Adoptive Transfer of Immunity to Hepatitis B Virus by Liver Transplantation in Rats

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Dedicated to

my parents

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CONTENTS

1. INTRODUCTION

1.1. Hepatitis B Virus Reinfection after Liver Transplantation

1.1.1. Clinical situation

Generally, transplantation is an effective treatment for patients with liver failure due to acute and chronic liver diseases. There are subgroups of patients, such as those with hepatitis B virus (HBV) infection, who historically have done poorly with transplantation. In absence of specific prophylactic treatments, the 1-year survival rate of HBV infected patients is 50%- 60%, compared with survival rates of 70%-85% for patients with alcoholic or cholestatic liver diseases (O' Grady JG 1992).

Reduced graft and patient survival in patients with pre-transplant HBV infection is largely related to the development of recurrent liver disease (Todo S 1991; Samuel D 1993). Before there was a specific therapy for hepatitis B, the recurrence rate of HBV after orthotopic liver transplantation (OLT) was up to 90% (Demetris AJ 1990; Hart J 1990). Circulating HBV in serum and free viral HBV DNA in extra hepatic sites, such as peripheral mononuclear blood cells or bone marrow cells, are supposed to lead to liver graft reinfection (Feray C 1990). Early studies from Europe and the United States suggested that the level of viral replication before transplantation is an important parameter for determining the risk of recurrent disease (Todo S 1991; Feray C 1990; Levy GA 1989). In a series of 334 patients from 17 different European centers, the risk of reinfection, defined by the presence of HBsAg in serum, was highest for patients with HBV related cirrhosis, lowest in patients with fulminant hepatitis B, and intermediate for patients with hepatitis D virus related infection. Within the group of patients with chronic HBV related cirrhosis, the risk of

reinfection was also related to the level of viral replication before transplantation. Around 83% of those patients who were HBeAg positive and HBV DNA positive developed a recurrent infection compared with 58% of patients who were negative in both assays (Samuel D 1993).

Most recipients of liver transplants, who become HBsAg positive after transplantation, have histological evidence of disease and, in general, the histological features are similar to those seen in non-transplant patients (Demetris AJ 1990). The rate of histological progression is accelerated in some patients, with cirrhosis development within two years of transplantation. Additionally, a rare histological variant called fibrosing cholestatic hepatitis has been described, which is characterized by the presence of periportal and perisinusoidal fibrosis, ballooned hepatocytes with cell loss, pronounced cholestasis, and a paucity of inflammatory activity (Demetris AJ 1990; Davies SE 1991). Immunohistochemical staining shows high cytoplasmic expression of viral antigens, suggesting that liver injury is due to a direct cytopathic effect of the virus. In addition, reduced endogenous interferon production has been observed in these patients, resulting in a greater risk of sepsis and leading to an increased mortality (Davies SE 1991; Martin P 1992). Patients with fibrosing cholestatic hepatitis frequently show a progressive course of the disease and the outcome is usually fatal (Benner KG 1992). Repeated liver transplantation in patients with HBV recurrence is associated with an even more truncated natural history and shorter period until graft failure (Crippin J 1994; Benner KG 1992; Van Thiel DH 1994).

1.1.2. Current strategies against hepatitis B virus reinfection

Efforts to improve the outcome of HBV infected patients undergoing liver transplantation have focused on strategies to prevent reinfection. Currently, three strategies against HBV recurrence are employed: 1) passive immunization with high dose of anti-HBs immune globulin; 2) antiviral treatment using agents such as Interferon-α (IFN-α) and nucleoside analogues; 3) active immunization with hepatitis B vaccine (HBVac).

1.1.2.1. Passive immunoprophylaxis

Several series have shown the effectiveness of hepatitis B immune globulin (HBIg) in reducing the rate of recurrent HBV infection after transplantation (Lauchart W 1987; Samuel D 1991; McGory RW 1996). In a large multicentre study from Europe, the rate of recurrent HBV infection after three years was only 30% in patients receiving long term prophylactic HBIg treatment (for at least six months) compared with 67% in those given non prophylaxis (Samuel D 1993). In most centers, HBIg is given during the anhepatic phase and daily for the first week after transplantation (10000 IU/day i.v.). Subsequent schedules of administration, e.g. once or twice monthly, are dependent on the titer of anti-HBs in serum. Administration of HBIg at intervals sufficient to achieve anti-HBs titers of 500 mIU/ml in patients who are positive for either HBeAg or HBV DNA and 100 mIU/ml in low-risk patients resulted in an overall recurrence rate of 20-50%. Additionally, long term HBIg treatment seems to be important, since the risk of recurrence was only 36% in patients receiving HBIg for more than six months. Those patients, however, who were treated for only two months or less, showed a substantially higher risk (74%) (Samuel D 1993).

Despite the clear efficacy of prophylactic HBIg therapy, this treatment has limitations. The principal disadvantages of a long-term HBIg regimen are the high expenses, which add $10000- $50000 to the first year' s charges for a liver transplant and $5000- $20000 to each subsequent year (Samuel D 1991; Terrault NA 1997).

1.1.2.2. Antiviral treatments

Antiviral drugs may be used as pre-emptive or as post-transplant treatment for patients with overt recurrence of HBV infection. Pre-emptive treatment begins before transplantation and continues for variable duration after transplantation (Terrault NA 1996). It is an attempt to decrease HBV replication to undetectable levels. Once achieved this result, it is supposed to reduce substantially the rate of post-transplant infection. In some cases, successful treatment before transplantation may delay or even obviate the need for liver transplantation.

Interferon Interferon-α (IFN-α) is an effective antiviral agent in immunocompetent patients with chronic hepatitis B. It acts by inhibiting the viral replication and augmenting the host immune response (i.e. the cytotoxic T cells) to HBV. The current recommended dose of interferon is 5 million units injected subcutaneously each day or 10 million units injected subcutaneously three times per week, for a period of 16 weeks. A meta-analysis of 16 randomized, controlled trials found that loss of HBeAg and HBsAg occurred in 33% and 37% of interferon-treated patients compared with only 12% and 17% in controls, respectively (Wong DK 1993). Histological improvement was seen more often in treated patients, in association with serologic responses (loss of HBeAg and HBV DNA).

IFN-α has been used both before and after liver transplantation (Marcellin P 1994; Terrault NA 1996). In a controlled study pre-emptive IFN-α treatment failed to reduce the rate of HBV reinfection (Marcellin P 1994). Rates of recurrence were, however, lower in treated patients who lost HBV DNA prior to transplantation, than in those with ongoing viral replication (17% vs. 78%, $p<0.05$). Controversially, it was demonstrated by other authors to be of little value in reducing the rate of reinfection (Todo S 1991; Rakela J 1989).

IFN-α treatment is mainly limited by its significant side effects including flu-like symptoms, fever, myalgia, mild bone marrow suppression, and thyroid abnormalities. Therefore it is poorly tolerated in patients with decompensated disease. Dose reduction is frequently necessary, which in turn, limits the antiviral efficacy of the drug. IFN-α enhances the human leukocyte antigen (HLA) expression on epithelial cells of the bile duct and increases leukocyte activity, which might promote allograft rejection (Marcellin P 1994; Terrault NA 1996). The magnitude of this risk is discussed controversially and cannot be quantified at present.

Nucleoside analogues Several new antiviral agents, such as lamivudine, famciclovir, gancyclovir, adefovir dipivoxil or lobucavir, have recently been developed. These drugs can decrease hepatitis B virus load by two to three logarithmic folds. Lamivudine is the most widely studied of these new agents. It acts as a reverse transcriptase inhibitor preventing HBV DNA synthesis through chain termination of the nascent proviral DNA. Extensive phase II and III studies in patients with chronic hepatitis B are in progress (Dusheiko G 1999). Preliminary results showed that 65% of lamivudine-treated patients became HBV DNA negative and had normal serum aminotransferases after a year of treatment. Histological improvements like attenuated hepatic necroinflammatory activity and fewer progressive hepatic

fibrosis have been noted in 38%-52% of lamivudine-treated patients, including HBeAg-negative/HBV DNA-positive patients -- a group that generally does not respond well to IFN-α (Tassopoulos NC 1999).

