. The adult recipients did not differ in the amount of liver tissue transplanted, but the graft-to-recipient weight ratio in the adult groups was only about 0.52 times the ratio in the younger groups. There were significantly more HLA mismatched loci in Group V than in Groups I and III. The duration of cold ischemia was slightly longer in Group IV and V recipients.

Figure 2 shows changes of the effector T cell proportion in circulating $CD8^+$ T cells with advancing age in 112 transplant recipients and 54 healthy individuals. There was no correlation (r = 0.39) between the effector T cell proportion and advancing age in healthy individuals, but a weak correlation (r = 0.55) was found in the recipients. In the healthy individuals, the proportion of effector T cells was lower (13.71 ± 1.92, mean ± standard error) in pediatric recipients (under 18 yr of age) than in adult recipients (30.19 ± 1.83); $P < 0.001$. In adult recipients there was no significant difference in the effector T cell proportion between healthy individuals and Group III or IV recipients ($P = 0.14$ and $P = 0.07$, respectively). In contrast, the difference between healthy individuals and Group V recipients was significant ($P < 0.0001$). The proportion of effector T cells was considerably higher in Group IV (13%) and Group V (44%) than the upper limits for healthy individuals.

Postoperative Complication in the 5 Groups

Figure 3 (left) shows Kaplan-Meier curves for the recipient's probability of survival in the 5 groups. The 2-yr survival was 96% in Group I, 89% in Group II, 100% in Group III, 89% in Group IV, and 74% in Group V (Group V vs. Group III, $P < 0.01$). The Eastern Cooperative Oncology Group performance status[25] was assessed objectively for surviving patients up to 1 yr after LDLT. The proportion of recipients with Grade 0 Eastern Cooperative Oncology Group performance status (fully active and able to carry on all predisease activities without restriction) decreased progressively from Group I to Group V, in which it was only 9% (Fig. 3, right). Table 4 shows the frequencies of rejection and infection in the 5 groups. Rejection frequencies were higher in pediatric recipients than in adults. The phenotypic and functional profiles prior to LDLT in pediatric Group II were quite similar to those of adult Group III. However, the incidence of rejection tended to be higher in Group II than in Group III ($P = 0.112$, Fisher's exact test). In Group II recipients, the $CD8^+$ naive T cell proportion prior to LDLT was low compared with that in Group I, but promptly upregulated to high levels, similar to Group I, following tacrolimus administration 24 hours after LDLT; there was corresponding downregulation of effector T cells and cytolytic activity (data not shown). This rapid restoration of naive T cells seems to depend on intact thymic function during early life. In adult Croup III, in contrast, the naive T cells could not be restored to high levels because of the involvement of the thymus by advancing age. It is not clear why $CD4^+$ and $CD8^+$ T cells with high levels of naive T cells are more closely related to rejection than T cells with lower naive T cells. It is likely that the incidence of rejection in Group II are similar to those of Group I, but are higher than in Group III. On the other hand, there was no significant ($P = 0.686$, Fisher's exact test) difference in infection rate between Groups II and III. Group V recipients clearly had a higher infection rate than Group III.

Donor age has been reported to be an important factor affecting the severity of liver disease following liver transplantation.[1] Specifically, an adverse effect has been reported of advanced donor age (>40 yr) on the

TABLE 3. Recipient, Donor, and Operation Profiles

Recipients Group (n) male/female	Original liver diseases (n)	MELD score	Donor source (n)	HLA mismatch (n)	ABO blood type combination	Operation profiles Ischemic time (minutes)* Cold	Warm
Group I (24) 9/15	BA (17), Byler (1), Alagille syndrome (1) hepatoblastoma (2), FHF (1), primary hyperoxaluria (1), tyrosinemia (1)	—	Parent (23), uncle (1)	1 (5), 2 (10), 3 (4)	Identical (16), compatible (6), incompatible (2)	89 55	53 ± 26
Group II (9) 5/4	BA (4), chronic rejection (3, BA), hepatoblastoma (1), FHF (1)	—	Parent (8), aunt (1)	1 (3), 2 (3), 3 (1), 5 (1)	Identical (5), compatible (3), incompatible (1)	83 ± 117	40 ± 10
Group III (26) 12/14	PBC (3), PSC (2), FHF (6), polycystic disease (1), HBV (6 with 2 HCC), HCV (8 with 3 HCC)	13 ± 6	Parent (2), offspring (6), Spouses (7), sibling (10), cousin (1)	0 (4), 1 (4), 2 (4), 3 (5), 4 (4)	Identical (17), compatible (5) incompatible (4)	76 ± 51	51 ± 19
Group IV (30) 21/9	PBC (4), Alcholic LC (1), AIH (1), Caroli (1), HBV (10 with 6 HCC), HCV (12 with 10 HCC) HBV + HCV with HCC (1)	13 ± 6	Parent (1), offspring (15), spouses (7), sibling (6), nephew (1)	0 (2), 2 (12), 3 (7), 4 (1), 5 (1)	Identical (23), compatible (5), incompatible (2)	115 ± 64	68 ± 55
Group V (23) 11/12	BA (2), PBC (2), Wilson's disease (1), alcholic LC (1), polycystic disease (1) HBV (5 with 4 HCC), HCV (11 with 7 HCC)	16 ± 11	Parent (3), offspring (6), spouses (6), sibling (7), nephew (1)	0 (1), 2 (4), 3 (5), 4 (2), 5 (4), 6 (1)	Identical (11), compatible (3), incompatible (9)	133 ± 87	56 ± 21

Abbreviations: BA, biliary atresia; FHF, fulminant hepatic failure; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; LC, liver cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; AIH, autoimmune disease.
*Values expressed as mean ± SD.

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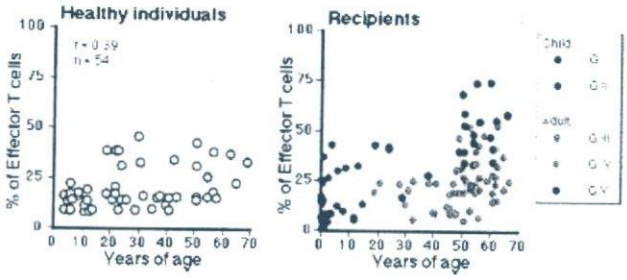

Figure 2. Changes in the proportion of circulating effector T cells with age in healthy individuals and transplant recipients. Each group is specified by a color marker: red, Group I; green, Group II; blue, Group III; brown, Group IV; and black, Group V.

outcome of transplantation for HCV.[26-30] In the present study, the mortality rates related to donor age were 8.6% (5/58) under 40 yr and 11.1% (6/54) over 40 yr ($P = 0.756$, Fisher's exact test).

During the study period, 11 (9.8%) of our 112 recipients died. Two of these were pediatric recipients; 1 suffered fulminant hepatic failure due to de novo autoimmune hepatitis, and the other suffered biliary atresia with retransplantation due to acute rejection. Nine adult recipients died (6 with either HCV or HBV infection, and 1 each with primary biliary cirrhosis, biliary atresia, and polycystic liver). Of 18 ABO blood group-incompatible LDLT, 3 (16.7%) died. The median age of the recipients was 48 yr (range, 1 month to 67 yr). The median age of donors was 38 yr (range, 21 to 64 yr). The median time from LDLT to death was 65 days (range, 13 to 351 days). Two recipients underwent retransplantation. Four of the deceased recipients were complicated by acute cellular rejection.

DISCUSSION

In Group I and II recipients, the survival probability was high and the Eastern Cooperative Oncology Group performance status was very good, but acute rejection occurred in approximately 60% (Fig. 3; Table 4). In adult recipients, in contrast, postoperative complications increased progressively from Group III to Group V. More postoperative complications developed in Group V recipients, leading to significant reduction in the survival probability and markedly reduced Eastern Cooperative Oncology Group performance status. These recipients were compromised by a high rate of life-threatening infection, rather than acute rejection.

Enhancement of $CD8^+$ Cytolytic Activity and Cytokine Production in Group IV and V Recipients

The outcome of the infection depends on how effectively the defensive mechanisms of the host resist the offensive tactics of the bacteria and virus.[31] In the present study, circulating $CD8^+$ T cells with a $CD45RA^+$-$CCR7^-$, combined with marked downregulation of $CD27^+CD28^+$, resembled cytolytic effector T cells. We have found that the interleukin-12 receptor β1 subunit in $CD8^+$ T cells upregulates positively with the proportion of effector T cells and IFN-γ-producing cells immediately after LDLT in Group IV and V recipients (data not shown). It is possible that interleukin-12 is important in promoting Th1-type immune response and cytotoxic T lymphocyte (CTL) activity after LDLT. Moreover, the preferential increase of effector T cells in Group IV and V was accompanied by marked up-regulation of IFN-γ and tumor necrosis factor-alpha (Table 1). Their enhanced expression of perforin plays a critical role in this cytolytic effector, since it can polymerize to form channel-like structures in the target cell membrane, through which granzymes can enter and subsequently activate the death machinery.[32,33] We found here that granule exocytosis by perforin is already operational in circulating effector-type $CD8^+$ cells prior to LDLT. More importantly, $CD27^+CD28^+$ expression was used to distinguish between subsets of differentiated $CD8^+$ T cells at different stages immediately after LDLT. These subsets can be assigned a position on a $CD8^+$ T cell differentiation pathway along which sequential downregulation of $CD27^+CD28^+$ subsets occurs, accompanied by upregulation of cytotoxic factors. Downregulation of the levels of $CD27^+CD28^+$ subsets therefore indicates that the activity of CTLs in eliminating virus-infected self-cells increases progressively from Group III to Group V recipients prior to LDLT. Group III recipients were able to mount an immune response that might help to clear HCV-ribonucleic acid even during immunosuppressive therapy, probably involving sustained viral clearance irrespective of small increases in IFN-γ. In Group V recipients, in contrast, the high effector T cell proportion is probably associated with the greatly enhanced cytotoxic activity, but could not adequately eliminate viral-infected cells.

