one should be used for reconstruction, and an adequate arterial flow to the nonanastomosed arterial branches should be confirmed using the following criteria. First, during the completion of a donor hepatectomy, when smaller branches of the hepatic artery are cut, pulsatile back-bleeding is observed. Second, on the bench, when perfusion fluid is flushed though the largest artery, it should be observed to flow out from the smaller arterial branches[69] Third, arterial flow can be confirmed during the recipient's operation following the reconstruction of the largest hepatic artery by the presence of pulsatile back-bleeding from the stump of the other graft's arteries. Finally, the hepatic arterial signal can be checked by Doppler ultrasonography of each segment of the liver graft.

BILIARY RECONSTRUCTION

The current standard for biliary reconstruction in whole cadaveric liver transplantation is a duct-to-duct choledochocholedochostomy. The preferred technique in adult LDLT is currently shifting from a hepaticojejunostomy to duct-to-duct anastomosis.

Duct-to-duct biliary reconstruction

Duct-to-duct biliary reconstruction has been presented in some institutions.[70-72] These reports advocate the advantages of duct-to-duct biliary reconstruction over a hepaticojejunostomy, i.e., the procedure might preserve physiologic bilioenteric and bowel continuity, thus preventing a delayed bowel movement. Duct-to-duct reconstruction allows for easy endoscopic access to the biliary tree for diagnostic and therapeutic instrumentation and management, and it prevents ascending cholangitis.

The rationale for using a hepaticojejunostomy in LDLT is based on the small size of the recipient's bile duct and the inadequate length of the donor's bile duct. Although size and length are not restriction factors for adult patients, as they are for pediatric patients, an underlying liver disease (e.g., biliary atresia) often mandates the use of a hepaticojejunostomy. Since 2000, we have used duct-to-duct anastomosis in patients without diseases involving the bile duct, such as biliary atresia or primary sclerosing cholangitis. However, long-term postoperative observations and technical modification are still necessary,[70,73] to determine the success rate.

Devices used during the operation

The rate of biliary complications after LDLT is approximately 40%, suggesting that biliary reconstruction remains a technically demanding and challenging problem in LDLT.[70,74,75] An intraoperative cholangiography is essential for visualizing biliary anatomy and anomalies (i.e., a right lateral sector bile duct originating from the left bile duct), and identifying the precise site of division.[29,76] To avoid narrowing of the common bile duct of the donor, there should be no attempt to obtain a single duct orifice in the graft. It is very important to maintain an adequate blood supply from the hepatic arteries and gastroduodenal artery to the bile duct.[77] Thus, meticulous and sharp dissection of the recipient's bile duct, preserving as much surrounding tissue as possible,[70] is indispensable for the safety of duct-to-duct reconstruction.

The existence of multiple bile duct orifices on the graft side is common. Intermittent suturing or tying them off during the donor operation, or on the bench, may be performed.[78] To identify the orifice of the bile duct to each hepatic segment, a surgical probe can be inserted individually into each bile duct under the guidance of ultrasonography.[79]

Postoperative complications

A surgical revision of bile duct stenosis is technically demanding if the endoscopic approach is not possible or unsuccessful. In repairs using a T-tube, an intraoperative cholangiography should be used for appropriate localization, which allows for sufficient bile juice drainage. Converting duct-to-duct anastomosis to a hepaticojejunostomy is another option.[70]

The raw surface of the liver graft or biliary anastomosis is a common site of bile leakage, which can result in fluid collection or an abscess. Careful ligation of all bile ducts on the raw

surface and placing a closed suction drain along the cut surface of the liver graft is important.

Scatton[80] reported an increase in the biliary complication rate in a T-tube group. In LDLT, a T-tube helps to decompress the bile duct, but it will not prevent stenosis at the anastomotic site. Additionally, there are often multiple duct orifices in the graft and a size difference between the common bile duct and the duct orifice in the graft, so it is difficult to put the tip of a T-tube across the anastomotic site. A transanastomotic external tube can theoretically help decrease the intrahepatic biliary pressure caused by edema and the consequent partial obstruction of the anastomosis.[81] The transanastomotic external tube will also facilitate a postoperative imaging study. The advantage over not stenting, however, has not been established.[70]

UNIVERSITY OF TOKYO EXPERIENCE

Between 1996 and September 2003, 167 adult patients underwent LDLT at the Tokyo University Hospital. Donor candidates consisted of 71 children, 34 siblings, 23 parents, 23 spouses, 11 who composed of aunts, uncles, nieces and nephews and 5 others. Their ages ranged from 20 to 65. The LDLT donors underwent a left hepatectomy (n=16), a left liver with caudate lobectomy (n=59), and a right hepatectomy (n=76), a right lateral sector (n=16). The actual graft weight ranged from 289g to 924g (median 534g), and its ratio to the recipient's SLV ranged from 31% to 88% at the time of the transplantation. All the LDLT donors recovered well and returned to normal lives with a mean hospital stay of 15 days (11-56 days). Only minor donor complications occurred. Seven donors (3%) underwent a reoperation with good results. The indications for the reoperation were bile leakage in six donors and peritonitis in one. None of the donors required a banked-blood transfusion.

As for the recipients, three patients required re-transplantation and there were nine early deaths. Primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis were the most common indications (33%), followed by hepatocelluar carcinoma with hepatitis (24%). The most common complication was acute rejection (45%), followed by biliary complications (25%). Patient and graft survival were 91% and 90%, respectively. Several considerations and techniques for performing LDLT and the experiences at Tokyo University were reviewed. The results confirmed that LDLT was satisfactory in adult patients with 3-year accumulated survival rates of 90%, and it can be performed with a low incidence of complications.

CONCLUSION

Taking into account the worldwide shortage of cadaveric organ donations, LDLT offers hope to patients with end-stage liver disease and its use will become increasingly more important. This procedure should be performed by an expert surgical team only after careful consideration of donor safety and recipient outcome. The long-term success of LDLT requires careful, thoughtful application of this procedure, as well as accumulated technical improvements in the field of hepatobiliary surgery.

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Defective development of splenic and epidermal CD4$^+$ dendritic cells in mice deficient for IFN regulatory factor-2

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Communicated by Tadatsugu Taniguchi, University of Tokyo, Tokyo, Japan, January 27, 2004 (received for review December 22, 2003)

Dendritic cells (DCs) play important roles in the initiation and regulation of immune responses. Although several subsets of DCs were identified according to their expression of surface molecules such as CD4, CD8, and CD11b, the regulatory mechanism for the development and homeostasis of these DC subsets remains unclear. Here we show that mice lacking IFN regulatory factor-2 (IRF-2$^{-/-}$ mice) exhibited a marked and selective defect in splenic CD4$^+$CD11b$^+$DCs, instead of CD8α^+CD11b$^-$DCs that were reported to be missing in mice lacking the related transcription factor IRF-8. Furthermore, the numbers of epidermal Langerhans cells in IRF-2$^{-/-}$ mice were reduced at least in part because of the lack of the CD4$^+$CD11b$^+$ subset. Studies with radiation bone marrow chimeras as well as *in vitro* retrovirus-mediated gene transduction showed that IRF-2 was required cell-autonomously for the development of myeloid-related DCs. Notably, these abnormalities in DCs diminished in mice lacking both IRF-2 and the IFN-α/β receptor, indicating that IRF-2 acted through negatively regulating IFN-α/β signals. In contrast, natural killer cells still showed developmental arrest in these double mutant mice, indicating that the mode of action of IRF-2 for CD4$^+$DC development is distinct from that for natural killer cell development. Our current findings thus pointed to a previously unknown unique cell-type-selective multimode function of IRF-2 in the regulation of lymphohematopoiesis.

Dendritic cells (DCs) play pivotal roles not only in the initiation but also in the determination of the direction, toward either type 1 or 2, of T cell-mediated immune responses against infection (1). In response to pathogens, DCs undergo differentiation from immature to mature DCs that act as principal antigen-presenting cells (APCs) in secondary lymphoid organs. Maturation of DCs is induced not only by microbial products acting through Toll-like receptors (2) but also by cytokines produced on infection with pathogens (1). Among such cytokines are type I IFNs (IFN-α/β) produced by cells infected with viruses and in response to microbial products such as lipopolysaccharide (LPS). However, there are other reports showing that IFN-α/β act suppressively on the differentiation of DCs (3, 4). There might therefore be yet-unrecognized regulatory mechanisms operating to control the negative and positive effects of IFN-α/β on the differentiation and functions of DCs.

Murine splenic DCs have been classified into three major subsets based on their surface expression of CD4 and CD8α molecules (5, 6). Although these cells were originally thought to represent distinct cell lineages, reports demonstrating that all three subsets can be generated from either common myeloid or lymphoid progenitors cast skepticism on this lineage hypothesis (7). Nevertheless, there appear to be differences, albeit not necessarily absolute, among these subsets in terms of immunological function such as production of IL-12, crosspriming of CD8$^+$ T cells, and maintenance of self tolerance as well as anatomical localization within lymphoid organs (8, 9). In addition to these three major DC subsets, a rare DC subset with plasmacytoid characteristics has been identified recently (10, 11).