In an attempt to improve outcomes after transplantation, lamivudine has been administered alone or in combination with HBIg in several nonblinded, noncomparative studies in liver transplant candidates with HBV-associated end-stage liver disease. The results were summarized by Jarvis recently: at least two weeks prior to transplantation, patients started to receive oral lamivudine 100 or 150 mg/day. HBIg was administered during surgery and was continued for at least 6 months after transplantation. Studies, in which lamivudine was used alone, showed that HBV DNA became undetectable by polymerase chain reaction in post-transplant liver biopsies (12 to 104 weeks) in more than 64% of patients. HBcAg was absent from liver biopsy specimens in most of these patients. Other three studies of lamivudine in combination with evidence of hepatitis B (e.g. HBV DNA, or HBeAg in the serum) in this review showed that replication was observed in only 2 out of totally 83 patients (Jarvis B 1999). Post-transplantation recurrence of hepatitis B after failed HBIg immunoprophylaxis also responded to lamivudine therapy. It inhibited HBV replication and improved the poor outcome in fibrosing cholestatic hepatitis, although the majority of patients remained HBsAg positive (Ben-Ari Z 1997).

Despite promising results, approximately 30% of patients became resistant to lamivudine as a result of a mutation at the *YMDD* locus of the HBV polymerase gene (Bartholomew MM 1997). Moreover, HBV DNA rebound was found in most patients after discontinuation of the treatment (Tyrrell D 1993). The optimal length of treatment remains unknown.

1.1.2.3. Active immunization by HBV vaccination

Active immunization with hepatitis B vaccine (either plasma-derived or recombinant vaccine) has been performed to prevent infection and reinfection of hepatitis B in liver transplant patients. Pretransplant vaccination resulted in a response rate between 16%-54% (Van Thiel DH 1992; Chalasani N 1998), while the rate was only 6.7%-23% when vaccination was performed after liver transplantation (Carey W 1990; Loinaz C 1997; Chalasani N 1998). The low response rate was considered to be due to the underlying liver disease as well as the immunosuppressive treatment after transplantation. Clinical trials of active immunization with HBV vaccination did not demonstrate any additional beneficial effects in preventing graft reinfection or alleviating HBV-induced liver disease (Muller R 1998).

Combination of therapies against HBV reinfection, such as administration of HBIg and lamivudine, or HBV vaccination and administration of human immunoglobulins showed encouraging results so far, although the numbers of patients included in these studies are still too small to draw any conclusion (Markowitz JS 1998; Jarvis B 1999). Despite all efforts, hepatitis B reinfection after liver transplantation still remains a relevant clinical problem.

1.2. Adoptive Transfer of Immunity

Since Landsteiner first described in 1942 that specific immunity was transferred through lymphocytes from one individual to another (Landsteiner K 1942),

it has been found that this adoptive transfer of immunity can be achieved by transferring cells from various sources, such as mesenteric lymph node cells, gut intraepithelial lymphocytes, peritoneal cells, spleen cells and bone marrow cells (Ghadirian E 1983; Iverson M 1983; McDonald V 1996). Adoptive transfer of immunity to various infectious agents has been investigated in bone marrow transplantation (BMT) and it has led to potential clinical applications: specific immunization of the donors can protect bone marrow transplant recipients from certain pathogens, such as varicella-zoster-virus or Pseudomonas aeruginosa (Saxon A 1986; Gottlieb DJ 1990; Kato S 1990). In the study by Shouval and Ilan, bone marrow recipient mice, conditioned by sublethal irradiation, were injected intravenously with bone marrow cells obtained from syngeneic anti-HBs antibody-positive immune donors. Seroconversion to anti-HBs occurred in more than 80% of BMT recipient mice within the third week after transplantation (Shouval D 1993). Similar results were obtained in HLA-matched BMT patients within 9-42 days after transplantation: a clearance of HBV infection was observed in an asymptomatic HBsAg+/HBV DNA+ carrier with leukemia, who underwent BMT from his HLA-matched brother who was anti-HBc+/anti-HBs+ at the time of bone marrow donation (Ilan Y 1993).

The benefits of transferring donor' s HBV-specific immunity into HBV-infected recipients after bone marrow transplantation encourage the investigations on similar applications in liver graft recipients: can donor' s specific immunity be transferred by liver transplantation? Can liver recipients benefit from this immune transfer to reduce the risk of HBV reinfection?

It is well described that the liver is the major site of hematopoiesis during fetal life (Lydyard P 1998). Even in adults, extra-medullary hematopoiesis is known to take place in the liver when the function of the bone marrow is severely suppressed as in

accidental radiation exposure. Taniguchi and Murase provided the evidence that multilineage hematopoietic reconstitution of supralethally irradiated mice or rats could be achieved by syngeneic liver transplantation (Murase N 1996; Taniguchi H 1996; Taniguchi H 1997). It was also demonstrated that donor rat hematopoietic progenitor cells were present in bone marrow of recipient rats after allogeneic liver transplantation and immunosuppressive treatment (Sakamoto T 1997). Therefore, it is hypothesized that liver transplantation, in some point regarded as "mini-version of bone marrow transplantation" (Ricordi C 1992), could also transfer donor' s specific immunity into the liver graft recipient.

Several clinical cases with unexpected complications after liver replacement have been documented: 1) several cases of immune-hemolytic anemia, which were caused by anti-recipient ABO antibody after blood-group-compatible but nonidentical liver transplantation (Ramsey G 1984); 2) a case of autoimmune thrombocytopenia after liver transplantation from a donor with such a disease. The patient was released after receiving a second liver transplantation and treated with plasma exchange (Friend PJ 1990). In those reports, adoptive transfer of donor derived immune cells was suspected to be responsible for the unexpected consequence in the recipient. Unlike in bone marrow transplantation, however, the transfer of humoral immunity through liver replacement has not been studied systematically up to now. Possible benefits of immune transfer by organ transplantation have not been investigated.

Using a rat model, it has been demonstrated that cellular and humoral immunity could be successfully transferred from skin-sensitized donors to their recipients by means of liver transplantation (Dahmen U 1997). In this study, it was investigated whether the immunity against HBV can be transferred by liver transplantation. One potential application of clinical relevance is the reduction of the high rate of HBV reinfection in liver transplantation patients by adoptively transferring

immune cells from vaccinated donors. If this specific immunity of donors could be transferred, the liver recipients can be protected from HBV infection. Following this hypothesis, a reduction of the HBV recurrence rate might be achieved.

1.3. Aim of the Study

Using a rat model, this animal experiment was designed to evaluate the efficacy of adoptively transferring anti-HBs immunity from HBV vaccinated donors to recipients by liver transplantation. The humoral immune response to HBsAg was investigated by using the anti-HBs titer as the main parameter.

The study consisted of two parts. In the first part, the following questions have been addressed:

1) Can donor‘ s humoral response to HBsAg be transferred to the recipient after liver transplantation?
2) How do different genetic backgrounds, represented by different rat strain combinations, influence the efficacy of adopted immunity?
3) Does immunosuppressive treatment influence the antibody titer duration and height?

In the second part, two different strategies to enhance donor derived anti-HBs immunity in the recipient were investigated:

1) enhancing the efficacy of adoptive immune transfer by simultaneous bone marrow transplantation, which possibly increases the number of antibody-secreting cells
2) restimulating the donor-derived immune cells by postoperative HBV vaccination (HBVac), which possibly induces a secondary immune response.

2. MATERIAL AND METHODS

2.1. Experimental Design

2.1.1. Animal model

Three major histocompatibility complex (MHC) fully mismatched rat strains, namely ACI ($RT1^a$), Brown Norway (BN, $RT1^n$) and Lewis (LEW, $RT1^l$), were involved in this study. RT1 written in the parentheses above is the technical term for rat major histocompatibility complex and is the counterpart of H-2 system in mouse and HLA system in man. The superscription indicates the specific haplotype of RT1.