Viral-infected recipients were characterized in the present study by enrichment of $CD8^+$ T cells having differing phenotypes between groups during chronic infection. These differences in $CD8^+$ T cell phenotype may relate simply to the differential properties necessary to control a virus. The virus load increases at least 10-fold after liver transplantation,[34] so that such viral replication may contribute further to the development and maintenance of the increased effector T cell proportion after LDLT. Therefore, when there is high HCV messenger ribonucleic acid, the enhanced cytotoxic activity may relate to the high viral load, leading to marked suppression of the host-effector immune response that usually controls HCV replication.

Of the 9 deceased adult recipients, 6 had chronic or HCV or HBV infection and the remaining 3 had other diseases. It follows that the recipient's immune response, characterized by a high effector T cell population, is not specific for chronic viral infection, and apparently plays a critical role in controlling not only liver damage but also infections such as fungi and bacteria. The immunosuppressive cascade would also have a greater and catastrophic effect on these recipients. Importantly, there were marked differences in clinical outcome and CTL generation according to the $CD8^+$ naive T cell proportion prior to LDLT. In Groups I, II, and III, $CD8^+$ T cells with a high naive T cell proportion had low

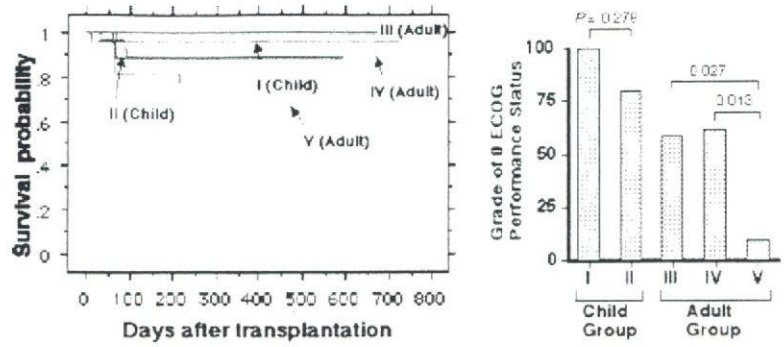

Figure 3. Kaplan-Meier curves showing recipient survival probability in the 5 (Groups I and II in child; Groups III, IV, and V in adult) groups (left). Percentage of grade 0 Eastern Cooperative Oncology Group performance status at 1 yr after LDLT (right).

TABLE 4. Comparison of Frequencies of Rejection and Infection in 5 Groups

Group	*n*	Rejection (%)	*P**	Infection (%)	*P**
Child					
I	24	58.3	—	54.2	—
II	9	66.7	1.000 (vs. group I)	44.4	0.708 (vs. group I)
Adult					
III	26	30.8	—	30.8	—
IV	30	23.3	0.561 (vs. group III)	46.7	0.279 (vs. group III)
V	23	30.4	1.000 (vs. group III)	69.6	0.010 (vs. group III)

*P-values are based on Fisher's exact test.

cytotoxic activity. In Groups IV and V, in contrast, $CD8^+$ T cells with very low proportions of naive T cells already had high cytotoxic activity prior to LDLT. The greater the $CD8^+$ CTL activity prior to LDLT, the smaller the capacity to generate CTLs for new invasion of bacteria and virus after LDLT (data not shown). Accordingly, the capacity to generate CTLs for infection after LDLT decreases progressively from Group I to Group V recipients, indicating progressive reduction in the latent ability to generate CTLs for clearance of new antigen. As a result, frequencies of postoperative complications are highest in Group V.

Current immunosuppressive induction protocols involve calcineurin inhibitors (cyclosporin, tacrolimus), corticosteroids, mono- and polyclonal antibodies, azathioprine, and mycophenolate mofetil. Use of steroids significantly increased the level of viremia in HCV-positive patients. Gane et al.[35] showed clearly that steroid pulse therapy is associated with a 4- to 100-fold increase in HCV-ribonucleic acid levels and subsequent development of acute hepatitis. In the present study, a Group IV recipient developed graft failure following repeated injection with steroid. On the other hand, in Groups IV and V, various immunosuppressors such as tacrolimus, cyclosporin, and others did not reduce preexisting CTL levels prior to transplantation. These levels had been reached during a lifetime of antigen exposure; there was also reduced thymopoiesis, characteristic of advancing age. In this regard, various conventional immunosuppressive agents remain limited in their ability to reduce preexisting CTLs. Drastic lymphocyte-depleting agents, such as rabbit anti-thymocyte globulin and anti-interleukin-2 antibodies, are able to induce cell regeneration by homeostasis-driven proliferation of T cells, and consequently provide the conditions involved in lymphocyte repopulation, favoring phenotypes with a low CTL activity with high naive T cells. Use of a preoperative shot pulse of antibody followed by low-dose immunosuppressive maintenance monotherapy may be reasonable compromise for a given transplant patient, based on preoperative high CTL activity.[36,37] However, we routinely perform antiinfective prophylaxis with less immunosuppression in Group V. It is very difficult to adjust the dose of immunosuppressive drugs specific for each recipient during infection.

Although this study had a short posttransplantation follow-up, the effect of the immunological status of T cells on the outcome has been definitively settled. Longer follow-up of larger cohorts is needed to decide whether the impairment of innate and adaptive immunoresponses by various dangerous factors after LDLTs has a significant adverse effect on long-term graft and patient survival.

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REFERENCES

1. Busquets J, Xiol X, Figueras J, Jaurrieta E, Torras J, Ramos E, et al. The impact of donor age on liver transplantation: influence of donor age on early liver function and on subsequent patient and graft survival. Transplantation 2001;71:1765-1771.
2. Kiuchi T, Kasahara M, Uryuhara K, Inomata Y, Uemoto S, Asonuma K, et al. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation 1999;67:321-327.
3. Timm JA, Thoman ML. Maturation of CD4+ lymphocytes in the aged microenvironment results in memory-enriched population. J Immunol 1999;162:711-717.
4. Kalams SA, Walker BD. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. J Exp Med 1998;188:2199-2204.
5. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a $CD4^+$ T-helper and a T-killer cell. Nature 1998;393:474-478.
6. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401: 708-712.
7. Appay V, Dunbar PR, Callan M, Klenerman P, Gillespie GM, Papagno L, et al. Memory $CD8^+$ T cells vary in differentiation phenotype in different persistent virus infections. Nat Med 2002;8:379-385.
8. Ozawa K, Uemoto S, Tanaka K, Kumada K, Yamaoka Y, Kobayashi N, et al. An appraisal of pediatric liver transplantation from living relatives. Initial clinical experiences in 20 pediatric liver transplantations from living relatives as donors. Ann Surg 1992;216:547-553.
9. Tanaka K, Uemoto S, Tokunaga Y, Fujita S, Sano K, Yamamoto E, et al. Living related liver transplantation. Transplant Proc 1992;24:2252-2253.
10. Yamaoka Y, Tanaka K, Ozawa K. Liver transplantation from living-related donors. Clin Transpl 1993;179-183.
11. Inomata Y, Uemoto S, Asonuma K, Egawa H. Right lobe graft in living donor liver transplantation. Transplantation 2000;69:258-264.
12. Kiuchi T, Tanaka K. Living donor adult liver transplantation: status quo in Kyoto and perspectives in the new millennium. Acta Chir Belg 2000;100:279-283.
13. Nakamura T, Tanaka K, Kiuchi T, Kasahara M, Oike F, Ueda M, et al. Anatomical variations and surgical strategies in right lobe living donor liver transplantation: lessons from 120 cases. Transplantation 2002;73:1896-1903.
14. Rosen HR. Hepatitis C in the liver transplant recipients: Current understanding and treatment. Microbes Infect 2002;4:1253-1258.
15. Ghobrial RM, Amersi F, Farmer DG, Chen P, Anselmo DM, Baquerizo A, et al. Rapid and severe early HCV recurrence following adult living donor liver transplantation (abstract, 228). Transplantation 2002;74(Suppl):88.
16. Taniguchi M, Trotter J, Wachs M. Feature of hepatitis C recurrence in living donor liver transplant recipients (abstract 229). Transplantation 2002;74(Suppl):88.
17. Berenguer M, Ferrell L, Watson J, Prieto M, Kim M, Rayon M, et al. HCV-related fibrosis progression following liver transplantation: increase in recent years. J Hepatol 2000; 32:673-684.
18. Tanaka K, Inomata Y, Kaihara S. Living-donor liver transplantation. Surgical techniques and innovation. Barcelona, Spain: Prous Science, 2003.
19. Miyagawa-Hayashino A, Haga H, Egawa H, Hayashino Y, Sakurai T, Minamiguchi S, et al. Outcome and risk factors of de novo autoimmune hepatitis in living-donor liver transplantation. Transplantation 2004;78:128-135.
20. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997;25:658-663.
21. Ohno T, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997;35:201-207.
22. Waldrop SL, Pitcher CJ, Peterson DM, Maino VC, Picker LJ. Determination of antigen-specific memory/effector CD4+ T cell frequencies by flow cytometry. J Clin Invest 1997;99:1739-1750.
23. Everitt BS, Landau S, Leese M. Hierarchial clustering. In: Everitt BS, Landau S, Leese M, eds. Cluster Analysis, 4th ed. London: Arnold, 2001:55-89.
24. Yu AS, Ahmed A, Keeffe EB. Liver transplantation: evolving patient selection criteria. Can J Gastroenterol 2001; 15:729-738.
25. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.
26. Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C and liver transplantation. J Hepatol 2001;35:666-678.
27. Neumann UP, Berg T, Bahra M, Puhl G, Guckelberger O, Langrehr JM, Neuhaus P. Long-term outcome of liver transplants for chronic hepatitis C: a 10-year follow up. Transplantation 2004;77:226-231.
28. Wali M, Harisson RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248-252.
29. Burak KW, Kremers WK, Batts KP, Wiesner RH, Rosen CB, Razonable RR, et al. Impact of cytomegalovirus infection, year of transplantation, and donor age on outcomes after liver transplantation for hepatitis C. Liver Transpl 2002; 8:362-369.
30. Berenguer M, Prieto M, San Juan F, Rayon JM, Martinez F, Carrasco D, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. Hepatology 2002;36:202-210.
31. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol 2001;19:65-91.
32. Shresta S, Pham CT, Thomas DA, Graubert TA, Ley TJ. How do cytotoxic lymphocytes kill their targets? Curr Opin Immunol 1998;10:581-587.
33. Henkart PA. Mechanism of lymphocyte-mediated cytotoxicity. Annu Rev Immunol 1985;3:31-58.
34. Chazouilleres O, Kim M, Combs C, Ferrell L, Bacchetti P, Roberts J, et al. Quantitation of hepatitis C virus RNA in liver transplant recipients. Gastroenterology 1994; 106:994-999.
35. Gane EJ, Naoumov NV, Qian KP, Mondelli MU, Maertens G, Portmann BC, et al. A longitudinal analysis of hepatitis C virus replication following liver transplantation. Gastroenterology 1996;110:167-177.
36. Starzl TE, Murase N, Abu-Elmagd K, Gray EA, Shapiro R, Eghtesad B, et al. Tolerogenic immunosuppression for organ transplantation. Lancet 2003;361:1502-1510.
37. Tector AJ, Fridell JA, Mangus RS, Shah A, Milgrom M, Kwo P, et al. Promising early results with immunosuppression using rabbit anti-thymocyte globulin and steroids with delayed introduction of tacrolimus in adult liver transplant recipients. Liver Transpl 2004;10:404-407.