Given the potential importance of DC subset differentiation in the regulation of immune responses, it is critical to understand the molecular nature of the factors regulating murine DC subset differentiation. Studies using gene-disrupted mice have started to shed light on the mechanism of DC subset regulation. Recently, among them, two groups have shown that mice lacking IFN consensus sequence-binding protein (ICSBP), also called IFN regulatory factor (IRF)-8, exhibited a defect in CD8α^+, plasmacytoid DCs, and epidermal Langerhans cells (LCs) (12–14). As we have shown previously, another member of the IRF family, IRF-2, attenuates signals evoked by spontaneously produced IFN-α/β, thereby preventing a CD8$^+$ T cell-mediated skin inflammation (15, 16). The function of IRF-2 might not be confined to those as a transcriptional repressor, and direct gene activation was also known to be induced by IRF-2 for several genes, such as those encoding vascular cell adhesion molecule-1 and gp91phox (17, 18). Moreover, IRF-2 was shown to be required for natural killer (NK) cell development (19). In terms of DC biology, IRF-2 is of great interest, because it was reported that IRF-2 and ICSBP/IRF-8 not only formed complexes but also acted cooperatively, for instance, in the expression of the *IL-12p40* gene (20). Here we examined the roles of IRF-2 in the development and functions of DCs using mice lacking this transcription factor (IRF-2$^{-/-}$ mice, ref. 21). Contrary to the case in IRF-8$^{-/-}$ mice, we found that IRF-2$^{-/-}$ mice exhibited a selective cell autonomous deficiency in the CD4$^+$ DC subset, including splenic CD4$^+$CD11b$^+$ DCs and epidermal CD4$^+$ LCs. Inactivation of the IFN-α/β receptor restored the development of both CD4$^+$ DCs and epidermal LCs, but not NK cells, in IRF-2$^{-/-}$ mice. Thus, IRF-2 is a unique regulator of lymphohematopoiesis, acting differently in CD4$^+$ DCs and NK cells in terms of its relationship to IFN-α/β signals.

Materials and Methods

Mice. IRF-2$^{-/-}$ mice kindly provided by Tak W. Mak (University of Toronto, Toronto) (21) were backcrossed 6 or 10 times to C57BL/6 (BN6 and BN10, respectively). These two lines of backcrossed mice gave identical results, and we did not discriminate these two series of mice in this work. IFNAR1$^{-/-}$ mice were purchased from B&K Universal (Hull, U.K.) and backcrossed 10 times to C57BL/6. IRF-2$^{-/-}$IFNAR1$^{-/-}$ double mutant mice were generated by crossing IRF-2$^{-/-}$BN10 and IFNAR1$^{-/-}$BN10 mice. IRF-2$^{-/-}$H-2^d mice were established by intercrossing F_1 progenies of IRF-2$^{-/-}$BN10 × B10.D2 (SLC,

Abbreviations: DC, dendritic cells; LC, Langerhans cells; IRF, IFN regulatory factor; BM, bone marrow; LPS, lipopolysaccharide; APC, antigen-presenting cell; NK, natural killer.

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Shizuoka, Japan) breeding. DO11.10 transgenic (tg) B10.D2 mice were established by backcrossing original DO11.10 tg BALB/c mice at least five times to B10.D2 mice. B6-Ly5.1 mice were purchased from Sankyo (Tsukuba, Japan). All mice were maintained under specific pathogen-free conditions and used at 8–12 wk of age. All experiments were performed according to institutional guidelines.

Antibodies and Reagents. Fluorochrome- and biotin-conjugated mAbs and streptavidins used in this study (FITC-anti-CD11c, FITC-anti-CD43, APC-anti-CD11b, APC-anti-NK1.1, APC-anti-CD8α, APC-anti-B220/CD45R, PE-anti-CD4, PE-anti-CD11b, PE-anti-I-A^b, PE-anti-CD86, PE-Cy7-anti-CD11b, biotin-anti-CD40, biotin-anti-Ly5.1, biotin-anti-CD19, and PerCP-streptavidin) were purchased from BD Pharmingen except for FITC-anti-Ly5.2, which was from e-Bioscience (San Diego). Biotin-labeled antibodies were developed with APC-streptavidin. OptEIA kits for measuring mouse IL-6 and IL-12p40 were from BD Bioscience.

Bone Marrow (BM) Chimeras. Radiation BM chimeras were established by transferring 5–10 × 10^6 red cell-depleted BM cells i.v. via the tail vein into 8- to 10-wk-old B6-Ly5.1 mice that had been irradiated by 9.0 Gy and analyzed 8–10 weeks later.

BM-Derived and Splenic DCs. BM cells were cultured *in vitro* in the presence of granulocyte–macrophage colony-stimulating factor (R & D Systems) for 8 days (22). Recovered cells were analyzed directly or purified by using FITC-anti-CD11c antibody and anti-FITC microbeads with MACS columns (Miltenyi Biotec, Auburn, CA). Purified cell preparations contained constantly >90% $CD11c^+$ cells. These cells were stimulated with 1 μg/ml LPS (*Escherichia coli* O55, Wako Biochemicals, Osaka) or with 1 μM CpG DNA (TCCATGACGTTCCTGATGCTT, completely phosphorothioate-modified; Qiagen, Valencia, CA) for 24 h. The supernatants and cells were collected for ELISA assays and for analyses of the expression of surface markers, respectively. Magnetically purified $CD4^+$ T cells from DO11.10 transgenic B10.D2 mice (1 × 10^5) were cultured with graded numbers of BM-DCs established from IRF-$2^{-/-}$H-2^d mice and control littermates in the presence of an OVA peptide as described (23), and T cell proliferation was measured by using CellTiter 96 Aqueous One kit (Promega). Splenic $CD11c^+$ cells were prepared by digesting with collagenase D (2.5 mg/ml, Roche Diagnostics).

Retroviral Transduction of IRF-2 cDNA. Mouse IRF-2 cDNA was amplified and cloned into the *Bam*HI-*Xho*I site of pMX-IRES-EGFP (a kind gift from T. Kitamura, University of Tokyo; ref. 24). The recombinant vector was transfected into a packaging cell phoenix (a kind gift from G. P. Nolan, Stanford University, Stanford, CA). On days 2, 3, and 4 of the granulocyte–macrophage colony-stimulating factor-assisted BM culture, BM cells were incubated in the virus-containing medium in the presence of 8 μg/ml polybrene (Sigma–Aldrich) under centrifugation (300 × *g*) for 2 h at 32°C. CD11c expression of the cells was analyzed on day 7.

Epidermal Sheets and LCs. Low-density single-cell suspension from epidermal layers was prepared as described (25). Briefly, ears were divided into dorsal and ventral halves with forceps. These halves were trypsinized and then split into epidermal and dermal layers. Single-cell suspension from epidermal layers was prepared by mechanical disaggregation through a stainless steel strainer. Low-density cells were collected by centrifugation by using 30% BSA solution. Epidermal cells were permeabilized by using the cell permeabilization kit FIX & PERM (Caltag, South San Francisco, CA) for intracellular staining because surface CD4 molecules were removed by trypsinization. Epidermal sheets were obtained as described (26), fixed by acetone, and subjected to immunohistochemistry.

Flow Cytometry. Cells were stained with fluorochrome-conjugated antibodies and analyzed by using Cytomics FC500 (Beckman Coulter) for the analyses of NK cells and a FACSCalibur cytometer (BD Bioscience) for others. Data analyses were performed by using RXP analysis software (Beckman Coulter) or CELLQUEST software (BD Biosciences). Dead cells were gated out by propidium iodide staining.

Results

Severe Reduction in $CD4^+CD11b^+$ DCs in IRF-$2^{-/-}$ Mice. Flow cytometry showed that the frequencies of $CD11c^{high}$ cells in IRF-$2^{-/-}$ mice were slightly lower than were those in control littermates (Fig. 1*A*; 29.0 ± 1.1 × 10^5 vs. 17.8 ± 9.6 × 10^5 per spleen for control and IRF-$2^{-/-}$ mice, respectively). It was also found that $CD4^+CD11b^+$ DCs, referred thereafter as $CD4^+$ DCs, were reduced in IRF-$2^{-/-}$ mice, whereas the percentage of $CD8\alpha^+CD11b^-$ DCs ($CD8^+$ DCs) were increased (Fig. 1 *B* and *C*). Accordingly, the numbers of $CD4^+$ and $CD8^+$ DCs per spleen were 12.6 ± 1.8 × 10^5 and 8.4 ± 0.5 × 10^5, respectively, for control and 2.4 ± 0.7 × 10^5 and 11.0 ± 7.7 × 10^5, respectively, for IRF-$2^{-/-}$ mice. The frequencies of $CD4^-CD8\alpha^-$ DCs were not dramatically altered in these mice compared with control littermates. These results suggested that $CD4^+$ DC development was defective in IRF-$2^{-/-}$ mice. The frequencies of yet another type of DCs, plasmacytoid DCs, defined as $CD11c^{dull}CD11b^-B220^+$ in the spleen, were not altered significantly in IRF-$2^{-/-}$ mice (E.I., unpublished data).

Next, cells bearing MHC class II (I-A) in epidermal sheets prepared from the ears were enumerated. As depicted in Fig. 2 *A* and *B*, the densities of epidermal $I\text{-}A^+$ cells representing LCs in IRF-$2^{-/-}$ mice were lower than those in control littermates. Epidermal cells recovered from IRF-$2^{-/-}$ mice contained consistently fewer numbers of $CD11b^+I\text{-}A^+CD11c^+$ cells representing LCs than those from control mice (Fig. 2*C*). Notably LCs positive for cytoplasmic CD4 ($cCD4^+$ LCs) were almost completely missing in IRF-$2^{-/-}$ mice (Fig. 2*C Lower*). Thus, the reduction of the density of $I\text{-}A^+$ epidermal cells was largely due to the absence of $cCD4^+$ LCs, although the impairment of $CD4^-CD8^-$ LCs might also have contributed to the reduction.

