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Eric Savier
John J. Lemasters
Ronald G. Thurman

Kupffer cells participate in rejection following liver transplantation in the rat

E. Savier · R. G. Thurman (✉)
Laboratory of Hepatobiology and Toxicology, Department of Pharmacology, Faculty Laboratory Office Building, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365, USA

J. J. Lemasters
Laboratory for Cell Biology, Department of Cell Biology and Anatomy, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365, USA

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Abstract Recognition of foreign antigens involves macrophages which release mediators such as immunoactive interleukins, and in the liver, the resident macrophages (Kupffer cells) are activated following transplantation. Therefore, we evaluated the hypothesis that Kupffer cells participate in the rejection reaction following transplantation. Orthotopic liver transplantation was performed between different syngenic rat strains. Livers from Lewis rats were stored in lactated Ringer's solution for 1 h to minimize cold ischemic injury and transplanted into PVG recipients. At 24 h postoperatively, transaminases (AST) were elevated to values around 2000 U/l, total bilirubin was increased to values around 20 μmol/l, and five of six rats died within 3 days. Macroscopic and histological examination showed large areas of necrosis without cellular infiltration, characteristic of rejection. When donor rats were treated with gadolinium chloride ($GdCl_3$, 10 mg/kg i.v. 24 h before storage of the liver) to inactivate the Kupffer cells, AST levels only rose to around 700 U/l, and the total bilirubin level was in the normal range (< 4 μmol/l). Survival was improved significantly by $GdCl_3$, with five of seven rats surviving more than 1 month ($P < 0.05$) and four of seven rats surviving for at least 100 days without immunosuppressive drug therapy. Rejection was not totally prevented, however, since the surviving rats had elevated AST and bilirubin levels, and cellular infiltration in portal areas along with proliferation of bile canaliculi was observed. These data are consistent with the hypothesis that Kupffer cells participate in mechanisms of early rejection following liver transplantation.

Key words Kupffer cells
Orthotopic liver transplantation
Early rejection reaction

Introduction

During the last decade, clinical liver transplantation has improved considerably and now allows lethal hepatic diseases to be cured. However, transplantation necessitates permanent immunosuppressive therapy, and the cumulative secondary effects of each drug must be carefully monitored. Ideally, immunosuppression should be directed specifically against the cells involved in graft rejection. Some experimental treatments directed against dendritic cells and macrophages of tissues or of the organ donor prior to transplantation allow survival of the graft

to be prolonged significantly in the absence of postoperative immunosuppressive therapy [4, 7, 9].

In the liver, evidence suggests that Kupffer cells may play an important role in the immunological response. First, Kupffer cells carry class II antigens of the major histocompatibility complex [10], which are potent stimulators of lymphocytes. Second, Kupffer cells overexpress class II antigens following liver transplantation [10], and third, immediately following transplantation, Kupffer cells release cytokines [8] which are involved in rejection reactions [2, 5, 11]. Therefore, the purpose of this study was to evaluate the hypothesis that inhibition of Kupffer cells before transplantation would minimize rejection of the liver graft following surgery.

Materials and methods

Rejection reactions were elicited by transplanting livers from one strain of syngenic rat into another. Livers from PVG rats (Bantin and Kingman; 200–225 g, female, RT-1^c, < 200 days old) were transplanted into Lewis rats (Harlan; 180–220 g, female, RT-1^l) according to procedures described by Zimmermann et al. [12] and Kamada and Caine [6]. Briefly, rats were anesthetized with ether, and surgery was performed under clean conditions. Dissected livers were rinsed in situ with 20 ml of lactated Ringer's solution at 4 °C and immersed at 1 °C for 1 h. Grafts were rinsed with 10–20 ml of lactated Ringer's solution at room temperature before reperfusion.

Blood was collected from the tail vein postoperatively, and sera were stored at −20 °C until assay. Serum transaminase (AST) activity was measured enzymatically [1], and total bilirubin was determined in sera by direct spectrophotometry at 454 nm.

Gadolinium chloride ($GdCl_3$; 10 mg/kg of body weight) was diluted in 1 ml of acidified saline solution and injected into Lewis donor rat via the tail vein 24 h prior to liver transplantation. Livers were fixed by immersion in 2% paraformaldehyde in Krebs-Henseleit buffer, embedded in paraffin, and processed for light microscopy.

Following randomization, a control group was compared to a $GdCl_3$-treated group. Data are presented as mean ± SEM. Statistical analyses were performed using chi-square and Student's *t*-test. The criterion for significance was $P < 0.05$.

Results

Gadolinium chloride treatment improves survival in a rejection model

When livers from Lewis rats were transplanted into PVG rats, five of six (83%) rats died within 3 days (Fig. 1). The first day following transplantation, AST levels reached nearly 2000 U/l (Fig. 2A), and the total bilirubin level was elevated to about 25 µmol/l (Fig. 2B). Macroscopic autopsy revealed yellow necrotic areas within hetero-

