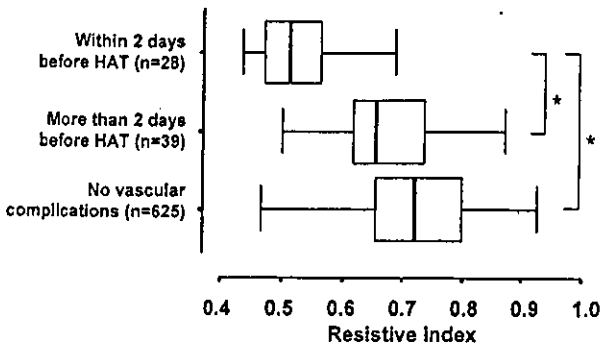

Fig. 3. Distribution of RI values in box-and-whisker plots. In patients with HAT, the RI values 2 days before the onset of HAT were significantly lower than those 2 to 7 days before the onset of HAT or those in patients without HAT. *$p <$ 0.001. *n* indicates the number of the US records.

Table 1. Sensitivity and specificity for HAT diagnosis for multiple discriminatory thresholds of RI

Threshold	TN	TP	FN	FP	Sensitivity (%)	Specificity (%)
< 0.4	660	1	29	2	3	100
< 0.5	652	13	17	10	43	99
< 0.6	564	25	5	98	83	85

FN, false negative; FP, false positive; HAT, hepatic artery thrombosis; RI, resistive index; TN, true negative; TP, true positive

10], however, Doppler US was not performed routinely and the indications for Doppler US were not clarified.

Recent studies [58] have indicated that protocol Doppler US is useful for early HAT detection. Urgent thrombectomy and revascularization based on the findings of protocol study significantly reduced the rate of late biliary complications and graft loss subsequently complicated by HAT. The present study differed from previous studies in which RI data were consecutively and prospectively collected. Nolten and colleagues [11] reported a qualitative change in the Doppler waveform over time. Although the waveform was initially normal in appearance, it progressed to an absent diastolic flow, dampening of the systolic peak, and, finally, loss of the hepatic arterial signal. The present study confirmed their findings, indicating that RI values of less than 0.6 predict HAT onset within 2 days with 83% sensitivity and 85% specificity.

In summary, we reviewed 692 Doppler US records in 70 pediatric patients. RI was a sensitive predictor for HAT during the first 2 weeks after LDLT. Thrombectomy and reanastomosis should be considered when RI values are less than 0.6 in protocol Doppler US.

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Revolution and Refinement of Surgical Techniques for Living Donor Partial Liver Transplantation

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Living donor liver transplantation (LDLT) was first successfully performed on a child in 1990 and the Shinshu group performed the same procedure on an adult for the first time in 1994. Over the past few years adult LDLT has been increasing worldwide because of the severe shortage of cadaveric organs, especially in locations where the transplantation of organs from brain-dead donors is rarely practiced. The surgical procedures for LDLT are more technically challenging than those for cadaveric whole liver transplantation. LDLT requires a full understanding of hepatobiliary anatomy and continuous technical refinement of the procedure. The development of innovative techniques is a key factor for a successful LDLT. Some of the technical highlights include selective vascular occlusion techniques for donor hepatectomy, hepatic arterial reconstruction under the microscope, the introduction of intraoperative ultrasound, graft volume estimation, hepatic venous reconstruction using cryopreserved vascular grafts, and the use of the right lateral sector of the liver. These techniques have improved the success rate of LDLT over the past few years. This review focuses on the surgical techniques for LDLT on the basis of our experience with adult LDLT at the Tokyo University Hospital.

Key Words: Living donor liver transplantation, middle hepatic vein, right liver graft

INTRODUCTION

The use of live donors for liver transplantation was initiated more than a decade ago as a solution to the cadaveric donor shortage for pediatric recipients.[1] Since the first successful case of LDLT performed on an adult patient in 1994,[2] this procedure is now widely applied to adult recipients, especially in countries where the availability of brain-dead donors is severely restricted.[3] This includes the United States and European countries where there is a critical shortage of cadaveric organs. In attempts to meet the growing needs of recipients, transplant surgeons have had to develop innovative techniques and appropriate algorithms to overcome deteriorating conditions and complications such as outflow and biliary complications.

In this review, several considerations of LDLT, including donor and graft selection criteria, technical highlights, and critical points necessary for successful patient outcome are discussed on the basis of our experience at the Tokyo University Hospital.

DONOR SELECTION CRITERIA

The first priority when performing LDLT is donor safety. Donor characteristics are the primary determinant of the outcome for both patients. Therefore, careful evaluation and selection of the donor are obligatory. In our department, a preoperative donor evaluation consists of three stages. In the first stage, patients and their families are given explanations about LDLT, including the risk of death for LDLT donors. After evaluating the medical and family history, social support system within the family, and psychological fitness, the donor's understanding of the risks involved with a liver resection and accompanying invasive tests must be confirmed for each donor candidate. The age of acceptable donors at our center is between 20 and 65 years with a

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relation to the recipient within the third degree of consanguinity.

The second phase involves performing liver function tests, ABO compatibility testing, lymphocyte cross matching; negative serology for hepatitis B and C, human immunodeficiency and adult T-cell leukemia viruses, cardiopulmonary function tests, the determination of tumor markers of donors over 40 years old, and a pregnancy test for female donors. ABO blood group incompatibility and positive lymphocyte cross matching are not definite exclusion criteria.