Defective $CD4^+$ DC Generation from IRF-2-Deficient BM Cells *in Vivo*. Irradiated B6-Ly5.1 mice were reconstituted with BM cells from Ly5.2-expressing IRF-$2^{-/-}$ mice (IRF-2 chimeras) or control littermates (control chimeras). In these chimeras, >99% of splenic $CD11c^{high}$ cells were of donor origin, because they expressed surface Ly5.2 (E.I., unpublished data) but not Ly5.1 markers (Fig. 3*A*). The frequencies of $CD11c^{high}$ cells within the spleens in IRF-$2^{-/-}$ chimeras were regularly approximately one-third of those in control chimeras (15.2 ± 3.0 × 10^5 and 5.9 ± 1.0 × 10^5 per spleen for control and IRF-2 chimeras, respectively). In control chimeras, $CD4^+$ DCs occupied ≈60% of total splenic $CD11c^{high}$ cells, whereas in IRF-$2^{-/-}$ chimeras, this population was dramatically shrunk (Fig. 3 *B* and *C*). When the frequencies of these DC subsets in total spleen cells were compared between these two types of BM chimeras, a remarkable reduction of $CD4^+$ DC frequencies was apparent (Fig. 3*D*). Consistently, the numbers of $CD4^+$ and $CD8^+$ DCs were 9.0 ± 2.1 × 10^5 and 1.2 ± 0.3 × 10^5 per spleen, respectively, for control and 1.1 ± 0.3 × 10^5 and 3.3 ± 0.4 × 10^5 per spleen, respectively, for IRF-2 chimeras. Because chimeras generated by transferring wild-type BM cells into irradiated IRF-$2^{-/-}$RAG-$1^{-/-}$ mice showed splenic DC subsets indistinguishable from those in RAG-$1^{-/-}$ mice received wild-type BM cells (Fig. 6, which is published as supporting information on the PNAS web site), it

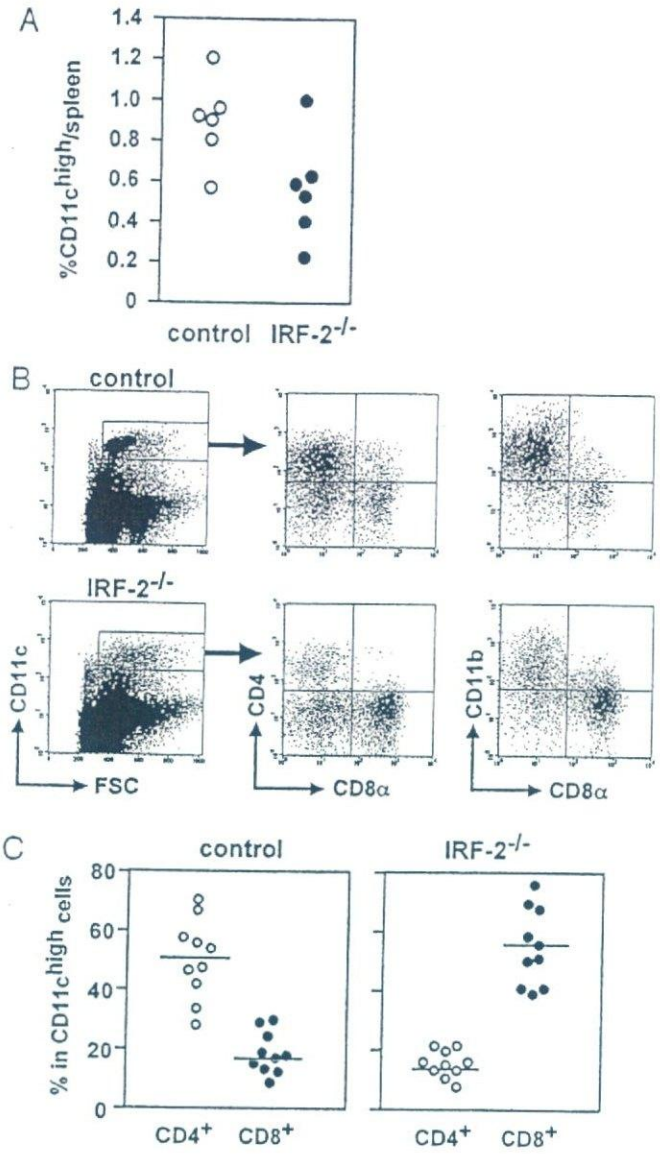

Fig. 1. Impaired splenic CD4$^+$ DC subpopulation in IRF-2$^{-/-}$ mice. (*A*) The percentages of CD11c^{high} cells in viable spleen cells, where filled circles represent control littermates and open circles represent IRF-2$^{-/-}$ mice, respectively. (*B*) CD11c^{high} cells gated as indicated were analyzed for CD4, CD8α, and CD11b expression (*C*). The frequencies of CD4$^+$ and CD8α$^+$ DC subsets within total CD11c^{high} cells were calculated. In *C*, filled circles denote CD4$^+$CD11c^{high} cells and open circles denote CD8α$^+$CD11c^{high} cells. Each dot represents the value obtained from an individual animal (*A* and *C*).

is clear that the nonhematopoietic environment did not play any role in CD4$^+$ DC development. These results indicated that IRF-2 deficiency affected selectively, if not exclusively, the potential of BM cells to develop into CD4$^+$ DCs, because of the defect intrinsic to BM progenitors.

Reduced Frequencies of the Generation of Mature DCs from IRF-2-Deficient BM *in Vitro*. BM cells isolated from IRF-2$^{-/-}$ mice and control littermates were cultured *in vitro* in the presence of granulocyte–macrophage colony-stimulating factor, a standard protocol to generate myeloid-related CD11b$^+$ DCs (22). Total cell numbers recovered from the cultures of IRF-2-deficient BM cells ranged from 50% to 100% of those from control cultures. We found that IRF-2-deficient BM cells gave rise to CD11c$^+$CD11b$^+$ DCs (BM-DCs) less efficiently than did control

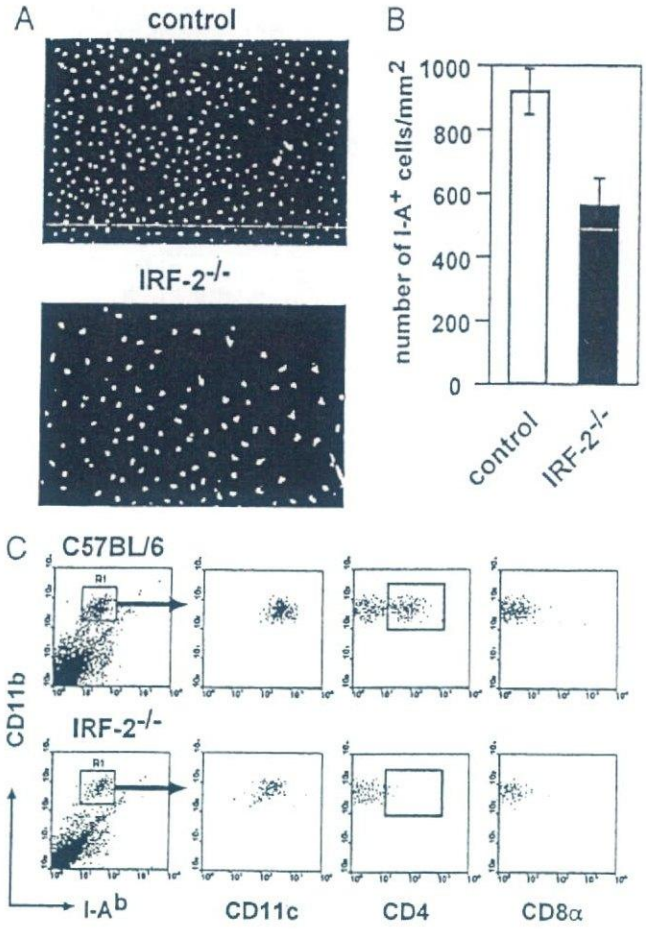

Fig. 2. Lack of CD4$^+$ LCs in the epidermis of IRF-2$^{-/-}$ mice. Epidermal sheets were stained for I-A (*A*), and the numbers of I-A$^+$ cells were counted (*B*). I-A$^+$CD11b$^+$ cells isolated from epidermis of control littermates (*Upper*) and IRF-2$^{-/-}$ mice (*Lower*) were gated as indicated and analyzed for the indicated cell surface markers and cytoplasmic CD4 (*C*).

BM cells (Fig. 4*A*). Moreover, the BM-DCs generated from IRF-2-deficient BM cells contained less CD86$^+$, CD40$^+$, and I-A$^+$ relatively mature DCs than control BM-DCs (Fig. 4*A*). These observations, together with another result in which retrovirus-mediated transduction of the IRF-2 cDNA restored the development of CD11c$^+$ cells from IRF-2-deficient BM cells (Fig. 4*B*), confirmed the notion that the developmental potential to myeloid-related DCs was impaired in IRF-2-deficient BM cells in a cell autonomous manner.

We stimulated magnetically purified BM-DCs with LPS or with unmethylated CpG DNA. As depicted in Fig. 4*C*, the amounts of IL-6 and IL-12p40 produced 24 h later did not differ between IRF-2-deficient and control BM-DCs. In contrast, although the up-regulation of CD86 and CD40 expression was observed on the vast majority of control BM-DCs on stimulation with LPS (Fig. 4*D*) and CpG (E.I., unpublished data), substantial fractions of IRF-2-deficient BM-DCs stayed to become CD86^{dull} and CD40^{dull} (Fig. 4*D* and E.I., unpublished data). In addition, antigen presentation to CD4$^+$ T cells by unstimulated IRF-2-deficient BM-DCs was less potent than that by control BM-DCs (Fig. 4*E*). Thus, IRF-2 seemed to be required not only for the generation but also, albeit partially, for the efficient functional maturation of DCs *in vitro*.