Three strain combinations were used: 1) syngeneic liver transplantation from LEW donor rats to LEW recipient rats; 2) allogeneic acutely rejecting combination from ACI to LEW rats; 3) allogeneic spontaneously tolerant combination from BN to LEW rats. In the syngeneic strain combination, LEW recipients do obviously accept the liver graft from another LEW rat. In the allogeneic acute rejection combination from ACI to LEW rats, the liver graft is rejected in a mean time of 12 days (Misumi M 1993; Okamura R 1989). Under daily immunosuppressive treatment, however, the ACI liver graft can be protected from acute rejection. In the allogeneic spontaneously tolerant combination from BN to LEW rats, LEW recipients accept BN liver grafts without immunosuppression although undergoing a transient rejection that resolves spontaneously (Houssin D 1979).

Rats of different strains were vaccinated with hepatitis B vaccine 6 weeks and boosted 2 weeks prior to organ donation. Orthotopic liver transplantations were performed between vaccinated donor rats and naive recipient rats. The animals were monitored weekly for the presence of anti-HBs antibodies (Figure 1).

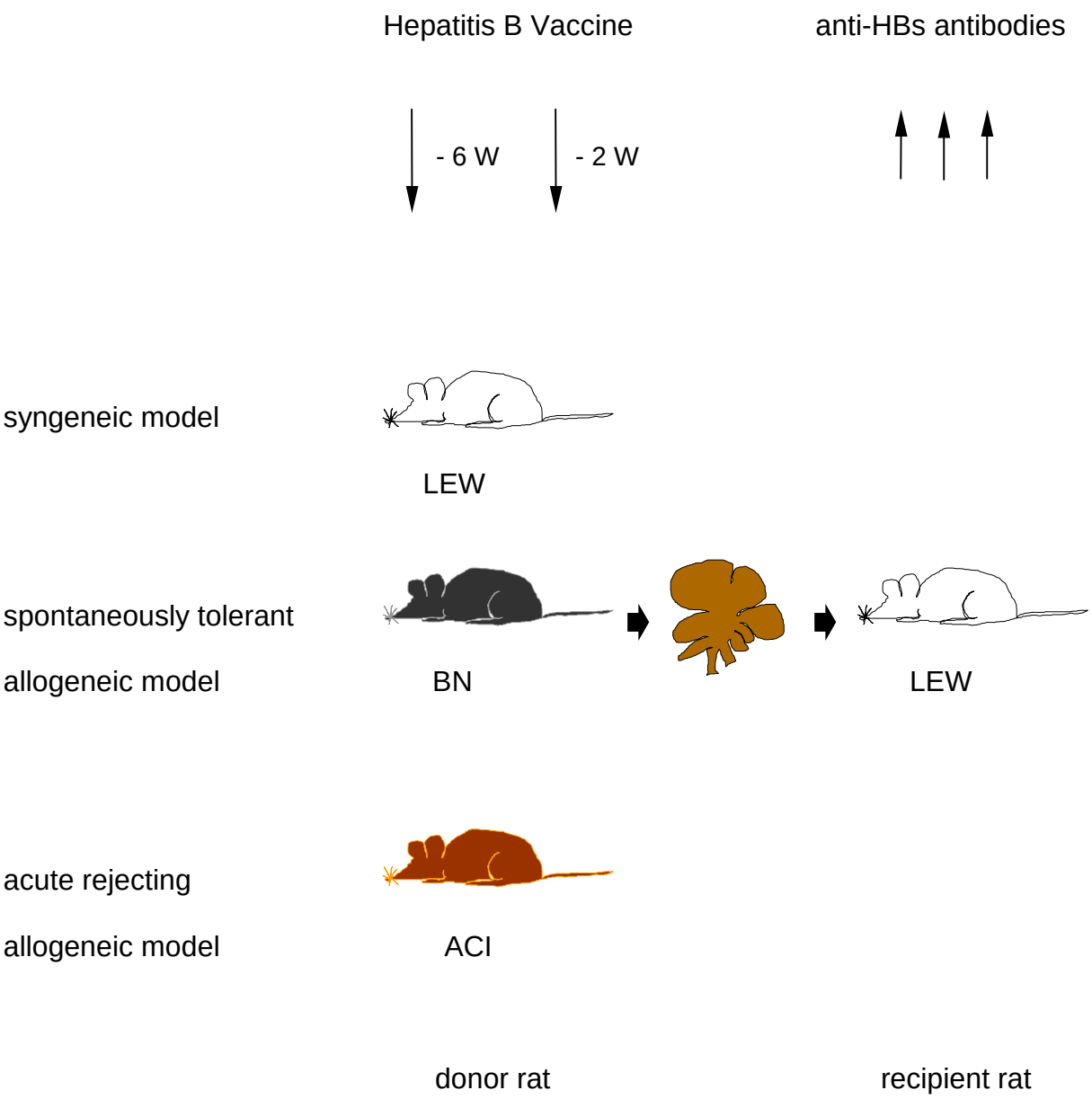

Figure 1. Animal models: three rat strain combinations were used, which represented a syngeneic liver transplantation model (LEW->LEW), a spontaneously tolerant allogeneic model (BN->LEW), and an acutely rejecting allogeneic model (ACI->LEW). Rats of three different strains were vaccinated with hepatitis B vaccine (HBVac) 6 weeks and boosted 2 weeks prior to organ donation. After liver transplantation, recipient rats were followed weekly for anti-HBs titer.

2.1.2. Animals

Male inbred LEW ($RT1^l$), BN ($RT1^n$), and ACI ($RT1^a$) rats (Charles River Wiga GmbH, Sulzfeld, Germany) at the age of 5-6 weeks (80-100 g/ rat) were chosen for vaccination. Six weeks later, they were used as organ donors. Male inbred LEW rats at the age of 10 to 11 weeks served as recipients. At the time of transplantation, the weight of each rat was within the range from 230-280 g.

The animals were housed under standard animal care conditions and were fed with rat chow ad libitum before and after operation. All procedures and housing were carried out according to the German Animal Welfare Legislation.

2.1.3. Experimental groups

Transplantations were performed according to the following groups:

Groups in part I Standard orthotopic liver transplantation in three rat strain combinations (LEW->LEW, BN->LEW, ACI->LEW): immunosuppressive medication with cyclosporine A (5 mg/kg/day, subcutaneously; Sandimmune, Sandoz Ltd., Basel, Switzerland) was applied in half of the liver recipients of each strain combination.

Groups in part II 1) Bone marrow augmented liver transplantation: aiming for augmenting the adoptive transfer of immunity, donor type bone marrow transplantation was performed at the time of liver transplantation in all three strain combinations. Immunosuppression was only applied in the two allogeneic models; 2) Postoperative vaccination: recipients from all three strain combinations were vaccinated against HBV at the 10th week post transplantation and were observed for another 10 weeks. Two groups were used as control: normal LEW rats receiving one

dose of HBVac, and the recipients of LEW liver grafts being vaccinated 10 weeks after liver transplantation.

2.2. Procedures

2.2.1. Vaccination of liver donors against hepatitis B surface antigen

Male inbred LEW, BN or ACI rats at the age of 5-6 weeks were chosen for vaccination. Each rat was first anesthetized by inhalation of methoxyflurane (Metofane, Janssen GmbH, Neuss, Germany). After sampling of 0.3 to 0.5 ml blood from the tail tip for serum antibody analysis, 0.2 ml HBV-DNA recombinant vaccine (Engerix*- B, SmithKline Beecham Pharma GmbH, Munich, Germany, containing HBsAg 20 μg /ml) was injected intramuscularly into the vastus medialis muscle using a 27 G needle (TERUMO, TERUMO EUROPE N.V. 3001, LEUVEN, BELGIUM). The animals were bled weekly (0.3- 0.5 ml) for anti-HBs titer assessment. Serum of each rat was separated and stored at -20°C until anti-HBs antibody titer was measured by microparticle-enzyme-immunoassay (MEIA). Four weeks after the first vaccination, all rats were boosted with another dose of HBV vaccine. They served as organ donor two weeks later.