If there is no anomaly, the donor candidate can proceed to the third stage; a Doppler ultrasound should be performed for hepatic artery, portal vein, and hepatic vein evaluation. Computed tomography (CT) is used to measure graft volume. Hepatic angiography can be performed to evaluate vessel anatomy. The donor's own blood and plasma are banked preoperatively. Throughout the course of the donor evaluation, the spontaneous willingness of the donor candidate is repeatedly confirmed.[4] Signed informed consent is obtained before the surgery.[5]

Preoperative liver biopsy and steatosis in graft

Preoperative determination of the extent of hepatic steatosis is important to ensure both donor and recipient safety. Donors with significant steatosis may not tolerate surgery as well as those with nonsteatotic livers and they tend to have increased postoperative morbidity, mortality, transfusion requirements, and surgical time.[6] A liver biopsy must be performed for a secure evaluation of the liver. The indications for a liver biopsy, however, must be determined carefully and some patients may require hospitalization after the procedure (5% frequency) or experience serious complications (1%).[7-9]

The degree of steatosis acceptable for LDLT remains controversial. Marcos et al. reported no impairment of function in either the donor or recipient when using grafts containing less than 30% steatosis.[10] Fan et al. do not use a right liver graft with steatosis of 20% or more,[11] whereas other groups use liver grafts with steatosis of less than 50% if the graft volume-to-standard liver volume (SLV) of the recipient ratio is 40% or more.[12] At our center, when hepatic steatosis is suspected by computed tomography and biochemical data (i.e., aspartate aminotransferase < alanine aminotransferase), a liver biopsy for evaluating steatosis is considered. If time permits, the potential donor should undergo a period of prescribed diet and exercise. Livers with less than 10% hepatic macrosteatosis are preferred.[7]

Estimation of the liver graft

A major concern for the application of LDLT to adults is graft size disparity. Small-for-size grafts are defined as functionally insufficient grafts for satisfying the recipient's metabolic demand, which will predispose the recipient to injuries characterized by cholestasis and histologic features of ischemia after implantation.[13] On the other hand, harvesting a larger graft puts the donor at higher risk.[14] The right liver is not indicated as a graft when the estimated volume in donors is over 70%, according to the criteria of Fan et al.[11] We established a method for estimatinggraft volume using CT and the following formula to calculate the SLV (optimal liver mass) in recipients from their body surface area:[15,16]

$$SLV\ (ml) = 706.2 \times (\text{body surface area } [m^2]) + 2.4.$$

The volume of each sector of the donor liver is evaluated by CT. The predicted graft volume/SLV ratio is then calculated.[17]

Principally, grafts with a weight/recipient SLV ratio of 40% are preferred for use in adult patients. In low-risk patients, a right liver graft with or without the middle hepatic vein (MHV) are considered. In other words, grafts with an SLV ratio of 40% or less may suffice only in the ideal situation of a good-risk patient as proposed by Lo and associates.[18] Our data indicate that[19] 96% of patients survive with a graft weight ratio of over 40%, while only 80% of patients survive with a graft weight ratio of 40% or less. High-risk patients include those with primary biliary cirrhosis with a Mayo risk score[20-22] of less than,[10] metabolic disease, and fulminant hepatic failure. For higher risk patients, a left liver with or without a caudate lobe should be evaluated by CT volumetric analysis.[23,24] If the volume of the right

lateral sector is greater than that of the left liver, the right lateral sector segments VI and VII, according to Coinaud's nomenclature for liver segmentation, should be considered for the graft.[5,22,23]

Objection against routine use of right liver graft

A right liver graft was first used for a pediatric case by Yamaoka et al.[25] It is now commonly used for adult patients. This procedure was followed by the introduction of an extended right liver graft, which includes the trunk of the MHV. This trend has grown rapidly.[26-28] At the same time, however, important ethical issues were raised regarding the execution of an extended hepatectomy on live donors.[3] Although graft size in living donors may be safely expanded, a multidisciplinary approach and meticulous donor evaluation are always necessary. We do not agree with the recent tendency to use a right liver graft routinely for almost all adult patients.[28] Based on our experience, the number of patients who inevitably need a right liver graft with the MHV is limited; we found that less than 10% of the recipients in our series required an extended right liver graft.

Evaluation of donor hepatic arterial anatomy

When planning a donor resection, a preoperative arteriography is necessary to assess the anatomy and quality of the vasculature of the resulting graft.[29] For example, upon performing a right liver LDLT, it is first necessary to determine which of the varied origins of the artery to segment IV is important for defining the optimal points for transection of the artery.[3] Although an angiography is a relatively invasive study with the potential for complications, the information it provides is essential for surgical planning and donor safety. Unfortunately, non-invasive techniques, such as magnetic resonance angiography or CT, are limited in their ability to demonstrate small vessels such as the accessory hepatic arterial branches.[28,30] The techniques for non-invasive imaging of smaller vascular structures are still under evaluation and are not yet sufficiently reliable for these purposes.

Intermittent inflow occlusion technique

In our department, a donor hepatectomy is routinely performed under Pringle's maneuver without any side effects. We postulate that the intermittent inflow occlusion acts as a preconditioning step and reduces blood loss during the hepatectomy.[31]

RECIPIENTS

During an evaluation of a liver transplant candidate for LDLT, there needs to be a balance between the severity of the liver disease and the adequacy of a partial graft for transplantation. Most of the complications associated with acute hepatic failure are reversible if the transplantation can be performed in the early stage.[32] Stable patients with chronic liver disease also benefit from living donors. Transplantation can be performed electively before decompensatory (i.e. fulminant hepatic failure with irreversible encephalopathy) complications occur.