Roles of IFN Signals in Impaired Lymphohematopoietic Development in IRF-2$^{-/-}$ Mice. We next asked whether the attenuator function of IRF-2 on IFN-α/β signals contributed to the efficient devel-

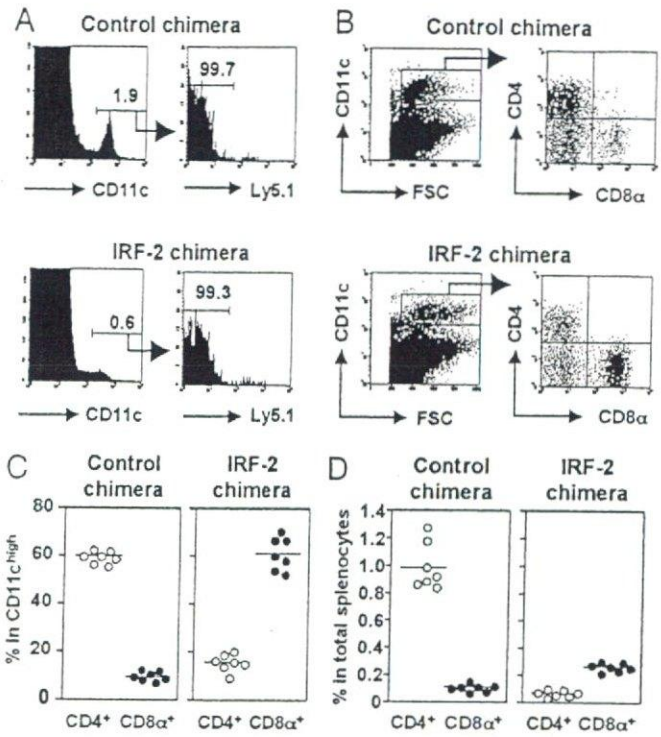

Fig. 3. Cell autonomous developmental defects in IRF-2-deficient BM cells. (*A*) In BM chimeras reconstituted with control BM cells (control chimera) or with IRF-2-deficient BM cells (IRF-2 chimera), >99% of splenic CD11c^{high} cells gated as indicated were negative for Ly5.1. (*B*) CD4 vs. CD8α profiles for CD11c^{high} cells are shown. (*C* and *D*) The percentages of either CD4$^+$ or CD8$^+$ DCs within CD11c^{high} cells (*C*) or total spleen cells (*D*) were plotted for control and IRF-2 chimeras. Vertical bars represent the means of the data obtained with seven chimeras generated in three independent transfers.

opment of splenic CD4$^+$ DCs, by generating mice concomitantly deficient for IRF-2 and the IFN-α/β receptor (IRF-2$^{-/-}$-IFNAR1$^{-/-}$ mice). Notably, the frequencies of CD4$^+$ DCs within splenic CD11c^{high} cells were restored in IRF-2$^{-/-}$-IFNAR1$^{-/-}$ mice to levels comparable to, if slightly lower than, those in control littermates (Fig. 5 *A* and *B*). In addition, the numbers of I-A$^+$ cells in the epidermis were restored in IRF-2$^{-/-}$IFNAR1$^{-/-}$ mice to levels seen in control littermates and IFNAR1$^{-/-}$ mice (Fig. 5*C*). These results together indicated that the function of IRF-2 relevant to the development of splenic CD4$^+$ DCs and epidermal LCs was to attenuate IFN-α/β signals.

We next asked whether the mechanisms by which IRF-2 regulates the development of CD4$^+$ DCs and NK cells were the same. As has been proposed recently, NK1.1$^+$ cells in the BM acquire CD11b and CD43 sequentially as they differentiate into mature NK cells (27). As can be seen in Fig. 5*D*, NK1.1$^+$CD3$^-$ cells in the BM of IRF-2$^{-/-}$ mice contained severely reduced numbers of CD11b^{high} and CD43$^+$ cells that were present abundantly in control littermates. This developmental arrest appeared to be due to IRF-2 deficiency within BM cells, because BM chimeras receiving IRF-2-deficient BM cells still showed identical developmental arrest (S.T., unpublished observation). Importantly, the developmental arrest of NK cells in the BM was not restored at all in IRF-2$^{-/-}$IFNAR1$^{-/-}$ mice (Fig. 5*D*), a contrasting situation to that observed for CD4$^+$ DCs (Fig. 5 *A* and *B*). Thus, although IRF-2 is required for the development of both CD4$^+$ DCs and NK cells, its mode of action seems to be different in these two cell types.

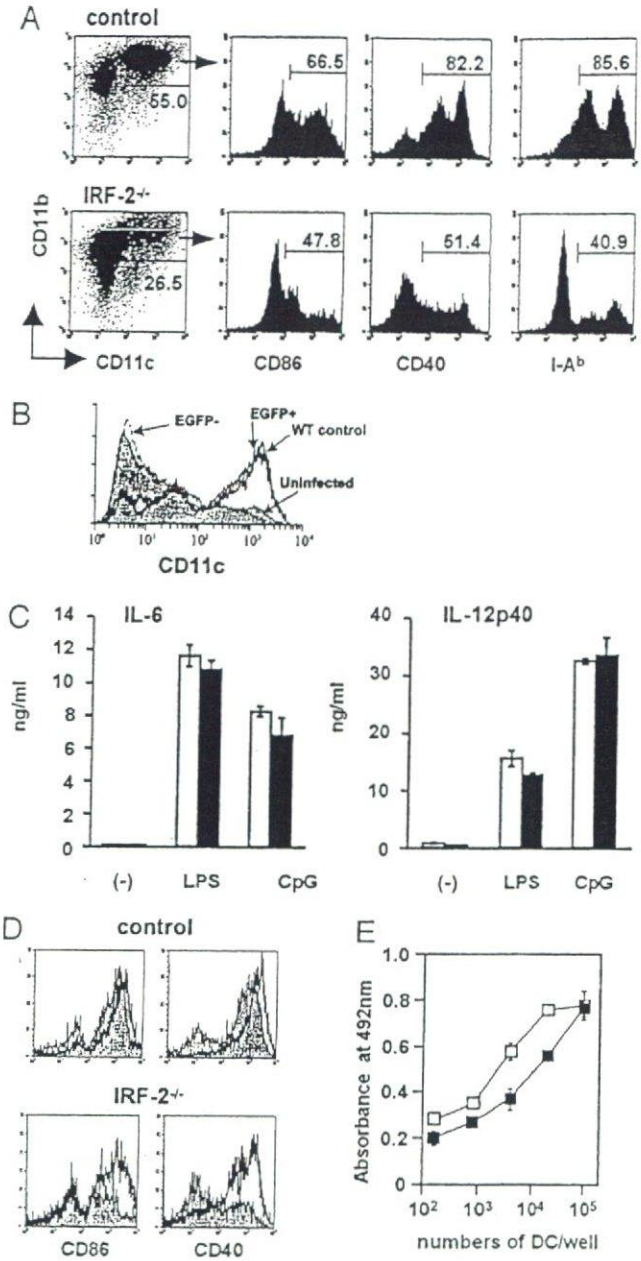

Fig. 4. Inefficient generation of mature DCs from IRF-2-deficient BM cells *in vitro*. (*A*) BM-DCs generated *in vitro* were stained for CD11c and CD11b, together with one of three activation markers, as indicated. Numbers indicate the percentages of cells within the gates (a representative result of more than five independent experiments). (*B*) BM cells were transduced with an expression vector for IRF-2 and enhanced GFP (EGFP) and cultured *in vitro* as above. The histograms "WT control," "uninfected," "EGFP+," and "EGFP−" represent control BM-DCs, uninfected IRF-2-deficient BM-DCs, IRF-2-deficient BM-DCs expressing EGFP-IRF-2, and those that failed to express EGFP-IRF-2, respectively. (*C*) The amounts of IL-6 and IL-12p40 produced by BM-DCs in response to medium alone (−), LPS, or CpG were measured. Open and filled columns represent the means and SD of triplicate cultures of control and IRF-2-deficient BM-DCs, respectively. (*D*) BM-DCs stimulated with LPS as in *C* were examined for CD86 and CD40. Shaded histograms were for BM-DCs cultured in medium alone and bold lines for those stimulated with LPS. Note that the CD86^{dull} or CD40^{dull} populations are remaining in IRF-2-deficient BM-DCs even after stimulation. (*E*) T cell proliferation induced by graded numbers of control (open squares) or IRF-2-deficient (filled squares) BM-DCs. Symbols and error bars represent the means and the SD of duplicate cultures, respectively. Where not seen, error bars were within the symbols.

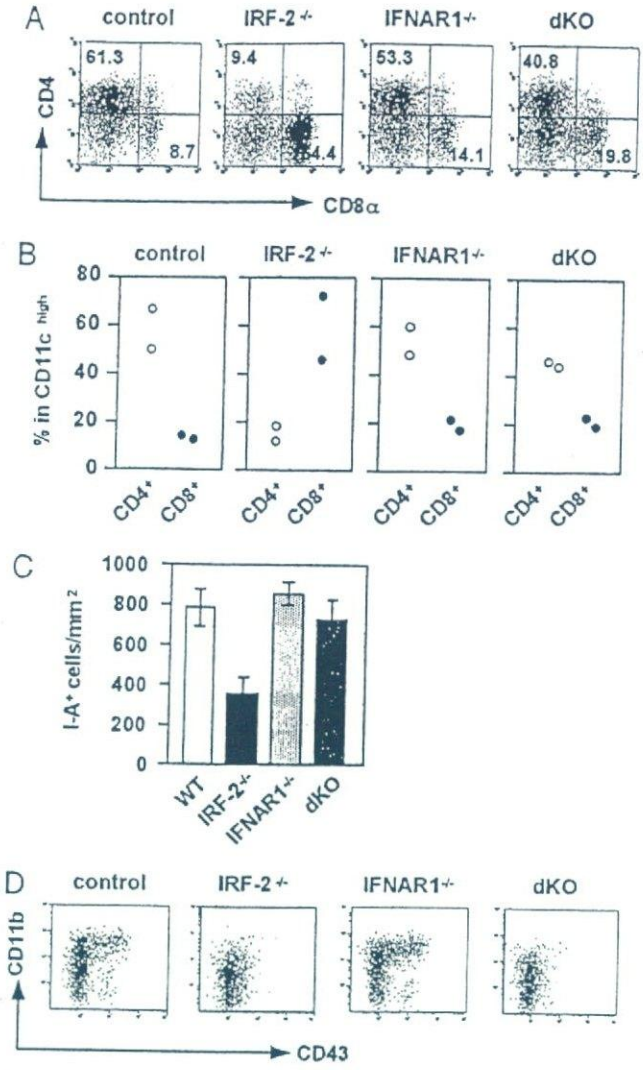

Fig. 5. Roles of IFN-α/β signals in the development of CD4$^+$ DCs and NK cells. (*A* and *B*) Splenic CD11c^{high} cells from IRF-2$^{-/-}$, IFNAR1$^{-/-}$, IRF-2$^{-/-}$IFNAR1$^{-/-}$ (dKO) mice and control littermates were analyzed for CD4$^+$ and CD8$^+$ DC subsets. Numbers indicate the percentages of cells within each quadrant (*A*). (*C*) The epidermal sheets of control (open), IRF-2$^{-/-}$ (filled), IFNAR1$^{-/-}$ (dotted), and dKO (hatched) mice were analyzed for I-A$^+$ cells. Data are shown as the mean numbers of I-A$^+$ cells per mm^2 with the SD. (*D*) BM cells isolated from the indicated mice were stained for NK1.1, CD3, CD43, and CD11b. Dot plots are shown for CD11b and CD43 on viable NK lineage cells (CD3$^-$NK1.1$^+$) (representative of three independent analyses).