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Beneficial Effects of Short-Term Lamivudine Treatment for *de novo* Hepatitis B Virus Reactivation After Liver Transplantation

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Clearance of hepatitis B surface antigen (HBsAg) by lamivudine is achieved in only a small proportion of patients with chronic hepatitis B virus (HBV) infection. We investigated the effect of lamivudine on *de novo* HBV reactivation after living-donor liver transplantation when the number of HBV was expected to be very small. Thirty-eight HBV-naive recipients who received liver grafts from antibodies to core antigen-positive donors receiving hepatitis B immunoglobulin (HBIG) were studied. HBsAg appeared in nine cases (23.7 %) despite receiving HBIG for 12–71 months (mean: 35.1 months) after transplantation. Lamivudine treatment was started in six recipients during the acute phase of HBV reactivation. Five of the six recipients achieved complete clearance of HBsAg in sera at a median of 4.6 months (ranging from 21 to 330 days) after lamivudine administration. Although lamivudine was stopped in four cases, all remained negative for HBsAg. Our findings suggested that short-term lamivudine treatment during acute phase of HBV reactivation could achieve complete clearance of HBsAg in a significant number of liver transplant recipients.

Key words: Anti-HBc, hepatitis B, hepatitis B immunoglobulin, lamivudine, liver transplantation

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Introduction

There is growing recognition that the majority of healthy individuals who are negative for hepatitis B surface antigen (HBsAg) but positive for antibodies to core antigen (anti-HBc), who had once been assumed to denote previous exposure to hepatitis B virus (HBV), have persistent viral infection of the liver tissues (1,2). Recently, we demonstrated that latent HBV infection is accompanied by ongoing viral replication in the livers, but not in the sera, of healthy anti-HBc-positive liver transplant donors (3). In support of the concept of occult HBV infection, HBV was transmitted from anti-HBc-positive donors to HBV-naive recipients at a high frequency via liver grafts in living-donor liver transplantation (LDLT) and orthotopic liver transplantation (4–8). Because of the persistent shortage of organs and the increasing number of patients awaiting transplantation, the use of liver grafts from anti-HBc-positive donors cannot be avoided, especially in areas where the prevalence of HBV is high (4). Thus, adequate prophylaxis against HBV is required to prevent viral reactivation in HBV-naive recipients after liver transplantation.

To date, strategies to prevent viral breakthroughs in the recipients of anti-HBc-positive livers have been empirical, and hepatitis B immunoglobulin (HBIG) has been widely used as the standard prophylaxis after liver transplantation (9). Several reports, including ours, suggested that HBIG prophylaxis was effective for preventing HBV exacerbation in recipients who received hepatic allografts from anti-HBc-positive donors (4,10). However, long-term passive immunization with HBIG is associated with problems, such as high cost, limited availability, and selection of viral strains containing mutations in the surface gene of HBV-DNA (9). Moreover, the difficulty of maintaining serum antibodies to HBsAg (anti-HBs) titer and the poor compliance of HBIG could result in viral reactivation after liver transplantation (7).

The aim of this study was to evaluate the efficacy of short-term administration of lamivudine for the treatment of *de novo* HBV exacerbation in transplant recipients with anti-HBc-positive donors.

Patients and Methods

Patients

From July 1995 to July 2004, 902 patients underwent LDLT at Kyoto University Hospital. Before operations, serological evaluation for HBV markers, including HBsAg, anti-HBs, anti-HBc, hepatitis B e antigen (HBeAg), and antibodies to HBeAg (anti-HBe), was carried out using commercial enzyme immunoassay kits (Dainabot, Tokyo, Japan). HBV-DNA was analyzed using a commercial polymerase chain reaction (PCR) assay (Amplicor HBV Monitor, Roche, Branchburg, NJ, USA). Among 902 donors, 121 (13.4%) were positive for anti-HBc in the absence of HBsAg. Of these, all recipients fulfilling both of the following criteria were included: (a) none of the HBV-related

serological markers positive before transplantation; (b) post-operative survival and well-tolerated prophylaxis with HBIG for longer than 6 months after LDLT. Accordingly, 55 recipients were excluded because they were positive for HBV-serological markers before LDLT. Twenty-six patients were also excluded from the study because of their short duration of survival, and all of these patients died from causes not related to the HBV reactivation. Two patients refused to receive HBIG for financial reasons, and were lost to follow-up. A total of 38 recipients were considered eligible for this study. These HBV-naive recipients with anti-HBc-positive donors underwent LDLT for the following liver diseases: biliary atresia (n = 21), hepatitis C virus (HCV)-related chronic liver disease (n = 3), primary biliary cirrhosis (n = 3), primary sclerosing cholangitis (n = 2), chronic rejection (n = 2), post-LDLT graft failure (n = 2) and others (n = 5). The male/female ratio was 1/1 and the age range was 0–58 years (mean age: 15.9 years, age ≤18 years: n = 28, age >18 years: n = 10). HBV reactivation after LDLT was diagnosed by confirming the appearance of HBsAg in sera of the recipients. Liver tissue and serum samples of all anti-HBc-positive donors were obtained at the time of operation and subjected to analysis for HBV-DNA. All subjects provided written informed consent, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Prophylaxis with HBIG and immunosuppressive protocol

HBIG monotherapy was given to all the recipients with grafts from anti-HBc donors, as reported previously (4). The first dose of HBIG at 200 IU/kg body mass was administered during the anhepatic phase of LDLT, and the same dose was given every day during the first 6 post-operative days. Subsequently, HBV-serological markers were examined at monthly intervals after the transplant operation and 1000 IU of HBIG was periodically administered to maintain serum anti-HBs titers at more than 200 IU/L throughout the follow-up period.

Immunosuppressive therapy for all recipients consisted of tacrolimus and low-dose steroids. Target trough levels of tacrolimus in whole blood were 10 to 15 ng/mL in the first week, and then 5 to 10 ng/mL during the first month after transplantation. Methylprednisolone (10 mg/kg) was administered intravenously (IV) during the anhepatic phase of surgery, followed by 2 mg/kg administered IV for the first 3 days, then tapered to 1 mg/kg for 3 days and converted to 0.3 mg/kg/day of prednisone, which was decreased gradually and discontinued between 3 and 6 months.

PCR amplification of HBV-DNA and sequencing of the surface gene

Preparation of DNA samples and detection of HBV genomes by nested PCR have been described previously (3). The nucleotide sequence spanning the S region was amplified by PCR using specific primer sets, followed by subcloning of PCR products using a pGEM-T Easy Vector System I (Promega, Madison, WI, USA). A total of 15 clones derived from each serum specimen were subjected to sequencing analyses (3).

Results

HBV reactivation despite HBIG prophylaxis

Post-operative HBIG prophylaxis was given to 38 HBV-naive recipients with grafts from anti-HBc-positive donors. Among them, 29 showed no evidence of HBV recurrence during the follow-up period (mean: 41.1 months, range: 10 months to 9.5 years). Unfortunately, in 9 of 38 cases (23.7 %), anti-HBs titer decreased concurrently with the appearance of HBsAg in the serum despite HBIG prophylaxis after LDLT. Table 1 shows the serological characteristics of the donors and pre-transplant status of the recipients who suffered from HBV reactivation despite HBIG administration. Baseline characteristics including age, gender and HBV-related serology were similar between these nine recipients and the remaining recipients without HBV recurrence (data not shown). Consistent with our previous analyses, 31 of the 38 donors with anti-HBc (81.6%) were positive for HBV-DNA in the liver specimens, indicating a high frequency of latent HBV infection in the livers of anti-HBc-positive individuals (3). In contrast, HBV-DNA was negative in the sera of all anti-HBc-positive donors. All allografts of nine recipients with HBsAg appearance were positive for HBV-DNA by PCR analyses, suggesting that the *de novo* HBV reactivation originated from the liver graft with latent HBV infection after LDLT.