Recipient surgery

The operative technique for recipients is based on the technique of whole liver resection with preservation of the inferior vena cava used for orthotopic liver transplantation.[33] A J-shape incision is made to open the abdominal space as is done for a right thoracotomy. Electrocautery is effective, time-saving, and useful for sharp dissection. An argon beam coagulator is useful to stop bleeding from the hepatic serosa. Each step of this operation requires meticulous maneuvers and great care to achieve an uneventful resection of the whole liver, while avoiding injury to the other visceral organs. It is important to make a large and long opening along the sides of the hepatic veins, and to maintain satisfactory portal, biliary, and hepatic arterial sources for the reconstruction. The right and left hepatic arteries should be dissected out as distally as possible, the left portal vein should be dissected up to the umbilical portion, which is just distal to the point of origin of the branch to segment,[2] and the right portal vein should be dissected up to its bifurcation into

the anterior and posterior branches.

In recipients with little portosystemic collateral circulation (i.e., familial amyloid polyneuropathy, citrullinemia, acute hepatic failure), the prevention of portal congestion is necessary during the anhepatic phase. A temporary shunt between the portal vein and the inferior vena cava should be made.[34] Briefly, the portal vein branch, which will not be used for reconstruction, is anastomosed end-to-side or connected by a tube to the inferior vena cava. Blood flow through this shunt is maintained until portal venous reperfusion to the graft is achieved by portal vein anastomosis.

HEPATIC VENOUS RECONSTRUCTION

The provision of an adequate outflow is indispensable for graft function, thus, it is necessary to obtain a wide ostium and a sufficient length of the hepatic vein for anastomosis.

Left liver

Early cases of LDLT entailed an end-to-side anastomosis for hepatic vein reconstruction. A longitudinal cavatomy was made along the anterior aspect, and the hepatic venous branches, which were joined on the bench, were anastomosed end-to-side to the caval window.[3,35] Takayama et al. cautioned,[36] however, that a direct anastomosis of the hepatic veins to a thin inferior vena cava can cause a bend in the inferior vena cava at the anastomotic side, which can result in outflow occlusion.

Currently, an end-to-end anastomosis[37] is preferred. In such cases, size matching is important. The left hepatic vein (LHV) and MHV in the recipient can be joined into one.[38] If the diameter of the joined veins is smaller than the left liver graft's hepatic vein, a wider orifice can be constructed by venoplasty of three hepatic veins[39] in the recipient (Fig. 1).

In the first method for venoplasty, the neighboring walls were simply sutured together. The second method involves the use of the pantaloon technique. The parenchyma around the venous branches is aspirated using an ultrasonic dissector, resulting in elongation of the venous

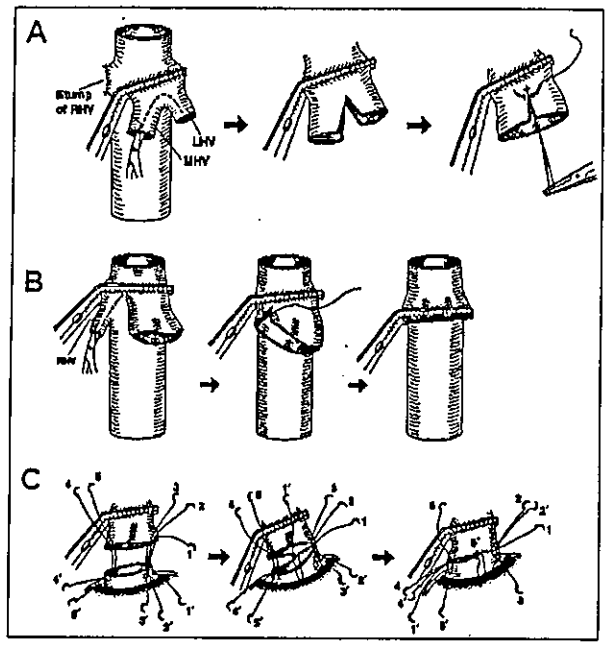

Fig. 1. Recipient venoplasty of the left hepatic vein (LHV) and middle hepatic veins (MHV) (A) or triple hepatic veins (B). (C) The anastomosis was made with continuous sutures (1-5).

branches. The branches are then cut longitudinally and sutured together. In the third method, the hepatic vein of the liver graft is cut in a perpendicular direction, and then a venous patch is anastomosed to the incised graft hepatic veins. De Villa et al.[40] detailed another venoplasty technique. They reported that when two hepatic veins are connected by a longer intervening septum, a venoplasty is made by an incision perpendicular to the septum by first removing the directly underlying liver parenchyma using a Cavitron ultrasonic surgical aspirator (CUSA).

Caudate vein reconstruction

Reconstruction of the caudate vein is technically demanding.[41] In the initial LDLT cases,[23] the drainage vein of the caudate lobe was not reconstructed. Takayama and associates[24] emphasized the importance of short hepatic vein reconstruction. According to the cast study by Couinaud, 91% (115/126) of the caudate veins entered directly to the vena cava,[42] thus indicating that one or two veins of the caudate lobe should be reconstructed to prevent venous congestion of the caudate lobe. The hepatic vein of the caudate lobe

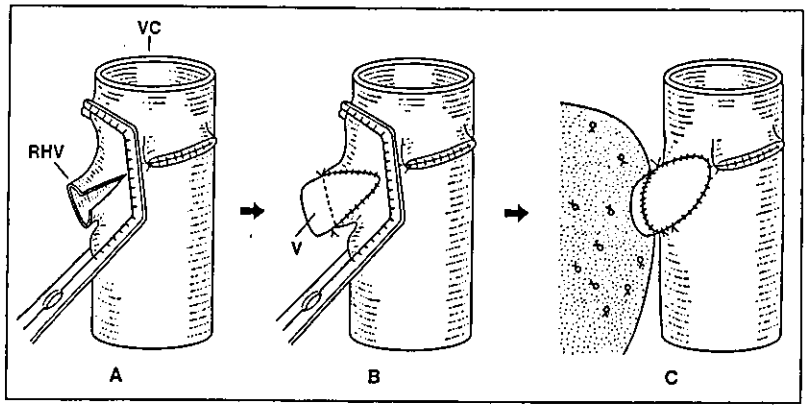

Fig. 2. (A, B) The V-shaped venous patch (V) was anastomosed to the incised anterior wall of the RHV of the recipient. (C) End-to-end anastomosis between the recipient and graft RHVs with continuous sutures.

