

Rapid Loss of Graft Immunogenicity and Transient Hyporesponsiveness to The Donor Antigen After Rat Liver Transplantation

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In orthotopic liver transplantation from Lewis (LEW) into Wistar Furth (WF) rats, a transient acute rejection response is usually followed by spontaneous tolerance of the graft. In this study, we investigated the alloimmunostimulatory ability of the graft and recipient spleen after liver transplantation, and studied the kinetic response of the recipient to the donor antigen. Alloimmunogenicity of the liver graft and recipient spleen, and kinetic response of the recipient were investigated in in vivo system. Alloimmunostimulatory ability of the liver graft disappeared by day 3 after transplantation. Alloimmunostimulatory capacity of recipient spleen was maximum 12 hours after grafting and rapidly disappeared thereafter. Challenge with normal LEW lymphocytes into recipient WF rats at 14, 28, 56 days after liver transplantation induced a progressive increase in rejection response. Our results suggest that rapid loss of graft immunogenicity and transient hyporesponsiveness to the donor antigen occur after grafting in our model.

Key words: antigen presenting cells, acute rejection response, graft immunogenicity, liver transplantation; rat, T cells response

Introduction

In alloantigen recognition, recipient T cells directly respond to major histocompatibility complex (MHC) class I and II molecules on donor antigen presenting cells (APCs).¹⁻³⁾ This direct recognition is responsible for the in vitro response to alloantigens, and likely relates to the acute rejection response in transplantation.⁴⁻⁶⁾

Dendritic cells are the most effective APCs⁷⁾ and are known to overexpress MHC class I and II antigens, adhesion and costimulatory molecules.⁸⁻¹⁰⁾ Furthermore, cells obtained from solid organs show potent alloimmunostimulatory activity in vitro.^{11,12)} On the other hand, donor passenger leukocytes disappear from the kidney,^{4,13)} heart¹⁴⁾ and skin¹⁵⁾ grafts, an event considered to be an essential mechanism for graft tolerance.^{16,17)} However, only a few studies have examined the kinetics of graft alloimmunogenicity.

Transplantation tolerance can be induced by various immunosuppressive modalities, although liver grafts are often spontaneously accepted in some strain combinations of laboratory animals.¹⁸⁻²⁰⁾ The underlying mechanisms involved in the spontaneous tolerance of the transplanted liver have been proposed.²¹⁻²⁹⁾ Studies from our laboratories have previously demonstrated that primed cytotoxic T lymphocyte precursors (CTLp) were eliminated or inactivated in long-term surviving recipient WF rats, while non-primed CTLp, which mediated the rejection response to the grafted liver, were present but graft immunogenicity disappeared in tolerant rats.³⁰⁾ We have suggested that these findings were responsible for the development and maintenance of tolerance.

In the present study, we examined kinetics of the immunostimulatory ability of the transplanted liver and immune response of the recipient to the donor antigen. Our results showed that the graft immunogenicity disappeared rapidly after liver transplantation. There was also a transient hyporesponsiveness of the recipient to the donor antigen during early phase of the transplantation.

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Materials and Methods

Animals

Male Lewis (LEW, RT-1A¹B¹D¹) rats weighing 230-280g were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). Male Wistar Furth (WF, RT-1A⁰B⁰D⁰) rats weighing 250-320g were obtained from a colony maintained in the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine. The experimental protocol was approved by the Ethics Review Committees for Animal Experimentation of the participating institutions.

Measurement of serum transaminase and total bilirubin

Blood samples were obtained from the tail vein of recipient WF rats at specific time intervals. Serum glutamic pyruvic transaminase (SGPT) and total bilirubin (TB) were measured using an autoanalyzer (UVIDEC-77, Japan Spectroscopic Co., Tokyo, Japan). Normal values for SGPT and TB are below 35IU/L and 0.4mg/dl, respectively.

