33. Van den Berg CW, Harrison RA, Morgan BP. The sheep analogue of human CD59: purification and characterization of its complement inhibitory activity. Immunology 1993; 78: 349.
34. Asch AS, Kinoshita T, Jaffe EA, Nussenzweig V. Decay-accelerating factor is present on cultured human umbilical vein endothelial cells. J Exp Med 1986; 163: 221.
35. Bryant RW, Granzow CA, Siegel MI, et al. Phorbol ester increases synthesis of decay-accelerating feactor, a phosphatidylinositol-anchored surface protein, in human endothelial cell. J Immunol 1990; 144: 593.
36. Bryant RW, Granzow CA, Siegel MI, et al. Wheat germ agglutinin and other selected lectins increase synthesis of decay-accelerating factor in human endothelial cells. J Immunol 1991; 147: 1856.
37. Berger M, Medof ME. Increased expression of complement decay-accelerating factor during activation of human neutrophils. J Clin Invest 1987; 79: 214.
38. Cho SW, Oglesby TJ, Hsi BL, Adams EM, Atokinson JP. Characterization of three monoclonal antibodies to membrane cofactor protein (MCP) of the complement system and quantification of MCP by radioassay. Clin Exp Immunol 1991; 83: 257.
39. Kojima A, Iwata K, Seya T, et al. Membrane cofactor protein (MCP,CD46) protects cells predominantly from alternative complement pathway-mediated C3-fragment deposition and cytolysis. J Immunol 1993; 151: 1519.
40. Oglesby TJ, Allen CJ, Liszewski MK, White DJG, Atkinson JP. Membrane cofactor protein (CD46) protects cells from complement-mediated attack by an intrinsic mechanism. J Exp Med 1992; 175: 1547.
41. Miyagawa S, Shirakura R, Matsumiya G, et al. Possibility for prevention of hyperacute rejection by DAF and CD59 in xenotransplantation. Transplant Proc 1994; 26: 1235.

Received 22 February 1994.
Accepted 21 April 1994.

0041-1337/94/5807-840$03.00/0
TRANSPLANTATION
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Vol. 58, 840–845, No. 7, October 15, 1994
Printed in U.S.A.

THE INDUCTION OF IMMEDIATE EARLY GENES IN POSTISCHEMIC AND TRANSPLANTED LIVERS IN RATS

ITS RELATION TO ORGAN SURVIVAL[1]

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The protein products of the immediate early genes (IEG)s have been proposed to play an important role in long-term tissue plasticity such as cell repair or programmed cell death. The expression of liver IEGs was studied following liver ischemia (LI) or OLT in rats. In LI, 60 min of warm ischemia was induced in shunted rats (shunt LI group; 100% survival) and nonshunted rats (nonshunted LI group; poor survival). In OLT, donor livers were transplanted into the recipients within 1 hr (fresh liver OLT group; 100% survival) or after 24 hr of storage using University of Wisconsin solution (preserved liver OLT group; poor survival). Using both models, IEG mRNAs (c-*fos* and c-*jun*) were analyzed by Northern blot hybridization at various times before and after reperfusion. The expression of liver IEGs was not induced by warm ischemia and cold preservation alone. Reperfusion of livers following warm ischemia or cold preservation resulted in a distinctly different pattern of gene expression in viable and nonviable livers. In shunted LI and fresh liver OLT groups (viable), c-*fos* and c-*jun* mRNAs increased markedly to a peak value within 1–2 hr of reperfusion, returning to basal level by 3 hr. In nonviable livers, the level of these mRNAs was detected continuously at 3 hr of reperfusion in the nonshunted LI model and also at 6 hr after reperfusion in the preserved liver OLT group.

Our data suggest that a protracted pattern of expression of c-*fos* and c-*jun* in the liver at the early stage of reperfusion might be correlated with the severity of liver transplant-related insults and subsequent graft failure.

Since the introduction of University of Wisconsin (UW)* cold preservation solution to liver transplantation, posttransplant liver function has improved and the viable pres-

[1] This work was supported by a grant from the Royal Brisbane Children's Hospital, Liver Transplant Fund, the Sasakawa Foundation, and Princess Alexandra Hospital Research and Development Foundation.

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* Abbreviations: IEG, immediate early gene; LI, liver ischemia; UW, University of Wisconsin.