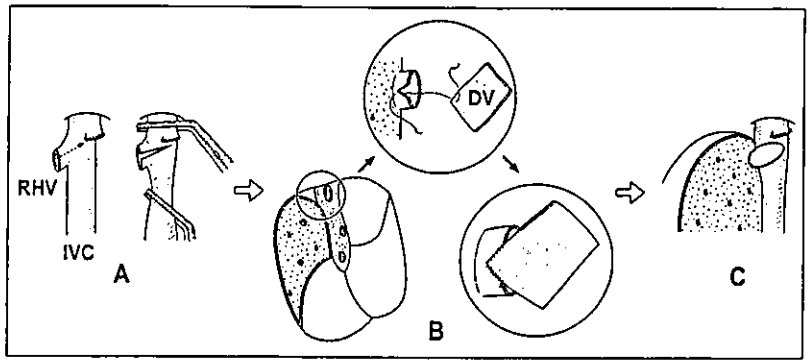

Fig. 3. (A) The anterior wall of the recipient right hepatic vein (RHV) was cut under cross-clamping of the inferior vena cava (IVC) (B) The diamond shaped venous patch (DV) was anastomosed to the incised anterior wall of the recipient's RHV. (C) End-to-end anastomosis was done between the recipient and graft RHV with continuous sutures.

can be resected with a cuff of the vena cava, which resembles a Carrel's patch. In the recipient operation, reconstruction of the caudate hepatic vein is performed and then the trunk of the left and middle hepatic vein of the recipient and the graft are anastomosed. When the orifice of the short hepatic vein is located near those of the LHV and MHV, the caudate vein with a cuff of the inferior vena cava can be sutured to the common orifice of the LHV and MHV (Fig. 4 and 5).[43]

Right liver

To overcome a size discrepancy between the right liver graft and the recipient's hepatic veins, the patch technique can be used. A vascular patch graft can be sutured separately or to both the RHV of the liver graft and the RHV of the recipient (Fig. 2 and 3). Three hepatic veins of the recipient can be joined to create a wide orifice for anastomosis.

The appropriate length for the reconstructed hepatic vein is still controversial and size matching between the liver graft and the recipient's hepatic veins is crucial.[44] Regeneration of the liver graft may compress the venous anastomotic site. Ghobrial et al. suggested that a short hepatic vein places undue tension on the anastomosis[44] and they cautioned that a long vein is predisposed to kinking after reperfusion. In contrast, we believe that it is necessary to obtain a wide ostium and sufficient length of the hepatic vein anastomosis to ensure adequate hepatic venous flow. We usually use a vein graft and make a long and wide anastomosis during the reconstruction.[45]

MHV reconstruction and cryopreserved vascular graft

An extended right liver graft is beneficial with regard to venous drainage of the graft because the MHV is the major draining vein of the right paramedian sector, and its role in the left paramedian sector is limited.[27] On the other hand, a right liver graft without the trunk of the MHV can cause severe congestion of the right paramedian sector (segments V and VIII) without MHV

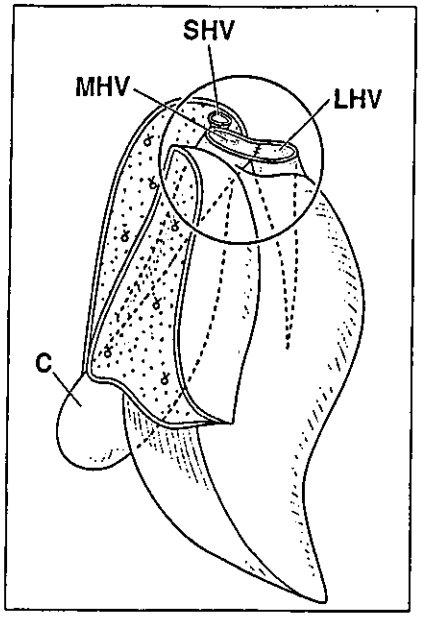

Fig. 4. The thickest hepatic vein (SHV) of the caudate lobe (C) was preserved, which was located near the orifice of the left hepatic vein (LHV) and middle hepatic vein (MHV).

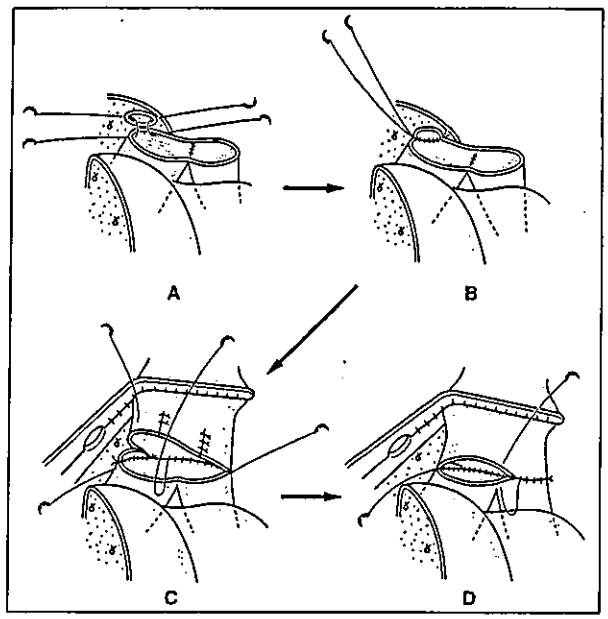

Fig. 5. Short hepatic vein of the caudate lobe sutured to the common orifice of the left and middle hepatic vein of the liver graft from the neighboring wall (A, B) End-to-end anastomosis between the common orifice of the left and middle hepatic vein and newly created hepatic venous orifices of the liver graft (C, D).