2.2.2. Surgical procedures of orthotopic liver transplantation (OLT) in rats

OLT procedure was performed according to the cuff technique of Kamada without arterial reconstruction (Kamada N 1983). Surgical procedures were

performed under 6-fold loop magnification. Clean, but nonsterile instruments were used**.**

Anesthesia. Inhalation of methoxyflurane was used for anesthesia, which has a short induction phase, is easily controlled and allows fast postoperative recovery. Anesthesia was induced by placing the animal in a closed glass cylinder with methoxyflurane. Anesthesia was maintained by positioning a 50 ml tube (50 ml PP-tube, Cellstar, Greiner labortechnik, Germany) containing methoxyflurane-soaked gauze in front of the rat. The depth of anesthesia was controlled by adjusting the distance between the tube and the nose of the animal (Figure 2).

Donor operation. The rat was immobilized in a supine position. After entering the abdomen through a transverse abdominal incision, the intestines were retracted to the left and covered with a saline soaked sponge. The falciform ligament, the gastrohepatic ligament, the triangular ligament, and the posterior attachments were severed. After ligating the left phrenic vein, the infrahepatic vena cava (IHVC) was divided from the lumbar vein and the right adrenal vessels. The right renal vein and artery were then ligated and divided individually. The hepatic artery, the pyloric, splenic, and inferior mesenteric vein were doubly ligated with 6-0 silk suture (RESORBA, Nuernburg, Germany) and divided. Donor bile duct cannulation was then performed. A 10 mm long tube (outer diameter 0.9 mm; 22-gauge FEP Teflon tube, KLINIKA Medical GmbH, Usingen, Germany) was inserted into the lumen of the distal bile duct and secured with a circumferential 6-0 silk suture (Figure 3). After the rat was heparinized with 200 IU heparin via the penile vein (Heparin-Natrium-250, 000-ratiopharm, Ratiopharm GmbH&Co. Ulm, Germany), the IHVC was cross-clamped and divided distal to the clip at the level of the left renal vein. The liver was perfused through the portal vein (PV) with physiological saline solution at 4°C (Figure 4). In order to minimize the influence of different volumes of blood remaining

Figure 2. Inhalation of methoxyflurane was used for anesthesia during the operation.

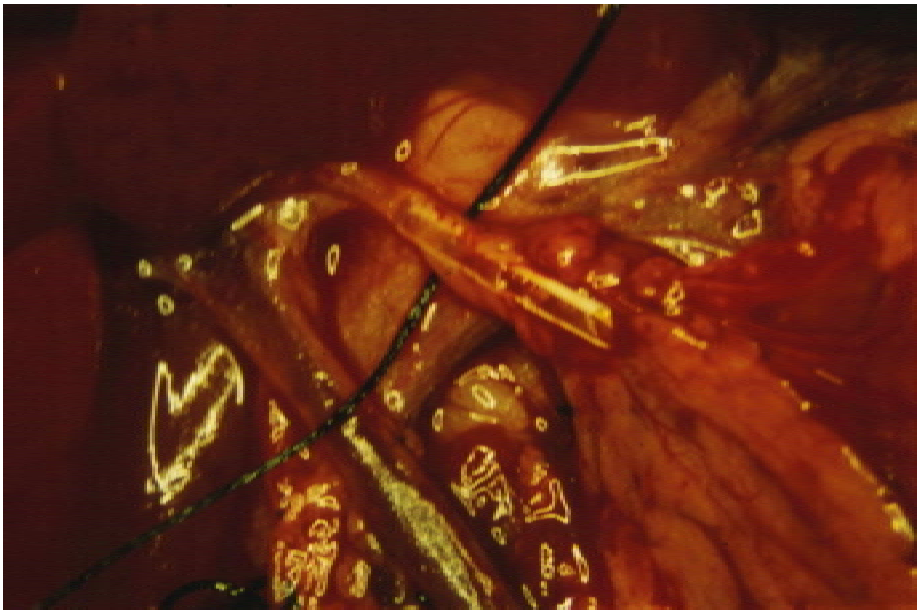

Figure 3. The donor bile duct was cannulated with a 22-gauge Teflon tube (6-fold magnification).

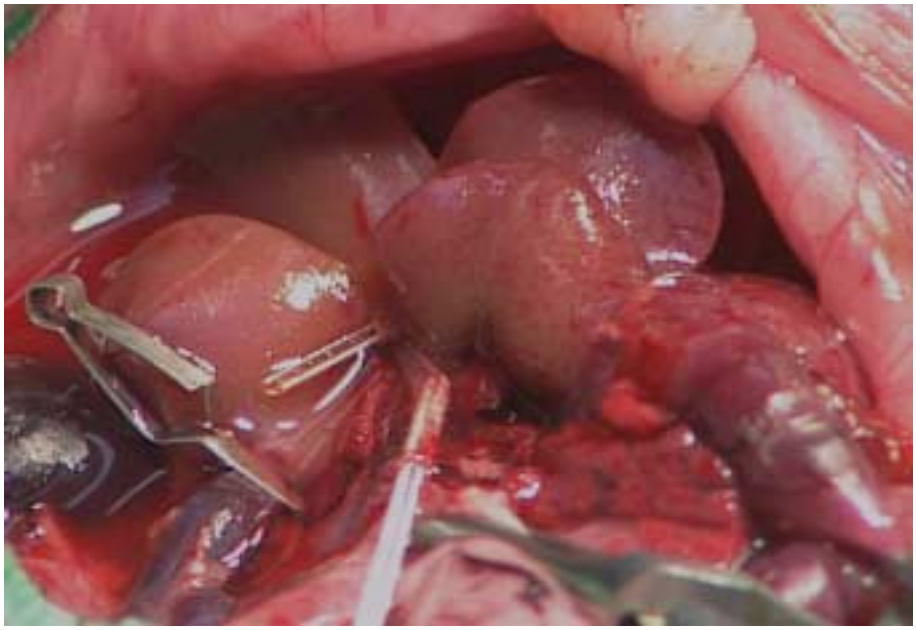

Figure 4. The donor liver was perfused through the portal vein with physiological saline solution at 4ºC.

in hepatic vessels or sinusoids, possibly containing primed lymphocytes and specific immunoglobulin, the liver graft was perfused with 10 to 15ml saline solution until the venous effluent become clear. The suprahepatic vena cava (SHVC) was then transected together with a small rim of diaphragm. The PV was divided at the level of the inferior mesenteric vein. The liver was placed in a Petri dish (100× 20 mm style, FALCON, Becton Dickinson Labware, Franklin Lakes, NJ, USA) with 4°C saline bath. The temperature was maintained by the surrounding cold water bath filled with crashed ice.

Ex vivo graft preparation. Bench operation was commenced immediately following the donor operation. It consisted of trimming the SHVC and attaching the cuffs to the PV and the IHVC. The cuff for the PV consisted of a cuff body and a cuff extension, both of which were 0.2 cm long (outer diameter 2.1 mm; 14-gauge polyethylene tube, Angiocath, Becton Dickinson GmbH, Heidelberg, Germany). For the IHVC it consisted of a 0.2 cm cuff body and 0.25 cm cuff extension (outer diameter 2.4 mm; 12-gauge polyethylene tube, Braunuele MT, B. Braun Melsungen AG, Melsungen, Germany). Attachment of cuff to both vessels was performed in an iced saline bath. First the cuff was slipped over the PV. The cuff extension, positioning towards the liver, was fixed by a bulldog clamp together with the PV. The bulldog clamp was then attached to the wall of the Petri dish. At this point the opening of the PV was grasped with two forceps on the right and left sides. The distal end of the PV was everted over the cuff body and secured in this position with a circumferential 6-0 silk suture. The same method was applied to the IHVC (Figure 5). Afterwards the SHVC was trimmed by resection of diaphragm. Placement of two stay-sutures using 7-0 polypropylene suture (Prolene, ETHICON GmbH & Co. KG, Norderstedt, Germany) on both sides of the SHVC completed the backtable procedure.