To define the factors associated with HBV reactivation in these nine recipients, variables related to the donors and recipients, transplant procedures, and HBIG prophylaxis were analyzed. The indications for LDLT in these recipients with HBV reactivation were biliary atresia (n = 7), Wilson's disease (n = 1) and primary sclerosing cholangitis (n = 1). The mean period between LDLT and *de novo* HBsAg appearance was 35.1 months (range: 12–71 months; Table 2).

Table 1: HBV-serological status of recipients with HBV reactivation post-LDLT and of their corresponding donors with anti-HBc

Recipient				Donor			
Case #	Age/Sex	Indication for LDLT	HBsAg/ Anti-HBs	Anti-HBc	HBsAg/ Anti-HBs	HBeAg/ Anti-HBe	HBV-DNA in liver graft
1	13/F	BA	−/−	+	−/+	−/+	+
2	3/M	BA	−/−	+	−/+	−/−	+
3	9/M	BA	−/−	+	−/+	−/+	+
4	22/F	BA	−/−	+	−/+	−/−	+
5	0/M	BA	−/−	+	−/+	−/+	+
6	16/F	BA	−/−	+	−/+	−/+	+
7	16/M	Wilson	−/−	+	−/−	−/+	+
8	23/F	BA	−/−	+	−/−	−/−	+
9	25/M	PSC	−/−	+	−/−	−/+	+

LDLT = living-donor liver transplantation; HBsAg = hepatitis B surface antigen; anti-HBs = antibody to HBsAg; anti-HBc = antibody to hepatitis B core antigen; HBeAg = hepatitis B e antigen; anti-HBe = antibody to HBeAg; BA = biliary atresia; PSC = primary sclerosing cholangitis.

Table 2: Clinical features of recipients with HBV reactivation

Case #	Anti-HBs titer[1] (mIU/mL)	Duration until HBV reactivation[2] (months)	Clinical features at the time of HBV reactivation		Possible reasons for HBV reactivation
			ALT (IU/L)	Histology	
1	N.D.	21	251	N.D.	Noncompliance
2	N.D.	32	190	CAH	Noncompliance
3	N.D.	16	13	N.D.	Noncompliance
4	23.8	71	699	N.D.	Immunosuppression
5	140.6	15	24	N.D.	Escape mutant
6	117	30	153	CAH	Escape mutant
7	11.7	12	1409	CAH	Unknown
8	N.D.	61	25	CAH	Unknown
9	34.7	58	65	CAH	Unknown

anti-HBs = antibody to HBsAg; ALT = alanine aminotransferase; N.D. = not determined; CAH = chronic active hepatitis; noncompliance = noncompliance of HBIG; escape mutant = emergence of surface escape mutant.
[1]Anti-HBs titer before HBsAg appearance.
[2]Period between liver transplantation and HBsAg appearance.

A liver biopsy was performed on five of the nine patients at the time of the *de novo* HBV recurrence, and all exhibited evidence of chronic active hepatitis accompanied by mild inflammatory activity and mild fibrosis. HBV appearance was attributed to the decrease in serum anti-HBs titer despite HBIG prophylaxis in four of nine recipients. Among them, three recipients (cases #1, #2 and #3) were considered to have suffered from HBV recurrence because of non-compliance of HBIG. Although post-operative HBIG prophylaxis was given to these three patients, they had a transient cessation of HBIG treatment for personal reasons 12, 7 and 11 months after LDLT. They experienced a decrease in anti-HBs titer and, consequently, HBsAg became detectable in the sera after cessation of HBIG treatment. The mean period between the cessation of HBIG treatment and the emergence of HBsAg in their sera was 12.3 months (range, 3–26 months). An immunosuppressive condition was presumed to be associated with viral activation, with consequent decreases of anti-HBs in one case (case #4). Recipient #4 showed an anti-HBs titer of less than 23.8 IU/L 2 weeks after the 2000 IU-HBIG infusion, followed by the appearance of HBsAg. Continuous medication with prednisolone for the treatment of chronic rejection suggested the underlying possible immunocompromised condition in this case.

In the remaining five cases (cases #5, #6, #7, #8 and #9), HBsAg eventually became positive despite the continuous treatment with periodical HBIG prophylaxis. HBV clones comprising mutations in the S gene have been reported in OLT recipients who developed recurrent hepatitis B despite HBIG prophylaxis (11). To ascertain whether the HBIG failure in these cases was associated with changes in antigenicity of the S protein, the S gene sequence of HBV-DNA was determined in the HBV strain of two patients whose sera at the acute phase of HBV exacerbation were available for further analyses (cases #5 and #6). The sequence analyses of the two cases revealed that the detected HBV clone contained several mutations, including G- to -A substitutions at nucleotide 586 (subtype adr) and 587 (subtype adw) within the 'a' determinant region on the HBsAg-encoding gene, suggesting that the cloned HBV variants might be responsible for HBV recurrence despite HBIG administration in these two cases. Unfortunately, we could not determine the factors that were related to HBV reactivation in cases #7, #8 and #9.

Short-term lamivudine treatment

Among recipients with *de novo* HBV recurrence, six (cases #1, #2, #4, #6, #7 and #9) of nine cases had elevated levels of serum alanine aminotransferase, suggesting that recurrent active hepatitis was present. Moreover, of these six cases, all four cases that were examined exhibited histological evidence of inflammation with lymphocytes infiltration around the portal area at the time of *de novo* HBV reactivation (Table 2). Lamivudine (100 mg) was given in six cases to suppress the viral activity (Figure 1). Of them, five patients started the treatment immediately after HBsAg appearance (average: 27 days; range: 1 day to 2 months). One patient (case #1) did not take lamivudine at the time of HBsAg appearance because of personal reasons, but finally received lamivudine therapy 23 months after HBV reactivation.

After the administration of lamivudine, HBsAg decreased in the sera of five out of the six recipients, and in all these five cases (cases #1, #2, #4, #7 and #9), HBsAg disappeared from the sera at a median of 4.6 months (range: 21–330 days) after the beginning of lamivudine treatment. Suppression of HBsAg by lamivudine treatment was invariably associated with a decline in serum transaminase levels in these five cases. After confirming the stable seroconversion to anti-HBs-positive status, lamivudine treatment was stopped in four of the five recipients after 60 months, 10 months, 1.5 months and 4 months (cases #1, #2, #7 and #9). The remaining individual (case #4) is currently receiving lamivudine treatment because she started treatment only 4 months ago; her most recent blood test

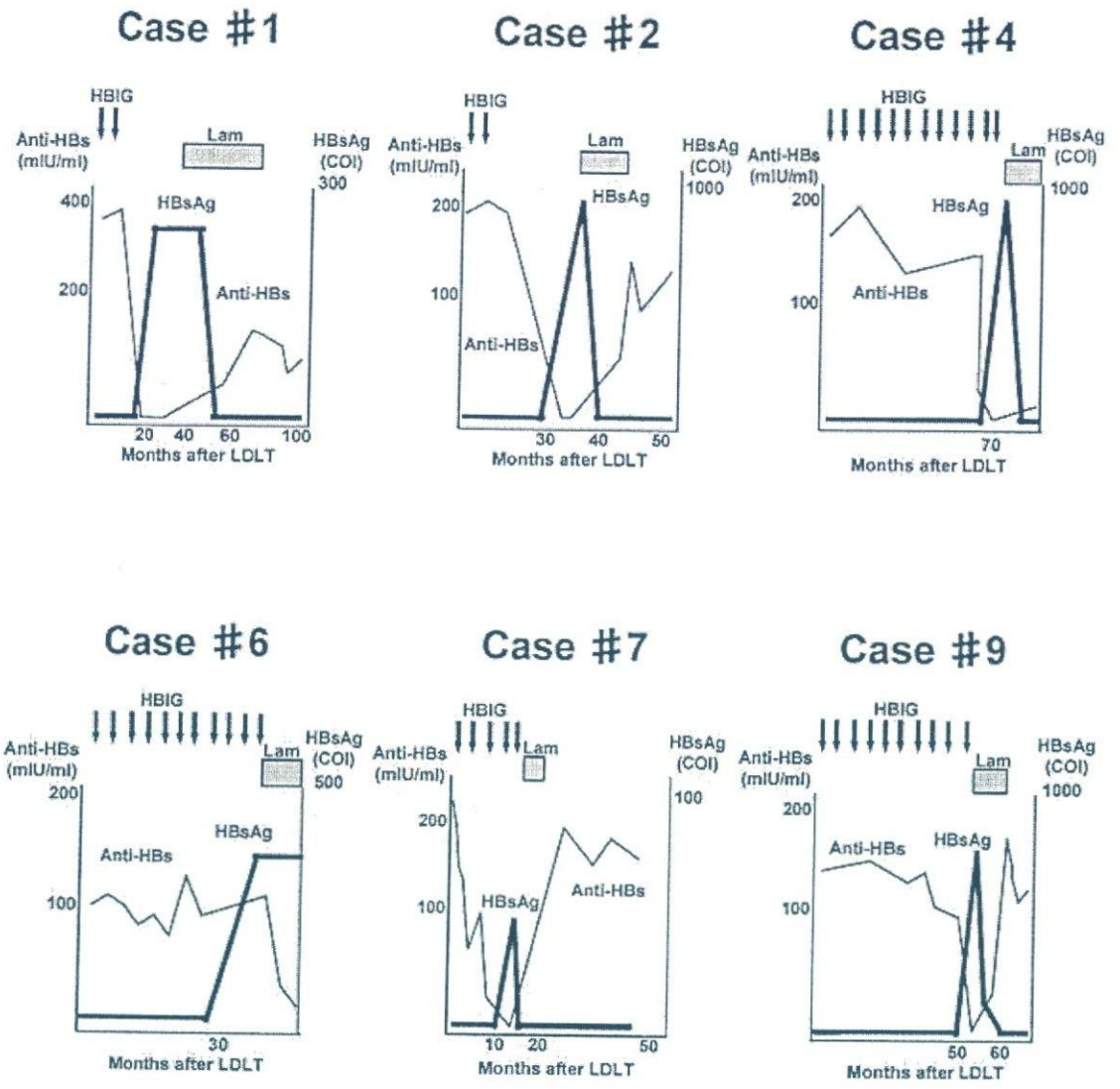

Figure 1: Clinical course of the six liver transplant recipients with HBV reactivation who received lamivudine treatment. The bold line represents HBsAg and the fine line represents anti-HBs. The treatment with HBIG is shown as arrows and treatment with lamivudine as shaded boxes. HBIG = hepatitis B immunoglobulin; Lam = lamivudine.