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ervation time of the liver has been extended to 24–30 hr, resulting in a significant increase in organ availability and sharing (*1*). Despite these advances, primary nonfunction and dysfunction of liver grafts following transplantation are still common problems which occur at a very early stage, preceding the immunological rejection by many days (*2, 3*). Although the precise mechanisms of such an allograft failure are unknown, the vulnerability of the liver to warm ischemia, cold preservation, and reperfusion injury has been suggested to be a key factor in immediate posttransplant graft function (*4*). We have reported the beneficial effects of various pharmacological agents or recipient manipulation on warm ischemia and preservation/reperfusion injury (*5–7*). These studies have raised several important questions about the mechanisms of liver transplant-related ischemia/reperfusion injury, including the identification and regulation of the molecular switch from reversible damage to irreversible ischemia/reperfusion injury. The use of molecular biological techniques in combination with a rat liver transplantation model facilitates the investigation of the gene expression that could underlie these mechanisms at a tissue level.

The immediate early genes (IEGs) (c-*fos*, c-*jun*, *jun*-B, NGFI-A, etc.) are rapidly and transiently induced in most tissues by a variety of extracellular stimuli, including ischemia/reperfusion injury (*8*). The IEGs c-*fos* and c-*jun* encode proteins that are members of the leucine zipper family of transcription factors. These proteins associate as dimers and recognize specific DNA sequences located in the regulatory region of target genes, possibly involved in cellular growth and differentiation (*9, 10*). In the rat liver, reperfusion after warm ischemia induces the transient expression of c-*fos* mRNA (*11, 12*), which has been suggested to be instrumental in the reprogramming of transcription for acute-phase proteins and subsequent tissue repair (*12, 13*). Conversely, recent studies indicate that continuous expression of 2 types of IEG (c-*fos* and c-*jun*) may be associated with programmed cell death (apoptosis) after damage to the nervous system and in various organs during development (*14, 15*). These findings have led to the hypothesis that the pattern of IEG expression may be useful as an important indicator of cell repair in some instances, and be a predictor of subsequent cell death in the liver graft after transplantation in others. In the present study, we investigated the expression of c-*fos* and c-*jun* genes in livers of rats subjected to liver transplant-related injuries such as warm ischemia, cold preservation, and reperfusion in relationship with the organ survival.

MATERIALS AND METHODS

Animals. In the liver ischemia (LI) model, nonfasting, male, outbred Wistar rats (250–300 g) were used. In the OLT model, male inbred PVG/c (RT1^c) rats, weighing 200–250 g, were used as both donors and recipients to prevent immunological rejection. Animals were allowed to take food and water ad libitum before and after the operation. All operations were performed under ether anesthesia.

Liver ischemia. A portosystemic shunt was induced by transposition of the spleen subcutaneously, according to Benmark's method, 3 weeks before the operation (*16, 17*). Within 3 weeks, collaterals were created from the convex surface and the hilum of the spleen with this method. After full skeletonization of the liver, normal rats (nonshunted) or shunted rats were subjected to 60 min of LI by clamping the portal vein and hepatic artery.

Orthotopic liver transplantation. In the fresh liver group, livers from untreated donors were flushed with 10 ml of ice-cold UW solution, preserved in 50 ml of UW solution at 4°C (storage time < 1 hr), and orthotopically transplanted into untreated recipients (fresh liver OLT group). In the preserved liver group, OLT was performed after the liver grafts were stored for 24 hr in UW solution at 4°C (preserved liver OLT group). In both groups, the liver grafts were rinsed with 5 ml of cold Ringer's lactate solution before implantation. Details of the technique of liver removal and OLT without arterialization have been described previously (*18, 19*). All recipients received 0.5 ml of Ringer's lactate and 0.5 ml of 8.4% sodium bicarbonate via the penial vein and an antibiotic (cefamandole, 100 mg/kg) was given intramuscularly. The average time of the recipient operation was 50 min.

Sham operation. One group of animals underwent sham operation; the abdomen was cut, the liver was exposed, and the abdomen was closed 60 min later.

One-week survival study. LI was generated in 12 rats (shunted rats, n=6; nonshunted rats, n=6), and 30 rat liver transplants (fresh liver OLT, n=12; preserved liver OLT, n=18) were performed for the preliminary 1-week survival study.

Northern blot analysis of IEG mRNAs in liver tissue. For the study of LI, 3 animals in each group were killed after 1 hr of LI without reperfusion (time 0) and at 30 min, 1 hr, and 3 hr after reperfusion. The liver was processed immediately. To measure IEG mRNA in livers during donor graft preservation (0–24 hr), a total of 15 donor livers were preserved with UW solution. Three livers were taken at 5 time points during preservation (0, 6, 12, 18, and 24 hr). For posttransplant analysis, a total of 30 rat liver transplants were performed. Three from each group were killed at 5 time points (30 min and 1, 2, 3, and 6 hr) following reperfusion. Two to 3 animals that underwent sham operation were killed at the same time points in both the LI and OLT studies. The liver specimens, taken from the median lobe (1–2 g wet weight) were promptly frozen in liquid nitrogen and stored at −70°C.