Preparation of alloantibody

Female LEW and WF rats (5-8 weeks old) were inoculated intraperitoneally with WF or LEW spleen cells ($1-2 \times 10^8$) once a week, respectively. Ten days after the 5th such inoculation, sera obtained from live rats were used as an antibody against MHC alloantigens.

Preparation of nylon-wool adherent and nonadherent cells

Spleen cells (2×10^8) from LEW liver-transplanted WF rats were incubated in a nylon-wool column for 30min at 37°C under 5% CO₂ in air. After addition of 50ml RPMI 1640 to the nylon-wool column, the cells obtained by crumpling the nylon-wool were used as the adherent cells and passed through the column were used as the nonadherent cells.

Cell panning

Petri dishes (60mm, Falcon 1016, Becton Dickinson, Meylan Cedax, France) were coated with 20 μ g/ml of rabbit anti-rat IgG (Fc) (Bethyl Laboratories Inc., Montgomery, AL) in 50mM Tris-HCl buffer (pH 9.5) for 120 min at room temperature. After washing twice with 0.15 M NaCl, the dishes were incubated overnight with 3ml BSA (1mg/ml) in PBS at room temperature. Spleen cells (3×10^8) suspended in RPMI 1640 were cultured with LEW anti-WF antiserum (diluted 1:20)

for 30 min at 4°C. After duplicate washing with RPMI 1640, cells were suspended in 0.5mM EDTA-PBS (Nacalai Tesque Inc., Kyoto, Japan) with 5% FCS and placed in dishes (2×10^7 /dish) followed by incubation for 120min at room temperature. Nonadherent cells ($2-4 \times 10^7$) in the dishes were cultured with LEW anti-WF antiserum and panned again. Nonadherent cells in the dishes were then gently harvested for flow cytometric analysis.

Flow cytometric analysis

Cells were incubated with WF anti-LEW antiserum, diluted (1:100) with Eagle's MEM for 30 min at 4°C. After washing twice with PBS, cells were incubated with FITC-labeled goat anti-rat IgG (Fc) (Chemicon International Inc., Temecula, CA), diluted 1:100) for 30 min at 4°C. After washing twice with PBS, cells were suspended in PBS and examined in a FACScan (Becton Dickinson, Mountain View, CA). Cells (1×10^4) were sorted and single histograms were obtained after gating on small lymphocyte population to concentrate the analysis.

Surgical preparation

Orthotopic liver transplantation was performed according to the cuff and nonarterialized method without immunosuppression.³¹⁾ Bile duct anastomosis was performed using a silastic stent tube (0.6mm OD). In the donor operation, after systemic administration of 100 units of heparin, the liver was first perfused through the portal vein with 10ml of ice-cold lactated Ringer's solution containing 10units/ml of heparin, then removed and placed in 4°C lactated Ringer's solution. Donor operation lasted approximately 30 min. Following removal of the recipient liver, the donor liver was orthotopically placed in the recipient and the suprahepatic inferior vena cava was anastomosed with 7-0 nylon, using a continuous suture technique. The unhepatic period was < 16 min and the recipient operation lasted approximately 60 min. All procedures were performed by the same surgeon (WG).

Skin transplantation was performed using fullthickness grafts, 1.5 x 1.5cm in size. Rejection of skin grafts was determined when 90% of the graft became necrotic.

Intraperitoneal challenge with homogenized liver or spleen cells into long-term surviving recipient WF rats with a LEW liver transplant

The liver was perfused through the portal vein with 20ml of ice-cold lactated Ringer's solution containing

10units/ml of heparin, Then it was removed and homogenized in a manual homogenizer. The homogenate pelleted by centrifugation for 5 min at 1,000 rpm was then injected intraperitoneally into recipient WF rats that survived for > 100 days after transplantation and showed no serological evidence of liver dysfunction. Liver homogenates consisted microscopically of parenchymal and nonparenchymal cells. After intraperitoneal injection of liver homogenates, blood samples were obtained on days 0, 5, 6 and 14. Spleen cells from recipient WF rats were also prepared and intraperitoneally injected into another recipient WF rats with LEW liver transplants.