Recipient operation. A transverse abdominal incision was performed under methoxyflurane anesthesia and the xiphoid process was elevated with a Mosquito clamp. The hepatic artery, the left phrenic vein, the right suprarenal vein as well as the lumbar veins were ligated and divided. The bile duct was transected at the proximal end. The IHVC was cross-clamped with a vascular clip at the level of the right renal vein and the PV was clamped at the level of the pyloric vein. When the liver had become pale, the SHVC was cross-clamped with a Satinsky clamp. Order of clamping is important to allow the outflow of the blood within the liver, which minimizes the blood loss in the recipient. These vessels were divided. Care was taken to ensure that the veins were kept as long as possible to facilitate the anastomosis by cuff or suture. The recipient liver was removed**.**

Liver implantation. The donor liver was removed from the iced saline bath and placed in orthotopic position, and the donor SHVC was anastomosed end-to-end with the recipient SHVC using a continuous 7-0 polypropylene suture (Figure 6). Portal vein anastomosis was then started. Tension in the recipient PV was maintained by traction applied by two polypropylene sutures on the right and left side of the opening. The PV was washed out with saline solution to prevent air embolism. Cuff of donor portal vein was inserted into the lumen of the recipient PV and secured with a circumferential 6-0 silk ligature (Figure 7). The clamp on the PV was released, followed by that on the SHVC. Anastomosis of the IHVC was performed in the same way using the cuff method. The bile duct anastomosis was accomplished by inserting the Teflon tube, which had been placed in the donor bile duct before, into that of the recipient. Anastomosis was secured with a circumferential 6-0 silk ligature, which was connected to the one on the graft side (Figure 8). Abdominal incision was closed with continuous 3-0 polyester suture (Mersilene, ETHICON, Norderstedt, Germany). All

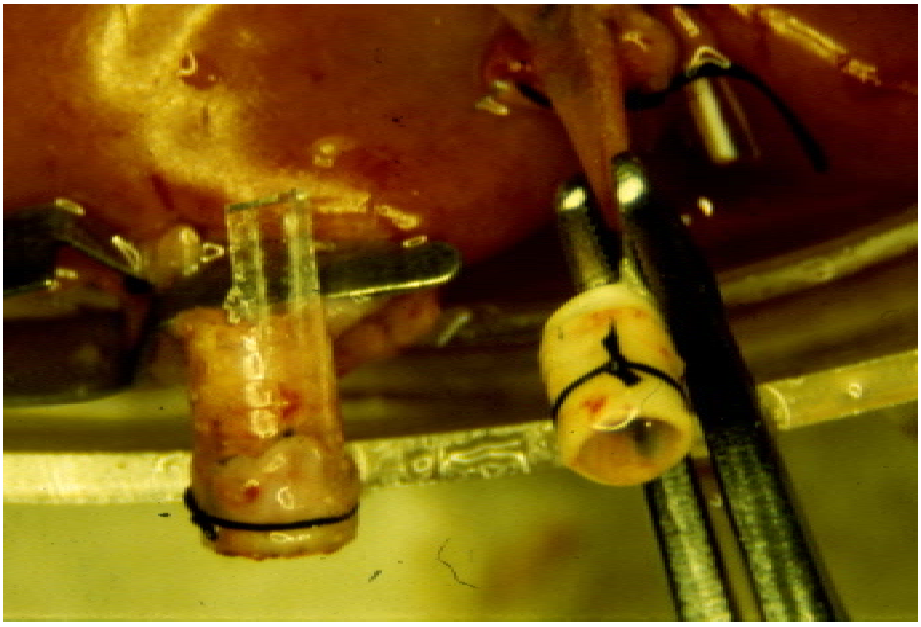

Figure 5. Cuff attachment into the infrahepatic vena cava (left) and the portal vein (right) of a liver graft (6-fold magnification).

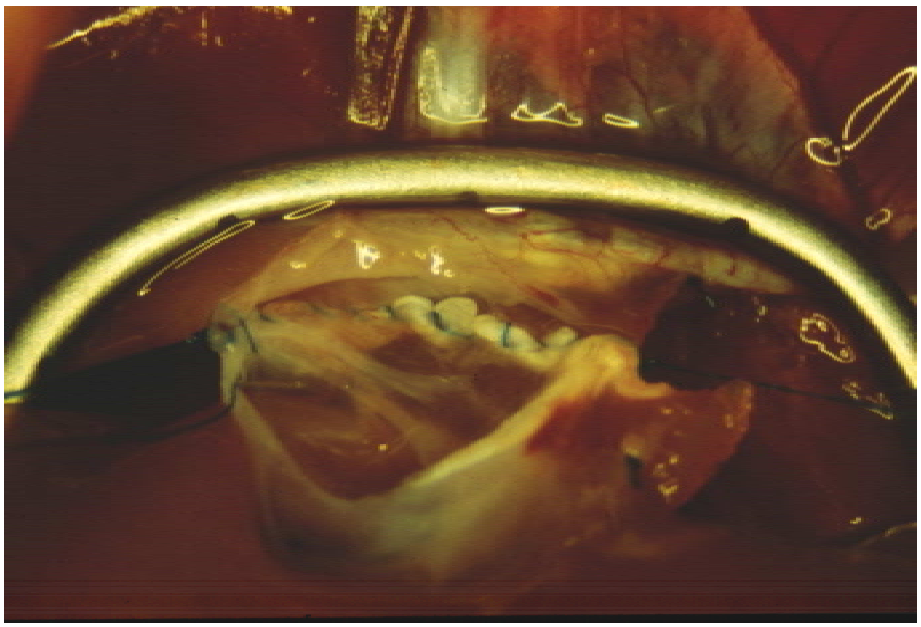

Figure 6. End-to-end anastomosis of the suprahepatic vena cava using a continuous 7-0 polypropylene suture (6-fold magnification).

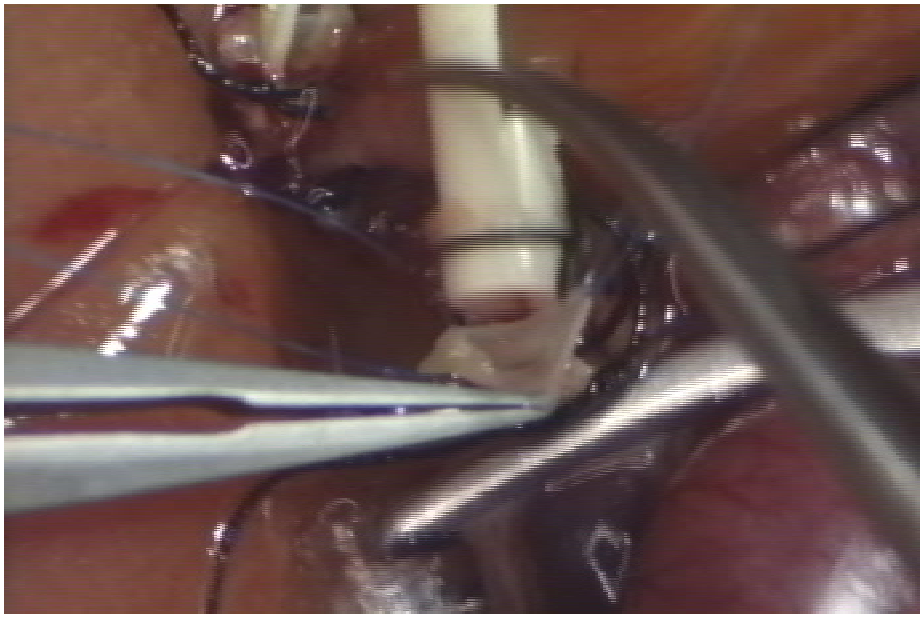

Figure 7. Anastomosis of the portal vein by inserting the cuff into the lumen of recipient’ s portal vein (6-fold magnification).