RNA preparation and Northern blot analysis were carried out as described previously (*20–22*). Total RNA (30 μg), extracted from liver tissue by a guanidinium thiocyanate method, was size fractioned by 1% (w/v) agarose-formaldehyde gel electrophoresis, transferred onto Hybond-N nylon membranes (Amersham, Sydney, Australia) by capillary transfer using 10 × standard saline citrate (1 × standard saline citrate is 0.015 M NaCl, 0.15 M sodium citrate), and UV cross-linked. The membranes were prehybridized for 15 min at 65°C in rapid hybridization solution (Amersham). DNA probes for c-*fos* (*20*) and c-*jun* (*22*) were labeled with [^{32}P]-dCTP (Bresatec, Adelaide, Australia) by the random multiprime method (Pharmacia, Sydney, Australia) and then added to the prehybridization solution. After hybridization at 65°C for 2 hr, the membranes were washed to a final stringency of 0.1% (w/v) SDS, 0.2 × sodium chloride sodium phosphate EDTA (SSPE) (1 × SSPE is 0.15 M NaCl, 10 mM NaH_2PO_4.H20, 1 mM EDTA) at 65°C for 30 min, and exposed to Kodak XAR-5 film with intensifying screens at −70°C for several days. Loading and transfer of RNA were monitored by reprobing the membranes with an oligonucleotide probe for 18S ribosomal RNA (*21*). Intensity of autoradiographic bands was measured by image analysis using the JAVA software package (Jandel Scientific, Corte Madera, CA) and adjusted for variation in the intensity of 18S ribosomal RNA.

Statistical analysis. Fisher's exact *t* test was utilized for determining significant differences in the survival rate, and Student's *t* test was used for IEG mRNA expression. A *P*-values less than 0.05 was considered to be a significant difference.

RESULTS

One-week survival in LI and OLT. Using the LI model, all 6 nonshunted rats that were subjected to 60 min of portal triad clamping died within 3 days after surgery. Two rats died

within 12 hr from irreversible intestinal congestion and hemodynamic shock. The remaining 4 animals died from liver failure as judged by mortal histological findings of extensive coagulation necrosis with neutrophil infiltration (data not shown). In contrast, all shunted rats subjected to the same insult survived more than 1 month (Table 1). Histologically, the liver revealed normal hepatic architecture.

In the OLT model, there was no significant difference in portal vein clamping time between the fresh and preserved liver groups (Table 2). Twelve animals grafted with fresh livers yielded a 1-week survival of 100% (12/12). No obvious hepatocellular injury was observed histologically in the fresh liver after reperfusion (data not shown). When the preservation period was lengthened to 24 hr, 1-week survival rate of rats grafted with preserved livers was poor (group A, 11.1% [2/18]). The cause of death in 4 animals was intra-abdominal hemorrhage. The remaining 14 rats died from hepatic failure as judged by mortal histological findings of extensive hepatocellular necrosis. At the level of the light microscope, histological architecture of the livers after 24-hr preservation appeared almost normal, although after 3-hr reperfusion, livers showed thrombosis in the sinusoidal lumen and vacuolization in the swollen hepatocytes without any obvious hepatocellular necrosis observed (data not shown).

IEG expression in LI model. LI alone failed to induce either c-*fos* or c-*jun* expression in the livers of shunted and nonshunted rats (data not shown). The basal expression of c-*jun* mRNA was considerably higher than that of c-*fos* in both groups. In shunted rats, there was a marked and transient induction of c-*fos* and c-*jun* expression that peaked 1 hr after reperfusion. The increase was short-lived and the level of c-*fos* and c-*jun* mRNA returned to basal levels within 3 hr after reestablishment of the blood circulation (Figs. 1 and 2). To investigate the relationship of hepatic IEG expression during the early stages of reperfusion to the severity of the liver insult, we measured IEG mRNAs in reperfused livers of nonshunted rats (poor survival model) after 60 min of liver ischemia. The pattern of liver c-*fos* and c-*jun* expression in nonshunted rats was distinctly different from that observed in shunted rats. It was maximal at 1 hr and persisted up to 3 hr after reperfusion (Figs. 1 and 2). We were unable to detect any significant change in expression of the IEGs at any time in the liver of sham-operated animals (data not shown).