In vivo administration of CsA

In some experiment, CsA (3mg/kg/day) was administered intramuscularly into naive WF rats on days 2, 1 and 0 before liver transplantation.

Statistical analysis

All data were expressed as mean \pm standard deviation (SD). Differences between groups were examined for statistical significance using the Student's *t*-test. A *P* value less than 0.05 denoted the presence of a statistically significant difference.

Results

Transient rejection response in recipient WF rats after orthotopic transplantation of LEW liver

Orthotopically transplanted LEW liver was accepted in WF rats without immunosuppressive therapy, and recipient rats survived for > 200 days except for technical failure. We first carried on the day course study of liver damage to examine the peak of serological rejection responses after transplantation. As shown in Fig. 1. SGPT and TB levels increased and reached peak values on day 4, but returned to normal levels within 14 and 28 days, respectively. When the LEW liver was transplanted into LEW rats, SGPT but not TB level slightly increased on one day but rapidly decreased thereafter. Histologically, cellular infiltrates around the portal tract and in sinusoids were observed in the grafted LEW liver in recipient WF, but not LEW rats 4 days post-transplantation (Fig. 2A, B). Cellular infiltration was most evident 2-3 weeks after grafting (Fig. 2C), but was markedly decreased in the grafted LEW liver obtained from long-term surviving recipient WF rats (Fig. 2D).

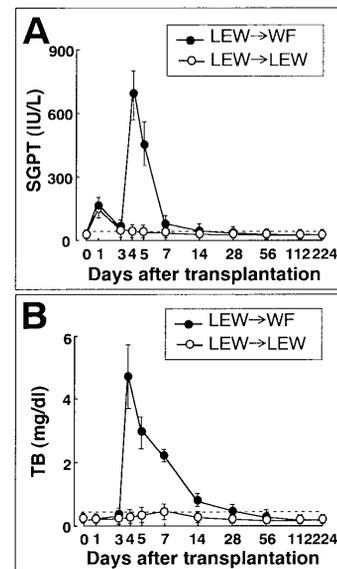


Fig. 1. (A) Serum levels of glutamic pyruvic transaminase and (B) total bilirubin after liver transplantation. For A and B, LEW livers were orthotopically transplanted into WF (●) and LEW (○) rats. Each group consisted of 6-8 rats.

Table 1. SGPT in the long-term surviving recipient WF rats after an intraperitoneal challenge with liver homogenates

| Inoculated tissues | SGPT (IU/L) ^a | | | |
|-----------------------------------------------------------------------------|--------------------------|--------|--------|-----------------------|
| | 0 | 5 | 6 | 14(days) ^b |
| Normal PVG liver homogenates | 30±4 | 35±4 | 31±6 | 25±3 |
| Normal LEW liver homogenates | | | | |
| 50% ^c | 24±4 | 235±57 | 242±43 | 19±2 |
| 10% | 30±4 | 244±52 | 202±68 | 28±4 |
| 5% | 23±5 | 97±39 | 199±23 | 27±7 |
| Grafted LEW liver homogenates from WF rat | | | | |
| 1 day ^d | 27±6 | 127±19 | 121±13 | 31±4 |
| 2 day | 24±3 | 40±11 | 83±19 | 26±5 |
| 3 day | 29±7 | 30±6 | 28±5 | 27±4 |
| 7 say | 30±5 | 27±4 | 31±2 | 29±3 |
| 14 day | 25±2 | 27±2 | 22±3 | 28±3 |
| Grafted LEW liver homogenates from LEW rat | | | | |
| 3 day ^d | 22±4 | 223±51 | 219±37 | 24±7 |
| 14 day | 24±5 | 182±57 | 215±48 | 24±4 |
| Grafted LEW liver homogenates from WF rat plus normal LEW liver homogenates | 29±5 | 215±59 | 179±46 | 32±4 |

^aValues represent mean \pm SD; n=3.

^bDays after intraperitoneal challenge.

^c% homogenates of the total liver volume from a normal LEW rat.