Discussion

We showed here that IRF-2$^{-/-}$ mice exhibited a selective cell-autonomous defect in splenic CD4$^+$ DC subset in a fashion that depends on the intact IFN-α/β signaling pathway. Because we did not observe any increase of DC numbers in several other lymphoid organs in IRF-2$^{-/-}$ mice including s.c. and mesenteric lymph nodes and the BM (unpublished observations), we consider that IRF-2 is critical to the development rather than the migration of CD4$^+$ DCs to the spleen. The numbers of CD8$^+$ DCs seemed to be slightly increased in IRF-2$^{-/-}$ mice and IRF-2 chimeras. Although we cannot exclude the possibility that the reduction of the CD4$^+$ DC population vacated the space in the spleen, thereby allowing the expansion of CD8$^+$ DCs, these observations may implicate a relatively minor function of IRF-2 acting negatively in CD8$^+$ DC development. In accordance with the positive role of IRF-2 in DC development *in vivo*, we observed that the generation of CD11c$^+$CD11b$^+$ DCs from IRF-2-deficent BM cells was also impaired in a cell-autonomous manner. Although BM-DCs hardly express CD4, and their relationship to splenic CD4$^+$ DCs was not clear, both of these DCs are CD11b$^+$ and are regarded as "myeloid-related" DCs. We envisage therefore that IRF-2 is required commonly for the efficient development of "myeloid-related" but not "lymphoid-related" DCs.

That IRF-8 is required for the development of lymphoid-related CD8$^+$ DCs instead of CD4$^+$ DCs (12–14), together with our current findings, indicates that distinct DC subpopulations use different IRF family transcription factors for their development. Notably, we also observed a nearly complete lack of cCD4$^+$ epidermal LCs in IRF-2$^{-/-}$ mice (Fig. 2). This observation, together with the simultaneous restoration of both CD4$^+$ splenic DCs and epidermal LCs in IRF-2$^{-/-}$IFNAR1$^{-/-}$ mice (Fig. 5), suggests the close relationship between these two types of DCs. It has recently been reported that IRF-8$^{-/-}$ mice showed a reduction by ≈50% in the numbers of epidermal LCs (28). Although epidermal LCs did not contain a CD8$^+$ subset (Fig. 2*C*), the remaining epidermal LCs in IRF-8$^{-/-}$ mice could be a sister population of splenic CD8$^+$, rather than CD4$^+$, DCs.

Currently, it is not clear how IRF-2 supports CD4$^+$ DC development selectively. One may argue that IRF-2 is selectively expressed in CD4$^+$ DCs by analogy with the observation that IRF-8 expression was restricted to CD8$^+$ DCs (14). However, because IFN-α/β receptors are thought to be expressed ubiquitously, such a simple selective expression model does not explain why CD8$^+$ but not CD4$^+$ DCs could tolerate the up-regulated IFN-α/β signals in IRF-2$^{-/-}$ mice (15), which appear to play a negative role in the development of CD4$^+$ DCs (Fig. 5). It is possible that CD4$^+$ and CD8$^+$ DCs may be different substantially in IFN-α/β-related signaling machinery, including IRF-2 expression. Understanding the mechanism for the cell type specificity of IRF-2 would thus provide a deeper insight into the regulation of DC subset differentiation.

Our current findings, together with the previous observations that RelB, Ikaros C, PU.1, and TRAF6 were required selectively for the development of "myeloid-related" DCs (29–33), implicated crosstalks between IRF-2/IFN-α/β signals and the pathways involving these signaling/transcriptional regulators. In this regard, an interesting report appeared recently in which RelB$^{-/-}$ mice developed an atopic dermatitis-like skin lesion that resembled the IFN-α/β-dependent skin inflammation in IRF-2$^{-/-}$ mice (15); both lesions developed in a T cell-dependent manner and showed several common pathogenic alterations such as thickening of the epidermis, keratinocyte proliferation at the basement membrane, and hair loss (34). On the other hand, NK cells in RelB$^{-/-}$ mice developed normally (35), and IRF-2$^{-/-}$IFNAR1$^{-/-}$ as well as IRF-2$^{-/-}$ mice exhibited an arrest in NK cell development (Fig. 5*D*), indicating that the role of IRF-2 in NK cell development was independent of IFN-α/β and RelB pathways. IRF-2 is thus a unique gene regulator that functions with distinct mechanisms in different cell types by attenuating IFN-α/β signals in CD4$^+$ DC development on the one hand and perhaps by directly activating a gene(s) promoting NK cell development on the other. Curiously, Id2$^{-/-}$ mice that lacked CD8$^+$ DCs were also reported to be defective in NK cell development (36). This raised an intriguing possibility that IRF-2 interacted with distinct transcription factors in CD4$^+$ DCs and NK cells.

Contrary to previous findings that IFN-α/β had adjuvant effects on immune responses likely by activating DCs (37, 38), we observed a defective maturation of DCs in IRF-2$^{-/-}$ mice despite the up-regulated IFN-α/β signals (Fig. 5 and ref. 15). Our observation agrees rather with a report showing that

IFN-α/β have an inhibitory effect on human DC maturation (3, 4). A speculation would hence be that developing DCs, likely myeloid-related subsets, might be sensitive to IFN-α/β at a certain stage(s) of maturation where IRF-2 normally protects them from maturation arrest as far as the amounts of IFN-α/β not exceeding the limit of its control. In this regard, IRF-2 is a regulator critical for efficient immune responses against pathogens by repressing the harmful effects of IFN-α/β on DC development and allows these cytokines to exert beneficial effects. Importantly, however, the defect in $CD4^+$ DCs was associated not with immunoinsufficiencies but with an autoimmune-like cutaneous inflammation (15). Evidence has accumulated recently for the suppressive activities of DCs (39), and our current and previous (15) findings together raise an interesting possibility that $CD4^+$ DCs suppressed $CD8^+$ T cell-mediated immune responses.

We thank Drs. Tak W. Mak, Toshio Kitamura, and Garry P. Nolan for IRF-$2^{-/-}$ mice, pMX-IRES-GFP vector, and phoenix cells, respectively. Ms. Namiko Azuta's technical assistance and Dr. Tsutomu Katsuyama's permission for access to the FACS facility at Shinshu University Hospital are also acknowledged. This work has been supported in part by Grants-in-Aid for Scientific Research on Priority Area 15019035 (to S.T.) and 13140202 (to K.I.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; Grants-in-Aid 15390159 (to S.T.) and 14370075 (to K.I.) from the Japan Science Promotion Society; and grants from the Novartis Foundation (Japan) and the Yamanouchi Foundation.

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Feasibility of Auxiliary Partial Orthotopic Liver Transplantation from Living Donors for Patients with Adult-Onset Type II Citrullinemia

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More than 20 patients with adult-onset type II citrullinemia have undergone liver transplantation, showing dramatic therapeutic effects. In Japan, living donor liver transplantation is the standard technique of liver transplantation because of the rare availability of cadaveric donors. The feasibility of auxiliary partial orthotopic liver transplantation (APOLT) for adult-onset type II citrullinemia to overcome the problem of a small-for-size graft in living donor liver transplantation has not been defined. We recently performed APOLT for patients with type II citrullinemia. Here, we present 2 patients: patient 1 was a 32-year-old man and patient 2 was a 43-year-old woman. Both patients suffered from hepatic encephalopathy, and laboratory data showed highly elevated plasma levels of ammonia and citrulline. In patient 1, the liver graft was obtained from a patient with familial amyloid polyneuropathy as a domino liver transplant. In patient 2, APOLT was performed after graft donation from her husband. The postoperative clinical courses of both patients were uneventful, and the neurological symptoms were completely resolved. The plasma concentrations of ammonia and citrulline normalized rapidly in both patients. APOLT can provide an adequate hepatocyte mass to correct the underlying enzyme deficiency in adult patients with type II citrullinemia. In addition, APOLT can be carried out safely to overcome the limitation of graft volume in living donor liver transplantation. (*Liver Transpl 2004;10:550–554.*)

Citrullinemia is a rare hereditary metabolic disorder characterized by highly elevated plasma levels of citrulline and ammonia, and is ascribed to a deficiency of argininosuccinate synthetase in the liver.[1] This disorder can be classified into 3 types: neonatal/infantile (types I and III) and adult (type II).[2] Most of the patients with type II citrullinemia have been reported in Japan, and the causative gene of this disorder, citrin, was recently identified.[3,4] In the past, most patients were treated with medication and died of severe brain edema within a few years after disease onset, but during the last decade a small number of patients have undergone liver transplantation,[5–8] showing dramatic therapeutic effects. In Japan, since the cadaveric donor pool is quite limited, living donor liver transplantation has become the standard alternative technique.[8] However, living donor liver transplantation requires a candidate living donor among the patient's family with sufficient liver graft volume.

We report the cases of 2 patients with type II citrullinemia who were treated successfully with auxiliary partial orthotopic liver transplantation (APOLT). This procedure was selected based on preoperative volumetric analysis of the liver graft, which did not reach 40% of the recipient's standard liver volume. The first patient underwent APOLT using a graft obtained from a patient with familial amyloid polyneuropathy (FAP) as a domino liver transplant. This is the first description of APOLT for patients with type II citrullinemia.

Case Reports

Patient 1

This patient was a 32-year-old Japanese man who had been found to have mild liver dysfunction at age 22, when a liver biopsy revealed steatotis. On January 17, 2003, he suddenly suffered severe consciousness disturbance after drinking a small amount of alcohol. Subsequently, he became irritable

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Abbreviations: APOLT, auxiliary partial orthotopic liver transplantation; FAP, familial amyloid polyneuropathy; ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS, carbamyl phosphate synthetase; OTC, ornithine transcarbamylase.