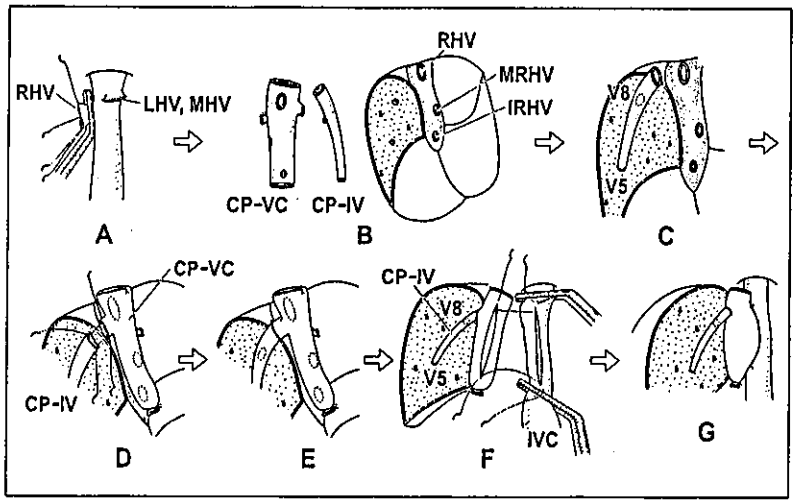

Fig. 6. (A) Left hepatic vein (LHV), middle hepatic vein (MHV) and right hepatic vein (RHV) of the recipient's liver were sutured at their roots. (B) Three side holes were created in the wall of the cryopreserved vena cava graft (CP-VC) for anastomosis with the RHV and the short hepatic veins [inferior right hepatic vein (IRHV) or middle right hepatic vein (MRHV)] of the graft. (C, D) Another cryopreserved vein graft (CP-IV) can be used for middle hepatic vein reconstruction. (E) The stump of the venous branch was anastomosed with a jumping vein graft for middle hepatic vein reconstruction. (F, G) Side-to-side anastomosis between the recipient's inferior vena cava (IVC) and CP-VC with continuous sutures was performed.

reconstruction. To provide a functioning liver mass comparable to an extended right liver, several methods have been devised for MHV reconstruction.[45,46] When a right liver graft has multiple short hepatic veins, use of a cryopreserved vena cava is recommended (Fig. 6).

The major concern in venous reconstruction using cryopreserved vein grafts is vein graft obstruction or the possibility of vein narrowing over the long-term. Mills et al.[47] reported a 51% complication rate after using cryopreserved vascular grafts. Kuang et al.[48] reported complications including an aneurysm, thrombosis, and stricture in 8 of 9 cryopreserved vein grafts that were used for portal vein and hepatic arterial interpositions. To date, we have not experienced any compli-

cations using cryopreserved vascular grafts, but previous discouraging results indicate that long-term follow-up is necessary to confirm the feasibility of their use.

Indication for MHV reconstruction

There is no consensus regarding the optimal strategy for MHV reconstruction. Some authors[49] claim that donor liver parenchyma transection without MHV tributary ligation is dangerous and that the reconstruction might increase the warm ischemia time. The development of the collateral circulation that drains the ligated MHV tributaries may occur in approximately 1 week,[50] but there is no evidence that these collaterals always occur or already exist in all patients.[51,52] Nakamura et al. clearly[53] demonstrated that the congestive area, which is due to hepatic vein ligation in the remnant liver, cannot be expected to function with the available parenchyma in the early postoperative period. They established that the congestive area resulted in histologic necrosis of the hepatic parenchyma approximately 24 hours after the ligation, although intrahepatic venous collaterals for draining the congestive area were observed through the sinusoids for 7 days after the ligation.[53]

A careful examination of the preoperative CT scan is useful to detect the number and diameter of the thick MHV tributaries draining the right paramedian sector of the donor liver. Anatomic variations, such as a venous variant type of small RHV with a large MHV might indicate the necessity for MHV reconstruction.[44] The indications for reconstruction of MHV tributaries can be determined based on our objective criteria.[54,55] First, discoloration of the liver surface should be observed after concomitant clamping of the MHV tributary and relevant hepatic artery for 5 minutes. Thereafter, only the hepatic artery is declamped and Doppler ultrasonography is performed. When hepatofugal portal flow is observed, the relevant area of the liver is confirmed to be congested. If the liver volume, excluding the area discolored by occlusion of the artery, is estimated to be insufficient for postoperative metabolic demand, (estimated graft volume less than 40% of the recipient's SLV), the MHV tributaries are reconstructed.

It is not rare to find thick, short hepatic veins during harvesting of a right liver graft (i.e., inferior right hepatic vein, middle right hepatic vein). Reconstruction of these vessels can be determined using the same criteria as for MHV reconstruction.

PORTAL VENOUS ANASTOMOSIS

In the preoperative evaluation of the donor, dynamic CT, visceral angiography, and dynamic CT with three-dimensional reconstruction provide detailed information about portal vein anatomy. Producing an anastomosis that is tension-free with wide enough orifices is a key determinant for successful portal vein anastomosis. Therefore, the portal vein on the recipient side should be dissected at the longest length possible during removal of the liver. On the donor side, a transverse portion of the portal vein has to have a long extrahepatic course to make it easier to obtain a longer portal vein in the left liver grafts than in the right liver grafts.