^dTime when grafted LEW liver homogenates were obtained after grafting.

Loss of alloimmunostimulatory ability of LEW liver grafts in recipient WF rats

In the next step, we investigated kinetics of the alloimmunostimulatory ability of the graft following LEW liver transplantation. As shown in Table 1. intraperitoneal challenge using 50% homogenates of the total liver volume from third-party PVG (RT-1A^cB^cD^c) rats into long-term surviving recipient WF rats did not induce a rise in SGPT levels although it efficiently

sensitized WF animals for PVG skin grafts (data not shown). Challenge with normal LEW liver homogenates (>5% volume) induced a transient rise in SGPT levels, which reached a peak level on days 5-6. In the following experiments, we used >50% homogenates of the total liver volume for intraperitoneal challenge.

Challenge with grafted LEW liver homogenates obtained from recipient WF rats on days 1 and 2, but not on days 3, 7 or 14 post-transplantation, induced a transient rise in SGPT levels. Grafted LEW liver homogenates obtained from syngeneic recipient LEW rats on days 3 and 14 after transplantation induced a transient elevation of SGPT levels. Challenge with grafted LEW liver homogenates from recipient WF rats obtained on day 3 together with the normal LEW liver

homogenate (volume ratio, 3 : 1) led to a transient elevation of SGPT levels. These results suggested absence of suppressive factors in the grafted LEW liver. Administration of normal LEW liver homogenates into naive WF and LEW rats did not increase SGPT levels (data not shown). Histological changes in the grafted LEW liver in long-term surviving recipient WF rats 7 days after intraperitoneal administration of normal LEW liver homogenates included cellular infiltration around the portal tract, focal liver necrosis and regeneration of hepatocytes (Fig. 2E). These changes were not observed in rats injected with grafted LEW liver homogenates obtained from recipient WF rats on day 3 post-transplantation (Fig. 2F).