IEG expression in fresh and preserved liver OLT. Injury to the liver graft during the transplantation procedure includes not only warm LI but preservation/reperfusion injury and rewarming injury (*4*). To determine whether hepatic gene expression in the immediate reperfusion phase after liver transplantation might be used as a predictor of allograft viability, the expression of the IEGs c-*fos* and c-*jun* at the level of mRNAs was analyzed in the rat OLT model with or without prior preservation. The basal expression of c-*jun* mRNA was considerably higher than that of c-*fos* in both groups. The expression of IEGs was not induced by preservation alone in both models (data not shown). However, reperfusion following transplantation of both fresh liver and preserved liver resulted in different patterns of gene expression (Figs. 3 and 4). In the fresh liver OLT group, c-*fos* and c-*jun* mRNA was

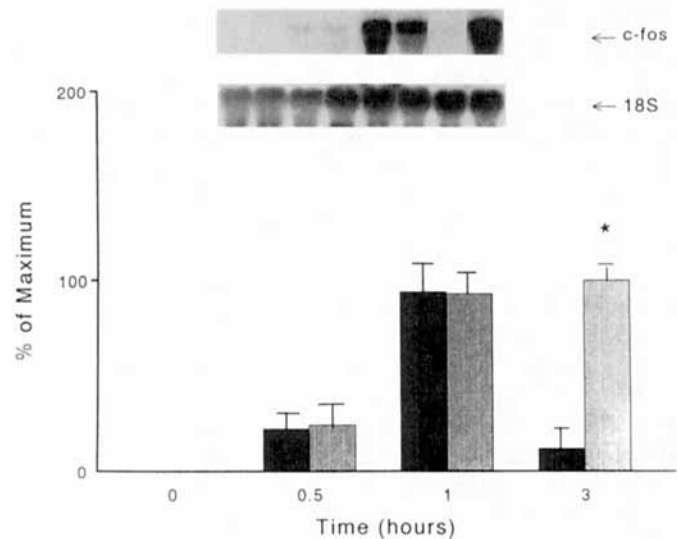

FIGURE 1. The expression of c-*fos* in livers of shunted rats (■) or nonshunted rats (▨) after 60 min of warm ischemia. Shunted rats or normal (nonshunted) rats were subjected to 60 min of warm ischemia by clamping the portal triad. Hepatic c-*fos* mRNA was analyzed by Northern blot hybridization at various times after reperfusion as indicated. A marked and transient expression of liver c-*fos* in shunted rats was observed at 1 hr and almost disappeared 3 hr after reperfusion. In contrast, c-*fos* expression in nonshunted rats peaked at 1 hr and persisted up to 3 hr after reperfusion. Values are mean ± SD of 3 separate experiments. *$P<0.05$. The Northern blot is representative of 3 paired studies.

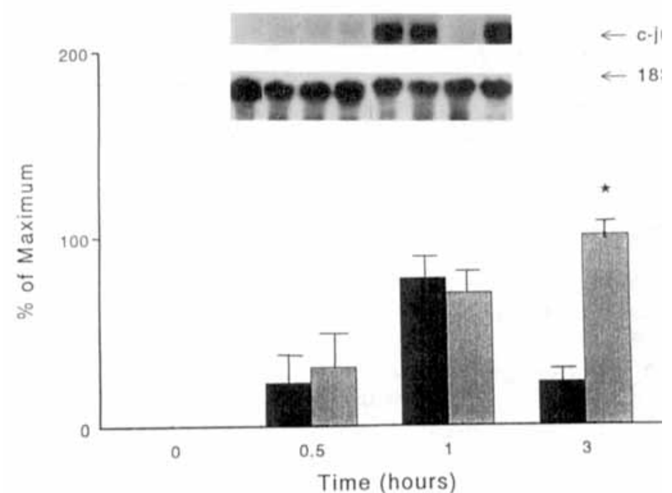

FIGURE 2. The expression of c-*jun* in livers of shunted (■) or nonshunted rats (▨) after 60 min of warm ischemia. The induction pattern of c-*fos* expression after reperfusion was similar to that of c-*fos* in both models as shown in Figure 1. Values are mean ± SD of 3 separate experiments. *$P<0.05$. The Northern blot is representative of 3 paired studies.

TABLE 1. One-week survival rates of shunted or nonshunted rats subjected to 60-min warm ischemia of the liver

	Shunted group	Nonshunted group
Number	6	6
Survival days	>7 (×6)	0 (×2), 1 (×3), 3 (×1)
One-week survival (%)	6/6 (100)	0/6 (0)[a]

[a] Fisher's exact test, $P<0.01$, as compared with the nonshunted group.