Portal venous thrombosis, sclerosis, and a size discrepancy between the graft and the recipient's portal vein are other issues that make it difficult or impossible to perform standard end-to-end anastomosis. These problems are usually overcome by use of an interposition vascular graft, vascular patch graft, or portal venoplasty.[56]

Trifurcation of portal vein

A common anomaly that requires attention during the donor operation is trifurcation of the portal vein in which the right lateral and right paramedian sectors are supplied separately. In this anatomic anomaly, the transverse portion of the portal vein is shorter than usual, and this necessitates a complete division of the portal vein tributaries to the caudate lobe when harvesting a left liver graft.

A right liver graft will have two portal branches. Some investigators excise the right paramedian and lateral portal vein with a side wall of the remaining donor portal veins as a patch. Defects in the remaining portal vein on the

donor side are repaired with a venous patch, by direct suturing of the defect, or with segmental resection and end-to-end anastomosis. These procedures add to donor risk and result in unsatisfactory portal reconstruction.[57] This anatomic variation can be overcome by one of three ways. First, venoplasty of these portal veins can be performed on the bench and anastomosed as one common orifice to the recipient's portal vein. Second, these branches can be separately anastomosed to the recipient's portal vein. Third, a cryopreserved vascular graft can be used.

ARTERIAL RECONSTRUCTION

The information provided by preoperative angiography is essential for surgical planning and donor safety.[58] Hepatic arteries are subject to many variations.[59,60] Basically, these variations can be summarized as follows: (1) an aberrant left hepatic artery originating from the left gastric artery; (2) an aberrant right hepatic artery originating from the superior mesenteric artery; and (3) aberrant accessory arteries, in addition to the original left (or original middle) hepatic artery, or in addition to the original or replaced right hepatic artery. Division and dissection of the hepatic artery should be planned and meticulously performed according to the preoperative and operative findings.

Hepatic arterial reconstruction in LDLT is technically difficult due to the existence of short and thin hepatic arteries on a liver graft. Marcos et al. reported that anastomosis under a microscope is usually unnecessary in adult recipients, especially with a right liver graft.[28] Hepatic artery thrombosis is a serious complication that occurs after orthotopic liver transplantation, and it might result in hepatic necrosis, biliary leakage, bacteremia, or mortality.[61] Mazzaferro et al.[62] reported a significant association between hepatic arterial thrombosis and the presence of hepatic arteries less than 3 mm in diameter. In LDLT, the median diameter of the arterial branch, especially in a left-sided graft, is less than 3 mm.[60] Thus, microsurgery has an inevitable and indispensable place in LDLT.[63,64]

Is reconstruction of all hepatic arterial branches necessary?

In the early series of LDLT, left liver grafts were mainly used, which had thin, short, and sometimes multiple arterial branches. Broelsch et al.[63] suggested that a double arterial supply to the liver graft is unsuitable for LDLT after two of three of their patients experienced hepatic artery thrombosis. To resolve this problem, Mori et al.[64] reconstructed all hepatic arteries of a liver graft. The Shinshu group,[65] however, demonstrated that reconstruction of all hepatic arterial branches was not necessary in their left liver graft series. Furthermore, Sakamoto revealed that the existence of aberrant hepatic arteries, especially in left liver grafts, allows the physician to obtain a thicker and larger hepatic artery for reconstruction.[60] An additional important note regarding the left liver is that dissection of the perivascular connective tissue around the umbilical portion of the portal vein must be avoided to maintain the collateral circulation among the segmental arteries.[65]

In our previous series, the frequency of multiple arterial orifices was 1% for right-sided liver grafts and 9% for left-sided liver grafts.[59] Whereas recent data from Marcos et al.[66] revealed that 12% (11/95) of consecutive right liver grafts have double arteries. Marcos et al. proposed that reconstruction of all arterial branches of right lobe liver grafts is necessary, claiming that no portion of right liver grafts is supplied by secondary arterial perfusion.[67] In their recent series, they anastomosed double arterial orifices with auto Y-shaped arterial grafts on the bench.[66] It is still controversial, however, whether all arterial stumps must be anastomosed in LDLT. We reported successful results with only one hepatic arterial reconstruction in both a left and right liver graft with multiple arterial stumps.[59,60,65] Redman[68] demonstrated that accessory hepatic arteries usually communicate with the original lobar arteries in the hepatic hilum, but they are not visualized on angiograms unless they actually function as collaterals.

Checking arterial communication in grafts

When multiple hepatic arteries exist, the largest

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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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