was negative for HBsAg. The presence of circulating exogenous anti-HBs derived from HBIG complicates detection of the endogenous anti-HBs, which reflect the development of anti-HBV immunity in recipients. Thus, we also gradually withdrew the HBIG prophylaxis after the administration of lamivudine, and confirmed the sustained positivity of anti-HBs without HBIG treatment in two recipients (cases #2 and #7). Since then, they consistently showed evidence of immunity against HBV with endogenous anti-HBs titers greater than 100 IU/L without any prophylaxis. Consequently, HBIG was not given to these two recipients, even after termination of lamivudine treatment. Serum HBsAg of these two recipients remained negative during the follow-up periods of 14 and 35 months after the withdrawal of HBIG and lamivudine. Recipient #7 died of progressive deterioration of liver function 35 months after the complete loss of HBsAg in sera, and the histological autopsy findings were consistent with chronic rejection in the liver graft. The remaining recipient (case #6) is currently being treated with lamivudine monotherapy. None of the recipients developed tyrosine-methionine-aspartate-aspartate (YMDD) mutants during the course of lamivudine therapy (data not shown).

Three patients (recipients #3, #5 and #8) did not receive lamivudine treatment after HBsAg appearance because the alanine aminotransferase levels of these patients showed no evidence of active hepatitis.

Discussion

In this study, we demonstrated that short-term lamivudine therapy for LDLT recipients with *de novo* HBV reactivation

could achieve a significant HBsAg seroconversion rate without inducing drug-resistant YMDD mutants. The important point to note is that after the transient use of lamivudine, some of the recipients who had been naive against HBV infection before transplantation maintained anti-HBs positivity despite withdrawal of HBIG even during immunosuppressive therapy, indicating that they were likely to acquire endogenous immunity to HBV infection.

Passive immunoprophylaxis with HBIG has been used to prevent *de novo* HBV recurrence after liver transplantation in HBV-naive recipients who received liver grafts from anti-HBc-positive donors (8). However, not only the efficacy, but also the safety of long-term HBIG treatment after liver transplantation has not been determined, including the risk of development of hepatitis B 'surface' escape mutants during passive immunoprophylaxis. In this study, we demonstrated that a considerable number of patients developed *de novo* HBV reactivation during the long-term course of HBIG prophylaxis. Our findings suggested that factors affecting the recurrence of *de novo* HBV in those recipients included non-compliance of HBIG, an immunosuppressive condition, and possible emergence of 'surface' escape mutants.

Lamivudine is a potent inhibitor of HBV replication, and several investigators have reported that lamivudine therapy is effective for hepatitis B treatment following liver transplantation (12,13). The present data show that early initiation of lamivudine treatment for post-transplant HBV *de novo* reactivation efficiently induced a sustained loss of HBsAg, suggesting that the effect of lamivudine on *de novo* HBV reactivation is stronger than its effect on chronic HBV infection. In general, HBV replication decreases immediately after administration of lamivudine; however, it has been shown that lamivudine treatment achieves clearance of HBsAg in only a very small proportion of cases. Indeed, loss of HBsAg occurred in only 0–2% of patients with chronic HBV infection that were treated with lamivudine (14). In transplant recipients with HBV-associated liver disease, lamivudine treatment induced the disappearance of HBsAg in only 3 of 52 cases (6%) by week 52, 2 of whom acquired anti-HBs (12). The better results observed in this study can be attributed to the fact that lamivudine treatment was commenced during the acute phase of *de novo* HBV reactivation when the number of HBV was expected to be very small. The beneficial effect of early lamivudine therapy for acute hepatitis has been reported previously (15,16). We suggest that the timing of the lamivudine administration in patients with HBV activation, specifically in the acute phase of HBV reactivation, is important to achieve complete viral suppression and successful seroconversion from HBsAg to anti-HBs.

It is well recognized that prolonged administration of lamivudine can lead to viral breakthrough because of the emergence of viral variants with reduced sensitivity to the drug resulting from one or more mutations in the YMDD locus of the HBV polymerase gene (17). Many investigators have reported a high rate of virological breakthrough with prolonged lamivudine therapy in liver transplant recipients with active HBV infection (17,18). In contrast, we confirmed the beneficial effect of lamivudine on *de novo* HBV reactivation and showed that it does not cause the emergence of YMDD mutants. Reduced risk for virological breakthrough during lamivudine therapy may be attributed to the short-term use of the drug and the low levels of HBV-DNA at the acute phase of *de novo* HBV reactivation, as prolonged use of lamivudine and high HBV-DNA levels before treatment were shown to be associated with the emergence of the drug-resistant mutants (19). Prevention of drug-resistant viral clones by transient use of lamivudine has also been demonstrated in patients with chronic HBV infection (20). Although short-term lamivudine therapy could reduce the incidence of YMDD mutants, the relapse rate was high after the withdrawal of lamivudine in HBV-related chronic liver disease patients (20). Thus, short-term lamivudine treatment might be applicable specifically to the acute phase of *de novo* HBV reactivation.

In conclusion, we have shown in this study that short-term use of lamivudine resulted in complete clearance of HBsAg in the majority of patients with *de novo* HBV reactivation, and that the effects of lamivudine were stronger in patients with *de novo* HBV reactivation than in patients with HBV-related chronic liver disease. More importantly, sustained clearance of HBV was obtained in two cases who stopped both HBIG and lamivudine administration, suggesting acquired immunity against HBV; however, a study of a large number of patients with *de novo* HBV reactivation is needed to reach any definitive conclusion. Thus, further studies with greater patient numbers are required to determine whether short-term lamivudine administration induces clearance of HBsAg in the sera, as well as acquired immunity against HBV in HBV-naive recipients receiving anti-HBc-positive allografts.

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References

1. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest 1994; 93: 230–239.
2. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: Clinically significant or purely "occult"? Hepatology 2001; 34: 194–203.
3. Marusawa H, Uemoto S, Hijikata M et al. Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. Hepatology 2000; 31: 488–495.
4. Uemoto S, Sugiyama K, Marusawa H et al. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in

living related liver transplants. Transplantation 1998; 65: 494–499.
5. Chazouilleres O, Mamish D, Kim M et al. "Occult" hepatitis B virus as source of infection in liver transplant recipients. Lancet 1994; 343: 142–146.
6. Dickson RC, Everhart JE, Lake JR et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. Gastroenterology 1997; 113: 1668–1674.
7. Dodson SF, Bonham CA, Geller DA, Cacciarelli TV, Rakela J, Fung JJ. Prevention of de novo hepatitis B infection in recipients of hepatic allografts from anti-HBc positive donors. Transplantation 1999; 68: 1058–1061.
8. Yen RD, Bonatti H, Mendez J, Aranda-Michel J, Satyanarayana R, Dickson RC. Case report of lamivudine-resistant hepatitis B virus infection post liver transplantation from a hepatitis B core antibody donor. Am J Transplant 2006; 6: 1077–1083.
9. Shouval D, Samuel D. Hepatitis B immune globulin to prevent hepatitis B virus graft reinfection following liver transplantation: A concise review. Hepatology 2000; 32: 1189–1195.
10. Roque-Afonso AM, Feray C, Samuel D et al. Antibodies to hepatitis B surface antigen prevent viral reactivation in recipients of liver grafts from anti-HBC positive donors. Gut 2002; 50: 95–99.
11. Ghany MG, Ayola B, Villamil FG et al. Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. Hepatology 1998; 27: 213–222.
12. Perrillo R, Rakela J, Dienstag J et al. Multicenter study of lamivudine therapy for hepatitis B after liver transplantation. Lamivudine Transplant Group. Hepatology 1999; 29: 1581–1586.
13. Loss GE Jr, Mason AL, Nair S et al. Does lamivudine prophylaxis eradicate persistent HBV DNA from allografts derived from anti-HBc-positive donors? Liver Transpl 2003; 9: 1258–1264.
14. Dienstag JL, Schiff ER, Wright TL et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999; 341: 1256–1263.
15. Andreone P, Caraceni P, Grazi GL et al. Lamivudine treatment for acute hepatitis B after liver transplantation. J Hepatol 1998; 29: 985–989.
16. Reshef R, Sbeit W, Tur-Kaspa R. Lamivudine in the treatment of acute hepatitis B. N Engl J Med 2000; 343: 1123–1124.
17. Bartholomew MM, Jansen RW, Jeffers LJ et al. Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet 1997; 349: 20–22.
18. Mutimer D, Pillay D, Shields P et al. Outcome of lamivudine resistant hepatitis B virus infection in the liver transplant recipient. Gut 2000; 46: 107–113.
19. Lok AS, Lai CL, Leung N et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology 2003; 125: 1714–1722.
20. Song BC, Suh DJ, Lee HC, Chung YH, Lee YS. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 2000; 32: 803–806.

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Case Report

Liver Transplantation from an Identical Twin without Immunosuppression, with Early Recurrence of Hepatitis C

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Hepatitis C virus reinfection after liver transplantation is universal and more severe than in nontransplant patients. Rejection episodes and immunosuppressive agents are considered risk factors for deterioration of recurrent hepatitis C. We report 2 cases of living donor liver transplantation for patients with hepatitis C-related cirrhosis who received right-lobe grafts from an identical twin. Thanks to genetic identity, no immunosuppressive drugs were administered during or after transplantation without rejection. Hepatitis C virus RNA kinetics showed a rapid increase following transplantation and liver biopsies 1 month after transplantation showed acute lobular hepatitis in both cases. Antiviral therapy using interferon α and ribavirin was started immediately, and both cases showed virological and histological response. In conclusion, avoidance of immunosuppression did not delay hepatitis C recurrence following transplantation, while early antiviral therapy without risk of rejection or immunosuppression led to successful viral eradication.