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TABLE 2. Characteristics and 1-week survival of the 2 OLT groups with different graft preservation time

	Fresh liver group	Preserved liver group
Number	12	18
Cold preservation time (hr, mean ± SD)	<1	24.2±0.6
Portal vein cross-clamping time (min, mean ± SD)	17.7±1.8	17.5±1.3
Survival days (no. of animals)	>7 (×12)	>7 (×2), 1 (×5), 2 (×6) 3 (×3), 4 (×1), 5 (×1)
One-week survival (%)	12/12 (100)	2/18 (11.1)[a]

[a] Fisher's exact test, $P<0.01$, as compared with the fresh liver group.

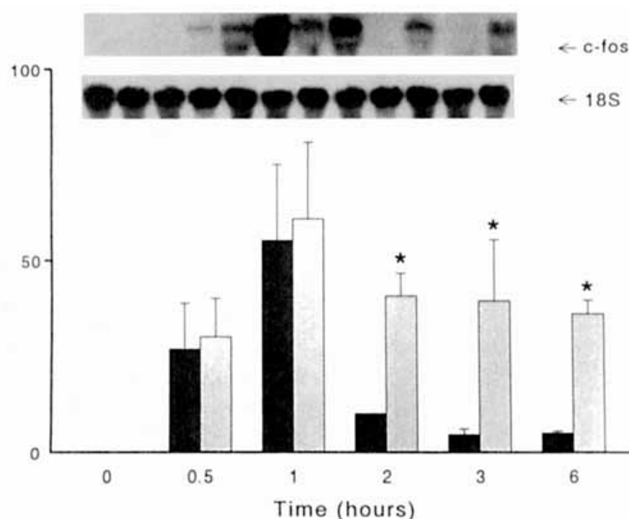

FIGURE 3. The expression of c-*fos* in livers transplanted within 1 hr (fresh liver OLT, ■) or after 24-hr cold preservation (preserved liver OLT, ▨). Donor livers were transplanted into the recipients within 1 hr or after 24 hr of storage using UW solution. IEG mRNAs (c-*fos* and c-*jun*) in the transplanted livers were analyzed by Northern blot hybridization at various times after reperfusion. In fresh liver OLT, the expression of liver c-*fos* appeared at 30 min with a peak at 1 hr, returning to the basal level 3 hr and 6 hr after reperfusion. In contrast, the expression in transplanted livers following preservation peaked at 1 hr and declined slightly at 2 hr after reperfusion. Further, the expression was still detected at up to 6 hr. Compared with c-*jun*, the induction of c-*fos* mRNA expression showed some variation for up to 3 hr after reperfusion. Values are mean ± SD of 3 separate experiments. $^*P<0.05$. The Northern blot is representative of 3 paired studies.

induced 30 min after reperfusion and increased rapidly and transiently with a peak at 1 hr after reperfusion, returning to basal level at 3 hr after reperfusion (Figs. 3 and 4). In contrast, the increased expression of c-*fos* and c-*jun* persisted for up to 6 hr after reperfusion in the preserved liver OLT group (Figs. 3 and 4). The induction of c-*jun* mRNA was consistent among the livers from 3 animals at each time point after reperfusion. The induction of c-*fos* mRNA, however, showed some variation at 3 hr after reperfusion, but was more consistent at 6 hr (Fig. 3). Namely, in the fresh liver OLT, the induction of c-*fos* mRNA was transient with a peak at 1 hr, becoming undetectable 3 hr after reperfusion. In the preserved liver OLT group, elevated expression of c-*fos* was observed in 2 of the 3 livers sampled 3 hr after reperfusion. At