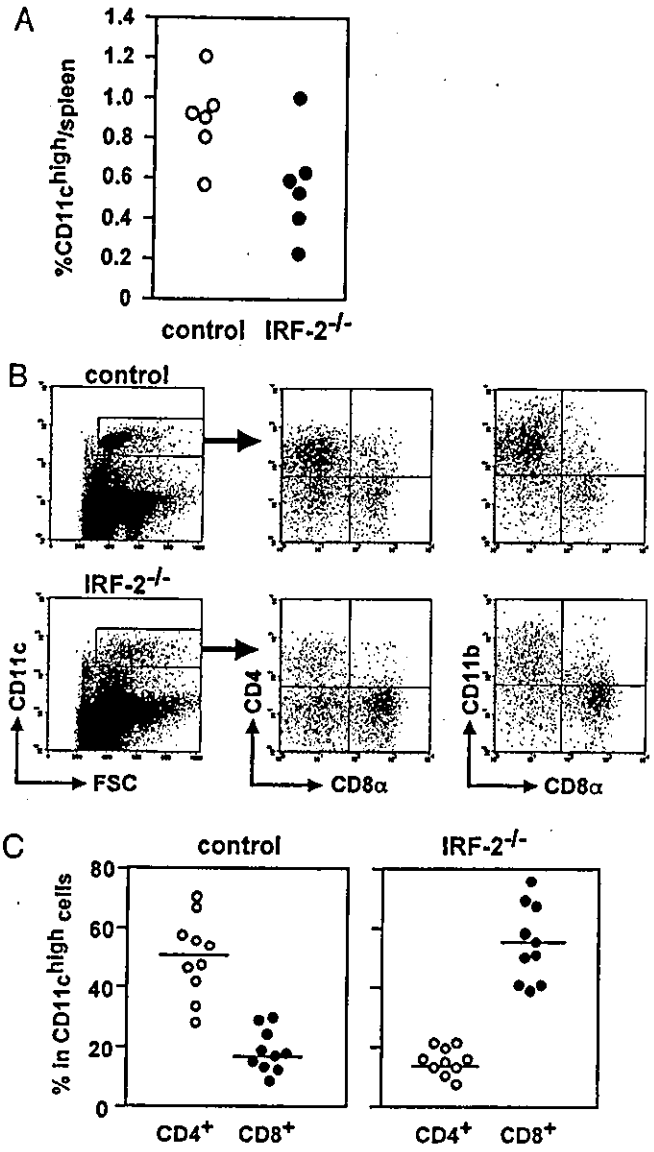

Fig. 1. Impaired splenic CD4+ DC subpopulation in IRF-2$^{-/-}$ mice. (*A*) The percentages of CD11c^{high} cells in viable spleen cells, where filled circles represent control littermates and open circles represent IRF-2$^{-/-}$ mice, respectively. (*B*) CD11c^{high} cells gated as indicated were analyzed 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*).

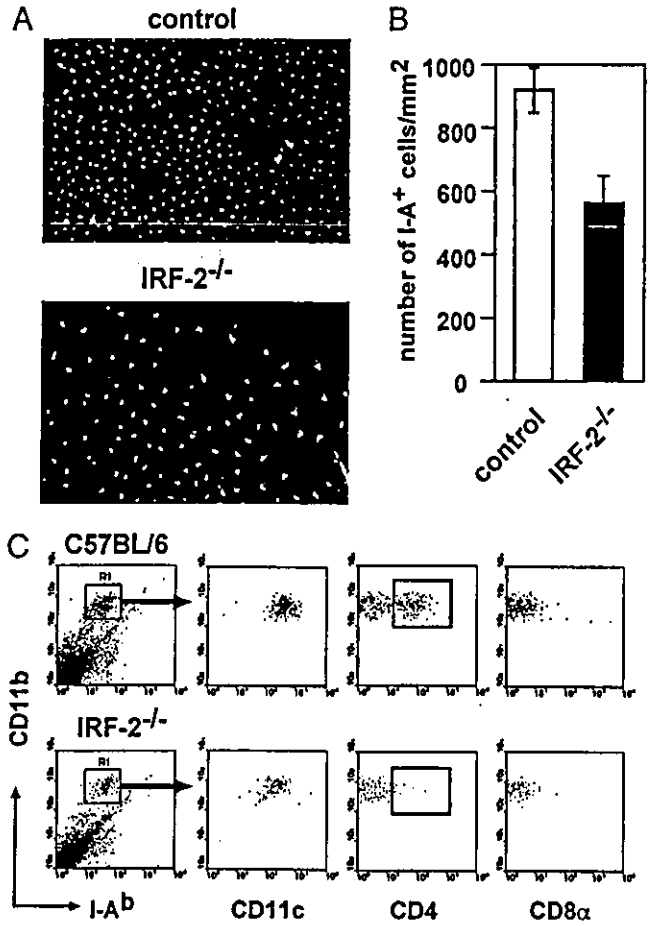

Fig. 2. Lack of CD4+ LCs in the epidermis of IRF-2$^{-/-}$ mice. Epidermal sheets were stained for I-A (*A*), and the numbers of I-A+ cells were counted (*B*). I-A+CD11b+ cells isolated from epidermis of control littermates (*Upper*) and IRF-2$^{-/-}$ mice (*Lower*) were gated as indicated and analyzed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

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 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-

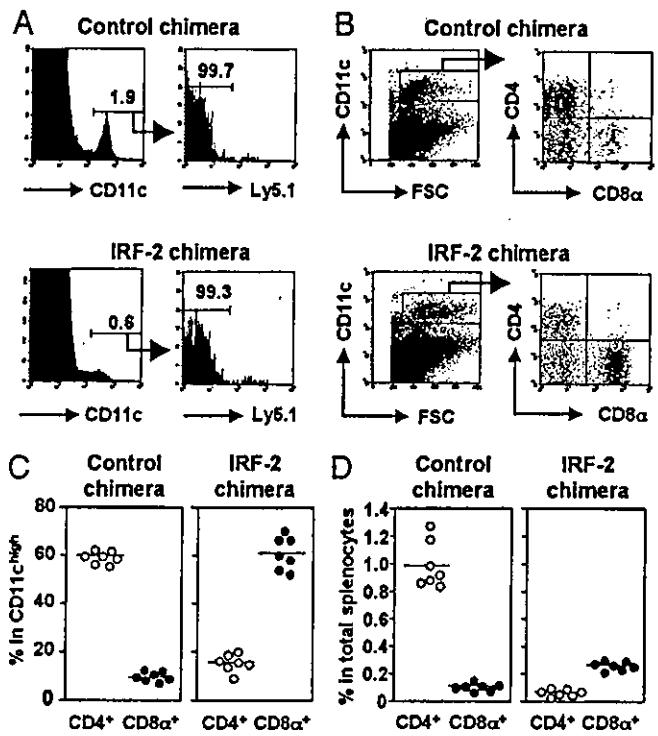

Fig. 3. Cell autonomous developmental defects in IRF-2-deficient BM cells. (*A*) In BM chimeras reconstituted with control BM cells (control chimera) or with IRF-2-deficient BM cells (IRF-2 chimera), >99% of splenic CD11c^{high} cells gated as indicated were negative for Ly5.1. (*B*) CD4 vs. CD8α profiles 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.