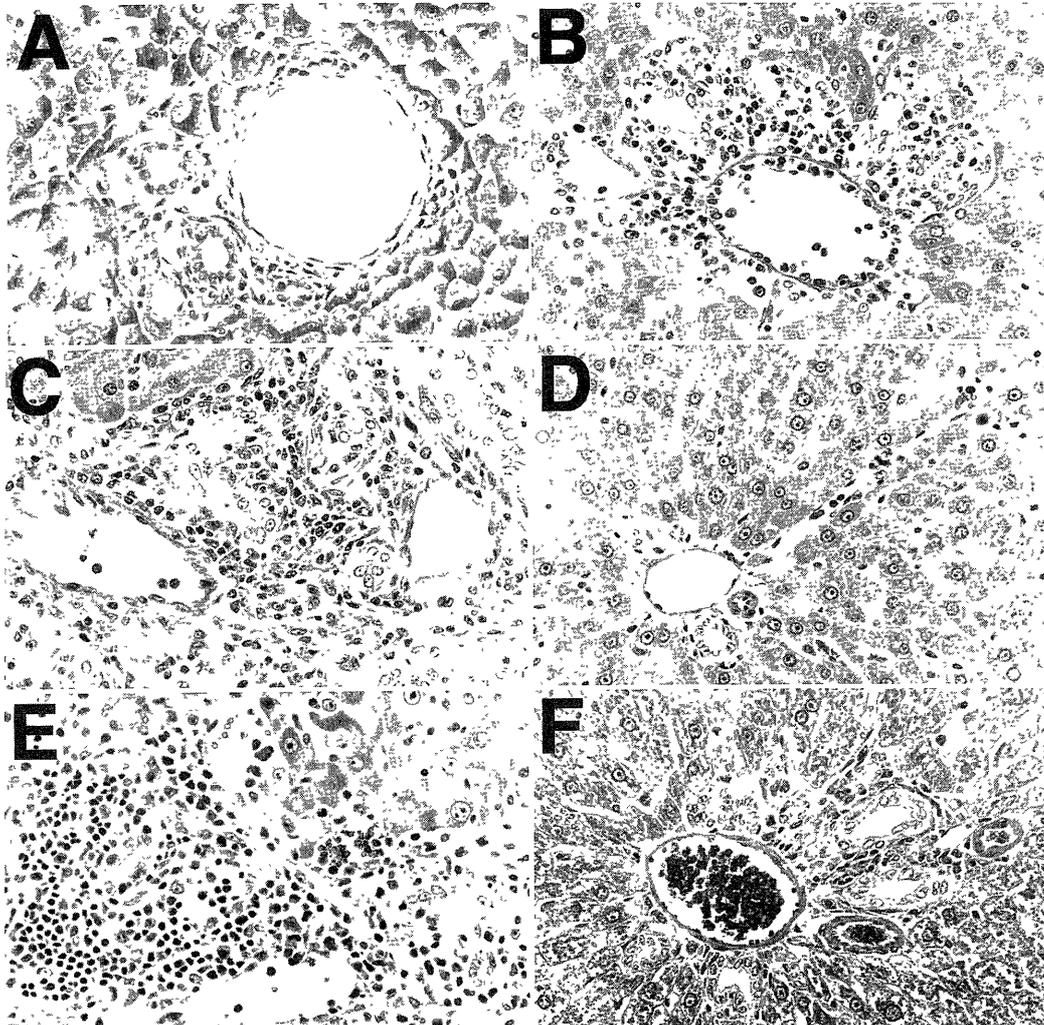


Fig. 2. Histological appearance (hematoxylin and eosin staining) of grafted LEW liver. Grafted LEW livers from recipient LEW (A) and WF (B) rats were obtained on day 4 after transplantation. In C, grafted LEW livers from recipient WF rats were obtained on day 14 after transplantation. In LEW to WF rat combination, grafted LEW livers were obtained from long-term surviving recipient WF rats before (D) and 7 days after (E) intraperitoneal administration of normal LEW liver homogenates. The same animals were used for this kinetic study (D and E). For F, grafted LEW livers were obtained from long-term surviving recipient WF rats on 7 days after intraperitoneal administration of grafted LEW livers, which were gained from recipient WF rats on day 3 after transplantation. (Magnification, A, B, C, D, E, F, x100).

Alloimmunostimulatory ability of recipient WF splenocytes after LEW liver grafting

We examined alloimmunostimulatory ability of recipient WF spleen cells (Table 2). No elevation of SGPT levels was observed following intraperitoneal administration of spleen cells (6×10^8) from naive WF rat into long-term surviving recipient WF rats. Intraperitoneal challenge with spleen cells (2×10^7) from naive LEW rat induced a transient elevation of SGPT levels. In the following experiments, we used spleens from two WF recipient rats.

Table 2. SGPT in the long-term surviving recipient WF rats after an intraperitoneal challenge with spleen cells

| Inoculated tissues | SGPT (IU/L) ^a | | | |
|----------------------------------------------------|--------------------------|--------|--------|-----------------------|
| | 0 | 5 | 6 | 14(days) ^b |
| Normal WF spleen cells | 27±5 | 29±4 | 21±4 | 25±3 |
| Normal LEW spleen cells | 24±6 | 250±69 | 296±42 | 26±7 |
| Recipient WF spleen cells after LEW liver grafting | | | | |
| 3 hours ^c | 24±3 | 29±4 | 36±3 | 33±3 |
| 12 hours | 24±9 | 128±21 | 96±27 | 24±6 |
| 12 hours (nylon-wool adherent cells) | 29±3 | 106±23 | 132±29 | 23±7 |
| 12 hours (nylon-wool nonadherent cells) | 26±3 | 31±5 | 28±4 | 27±4 |
| 24 hours | 27±8 | 43±25 | 60±17 | 25±3 |
| 2 day | 22±4 | 27±3 | 30±5 | 26±6 |
| 2 day (CsA-treated) | 27±5 | 65±5 | 73±21 | 30±5 |
| 3 day | 24±5 | 22±2 | 30±5 | 29±4 |

^aValues represent mean ± SD; n=3.