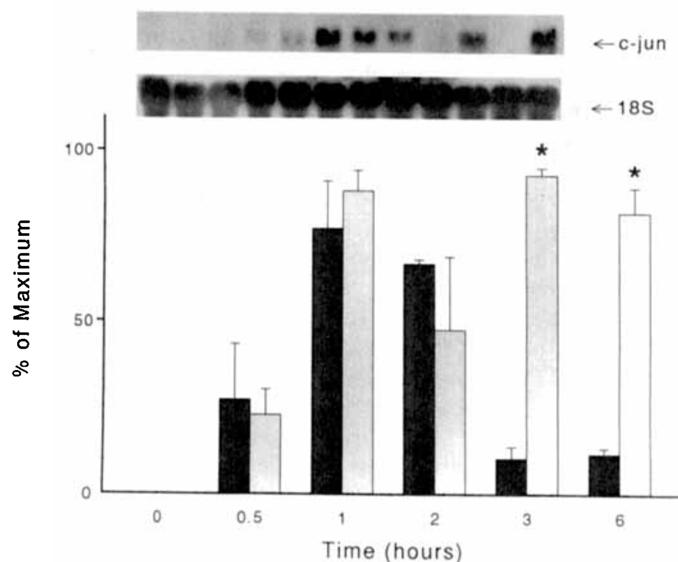

FIGURE 4. The expression of c-*jun* in livers transplanted within 1 hr (fresh liver OLT, ■) or after 24-hr cold preservation (preserved liver OLT, ▨). The basal expression of c-*jun* was considerably higher than that of c-*fos*. In fresh liver Tx, the expression of liver c-*jun* peaked at 2 hr after reperfusion and returned to the basal levels afterward. In preserved liver Tx, the liver c-*jun* expression was observed consistently, with greater signal intensity 3 hr and 6 hr after reperfusion. Values are mean ± SD of 3 separate experiments. $^*P<0.05$. The Northern blot is representative of 3 paired studies.

6 hr after reperfusion, induction of c-*fos* was consistently observed in the 3 livers from 3 preserved liver-transplanted rats. We were unable to detect any significant change in expression of the IEGs at any time in the liver of sham-operated animals (data not shown).

DISCUSSION

All liver grafts are damaged to some extent by the warm ischemia, cold preservation, rewarming, and reperfusion that occur during the transplantation process. Most transplanted livers recuperate spontaneously, but some are so severely injured during harvesting, storage, and reperfusion that they are unable to function during the early postoperative period, resulting in graft death (primary nonfunction) (*2*, *3*). The transition from reversible to irreversible liver damage may be a reflection of a delicate balance among the processes of proliferation, differentiation, cell repair, and cell death in the liver graft. The induction of hepatic IEG has been suggested to be associated with cell repair (*11*, *12*) or programmed cell death (*14*, *15*). The response to several types of cell injury during the transplantation process could involve the activation of IEGs soon after liver transplantation. The pattern of this gene expression may represent the status of the tissue and its likely long-term response to insult.

The transient induction of c-*fos* expression in liver has been reported in an experimental model of LI (*11*, *12*). From the surgical point of view, the ischemic insults described by these authors were not severe enough to affect the survival of animals after surgery. There was, however, transient induction of c-*fos* expression within 1–2 hr after reperfusion. Using these LI models, the purpose of our preliminary study was to determine whether the IEG expression pattern might reflect

prognosis after surgery for the perfect survival model and the poor survival model. After we could consistently confirm the transient pattern of hepatic IEG expression in a short-time liver ischemia model (100% survival model) as others have reported, we extended the liver ischemia time to lethal time (60 min, poor survival model), resulting in a protractive pattern of IEG expression. Concomitantly, the LI model with prior shunting was set as a perfect survival model with same time of liver ischemia. In another study of LI, an inverse relationship between the rate of ATP recovery and the degree of c-*fos* and c-*jun* expression in livers after reperfusion was described (*11*). Thus, the pattern of IEG expression pattern in reperfused livers may be associated with differences in the severity of warm ischemia/reperfusion injury during the liver transplantation process. Therefore, to determine whether this concept could be applied to experimental liver transplantation, the pattern of IEG mRNAs expression was analyzed in the rat OLT model, which includes several types of liver injuries.

Our OLT study demonstrates that the expression of the IEGs was not induced by preservation alone, but by reperfusion of both fresh and preserved liver. This treatment resulted in a distinct pattern of gene expression in transplanted livers: transient expression pattern of liver IEGs in fresh liver OLT (viable graft) and protracted pattern of c-*fos* and c-*jun* expression in transplanted livers after 24-hr preservation (almost nonviable graft). The induction of c-*jun* mRNA in transplanted livers following reperfusion, which was more consistent than that of c-*fos* mRNA, may specifically reflect the differences in the severity of the liver transplant-related insults and the graft prognosis. The reason for the inconsistency of liver c-*fos* expression at 3 hr after reperfusion is unclear but may be due to the differences of gene response to the severity of the preservation/reperfusion injury.