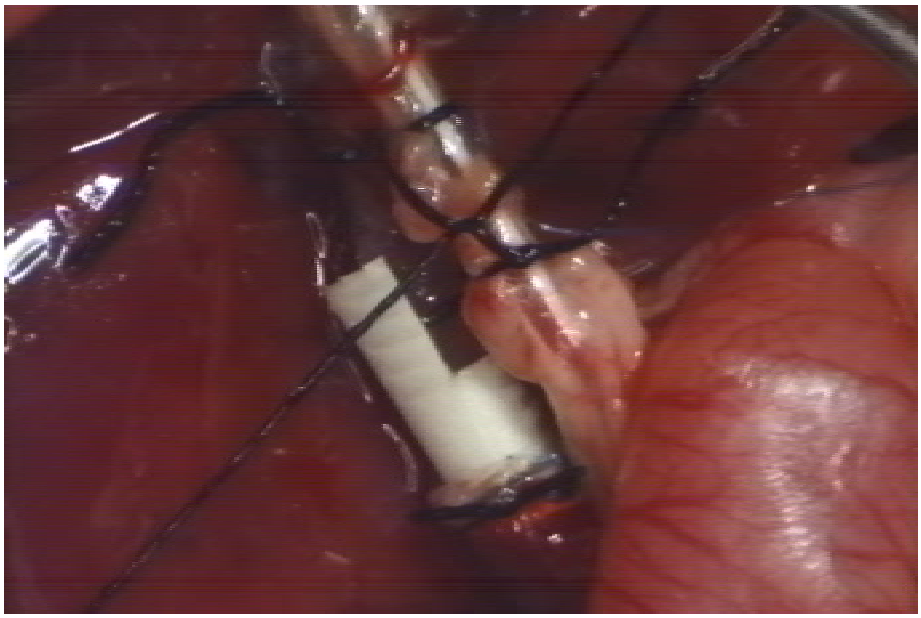

Figure 8. Anastomosis of the bile duct by inserting the Teflon tube into the lumen of recipient’ s bile duct (6-fold magnification).

recipients received a bolus of 100 mg/kg Mezlocillin (Baypen, Bayer AG, Leverkusen, Germany) as antibiotic prophylaxis by intramuscular injection after operation.

2.2.3. Bone marrow augmented liver transplantation in rats

Preparation of bone marrow cells. Donor rats were euthanized with methoxyflurane. The femur and the tibia were harvested using aseptic technique. After severing the bones between the joints, the bone marrow cavity was flushed with a 22-gauge needle using 10 ml RPMI 1640 supplemented with 2 mmol/L L-Glutamine, penicillin (50 U/ml) and streptomycin (50 μg/ml) (GIBCO/BRL, Life Technologies LTD, Paisley, Scotland) until the cavity appeared pale. The marrow was mechanically resuspended in medium by gentle aspiration through sterile nylon mesh. After cells were pelleted (at 1600 rpm for 10 minutes in room temperature), they were adjusted to a concentration of 80-100$\times 10^6$ cells/ml. Cell viability was determined by trypan blue exclusion which was routinely found to be over 95%.

Bone marrow cells infusion. The bone marrow cells were stored at 4°C for one hour until they were injected via the penile vein to the liver recipient immediately after transplantation. For each recipient, both the liver graft and the bone marrow cells were harvested from the same donor. Each recipient received 1 ml of 80- 100$\times 10^6$ /ml bone marrow suspension. There was no ablation of recipient' s immune system before transplantation.

2.2.4. Postoperative management

Each group was followed for more than 20 weeks for serum anti-HBs level. 0.3 to 0.5 ml peripheral blood were drawn from the tail tip weekly under methoxyflurane

anesthesia. The serum was separated and stored at -20°C until measurement of anti-HBs antibody titer by MEIA.

In each strain combination half of the recipients were given daily immunosuppressive treatment (5 mg/kg/day Cyclosporine A by subcutaneous injection) (Okamura R 1989). Ten weeks after organ transplantation, liver recipients were vaccinated once with 0.2 ml recombinant hepatitis B vaccine by intramuscular injection. Six LEW rats, receiving liver grafts from naive LEW donors, were also vaccinated 10 weeks after transplantation to serve as a control group.

Animals whose general condition deteriorated within the first postoperative week were sacrificed and excluded from further analysis.

2.3. Measurement of Anti-HBs Titer

A fully automated microparticle enzyme immunoassay (MEIA) was used for detection and quantification of rat serum antibody against hepatitis B surface antigen (anti-HBs), which is described by Abbott Laboratories (Ostrow DH 1991). No operator intervention was required other than pipetting of the samples into the specimen wells, placing the reagent vials into the analyzer and initiating the assay. Principally, in the first step of the assay, microparticles coated with rHBsAg were added to the specimen. An aliquot of the reaction mixture was transferred to a glass fiber matrix. The microparticles bound irreversibly to the matrix and unbound material was washed through the matrix. Biotinylated rHBsAg was then added to the matrix to react with captured anti-HBs. Anti-biotin/alkaline phosphatase conjugate was added to the matrix to react with the biotinylated rHBsAg/captured latex microparticle complex. Unbound conjugate was removed by washing the matrix. The bound

conjugate complex was detected by incubation with the fluorogenic substrate 4-methylumbelliferyl phosphate. The rate of fluorescence signal generation was proportional to the amount of anti-HBs bound to the microparticle/matrix solid phase. Anti-HBs concentrations in specimens were calculated automatically by comparison of the specimen rate to value determined from a stored standard curve.

2.4. Histological Study

Autopsy was performed in all animals post-mortem. Tissue samples from organ grafts were excised, fixed in 4% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin prior to histopathological analysis.

Judgment and grading of liver allograft rejection were done according to an internationally accepted grading system, namely, Banff schema (Demetris AJ 1997). Three specific features, portal inflammation, bile duct inflammation/damage, and venular inflammation, were semiquantitatively scored on a scale from 0 to 3 (mild, moderate, and severe), according to the criteria listed in Table 1. The results of the individual score in the categories were added in order to obtain the final rejection activity index (RAI). RAI of 3 or 4 equals to mild acute rejection. RAI between 5 to 7 refers to moderate rejection. Severe rejection is indicated by RAI above 8.

Table 1. Grading of acute liver allograft rejection (Demetris AJ 1997)

Category	Score	Criteria
Portal Inflammation	1	• Mostly lymphocytic inflammation involving, but nor noticeably expanding, a minority of the triads
	2	• Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophiles and eosinophils
	3	• Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma
Bile Duct Inflammation/Damage	1	• A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear: cytoplasmic ratio of the epithelial cells
	2	• Most or all of the ducts are infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium
	3	• As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption
Venous Endothelial Inflammation	1	• Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules
	2	• Subendothelial infiltration involving most or all of the portal and/or hepatic venules
	3	• As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis.

2.5. Statistical Analysis

The antibody titers of individual rat in each group were calculated as geometric mean titers (GMT) in "mIU/ml" (Jilg W 1989; Shouval D 1993). Calculations regarding GMT and titer duration were based on the seroconverted animals. A two-tailed student t-test analysis was employed to assess the differences within each group. Quantitative data were compared using chi-square test. A p value below 0.05 was considered to be statistically significant. All data were analyzed using the Microsoft Excel Statistic Program.

3. RESULTS

3.1. Efficacy of Vaccination of Organ Donors

100 animals were vaccinated with recombinant HBV vaccine (rHBVac). 7 of them died due to anesthesia complications during weekly blood sampling. A level of 10 mIU/ml of hepatitis B surface antibody is widely accepted as an effective immune response and is used as the threshold for a positive response to rHBVac (Centers for Disease Control and Prevention 1987; Sokal EM 1992; Berner J 1993; Katkov WN 1996). Based on this definition, the response rate to rHBVac was 95.7% (89/93) after the first vaccination. A secondary response was observed after the second vaccination with rHBVac: the anti-HBs titer increased 30- to 70- fold and one more animal seroconverted, resulting in an overall response rate of 96.8% (90/93).