^bDays after intraperitoneal challenge.

^cTime when recipient WF spleen cells were obtained after grafting.

In long-term surviving recipient WF rats, intraperitoneal challenge with spleen cells from different recipient WF rats obtained 3 hours after LEW liver transplantation did not induce a transient elevation of SGPT level. However, spleen cells obtained 12 and 24 hours after grafting induced a transient elevation of SGPT level. Challenge with spleen cells obtained 12 hours after grafting was associated with the highest SGPT levels. Intraperitoneal challenge using spleen cells obtained at 2-3 days after LEW liver grafting did not induce a rise in SGPT level. We also investigated the immunostimulatory ability of spleen cells that had been passed through a nylon-wool column. Nylon-wool adherent but not nonadherent recipient WF spleen cells obtained 12 hours after LEW liver transplantation induced a transient elevation of SGPT level, while intraperitoneal challenge, using spleen cells obtained on day 2 after grafting from CsA-treated recipient WF rats elicited a rise in SGPT level. These findings suggested that the transient elevation of SGPT was due to donor APCs, but not due to donor and host T cells.

Sensitization of third party PVG rats against LEW by challenge with LEW-liver carrying WF spleen cells

Sensitization against LEW by challenge with LEW-liver carrying WF spleen cells was investigated using skin grafts. Spleen cells were injected intraperitoneally into PVG rats 3 days before skin grafting. As shown in Table 3, intraperitoneal challenge with LEW-liver carrying WF spleen cells obtained 12 hours, but not on day 3 after transplantation, sensitized PVG rats against LEW.

Table 3. Survival of the skin grafts on PVG rats after an intraperitoneal challenge with spleen cells obtained from LEW-liver carrying WF rats

| Challenge with spleen cells | Skin grafts | Skin graft survival (days) | MGS ^a (days±SD) |
|--------------------------------------------------------|-------------|----------------------------|---------------------------------|
| none | LEW DA | 8,8,8,9, 8,8,9, | 8.2±0.5 8.3±0.6 |
| naive WF spleen cells (3×10^8) ^b | LEW DA | 8,8,8,9, 8,8,8, | 8.2±0.5 8.0±0.0 |
| 12h ^c (1×10^7) | LEW DA | 5,5,5,5,6,6, 8,8,8, | 5.3±0.5 ^d 8.0±0.0 |
| 12h (3×10^8) | LEW DA | 5,5,5,5,6,6, 8,8,9, | 5.3±0.5 ^d 8.3±0.6 |
| 3 days (1×10^7) | LEW DA | 8,8,8,9,9,9, 8,8,9, | 8.4±0.5 ^d 8.3±0.6 |
| 3 days (3×10^8) | LEW DA | 8,8,8,8,9,9, 8,8,9, | 8.3±0.5 ^d 8.3±0.5 |

^aMGS: Mean graft survival.

^bCell number.

^cTime when the host spleen cells were obtained after LEW liver transplantation.

^d $P < 0.01$.

Flow cytometric analysis

We also investigated the presence of donor LEW cells in the recipient WF spleen by flow cytometry. As shown in Fig. 3, after panning WF cells, LEW cells were detected 12 hours after transplantation. The number of LEW cells markedly reduced on day 3. The number of cells is 1350 ± 220 and 69 ± 26 events, respectively (n=3).