Although in situ hybridization and immunohistochemical study have not been performed in the present study, it would be of much interest to examine the precise localization of gene expression at the cellular level in response to warm ischemia or cold preservation/reperfusion insult. Unlike the warm liver ischemia/reperfusion injury, cold preservation/reperfusion injury involves endothelial cells as a primary target, and subsequent microcirculatory disturbances lead to liver necrosis and graft failure in liver transplantation (*23–25*). The morphological study indicated serious injury to sinusoidal lining cells and mild injury to the hepatocytes during the first 1–3 hr after transplantation following 24-hr UW storage (*19*, *26*). However, we have indicated that 24-hr UW preservation of the rat liver resulted in extensive hepatocellular necrosis and poor graft survival at a later stage of liver transplantation. These results indicate that cell death may first occur at the sinusoidal lining cell level and then in the parenchymal cells of the liver graft until degenerative death or necrosis is completed. The continuous expression of IEGs in transplanted livers following extended preservation may reflect this serial step of cell repair or cell death in different types of cells in the liver allograft. A continuous pattern of c-*fos* and c-*jun* expression has been suggested to be associated with programmed cell death, including apoptosis (*14*, *15*). It is well known that an apoptotic type of cell death occurs after hepatic ischemia and hypertrophy (*27*). It is possible, therefore, that this type of cell death contributes to tissue damage encountered in our transplantation model.

In conclusion, our results suggest that the induction pattern and the level of c-*fos* and c-*jun* expression in postischemic livers and transplanted livers with various conditions may reflect the differences in severity of the transplant-related insults and the differences in subsequent graft survival upon transplantation. Analysis of inducible hepatic gene expression during liver transplants may elucidate more precisely the mechanism of primary graft nonfunction or dysfunction and allow application of gene-related therapy to liver transplantation.

REFERENCES

1. Todo S, Nevy J, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. JAMA 1989; 261: 711.
2. Greig PD, Woolf GM, Sinclair SB, et al. Treatment of primary liver graft non-function with prostaglandin El. Transplantation 1989; 48: 447.
3. Furukawa H, Todo S, Imventaraza O, et al. Effect of cold ischemia time on the early outcome of human hepatic allografts preserved with UW solution. Transplantation 1991; 51: 1000.
4. Clavien PA, Harvey RC, Strasberg SM. Preservation and reperfusion injuries in liver allografts. Transplantation 1992; 53: 957.
5. Goto S, Kim YI, Kodama Y, et al. The beneficial effect of a stable prostacyclin analogue (OP41483) on rat liver preserved for twenty-four hours with lactobionate solution. Transplantation 1991; 52: 926.
6. Goto S, Kim YI, Shimada T, Kawano K, Kobayashi M. The effects of pre-transplant cyclosporine therapy on rats grafted with twelve-hour cold-stored liver. With special reference to reperfusion injury. Transplantation 1991; 52: 615.
7. Kawano K, Kim YI, Goto S, Ono M, Kobayashi M. A protective effect of FK506 in ischemically injured rat livers. Transplantation 1991; 52: 143.
8. Morgan JI, Curran T. Stimulus-transcription coupling in the nervous system: involvement of the inducible protooncogenes *fos* and *jun*. Annu Rev Neurosci 1991; 14: 421.
9. Curran T, Franza BR. Fos and jun: The AP-l connection. Cell 1988; 55: 397.
10. Franza BR, Rauscher FJ, Josephs SF, Curran T. The fos complex and fos related antigen recognize sequence elements that contain AP-lsites. Science 1988; 239: 1150.
11. Marterre WF, Kindy MS, Carney JM, Landrum RW, Strodel WE. Induction of the protoncogine *c-fos* and recovery of cytosolic adenosine triphosphate in reperfused liver after transient warm ischemia: effect of nitrone free-radical spin-trap agents. Surgery 1991; 110: 184.
12. Schiaffonati L, Rappocciolo E, Tacchini L, Cairo G, Bernelli-Zazzera A. Reprogramming of gene expression in postischemic rat liver: induction of proto-oncogenes and hsp 70 gene family. J Physiol 1990; 143: 79.
13. Schiaffonati L, Cairo G, Tacchini L, et al. Protein synthesis and gene expression in transplanted and postischemic livers. Transplantation 1993; 55: 977.
14. Smeyne RJ, Yendrell M, Hayward M, et al. Continuous *c-fos* expression precedes programmed cell death in vivo. Nature 1993; 363: 166.
15. Dragnunow M, Young D, Hughes P, et al. Is *c-jun* involved in nerve cell death following status epilepticus and hypoxic-ischaemic brain injury? Mol Brain Res 1993; 18: 347.
16. Bengmark S, Brojesson B, Olin T. Development of portosystemic shunt after subcutaneous transposition of the spleen: an ex-