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Supported in part by grants-in-aid 08457297 and 09877243 for scientific research from the Ministry of Education, Science and Culture of Japan.

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Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/lt.20131

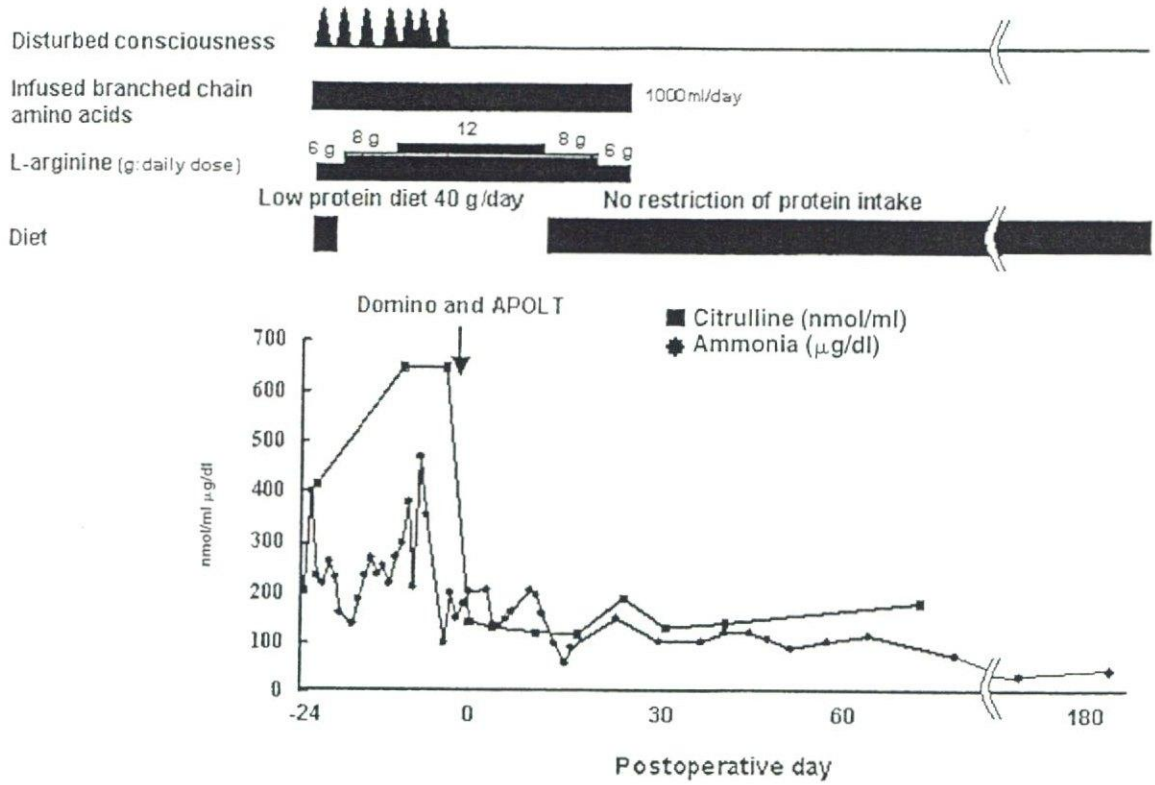

Figure 1. Clinical course and serial changes in plasma citrulline and ammonia levels before and after liver transplantation in patient 1.

and began to verbally abuse his family. He also showed disorientation and became very forgetful. He was admitted to a local hospital on January 23, and a laboratory examination revealed a raised plasma level of ammonia. He was referred to our hospital for further examination on January 25.

The patient was 170 cm in height and 52 kg in weight. He was highly irritable and confused, and showed flapping tremor. His plasma levels of ammonia and citrulline were highly elevated to 192 μg/dl (normal < 80 μg/dl) and 416.4 nmol/ml (normal < 60 nmol/ml), respectively. The plasma arginine concentration was mildly elevated to 147.7 nmol/ml (normal < 130 nmol/ml). Computed tomography showed a diffuse low-density area in the liver, suggesting severe steatosis. An electroencephalogram showed diffuse slow waves including frequent discharge of triphasic waves. DNA analysis of the citrin gene revealed that the patient was homozygous for the Ser225X mutation,[3] and, therefore, he was diagnosed as having type II citrullinemia. A low protein diet (protein 40 g/day) and oral administration of lactulose, kanamycin (1500 mg/day) and L-arginine (12 g/day)[9] were started. Subsequently, intravenous hyperalimentation with branched chain amino acids was also begun instead of the diet. Despite these treatments, his consciousness disturbance with disorientation and abnormal behavior did not improve and his plasma concentrations of ammonia and citrulline continued to increase (Fig. 1). Consequently, urgent liver transplantation seemed the only option. Although we considered living donor liver transplantation, unfortunately, there was no appropriate donor candidate in his family. Moreover, cadaveric liver transplantation seemed improbable because of the serious shortage of cadaveric donors in Japan. Therefore, we finally planned to perform combined domino transplantation and APOLT using a graft from a 52-year-old female patient with FAP (a Val30Leu transthyretin gene mutation), for whom living donor liver transplantation had already been planned. Informed consent was obtained from both the patient with type II citrullinemia (recipient) and the patient with FAP (domino transplant donor), and liver transplantation was performed on February 18, 2003, after approval by the local ethics committee. The recipient's left hepatic lobe (segments 1–4 according to Couinaud's segmentation) was resected and the domino left liver graft (segments 2–4) was transplanted orthotopically. The right hepatic lobe of the recipient was not removed and the right anterior portal vein was ligated and transected, leaving the right posterior portal vein patent. This procedure causes hypertrophy of the left liver graft as well as atrophy of the recipient's right anterior segment. The domino left liver weighed 284 g, corresponding to only 26% of the recipient's estimated whole liver volume. The details of this surgical procedure have been reported elsewhere.[10–12] Histo-

Table 1. Results of Urea Cycle Enzyme Analysis (Unit/mg Protein) of the Resected Liver

	CPS	OTC	ASS	ASL	ARG
Patient 1	0.023	0.94	0.0055	0.049	6.6
Patient 2	0.08	1.97	0.007	0.071	11.9
Controls	0.036 ± 0.013	0.88 ± 0.35	0.033 ± 0.012	0.052 ± 0.025	15.8 ± 3.1

Abbreviations: CPS, carbamyl phosphate synthetase; OTC, ornithine transcarbamylase; ASS, arginosuccinate synthetase; ASL, arginosuccinate lyase; ARG, arginase.

logical examination of the resected left lobe revealed severe fatty change. Five urea cycle enzyme analyses of the resected liver revealed that only argininosuccinate synthetase activity was extremely low (Table 1). After transplantation, the patient began receiving routine immunosuppression therapy. All of his neurological symptoms including the consciousness disturbance and flapping tremor disappeared within 3 days after transplantation. The plasma concentrations of citrulline and ammonia declined rapidly without either any specific medication or nutritional support for hyperammonemia (Fig. 1). Electroencephalography on postoperative days 10 and 50 showed complete disappearance of abnormal waves. The postoperative course of this patient was uneventful. Computed tomographic volumetry of the graft on postoperative day 28 gave value of 409 ml. Postoperative analysis of serum transthyretin in the serum of patient 1 using matrix-assisted laser desorption ionization and time-of-flight mass spectrometry[13] revealed both wild-type transthyretin (Val30) and variant transthyretin (Leu30), the latter produced by the liver graft donated from the FAP patient (Fig. 2).

Patient 2

The patient was a 43-year-old Japanese woman who had started to experience occasional headaches, drowsiness, and abnormal behavior at age 40. She was admitted to a hospital because of consciousness disturbance at age 42. Laboratory examination revealed hyperammonemia (583 μg/dl) and hypercitrullinemia (214.9 nmol/ml). DNA analysis of the citrin gene revealed that the patient was homozygous for the Ser225X mutation.[3] She was, therefore, diagnosed as having type II citrullinemia, and oral administration of L-arginine (9 g/day) and sodium benzoate (9 g/day) was started. However, her plasma ammonia level continued to increase (866 μg/dl), necessitating referral to our hospital on January 27, 2003. At this time, her consciousness was clear and fine postural tremor of both hands was seen. Her plasma ammonia and citrulline concentrations were 201 μg/dl and 605.8 nmol/ml, respectively. Abdominal computed tomography demonstrated a diffuse low-density area in the liver, and electroencephalography showed frequent slow waves with triphasic waves. She was treated by low protein diet (30 g/day) and oral administration of L-arginine (16 g/day), sodium benzoate (9 g/day), kanamycin (1500 mg/day), and branched amino acids. However, her plasma ammonia level did not decrease, and occasional drowsiness was seen (Fig. 3). On April 6, her plasma ammonia level became highly increased, and intravenous hyperalimentation with branched chain amino acids was begun after withdrawal of the oral diet. These treatments failed to improve her condition, and, therefore, she underwent partial liver trans-

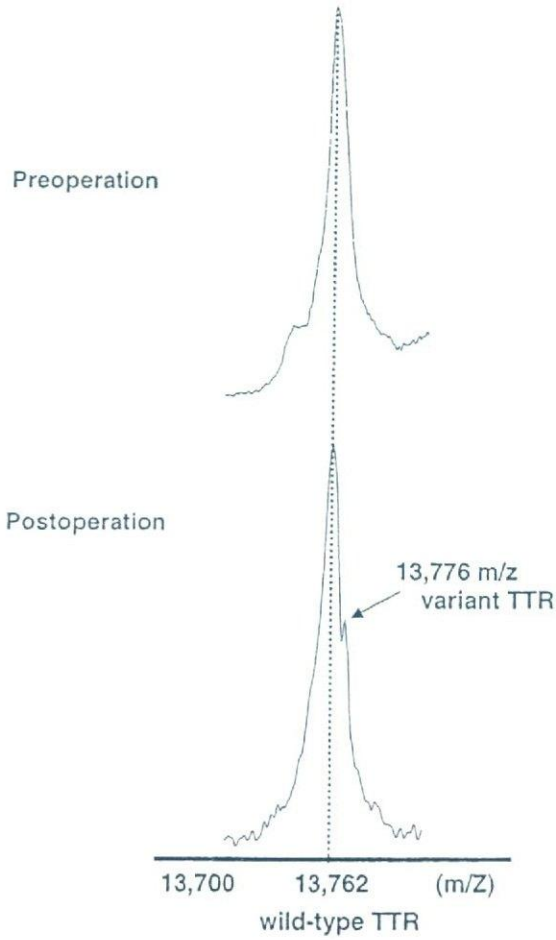

Figure 2. Mass spectra of immunoprecipitated serum transthyretin in patient 1, obtained by matrix-assisted laser desorption ionization and time-of-flight mass spectrometry.[13] Wild-type transthyretin (Val30) is identified as an ion peak of 13,762 m/z and the variant transthyretin (Leu30) is 14 m/Z larger than the wild-type transthyretin.