Key words: Hepatitis C, immunosuppression, liver transplantation, living-related liver donors, reinfection

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Introduction

The first successful kidney transplantation between identical twins was performed by Murray in 1954 (1). Solid-organ transplantation between identical twins has been successfully performed with the small intestine (2), kidney, pancreas (3), combined pancreas and kidney (4), parathyroid gland (5) and liver (6,7). This historical success has proved that for solid-organ transplantation from a syngeneic donor, allografts can be transplanted without immunosuppressive therapy. We performed living donor liver transplantation (LDLT) for two patients with hepatitis C virus (HCV)-related cirrhosis, using a right-lobe graft from an identical twin in each case without any immunosuppression.

HCV-related disease is one of the leading indications for liver transplantation worldwide. However, reinfection with HCV is immediate and universal following surgery, and jeopardizes both graft and patient survival (8). The spectrum of allograft injuries related to HCV recurrence ranges from no evidence of injury to graft failure requiring retransplantation in a subset of patients. Several factors have been proposed to explain these variable outcomes, including HCV viral load, genotype, rejection episodes and immunosuppression (9). Immunosuppression accounts for a major part of the accelerated progression. Steroid pulse therapy for rejection reportedly aggravates recurrent HCV (10). Furthermore, intraoperative bolus steroid injection has been shown to increase serum HCV RNA immediately after transplantation (11).

Liver transplantation for HCV is analogous to acute HCV infection, in that a never-infected liver is placed into a viremic host and inevitably becomes infected. Human liver transplantation thus offers a unique opportunity to study viral kinetics and the immunopathogenic mechanisms of acute HCV hepatitis. In the present cases of LDLT between identical twins, the influences of rejection and immunosuppression can also be excluded. We report herein the kinetics of serum HCV RNA levels, clinical course with antiviral therapy and pathological features for acute hepatitis C after LDLT free from both alloimmune response and immunosuppressive therapy.

Case 1

The recipient was a 51-year-old man diagnosed with HCV cirrhosis and multiple hepatocellular carcinoma. Genotype was 1b and HCV RNA level was 160 kIU/mL, while Child-Pugh classification was A. LDLT was performed using a right-lobe graft (graft-to-recipient weight ratio, 1.25%) from his identical twin. No immunosuppressive agent was administered, including intraoperative bolus steroid injection.

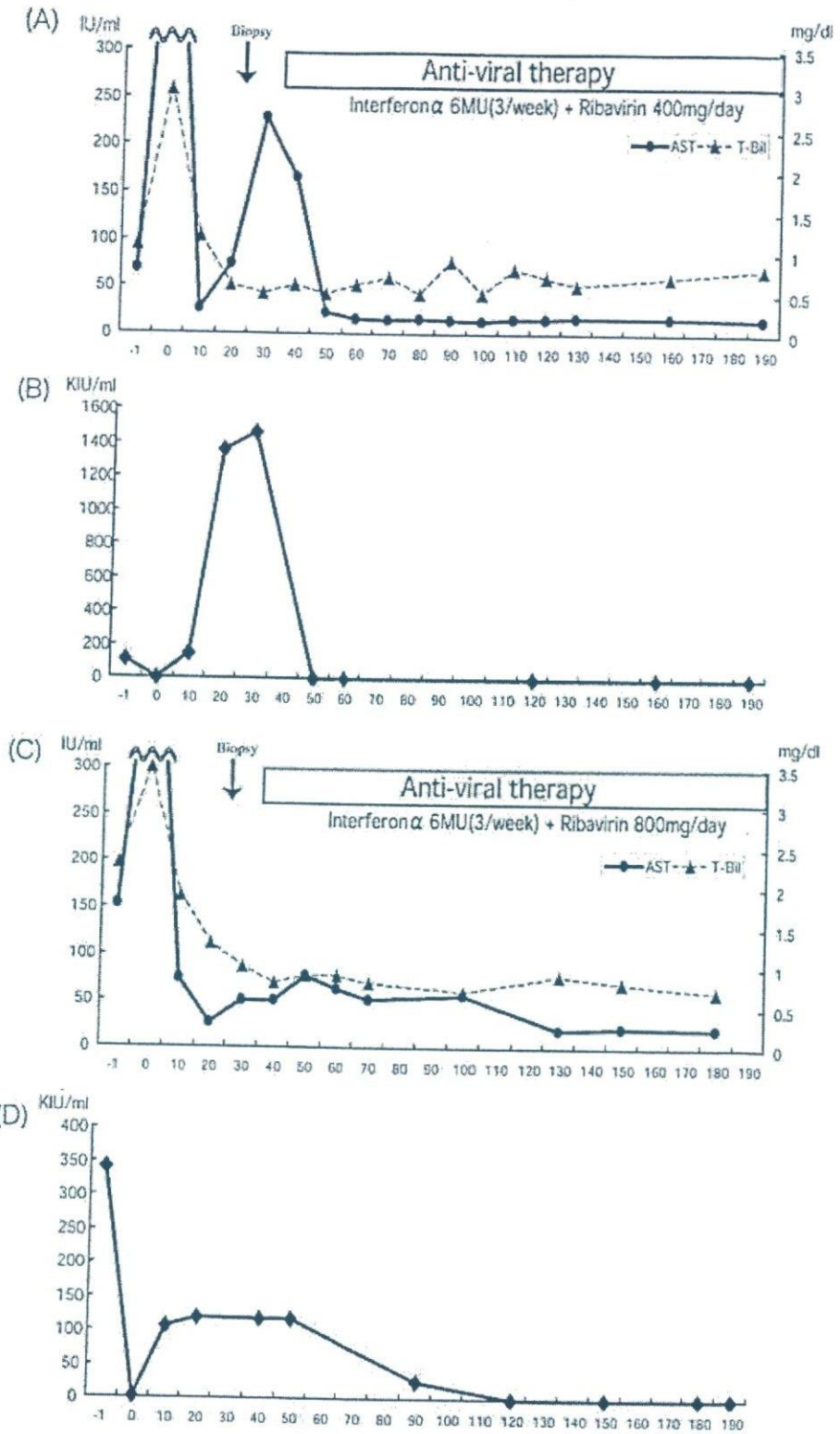

Figure 1: Results of liver function tests, serum HCV RNA level and clinical courses of Cases 1 and 2. (A) AST and total bilirubin (T-Bil) levels in Case 1. AST and T-Bil levels were elevated on POD 1 and decreased to within normal ranges by POD 10. On POD 17, AST elevated again and liver biopsy was performed on POD 24. Combined antiviral therapy was started from POD 31. (B) Kinetics of serum HCV RNA levels in Case 1. After reperfusion, HCV RNA could not be detected from serum. On POD 1, serum HCV RNA became positive and elevated continuously until peaking on POD 21 at 1600 kIU/mL. After antiviral therapy, serum RNA level decreased rapidly to be undetectable within 2 weeks. (C) AST and T-Bil levels in Case 2. AST and T-Bil elevated on POD 1 and gradually decreased. T-Bil gradually decreased to normal range after POD 20, but AST level remained abnormal until 3 months after antiviral therapy. T-Bil and AST have since remained within normal range. (D) Kinetics of serum HCV RNA level in Case 2. After reperfusion, serum HCV RNA became negative and continued until POD 7. On POD 10, serum HCV RNA level became positive and elevated to a peak of 100 kIU/mL on POD 27. After antiviral therapy started, serum HCV RNA gradually decreased and became negative on POD 120.

AST level was elevated to 1000 IU/mL on postoperative day (POD) 1, but subsequently improved before again elevating after POD 17 (Figure 1A). HCV RNA became negative after reperfusion, then turned positive on POD 1 and elevated continuously to a peak of 1600 kIU/mL on POD 21 (Figure 1B). Liver biopsy on POD 24 demonstrated the following findings: mild portal inflammation with infiltration of lymphocytes and eosinophils, but without bile duct