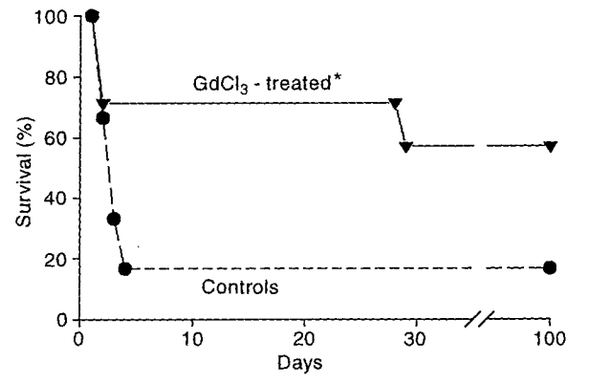

Fig. 1 Effect of ($GdCl_3$) treatment on survival following liver transplantation of Lewis to PVG rats. Controls: Livers from Lewis syngenic rats, stored for 1 h in cold lactated Ringer's solution, were transplanted orthotopically into a PVG syngenic rat without immunosuppressive therapy. *$GdCl_3$-treated:* Donor Lewis rat was treated intravenously with $GdCl_3$ (10 mg/kg) 24 h before surgery as described in methods. (* $P < 0.05$ for χ^2 test, $n = 6$ to 7 per group)

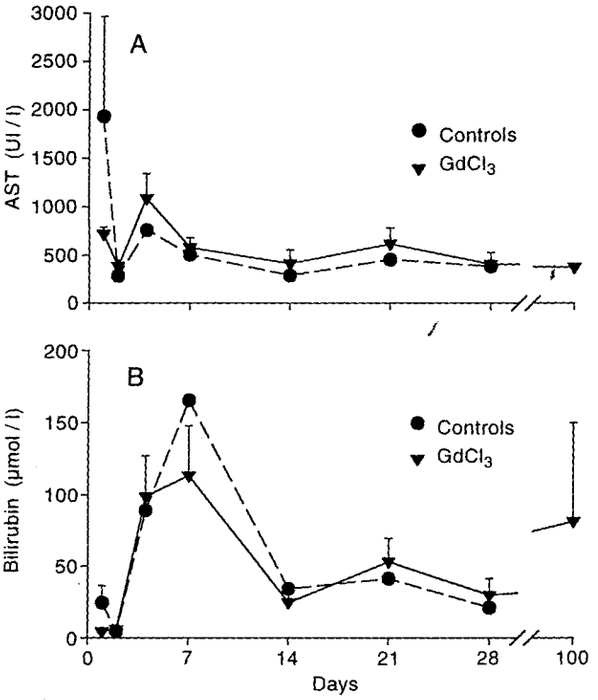

Fig. 2A,B Postoperative transaminases (AST) and bilirubin release in a rejection model. Sera from surviving PVG rats, orthotopically transplanted with livers from Lewis, rats as described in legend to Fig. 1 and in methods, were obtained by centrifugation from blood collected from the tail vein at times indicated on abscissa. **A** *AST*; **B** total bilirubin. ($n = 4$ to 7; means ± SEM)

geneous distribution between different lobes. Histological examination showed that the main regions of injury were areas of total necrosis that contrasted strongly with adjacent normal parenchyma. Necrosis was centered around the portal triad, which was devoid of cellular infiltration.

When the donor rat was treated with $GdCl_3$ 24 h before transplantation, five of seven (70%) PVG recipient rats survived for at least 1 month ($P < 0.05$; Fig. 1). The first day following transplantation, AST values were elevated to only 700–800 U/l, and total bilirubin level was in the normal range. On the first or second postoperative day, livers from transplant recipients which had received $GdCl_3$ appeared quite normal, and almost no necrotic areas were observed histologically, similar to untransplanted controls.

Liver injury was similar in control and $GdCl_3$-treated rats

One of six (17%) PVG recipient rats from the control group and four of seven (60%) from the $GdCl_3$-treated group survived for at least 100 days without immunosuppressive drug therapy (Fig. 1). For both of these groups, the time course of AST and bilirubin release was similar (Fig. 2). AST values increased to maximal values around 1000 U/l at day 4 postoperatively and remained elevated between 300 and 600 U/l (Fig. 2A), while bilirubin increased to values between 100 and 150 μmol/l for about 1 week. Subsequently, bilirubin levels stabilized at values between 20 and 150 μmol/l, which are above the normal range (Fig. 2B). Histological examination at 1 or 3 months revealed periportal infiltration by mononuclear cells, indicative of hepatitis, along with proliferation of biliary canaliculi. Thus, a rejection reaction occurred in $GdCl_3$-treated rats, but unlike controls, it was not fatal.

Discussion

The purpose of this study was to test the hypothesis that Kupffer cells are involved in the rejection reaction following liver transplantation. We employed a rat model of orthotopic liver transplantation in which 85% of recipients died within 3 days following surgery (Fig. 1). Autopsy and histological examination allowed us to conclude that an immunological reaction against the graft was the cause of death. A dramatic increase of survival with decreased injury following $GdCl_3$ treatment implicated donor Kupffer cells in the mechanism of toxicity [3]. It is reasonable to propose that a PVG antigen reacted with activated Lewis Kupffer cells, which then released toxic mediators. Further, injury predominated in the periportal regions, where Kupffer cells are mainly located.

In rats that survived immediate rejection (Fig. 1), infiltrates were observed in the portal areas, and AST and bilirubin levels were above normal (Fig. 2), yet no differences between control and $GdCl_3$-treated rats were evident. These results support the hypothesis that a chronic rejection reaction occurs which is not influenced by inhibition of the Kupffer cells. In another combination known to produce acute rejection (transplantation of DA to Lewis rat) [10], death of the recipient rat occurred during the second week following transplantation, and massive cellular infiltration of the graft was observed (E. Savier and R. G. Thurman, unpublished data). In this latter model, inactivation of the Kupffer cells with $GdCl_3$ had no effect on survival or cell injury. Therefore, the beneficial effect of Kupffer cell inactivation may apply only to early rejection.

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