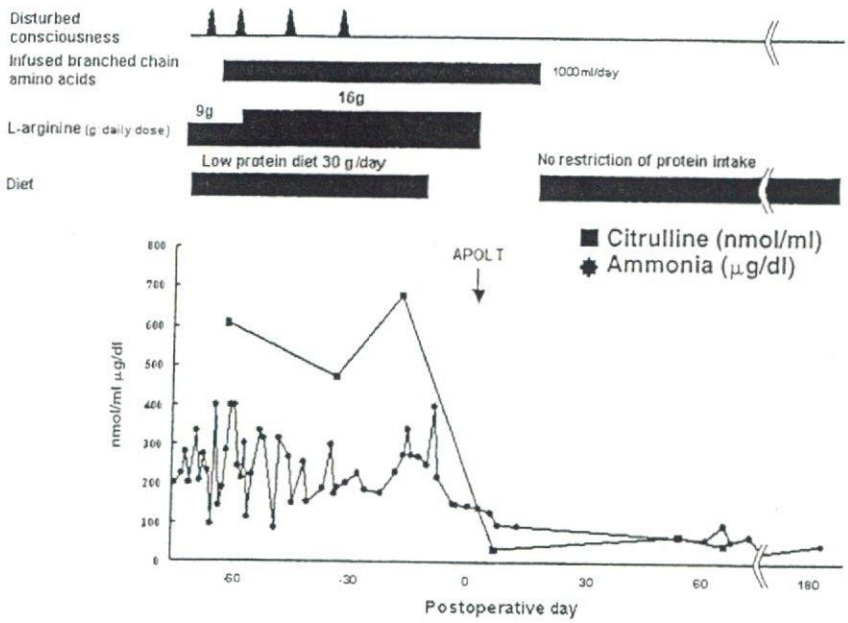

Figure 3. Clinical course and serial changes in plasma citrulline and ammonia levels before and after liver transplantation in patient 2.

plantation on April 15, 2003, using a left lobe graft from her husband. Since the graft volume obtained from her husband was insufficient (384 g), corresponding to 39.1% of the patient's standard liver volume (981 ml), we eventually decided to perform APOLT. The patient's left lobe (segments 1–4) was resected leaving her right hepatic lobe intact. The left liver graft (segments 2–4) was transplanted orthotopically and the recipient's right portal vein was ligated and transected. The postoperative course was uneventful, and the neurological symptoms were resolved on postoperative day 2. Histological examination of the patient's left lobe revealed severe steatosis. Urea cycle enzyme analysis of the liver demonstrated argininosuccinate synthetase deficiency (Table 1). The plasma concentrations of citrulline and ammonia normalized rapidly without any specific medication or nutritional support (Fig. 3). The computed tomography estimated graft volume on postoperative day 28 was 769 ml.

Discussion

We have described 2 adult patients with type II citrullinemia who underwent APOLT successfully with small left liver grafts from living donors.

The preoperative clinical condition of patient 1, including laboratory data, deteriorated rapidly despite several courses of intensive treatment for hepatic encephalopathy, and preoperative computed tomography volumetry showed that the left lobe of the FAP patient was small for use as a graft. We, therefore, decided to perform combined domino transplantation and APOLT for this patient. In preparing this procedure for patient 1, it was an issue of repeated discussion whether to divide the recipient's right portal vein completely or partially. We elected to divide his right anterior portal venous branch for the following 2 reasons. First, the possible development of FAP in patient 1 in the future (probably within 20 to 30 years after transplantation) would jeopardize his life. Considering the rapid advances in medical treatment for metabolic disorders including FAP and citrullinemia, division of his right portal venous trunk would eliminate the possibility of removing the transplanted left liver if gene treatment for citrullinemia were ever established in the future. Second, because the estimated graft volume was too small for patient 1 (corresponding to 26% of the recipient's standard liver volume), it was considered beneficial to leave the right posterior portal vein patent to avoid exposing the graft to portal venous hypertension, which is assumed to be an adverse factor in liver transplantation with a small-for-size graft.

In patient 2, we considered that the graft volume (384 g: 39.1% of the recipient's standard liver volume) was not sufficient for the recipient, and, therefore, decided to perform APOLT for her. In contrast to patient 1, however, we divided the right portal venous trunk because the graft volume was not too small and there would be little likelihood of needing to extirpate the transplanted normal liver graft.

APOLT was initially introduced as a temporary or

permanent support for patients with fulminant hepatic failure,[14] and its indications have been extended to liver-based metabolic disorders. At our institution, APOLT has been carried out for patients with FAP to guarantee a sufficient margin of safety for both donor and recipient when a living donor's left lobe volume is insufficient for the recipient.[10]

With regard to urea cycle disorders, APOLT has been performed for a few patients with ornithine transcarbamylase deficiency.[15] Kasahara et al.[15] proposed that, for such patients, removal of the whole native liver is unnecessary for 3 reasons: a partial liver segment with normal enzyme activity corrects the hyperammonemia; if graft failure occurs, the remnant native liver is an available backup; and the remnant liver could benefit in the future from potential advances in gene treatment. In both patients 1 and 2, we also decided not to remove the remnant native liver later, as mentioned by Kasahara et al.[15] In particular, although in patient 1 the graft volume was only 26% of the estimated standard liver volume, transplantation dramatically improved his systemic condition and laboratory, data as shown in Fig. 1. These results indicate that APOLT can provide an adequate hepatocyte mass to correct the underlying metabolic abnormality in adult patients with type II citrullinemia. Meanwhile, because around 8% of patients with type II citrullinemia are reported to develop hepatocellular carcinoma,[16] careful follow-up is mandatory.

In conclusion, APOLT can be a safe and effective treatment for patients with type II citrullinemia, especially those who have candidate donors with insufficient graft volume or who have a chance of receiving a domino liver graft.

Acknowledgment

The authors thank Dr. Mitsuyuki Watanabe, Department of Gastroenterology, Tokai University Hachioji Hospital, for referring patient 2 to our hospital.

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ELSEVIER

Conversion from Tacrolimus to Cyclosporine Microemulsion Therapy in Liver Transplant Recipients

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ABSTRACT

The calcineurin inhibitors cyclosporine and tacrolimus have distinct advantages and drawbacks. Therefore it is important to tailor their use to the patient's tolerance. In some patients, the need to ameliorate the adverse effects of tacrolimus may necessitate a switch to cyclosporine-based therapy. Rescue therapy with a cyclosporine microemulsion (Neoral)-based regimen for transplant patients intolerant of tacrolimus has been evaluated to assess the best method of switching and determine the initial and maintenance doses of Neoral in children and adults. Our aims were to evaluate not only these facets, but also the pharmacokinetics of Neoral in stable patients, including target 2-hour postdose blood concentrations (C_2) of cyclosporine in liver transplant recipients. Eighteen liver transplant patients switched from tacrolimus to Neoral underwent a program of cyclosporine blood level monitoring. The conversions were conducted safely; the incidence of acute rejection episodes was low (11.1%). Statistical analysis showed that the C_2 correlated with the area under the time-blood concentration curve of cyclosporine for 0 to 4 hours after dosing ($R = 0.970$). We determined the maintenance doses of Neoral for pediatric and adult patients as well as the feasibility of C_2 quantitated monitoring in liver transplantation.

THE CALCINEURIN INHIBITORS cyclosporine and tacrolimus represent the mainstays of current immunosuppressive regimens for the prevention of acute rejection after liver transplantation.[1-3] Because these drugs have their own advantages and drawbacks,[4,5] it is important to tailor their use to patient tolerance. From our experience in living donor liver transplantation, the incidence of acute cellular rejection is lower using tacrolimus- than cyclosporine-based regimens.[6] However, the adverse effects of tacrolimus have affected its clinical utility in some patients.[3-5] The need to ameliorate tacrolimus-associated adverse effects, such as gastrointestinal intolerance, neurotoxicity, and diabetes mellitus, may prompt a switch to cyclosporine-based therapy.[7] The validity of rescue therapy with a cyclosporine microemulsion (Neoral)-based regimen in transplant patients intolerant of tacrolimus has been evaluated in several centers.[7] Recently, Abouljoud et al[8] reported positive results from a multicenter, open-label, single-arm prospective cohort study. There is now a need to investigate the best method and any contraindications to switch patients from tacrolimus to Neoral, as well as to determine the initial and maintenance doses of Neoral in children and adults. We not only evaluated these issues, but also the pharmacokinetics of Neoral in stable patients, especially estimating target 2-hour postdose blood concentrations (C_2) of cyclosporine in liver transplant recipients.

PATIENTS AND METHODS

Between September 1993 and August 2003, 204 patients (mean age 23.5 ± 22.6 years) underwent primary liver transplantation under tacrolimus-based immunosuppression. Among them, 18 patients (aged 8 months to 61 years; 11 male, 7 female) were switched from tacrolimus to Neoral (at 18 to 2610 days after liver transplantation) and underwent monitoring of cyclosporine blood levels. Nine of the 18 subjects were 15 years or younger. The conditions necessitating

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Supported in part by grants-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan (08457297, 09877243).