The development of anti-HBs antibodies in LEW, BN and ACI rats is presented in Figure 9. Twenty-eight LEW rats, 40 BN rats and 25 ACI donor rats were evaluated in several independent experiments. Four weeks after the first vaccination the geometric mean titer (GMT) of anti-HBs was 411 mIU/ml in the responding LEW rats, 554 mIU/ml in BN rats and 881 mIU/ml in ACI rats. The booster vaccination led to anti-HBs GMT of 27897 mIU/ml in LEW rats, 27617 mIU/ml in BN rats and 31644 mIU/ml in ACI rats. No significant difference was observed between the anti-HBs GMT among the different rat strains ($p > 0.05$, Table 2).

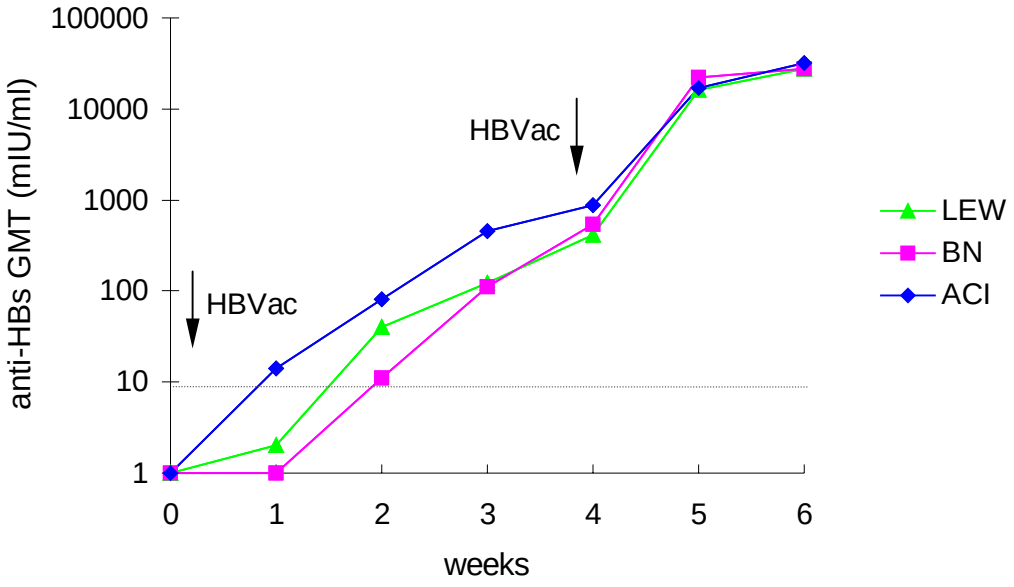

Figure 9. Response to hepatitis B vaccine (HBVac) in three different donor rat strains. Four weeks after the first vaccine (one dose of 4 μg HBsAg), all rats were boosted with another dose of HBVac. Two weeks later, at the time of organ donation, the anti-HBs titer increased 30- to 70- fold.

Table 2. Immune response to recombinant hepatitis B vaccine in rats

Strain	Total number	Number of animals with effective response (anti-HBs $\geq$ 10 mIU/ml)	Geometric mean anti-HBs titer* (minimum~ maximum)
LEW	n=28	n=28	27897 (1217~ 160872) mIU/ml
BN	n=40	n=37	27617 (673~ 265380) mIU/ml
ACI	n=25	n=25	31644 (220~ 312809) mIU/ml

* anti-HBs titers were measured at two weeks after the second vaccination

3.2. Survival and Postoperative Complications of Liver Recipients

From 90 rats who had seroconverted to HBVac, 9 rats were employed in other projects; the remaining 81 animals were used as organ donors in this study. 81 orthotopic liver transplantations were performed from immunized donors to naïve recipients (Table 3). 21 of these recipients received simultaneous bone marrow cells infusion. Another 6 liver transplantations were performed using non-vaccinated LEW rats as donors, which served as a control group for postoperative vaccination.

Twenty rats, which survived less than one week after transplantation, were excluded from the serological study. Most of them died due to perioperative complications, either caused by an anesthesia over dose (n=1) or caused by the surgical procedure, such as air embolism in the suprahepatic vena cava (n=1), prolonged clamping time of portal vein (n=3), bleeding or obstruction of anastomosed vessel (n=10). Autopsy did not reveal any specific findings explaining the postoperative death in the other five recipients.

Table 3. Animal numbers in the different groups of liver recipients.

Strain combination	Control (no CsA, no BMT)	CsA*	BMT**	BMT and CsA	Excluded***	Total
LEW->LEW +	6	5	-	6	11	28
BN->LEW +	9	8	4	7	5	33
ACI->LEW +	6	6	-	4	4	20
LEW->LEW ‡	6	-	-	-	-	6

+ donor rats were immunized to rHBVac

‡ donor rats were not immunized to rHBVac

* with daily cyclosporine A treatment

** with simultaneous bone marrow transplantation

*** recipients surviving less than one week

Lethal rejection of the allogeneic liver grafts was observed as expected in the six recipients without CsA treatment in the allogeneic rejecting strain combination (ACI-> LEW). The survival time was 11 ± 6 days (mean ± SD). Histological examination showed acute rejection by: 1) severe portal inflammation with extension of mononuclear cells into the parenchyma, gross expansion of the portal tracts due to edema and cellular infiltration; 2) widespread bile duct inflammation and damage; 3) severe venous endothelial inflammation (Figure 10). No lethal rejection occurred in the eight recipients without immunosuppression in BN to LEW strain combination. All long-term surviving recipients in this strain combination showed discrete signs of chronic dysfunction such as bile duct proliferation or bile duct loss (Figure 11). In the syngeneic strain combination no signs of rejection were detected but normal histological morphology (Figure 12). In immunosuppressed allogeneic liver recipients, bile duct proliferation was the predominant change (Figure 13).

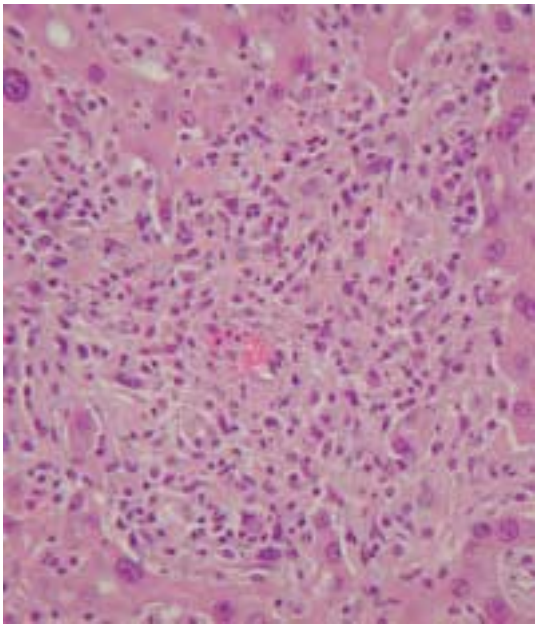

Figure 10. Acute rejection of a liver graft in ACI-> LEW recipient
(on postoperative day 11; HE, 200×)

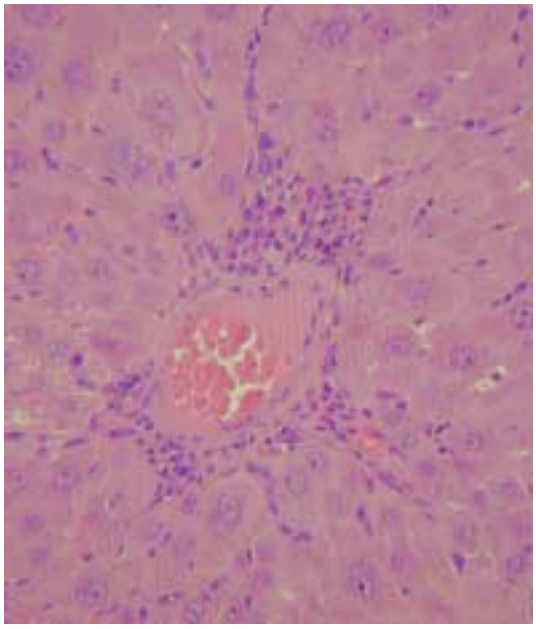

Figure 11. Bile duct loss in long-term surviving BN->LEW liver recipient (at postoperative week 12, HE, 200×)