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perimental study in the rat. Scand J Gastroenterol Suppl 1970; 7: 175.
17. Bengmark S, Brojesson B, Olin T. Development of portosystemic shunt after subcutaneous transposition of the spleen in the rat. Scand Am J Surg 1973; 125: 757.
18. Kamada N, Calne RY. A surgical experience with five-hundred-thirty liver transplantation in rats. Surgery 1983; 93: 64.
19. Goto S, Kim YI, Kodama Y, et al. The effect of a prostaglandin I2 analogue (OP41483) on energy metabolism in liver preservation and its relation to lipid peroxidative reperfusion injury in rats. Cryobiology 1993; 30: 459.
20. Le F, Wilce PA, Cassady I, Hume DA, Shanley BC. Acute administration of ethanol suppresses pentylenetetrazole-induced *c-fos* expression in rat brain. Neurosci Lett 1990; 120: 271.
21. Le F, Wilce PA, Hume DA, Shanley BC. Involvement of r-aminobutyric acid and N-methyl-D-aspartate receptors in the inhibitory effects of ethanol on pentylenetetrazole-induced *c-fos* expression in rat brain. J Neurochem 1992; 59: 1309.
22. Matsumoto I, Leah J, Shanley B, Wilce P. Immediate early gene expression in the rat brain during ethanol withdrawal. Mol Cell Neurosci 1993; 4: 485.
23. Caldwell-Kenkel J, Thurman RG, Lemasters JJ. Selective loss of nonparenchymal cell viability after cold ischemic storage of rat livers. Transplantation 1988; 45: 834.
24. Mckeown CMB, Edward V, Phillip MJ, Harvey PRC, Petrunk CN, Strasberg SN. Sinusoidal lining cell damage: the critical injury in cold preservation of liver allograft in the rat. Transplantation 1988; 46: 1795.
25. Thurman RG, Marzi I, Seitz C, Thies J, Lemasters JJ, Zimmerman F. Hepatic reperfusion injury following orthotopic liver transplantation in the rat. Transplantation 1988; 46: 502.
26. Sumimoto R, Jamieson NV, Wake K, Kamada N. 24-Hour rat liver preservation using UW solution and some simplified variants. Transplantation 1989; 48: 1.
27. Kerr JFR. A histochemical study of hypertrophy and ischemic injury of rat liver with special reference to changes in lysosomes. J Pathol Bacteriol 1965; 90: 419.

Received 25 January 1994.
Accepted 21 April 1994.

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0041-1337/94/5807-845$03.00/0
TRANSPLANTATION
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Vol. 58, 845–848, No. 7, October 15, 1994
Printed in U.S.A.

TRANSFUSION OF ONE HLA-DR ANTIGEN-MATCHED BLOOD TO POTENTIAL RECIPIENTS OF A RENAL ALLOGRAFT

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Patients awaiting a renal transplant were given 1 HLA-DR antigen-matched blood in order to compare the sensitization and graft outcome of these patients with those of control patients, who had been given random blood before transplantation. No differences in sensitization, measured by the formation of HLA antibodies, or in graft survival were found between the 2 groups. However, the incidence of rejection episodes was significantly reduced in the patients who received blood matched for 1 HLA-DR antigen compared with the patients who received random blood.

The beneficial effect of blood transfusion on allograft survival has been well documented (*1*). The policy at this center has been to transfuse all potential recipients of a kidney allograft with 2 U of blood before transplantation. However, the benefits of blood transfusion have been questioned as a result of improved graft survival in patients who did not receive transfusions (*2*), and many centers have eliminated the transfusion of potential recipients. One reason to avoid transfusions is that they may lead to the formation of HLA antibodies that make it more difficult for the patient to receive a cross-match-negative graft.

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It has been reported that giving transfusions with 1 HLA-DR antigen shared between the blood donor and the recipient is associated with a reduction in the sensitization rate and improval in graft survival, compared with recipients who were mismatched with their blood donors for both HLA-DR antigens (*3*). In an attempt to verify these findings, we have transfused patients before transplantation with blood matched for 1 HLA-DR antigen.

MATERIALS AND METHODS

Forty-six potential recipients of a renal allograft with no previous history of pregnancy, blood transfusion, or transplantation and no detectable HLA antibody in their serum were entered into the study. Twenty-nine patients have subsequently been transplanted with a cadaveric graft. An additional 4 patients have been transplanted but have been excluded because 3 did not receive CsA and 1 received a kidney from a sibling. Thirteen patients are still waiting for a graft. Each patient was given 2 U of blood (packed cells), with each unit sharing 1 HLA-DR antigen with the patient. In all cases, the 2 U of blood were matched for the same HLA-DR antigen and were given 15–60 days apart. Patients who received their first unit of blood, which in addition to having 1 HLA-DR antigen matched also had 1 HLA-DR antigen mismatched, were given a second unit that also contained 1 HLA-DR antigen mismatched. Patients who received their first unit of blood with 1 HLA-DR antigen matched but no HLA-DR antigen mismatched were given a second unit with no