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0041-1345/04/$-see front matter
doi:10.1016/j.transproceed.2003.12.037

Table 1. Mean Blood Concentrations of Cyclosporine After Neoral Administration in Living Donor Liver Transplant Recipients

	2–6 Months After Liver Transplantation				More Than 6 Months After Liver Transplantation			
		C_0	C_2	AUC_{0-4}		C_0	C_2	AUC_{0-4}
Ă	Dose of Neoral (mg/kg per day)	(ng/mL)			Dose of Neoral (mg/kg per day)	(ng/mL)		
Pediatric (≤15 y)	8.3 ± 3.9	150 ± 88	455 ± 374	1738 ± 1485	6.4 ± 3.4	124 ± 73	401 ± 157	1269 ± 415
Adult (>15 y)	2.6 ± 0.8	185 ± 108	652 ± 276	2200 ± 759	1.5 ± 0.5	118 ± 37	453 ± 197	1564 ± 613

C_0, trough blood concentration; C_2, blood concentration 2 hours after administration; AUC_{0-4}, mean area under the curve between trough and 4 hours after Neoral administration.

liver transplantation were biliary atresia (n = 6), liver cirrhosis due to hepatitis C virus (n = 3), fulminant hepatic failure (n = 3), familial amyloid polyneuropathy (n = 3), Alagille's syndrome (n = 2), and primary biliary cirrhosis (n = 1).

The initial tacrolimus dose of 0.05 mg/kg per day was adjusted to maintain whole blood trough concentrations (C_0) of 15 to 18 ng/mL during the first 2 weeks after transplantation, 10 to 15 ng/mL during the third and fourth weeks, and 5 to 10 ng/mL thereafter. All patients received a 20 mg/kg intravenous bolus of methylprednisolone before reperfusion of the liver graft. From postoperative day 1, methylprednisolone was tapered from 3 to 0.5 mg/kg within 7 days and thereafter to 0.06 mg/day at 6 months. The tacrolimus C_0 was measured by the microparticle enzyme immunoassay technique (Abbott Japan, Tokyo, Japan). When patients developed tacrolimus-related adverse effects, the tacrolimus was withdrawn and cyclosporine (twice a day orally) was commenced 12 hours later. The reasons for changing the medication were liver dysfunction (n = 6), renal dysfunction (n = 5), gastrointestinal toxicity (n = 3), diabetes (n = 2), neurotoxicity (n = 1), and hematological disorder (thrombotic thrombocytopenic purpura; n = 1), all of which were suspected to be tacrolimus-related. The mean tacrolimus C_0 at the onset of adverse reactions was 12.3 ± 5.0 (range 5.6 to 21.6) ng/mL.

The first Neoral dose ranged from 2 to 3 mg/kg per day in two divided doses. The doses of Neoral were titrated to maintain the cyclosporine C_0 within the target range as described elsewhere.[6] Following the change in their drug regimens, patients were evaluated every day for the first week; weekly for the next 3 weeks; and then at months 2, 3, 6, and 12 for therapeutic cyclosporine blood levels, acute rejection episodes, graft function, and assessment of the signs and symptoms that had led to tacrolimus withdrawal. Monitoring of the tacrolimus blood level was continued until it fell below 3 ng/mL. Each evaluation of cyclosporine blood levels included C_0, the 1-hour postdose blood concentration (C_1), C_2 and the 4-hour postdose blood concentration (C_4). The area under the time-blood cyclosporine concentration curve between C_0 and C_4 (AUC0 = 4) was measured by the linear trapezoidal rule. Correlations of single-point samples were done by Pearson's correlation test. In all subjects the efficacy of immunosuppression was assessed over the 6 months following the change to Neoral. The median follow-up time was 1001 days.

RESULTS

None of the 18 patients died during the follow-up period. Resolution or improvement of tacrolimus-related adverse effects was observed in all patients except three who had liver dysfunction. The time from withdrawal of the tacrolimus to resolution of the side effects was 1, 2, 3, 5, and 14 days for neurotoxicity; 8, 16, and 21 days for hepatotoxicity; 3, 5, and 9 days for gastrointestinal toxicity; 13 and 44 days for diabetes; 10 days for thrombotic thrombocytopenic purpura; and 3 days for nephrotoxicity. Two adult patients experienced rejection episodes—one at day 65 and another on day 201 after the change in drug regimen. All episodes responded to steroid pulse therapy.

Adequate trough concentrations were achieved with Neoral (Table 1). During the drug conversion phase, the blood concentrations of both tacrolimus and cyclosporine were monitored and the dose of Neoral titrated. For example, when the blood level of tacrolimus had declined to half the target level, the Neoral was titrated to achieve half the target cyclosporine trough level. As a result, no adverse effects from coadministration of the two drugs—including deterioration of renal dysfunction—were encountered. The maintenance doses differed considerably between pediatric and adult recipients (Table 1). Statistical analysis showed that C_2 correlated better with AUC0 = 4 (R = 0.970) than C_0 (R = 0.586), C_1 (R = 0.881), or C_4 (R = 0.768). However, in two infant recipients, the blood concentration curve did not peak at C_1 or C_2, but increased gradually to C_4. These two patients had undergone liver transplantation because of liver cirrhosis after a Kasai procedure for biliary atresia. The other three infant patients who underwent liver transplantation after a Kasai procedure had absorption profiles peaking at C_2. None of the recipients with biliary atresia developed gastrointestinal disorders or biliary complications.

DISCUSSION

We evaluated a protocol whereby the immunosuppressant drug therapy of 18 patients who had undergone liver transplantation was switched from tacrolimus to Neoral. The initial dose of Neoral was set relatively low at 2 to 3 mg/kg per day, and the drugs concentrations were monitored closely for 1 week. This resulted in fair outcomes without deterioration of nephropathy. We also investigated the pharmacokinetics of Neoral to assess the validity of C_2 monitoring with positive results. We were able to determine the target C_2 levels for liver transplant patients observing considerable differences in the requirements between children and adults (Table 1).

Neoral is a microemulsion formulation of cyclosporine with more consistent pharmacokinetic parameters and better bioavailability than the conventional oil-based formula-

tion of cyclosporine. Some studies have demonstrated that induction and maintenance of immunosuppression with Neoral are more effective than with Sandimmune, as demonstrated by a lower incidence of acute rejection with no additional toxicity. Our present study showed that C_2 may prove to be an excellent surrogate marker of AUC0 - 4, as described previously.[9] However, careful monitoring of C_2 is necessary for some infant recipients who have undergone the Kasai procedure for biliary atresia.

In summary, tacrolimus-related adverse effects can be treated successfully with a low incidence of rejection by switching patients to Neoral. We have determined the maintenance dose of Neoral in pediatric and adult liver transplant recipients as well as the recommended C_2 levels for drug monitoring.

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DELAYED DOMINO LIVER TRANSPLANTATION: USE OF THE REMNANT LIVER OF A RECIPIENT OF A TEMPORARY AUXILIARY ORTHOTOPIC LIVER TRANSPLANT AS A LIVER GRAFT FOR ANOTHER PATIENT

Domino liver transplantation (DLT) has been developed as a method for expanding the donor pool (*1*). In contrast, temporary auxiliary partial orthotopic liver transplantation (APOLT) has been used for selected liver diseases (*2*). In temporary APOLT, we routinely resect the left liver and transplant the donor's left liver, and thereafter ligate and divide the right portal vein. This causes hypertrophy of the graft in the recipient and atrophy of the remnant liver, which is then removed approximately 2 months after transplantation (*2*).

A 27-year-old man with familial amyloid polyneuropathy (patient 1) was referred to our institution for living-donor liver transplantation. Preoperative computed tomography of the donor candidate showed a left hepatic lobe volume of 322 mL, corresponding to only 28% of the recipient's standard liver volume. We decided to perform temporary APOLT for the patient, considering the safety of both donor and recipient. On July 4, 2001, the left hepatic lobe (segments I–IV according to Couinaud's nomenclature) was resected from patient 1 and the left liver graft (segments II–IV) was orthotopically transplanted. However, just after reperfusion, color-duplex ultrasonography demonstrated thrombosis of the graft hepatic vein and the portal vein. Continuous administration of heparin resulted in successful thrombectomy and reanastomosis, allowing recovery of graft perfusion. We then considered whether to ligate the right portal vein, as scheduled. Considering the possible recurrence of thrombosis and the need to modulate the portal venous flow to the recipient's remnant liver to induce compensatory hypertrophy of the left lobar graft, we decided not to ligate the trunk of the right portal vein, but to transect only the anterior branch of the right portal vein, leaving the posterior branch patent. The postoperative course was uneventful, and removal of the remnant native liver was planned 8 weeks after APOLT.

On August 10, 2001, a 61-year-old man with hepatocellular carcinoma (patient 2) was referred for possible living-donor liver transplantation. However, no suitable donor was available. We carefully discussed the adequacy of using the remnant liver of patient 1 as a graft for patient 2, on the basis of our experience with preoperative portal vein embolization for extended lobectomy of the liver (*3*). Taking care to avoid any coercion, we asked patient 1 whether he would be willing to approve use of the removed remnant liver as a graft for another patient. His reply was promptly affirmative. We then explained to patient 2 the delayed DLT procedure, including its untried aspects, uncertainty about the graft quality, and future onset of familial amyloid polyneuropathy. The patient expressed a strong desire to undergo this procedure. The entire process was reviewed and approved by the Ethical Committee of Shinshu University. On September 4, 2001, we removed the remnant right liver from patient 1 and transplanted it into patient 2. The postoperative courses in these patients were uneventful. The presented cases indicate that division of one of the two portal venous branches to the remnant right liver is feasible in APOLT and that delayed DLT could be an option in selected situations.

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DOI: 10.1097/01.TP.0000101004.83154.B4

This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan (08457297 and 09877243).

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Received 6 May 2003. Revision requested 10 June 2003. Accepted 22 July 2003.

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