In vivo kinetic response of recipient WF rats to donor antigen after LEW liver transplantation

In the next step, we examined the kinetics of in vivo response of recipient WF rats to donor antigen after liver transplantation. Following LEW liver transplantation into WF rats, the rise in SGPT decreased on day 7 (SGPT= 69.3 ± 14.2 ; n=8) and returned to the normal level within 14 days (SGPT= 30.1 ± 7.5 ; n=8) as described above. Then, intraperitoneal challenge with normal LEW spleen cells (1×10^8) was performed

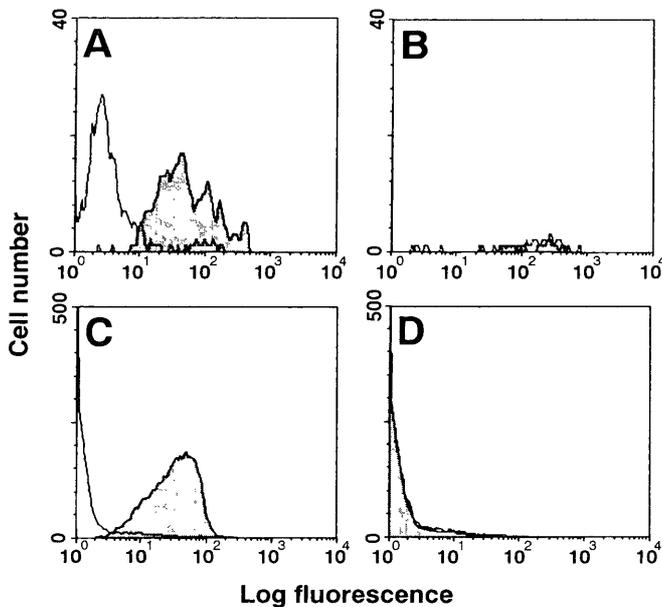


Fig. 3. Donor LEW cells in the spleen of recipient WF rats 12 hours (A) and 3 days (B) after liver transplantation, and specificity analysis of antiserum (C and D). Recipient spleen cells after removal of WF cells with panning procedure (A and B), normal LEW spleen cells (C) and normal WF spleen cells (D) were incubated with anti-LEW antiserum and FITC-labeled goat anti-rat IgG (Fc) (gray area). Open area, FITC-labeled goat anti-rat IgG only. Results of a single experiments, representative of three experiments with similar results.

in recipient WF rats after liver transplantation. As shown in Table 4, no response was observed in recipient WF rats 7 days after transplantation to a challenge with donor lymphocytes. However, challenge with the same cells into recipient WF rats 14, 28, and 56 days after liver transplantation showed a progressive increase in SGPT due to liver damage, and SGPT levels were maximum when LEW spleen cells were challenged into recipient WF rats over 100 days after grafting. These results suggested that recipient WF rats showed transient hyporesponsiveness to the donor antigen after liver transplantation and the immune re-

Table 4. SGPT in recipient WF rats after an intraperitoneal challenge with normal LEW spleen cells

| The days after grafting | SGPT (IU/L) ^a | | | |
|-------------------------|--------------------------|--------|--------|-----------------------|
| | 0 | 5 | 6 | 14(days) ^b |
| 7 POD ^c | 62±29 | 55±15 | 50±8 | 29±7 |
| 14 POD | 36±3 | 54±14 | 71±31 | 35±5 |
| 28 POD | 32±9 | 65±41 | 85±58 | 27±3 |
| 56 POD | 32±3 | 102±56 | 98±25 | 30±5 |
| 84 POD | 31±4 | 100±43 | 158±43 | 32±2 |
| 112 POD | 23±6 | 308±36 | 330±82 | 29±4 |

^aValues represent mean ± SD; n=3.

^bDays after intraperitoneal challenge.

^cPOD: postoperative days.

sponse of the recipient recovered later.