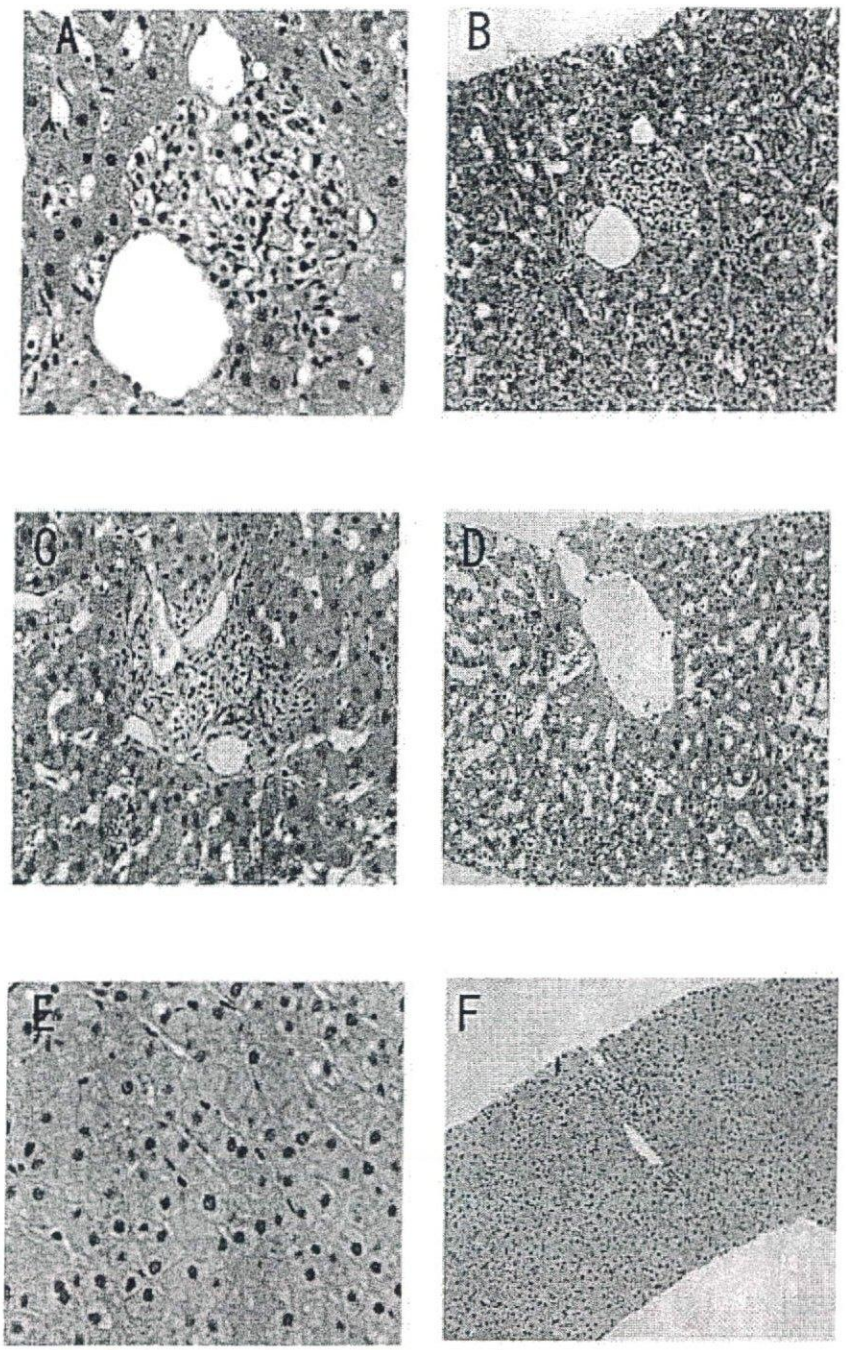

Figure 2: Histopathological findings from liver posttransplant biopsy. (A, B) Liver biopsy was obtained on POD 24 in Case 1 after LFTs elevated. (A) Mild portal inflammation with lymphocytes and eosinophils and no bile duct damage or endothelialitis. (B) Hepatocyte degeneration, steatosis and sinusoidal dilatation. (C, D) Liver biopsy was obtained on POD 27 in Case 2 after LFTs elevated. These biopsies show similar findings to Case 1. (E, F) Liver biopsy in Case 1 was obtained 7 months after transplantation, when HCV RNA was not detected in peripheral blood. These biopsies showed no signs of hepatitis.

damage or endothelialitis; lobular inflammation with acidophilic bodies; hepatocyte degeneration and steatosis and sinusoidal dilatation (Figure 2A, B). With a histological diagnosis of lobular hepatitis (A1, F0, according to METAVIR score (12)), antiviral therapy was started using interferon α-2b (6 MU 3 times/week) and ribavirin (400 mg/day) from POD 31. Ribavirin dose was reduced because the patient was anemic (Hb, 9.4 g/dL). HCV RNA turned negative within 2 weeks after starting treatment. Treatment was continued for 48 weeks, and sustained virological response has been maintained for >1 year after the cessation of therapy with good liver function. Liver biopsy results as of 8 months after LDLT showed no sign of hepatitis (Figure 2E, F).

Case 2

The recipient was a 38-year-old man diagnosed with HCV cirrhosis (Child-Pugh grade B, genotype 1b, HCV-RNA 340 kIU/mL). He had been treated using interferon

monotherapy for HCV-related chronic hepatitis at 20-years old, but this was ineffective and liver disease progressed to cirrhosis. The patient underwent LDLT using a right-lobe graft (graft-to-recipient weight ratio, 1.28%) from his identical twin without any immunosuppression. Serum HCV RNA rapidly decreased to undetectable levels after reperfusion and remained negative until POD 7. On POD 10, HCV RNA became positive and elevated to a peak of 100 kIU/mL on POD 25 (Figure 1D). Liver biopsy on POD 27 showed lobular hepatitis (A1, F0) with similar findings to those in Case 1 (Figure 2C, D). Combined antiviral therapy comprising interferon α-2b (6 MU 3 times/week), and ribavirin (800 mg/day) was administered from POD 34. Biochemical and virological responses were attained. After 12 weeks of therapy, HCV RNA was 0.57 kIU/mL. By 7 months after LDLT, the patient showed normal results on liver function test (LFTs) and HCV RNA was not detectable in serum. Liver biopsy 7 months after LDLT showed no signs of hepatitis. The patient remains on this treatment.

Discussion

These cases revealed three key findings. First, increased serum HCV RNA levels and histological findings of acute lobular hepatitis accompanied by mild elevation of LFT results were observed soon after LDLT despite the absence of immunosuppression. This absence of immunosuppression was expected to delay or alleviate HCV reinfection, if immunosuppression represents one of major factors in the progression of HCV recurrence. However, recurrence of hepatitis C proved inevitable after liver transplantation for HCV-infected patients, even without immunosuppression. A possible explanation for the early reinfection and progression of acute hepatitis was the genetic identity between donor and recipient. As HCV demonstrates cell tropism (13), a grafted liver from an identical twin would be more easily infected than an allograft. However, whether sharing the same HLA loci represents a risk factor for recurrence of HCV remains controversial. Another explanation is the graft type used in these cases. Right-lobe grafts inevitably undergo regeneration immediately after transplantation, and hepatocyte proliferation promotes viral replication (14). Whether hepatitis C recurrence occurs earlier and with greater severity for LDLT than for deceased donor liver transplantation has recently become a subject of debate (15,16).

The second key finding was that in the present 2 cases, liver biopsy results 1 month after LDLT showed similar features of acute lobular hepatitis, even though viral kinetics differed. Generally, early histopathological features of recurrent hepatitis C may be modified by immunosuppressive therapy and can be difficult to differentiate from acute rejection. The presence of eosinophils in portal inflammatory infiltrate is reportedly a significant variable associated with acute rejection (17,18). In the present cases where the possibility of acute rejection could be excluded, eosinophils were seen in areas of portal inflammation in both patients. Interestingly, this suggests that eosinophil infiltration is not necessarily specific to acute rejection, but also appears in early acute hepatitis. Sinusoidal dilatation, which is reported as the only specific feature of recurrent hepatitis C (17), was identified in both cases.

The third key finding was that, though sustained virological response could not be assessed in Case 2, antiviral therapy was effective in both cases. Antiviral therapy can reportedly stimulate immune responses and may in turn increase the risk of allograft rejection (19). However, in the present cases, treatment could be started without risk of rejection as soon as recurrent hepatitis was confirmed. Early treatment of acute hepatitis C with interferon is reportedly more effective compared to that for chronic infection (20). In terms of early introduction of antiviral therapy and remaining free from immunosuppression, the rapid response of these 2 patients with genotype 1b viruses may display some analogy to acute hepatitis C in nontransplant recipients.

In conclusion, we have reported 2 cases of LDLT between identical twins for HCV cirrhosis without any immunosuppressive drugs. Despite avoidance of immunosuppression, rapid increases in serum HCV RNA levels and histological recurrence of HCV by 1 month after LDLT were observed. However, antiviral therapy for acute hepatitis yielded good responses in both cases.

References

1. Tilney NL. Renal transplantation between identical twins: A review. World J Surg 1986; 10: 381–388.
2. Morris JA, Johnson DL, Rimmer JA et al. Identical-twin small-bowel transplant for desmoid tumour. Lancet 1995; 345: 1577–1578.
3. Sutherland DE, Gores PF, Farney AC et al. Evolution of kidney, pancreas, and islet transplantation for patients with diabetes at the University of Minnesota. Am J Surg 1993; 166: 456–491.
4. Benedetti E, Dunn T, Massad MG et al. Successful living related simultaneous pancreas-kidney transplant between identical twins. Transplantation 1999; 67: 915–918.
5. Segerberg EC, Grubb WG, Henderson AE. The first successful parathyroid transplant from an identical twin for the cure of permanent postoperative hypoparathyroidism. Surgery 1992; 111: 357–358.
6. Sugawara Y, Ohtsuka H, Kaneko J, Ohkubo T, Imamura H, Makuuchi M. Successful treatment of hepatitis C virus after liver transplantation from an identical twin. Transplantation 2002; 73: 1850–1851.
7. Liu LU, Schiano TD, Min AD et al. Syngeneic living-donor liver transplantation without the use of immunosuppression. Gastroenterology 2002; 123: 1341–1345.
8. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002; 122: 889–896.
9. Wiesner RH, Sorrell M, Villamil F. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. Liver Transpl 2003; 9: S1–S9.