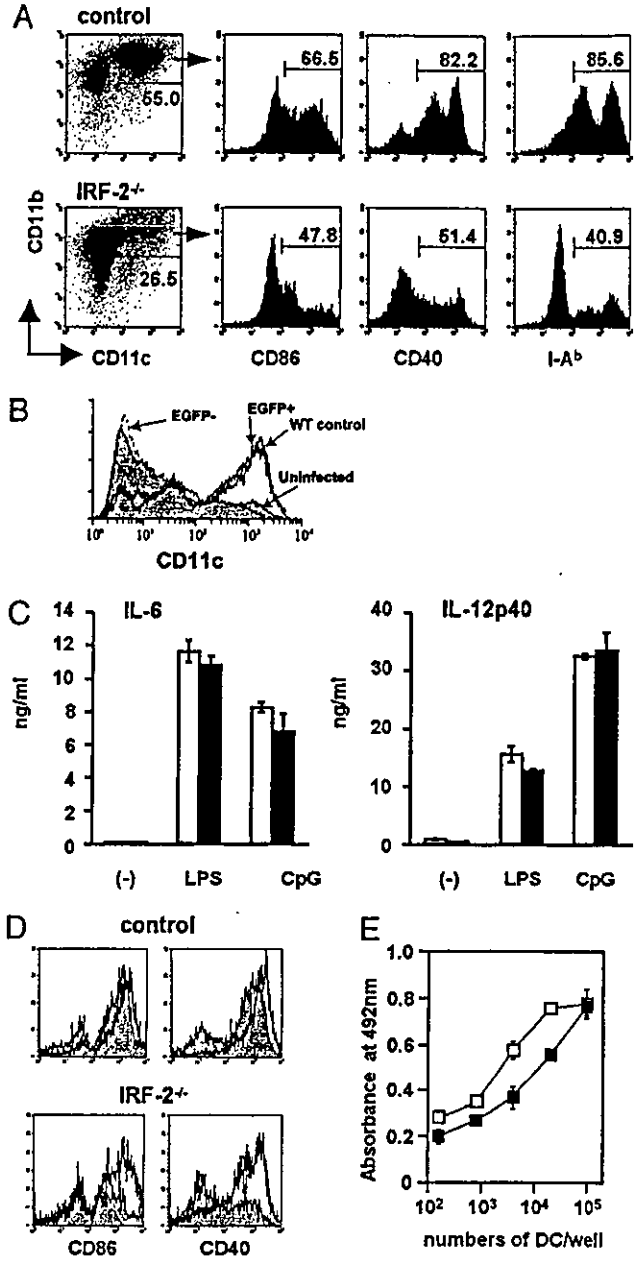

Fig. 4. Inefficient generation of mature DCs from IRF-2-deficient BM cells *in vitro*. (*A*) BM-DCs generated *in vitro* were stained for CD11c and CD11b, together with one of three activation markers, as indicated. Numbers indicate the percentages of cells within the gates (a representative result of 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.

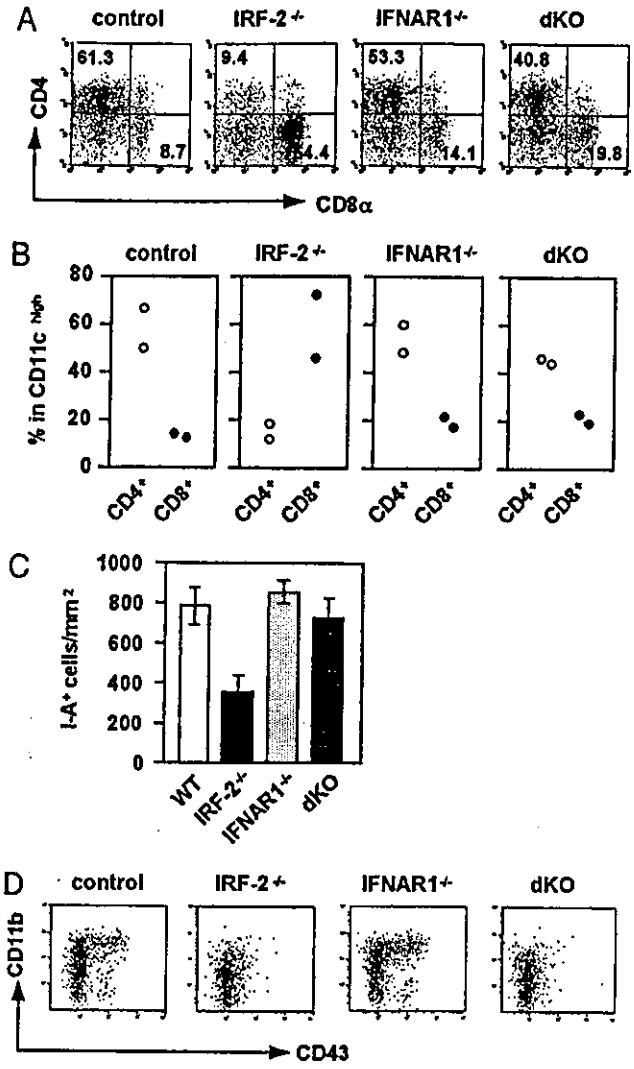

Fig. 5. Roles of IFN-α/β signals in the development of CD4+ DCs and NK cells. (*A* and *B*) Splenic CD11c^{high} cells from IRF-2$^{-/-}$, IFNAR1$^{-/-}$, IRF-2$^{-/-}$IFNAR1$^{-/-}$ (dKO) mice and control littermates were analyzed for CD4+ and CD8+ DC subsets. Numbers indicate the percentages of cells 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.

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