Discussion

Following transplantation, donor passenger leukocytes migrate from the graft to secondary lymphoid organs of the recipient, a process known to initiate the primary immune responses.^{14,15} In the present study, we showed that the alloimmunostimulatory ability of the grafted LEW liver disappeared in recipient WF rats by day 3 after transplantation. This was confirmed by the findings that 5% volume of normal LEW liver homogenates, but not total volume of grafted LEW liver homogenates, could induce transient rejection responses in long-term surviving LEW-liver carrying recipient WF rats (Table 1) and that grafted LEW liver parked for 3 days in primary WF recipients elicited no elevation of SGPT and TB levels when the graft was re-transplanted into secondary naive WF rats (n=3, data not shown). Furthermore, our results showed that the loss of alloimmunostimulatory ability was due to migration of donor APCs from the liver graft into the recipient. Loss of graft immunogenicity has also been shown in kidney transplantation. Batchelor *et al.*³² showed that long-term surviving (AS x AUG) F1 rat kidney allografts did not elicit strong alloimmune reactions when re-transplanted into secondary AS recipients of the same genotype. Those grafts did not contain bone marrow-derived passenger leukocytes of the donor.¹³ Dendritic cells from solid organs such as heart, kidney and liver showed potent allostimulatory ability *in vitro*.^{11,12} The time when grafted organs lose alloimmunostimulatory ability may vary depending on the grafts and strain combinations used. Chui *et al.*³³ showed that depletion of passenger leukocytes in kidney grafts was achieved by parking the graft for more than 14 and 50 days in recipient (AS x AUG) F1 to AS and AUG to AS strain combinations, respectively. Welsh *et al.*³⁴ suggested that the loss of immunogenicity of kidney graft was a slow process.

A number of immune mechanisms have been postulated for the spontaneous tolerance of the transplanted liver.²¹⁻²⁹ Sun *et al.*^{24,25} suggested that spontaneous liver transplant tolerance was a high-dose or activation-associated immune phenomenon. They argued that donor leukocytes appeared to be centrally involved in induction of liver acceptance. Our study demonstrated that immune activation after liver transplantation was due to donor APCs that migrated from the graft into the recipient lymphoid tissues and that allostimulatory ability of recipient spleen was at maximum in those obtained at 12 hours after grafting. Thus, a rapid anti-

gen presentation by a large number of donor APCs from the graft may result in immunological modulation in the recipient in liver transplantation. Interestingly, severe but transient hyporesponsiveness against the donor antigen occurred early after liver grafting in our model.

We noted a marked reduction of alloimmunostimulatory cells that migrated from the liver graft in the recipient spleen by day 3 post-transplantation. In comparison to our results, Bishop *et al.*²⁷⁾ reported the presence of donor cells in recipient lymphoid tissues, which reached a peak number on day 1 but not 12 hours after liver transplantation. Larsen *et al.*¹⁴⁾ reported that donor-derived dendritic leukocytes disappeared in the recipient spleen by day 6 after heart transplantation. Furthermore, this study showed that donor-type immunostimulatory ability in the recipient spleen was maintained much longer in CsA-treated recipient rats. Cytokine production and recipient CD8 T cells may be associated with the rapid depletion of donor cells.^{27,35)} On the other hand, we observed the restoration of response to the donor antigen in the recipients 14-28 days after liver transplantation, but most donor APCs from the graft had already been eliminated in the recipient by that time.

We showed that MHC antigens on the grafted LEW liver to be intact since a transient rejection response occurred in the long-term surviving grafts by challenge with donor LEW cells. We previously found that the transient rejection response was mediated by CD8 T cells.^{30,36)} Appropriate stimulation by donor APCs resulted in a transient rejection against the grafted liver. Primed cells are able to reject the graft without involving donor APCs,³²⁾ but those cells are eliminated or inactivated after liver transplantation as we previously demonstrated.³⁰⁾

Alloimmunostimulatory cells migrating from the liver graft were nylon-wool adherent, yet flow cytometric analysis showed a number of donor lymphocytes in the recipient spleen to be CD3⁺ cells (data not shown). Donor T cells may play a role in liver acceptance due to host T cell modulation in nonrejecter rat strain combination.^{24,25)} However, donor T cells do not seem to be responsible for deletion of primed T cells in the recipient. We previously showed that primed CTLs could be recovered from WF rats immunized with normal LEW liver homogenates containing donor T cells, but not from LEW liver-transplanted WF recipient rats,³⁰⁾ suggesting that the liver graft itself is involved in the elimination of primed T cells.²⁹⁾

In conclusion, a rapid reduction of donor APCs and transient low reactivity of the host immune system might explain the induction of tolerance in our model.

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