10. Gane EJ, Naoumov NV, Qian KP et al. A longitudinal analysis of hepatitis C virus replication following liver transplantation. Gastroenterology 1996; 110: 167–177.
11. Garcia-Retortillo M, Forns X, Feliu A et al. Hepatitis C virus kinetics during and immediately after liver transplantation. Hepatology 2002; 35: 680–687.
12. Bedossa P, Poynard T, for the METAVIR Cooperative Study Group. An algorithm for the grading of activity in chronic hepatitis C. Hepatology 1996; 24: 289–293.
13. Ikeda M, Kato N, Mizutani T, Sugiyama K, Tanaka K, Shimotohno K. Analysis of the cell tropism of HCV by using in vitro HCV-infected human lymphocytes and hepatocytes. J Hepatol 1997; 27: 445–454.
14. Zimmerman MA, Trotter JF. Living donor liver transplantation in patients with hepatitis C. Liver Transpl 2003; 9: S52–S57.
15. Garcia-Retortillo M, Forns X, Llovet JM et al. Hepatitis C recurrence is more severe after living donor compared to cadaveric liver transplantation. Hepatology 2004; 40: 699–707.
16. Takada Y, Haga H, Ito T et al. Clinical outcomes of living donor liver transplantation for HCV-positive patients. Transplantation 2006; 81: 350–354.
17. Petrovic LM, Villamil FG, Vierling JM, Makowka L, Geller SA. Comparison of histopathology in acute allograft rejection and recurrent hepatitis C infection after liver transplantation. Liver Transpl Surg 1997; 3: 398–406.
18. Nagral A, Ben-Ari Z, Dhillon AP, Burroughs AK. Eosinophils in acute cellular rejection in liver allografts. Liver Transpl Surg 1998; 4: 355–362.
19. Brown RS. Hepatitis C and liver transplantation. Nature 2005; 436: 973–978.
20. Jaeckel E, Cornberg M, Wedemeyer H et al. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001; 345: 1452–1457.

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ORIGINAL ARTICLE

Auxiliary Partial Orthotopic Living Donor Liver Transplantation for a Child with Congenital Absence of the Portal Vein

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Congenital absence of the portal vein (CAPV) is a rare malformation of the mesenteric vasculature in which visceral venous blood bypasses the liver, completely draining into the systemic circulation through a congenital porto-systemic shunt. Liver transplantation has rarely been indicated for patients with this disease. We present a child with CAPV who was managed successfully by living donor auxiliary partial orthotopic liver transplantation (APOLT), while preserving the right lobe of the native liver. In conclusion, APOLT for patients with CAPV is a feasible and ideal procedure because portal vein (PV) diversion is not necessary. *Liver Transpl 12:845-849, 2006.* © 2006 AASLD.

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Congenital absence of the portal vein (CAPV) and extrahepatic porto-caval shunt, also referred to as an Abernethy type 1 shunt, is a rare malformation of the mesenteric vasculature in which splanchnic blood bypasses the liver through a congenital shunt vessel, completely draining into the systemic circulation, such as the inferior vena cava and renal vein. It can cause a broad spectrum of clinical manifestations, including cardiac anomaly,[1] hypergalactocemia,[2] focal nodular hyperplasia of the liver,[3-6] hepatopulmonary syndrome,[7] and hepatic encephalopathy, which can result in poor prognosis.

Liver transplantation has rarely been indicated for patients with CAPV, with only a few cases having been reported sporadically.[8-11] Herein we report a child with CAPV who was managed successfully by living donor auxiliary partial orthotopic liver transplantation (APOLT). Furthermore, indication for liver transplantation in patients with CAPV and the technical feasibility of APOLT is discussed.

CASE REPORT

A 2-yr-old Japanese boy was referred to us as a liver transplant candidate. He tested positive for neonatal screening of galactosemia during neonatal mass screening, and CAPV was diagnosed by cardiography, which was performed when he was 1 yr old. He presented with mild mental retardation and persistent hyperammonemia. Imaging studies, including an abdominal computed tomography scan and abdominal angiography, revealed absence of the portal vein (PV) and a large extrahepatic porto-systemic shunt draining into the inferior vena cava. The superior mesenteric vein and splenic vein joined to form a common trunk, which directly entered the left renal vein (Fig. 1). Cardiography revealed neither cardiac abnormality nor pulmonary hypertension. Hyperintensity in the globus pallidus was observed in brain magnetic resonance imaging. No intrahepatic PV structure was found on liver biopsy. Pretransplant blood tests showed mild liver

Abbreviations: CAPV, congenital absence of the portal vein; APOLT, auxiliary partial orthotopic liver transplantation; LDLT, living donor liver transplantation; PV, portal vein.

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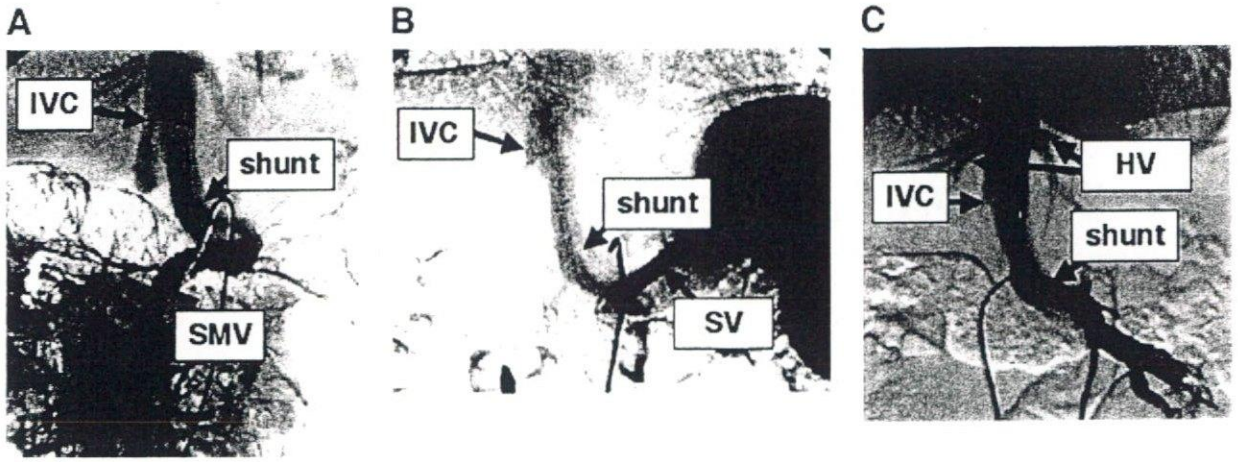

Figure 1. The venous phase of superior mesenteric (A) and splenic (B) arteriography revealed that both the superior mesenteric vein (SMV) and splenic vein (SV) drained into the left renal vein. This was confirmed by left renal venography (C). HV, hepatic vein; LRV, left renal vein; IVC, inferior vena cava.

dysfunction and elevation of ammonia level: total bilirubin, 0.9 mg/dL; aspartate aminotransferase, 49 IU/L; alanine aminotransferase, 18 IU/L; total bile acid, 40.8 μmol/L; ammonia level, 181 μg/dL; and prothrombin time (international normalized ratio), 50% (1.46). Living donor liver transplantation (LDLT) was indicated for the persistent hyperammonemia, hypergalactosemia, and mental retardation, as well as for the expected poor prognosis.

APOLT using a left lateral segment graft donated from his 28-yr-old mother was performed with preservation of his native right lobe. The left lateral segment graft was procured in the usual manner. Configuration of the donor PV, hepatic artery, hepatic vein, and bile duct was normal. The graft weighed 220 gm, which accounted for 1.52% of the graft-to-recipient weight ratio. The inferior mesenteric vein of the donor, 6 cm in length, was procured as a vascular graft and preserved in heparinized saline. In the recipient, the native liver looked normal in color and consistency; however, the right lobe of the liver was relatively small and atrophic compared to the left lobe. A large porto-caval shunt, approximately 2.0 cm in diameter, was identified dorsal to the hepatoduodenal ligament. A Kocher's maneuver and dissection around the shunt vessel revealed that the shunt vessel drained directly into the inferior vena cava. Cholecystectomy was performed and a catheter (4 Fr) was inserted through the cystic duct for later cholangiography or a leak test. The left hepatic artery was dissected and freed. The left hepatic duct was identified, minimally dissected, and cut distal to the bifurcation. As expected, there was no PV structure encountered in the hepatic hilum. The middle hepatic vein was confirmed by intraoperative ultrasonography. Parenchymal transection of the liver was performed along the right side of the middle hepatic vein utilizing the Cavitron Ultrasonic Surgical Aspirator (CUSA System 200; Valleylab, Boulder, CO) and electrocautery without any vascular inflow interruption on both sides of the liver. The middle hepatic vein was not preserved together

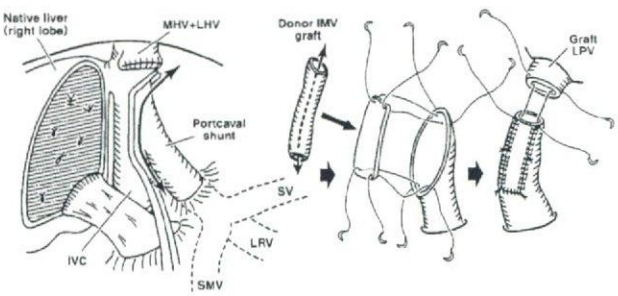

Figure 2. Schematic demonstration of portal reconstruction. Patch venoplasty using a donor inferior mesenteric vein graft was performed under total clamp of the shunt vessel. SMV, superior mesenteric vein; SV, splenic vein; LRV, left renal vein; IMV, inferior mesenteric vein; IVC, inferior vena cava.

with the remnant liver because middle and left hepatic vein confluence was required for later venous reconstruction. After completion of the parenchymal transection, the porto-caval shunt vessel was dissected and exposed. The inferior vena cava was partially clamped with a Satinsky clamp and the shunt vessel was detached from the inferior vena cava (Fig. 2). The inferior mesenteric vein graft of the donor was transected axially to make a patch graft, which was anastomosed to the shunt using 7-0 PDS-II continuous sutures (Johnson & Johnson K.K., Tokyo, Japan) to enlarge the caliber of the shunt. Implantation of the lateral segment graft was performed in the usual manner. The left hepatic vein of the donor was anastomosed to the middle and left hepatic vein confluence of the recipient using the triangulation method with 6-0 PDS continuous sutures. The shunt was then anastomosed to the graft PV in an end-to-end fashion using 7-0 PDS continuous sutures. Reperfusion of the liver was prompt and smooth. The total clamp time of the shunt vessel was 93 minutes, and the graft warm ischemic time was 40 minutes. The graft left hepatic artery was anastomosed to the recipient left hepatic artery using interrupted 8-0