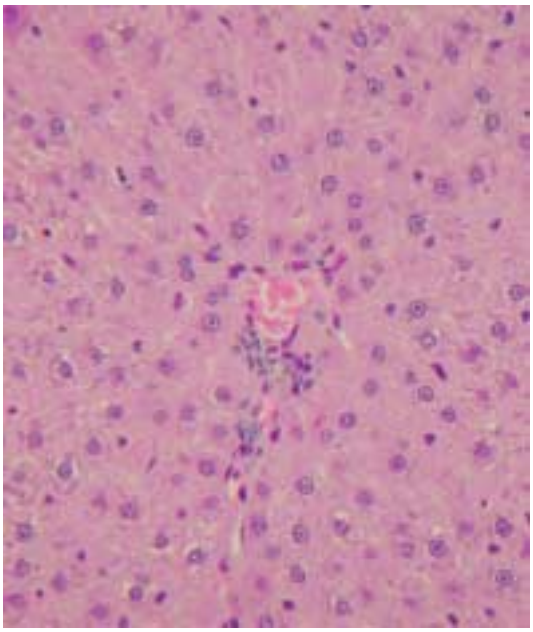

Figure 12. Maintenance of normal morphology of a liver graft in long-term surviving LEW->LEW recipient
(at postoperative week 25, HE, 200×)

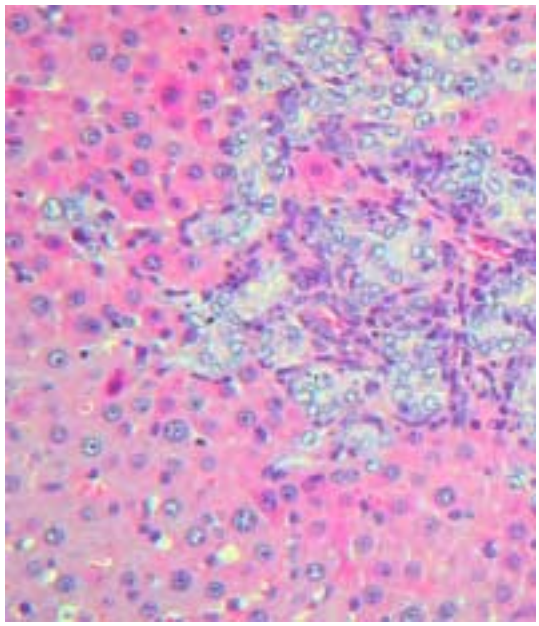

Figure 13. Bile duct proliferation of a liver graft in long-term surviving ACI->LEW recipient under immunosuppression
(at postoperative week 25, HE, 200×)

3.3. Adoptive Transfer of Immunity to HBsAg after Orthotopic Liver Transplantation

3.3.1. Development of anti-HBs in liver recipients from different strain combinations

LEW to LEW strain combination Among 11 LEW liver recipients, 6 were treated with CsA whereas the other 5 rats were left untreated (Table 4). 5 out of the 6 treated and 4 out of 5 untreated LEW recipients seroconverted to an effective anti-HBs titer ($\geq$ 10 mIU/ml) after transplantation. The geometric mean anti-HBs titer in the two groups was 42 mIU/ml (ranging from 14 to 108 mIU/ml) or 55 mIU/ml (19~ 311 mIU/ml) respectively. These titers represented 0.18% (0.10~ 0.27%) and 0.32% (0.10~ 0.76%) of the donor' s titer as measured on the day of liver donation. The effective titer in the two groups lasted for about 4 and 3 weeks, respectively. A titer between 0 to 10 mIU/ml was detected until postoperative week 6 (Figure 14).

BN to LEW strain combination In this strain combination 17 liver grafts were transplanted. 8 of these recipients were left untreated whereas the other 9 recipients were treated daily with CsA (Table 4). After the first postoperative week, 6 out of 8 and 8 out of 9 LEW recipients had developed an effective anti-HBs titer (Figure 15). The geometric mean anti-HBs titer in the two groups was 153 mIU/ml (16~ 854 mIU/ml) and 98 mIU/ml (42~ 414 mIU/ml), respectively. The titer represented 0.31% (0.13~ 1.90%) and 0.30 % (0.16~ 0.51%) of the donor' s titer as measured on the day of liver donation. The effective titer lasted significantly ($p< 0.01$) longer in the CsA treated group (5.5 weeks) compared with the untreated group (2.8

weeks). In the group with immunosuppressive treatment, one recipient rat had a detectable anti-HBs titer for more than eight weeks.

Table 4. Seroconversion to anti-HBs in rat liver recipients from three strain combinations.

Strain combination	Postoperative treatment	Seroconversion rate (animal number)	Mean value of Recipient-to-donor titer ratio* (range)	Weeks with anti-HBs titer $\geq$ 10 mIU/ml* (range)
LEW-> LEW	Without CsA	4/5	0.32% (0.10~0.76%)	4
	With CsA	5/6	0.18% (0.10~0.27%)	3 (2~5)
BN-> LEW	Without CsA	6/8	0.31% (0.13~1.9%)	2.8 (2~5)
	With CsA	8/9	0.30% (0.16~0.51%)	5.5 (4~9)
ACI-> LEW	Without CsA	6/6	0.10% (0.06~0.25%)	1
	With CsA	5/6	0.13% (0.07~0.30%)	5.3 (4~9)

* recipients, which did not seroconvert to anti-HBs titer over 10 mIU/ml, were not included in the calculation.

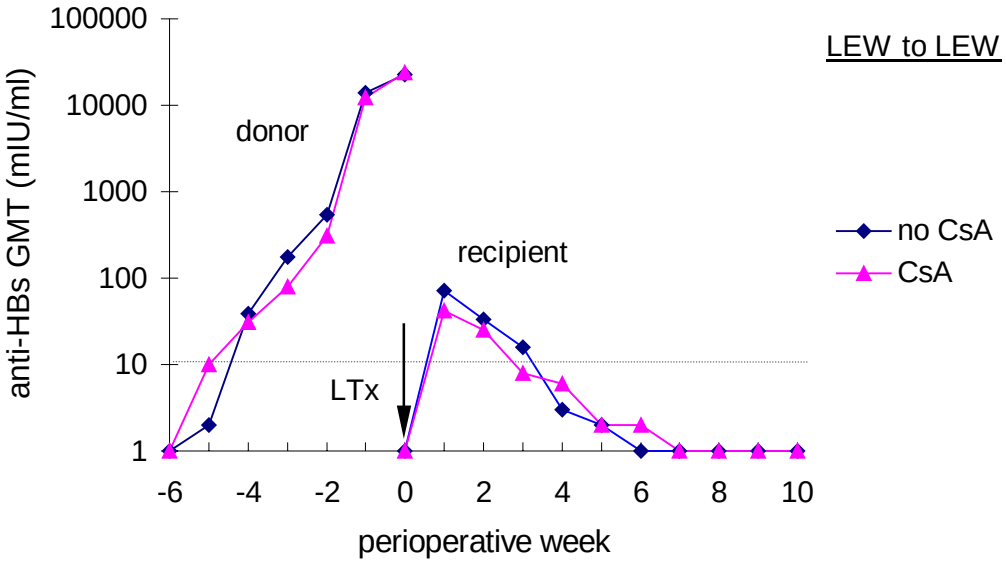

Figure 14. Anti-HBs titer development in LEW to LEW liver transplantation (LTx), shown as geometric mean titer (GMT) of donors and recipients in both cyclosporine A (CsA) treated (n=5), and untreated groups (n=4). The effective titer (≥10 mIU/ml) lasted for around 3 to 4 weeks in liver recipients.

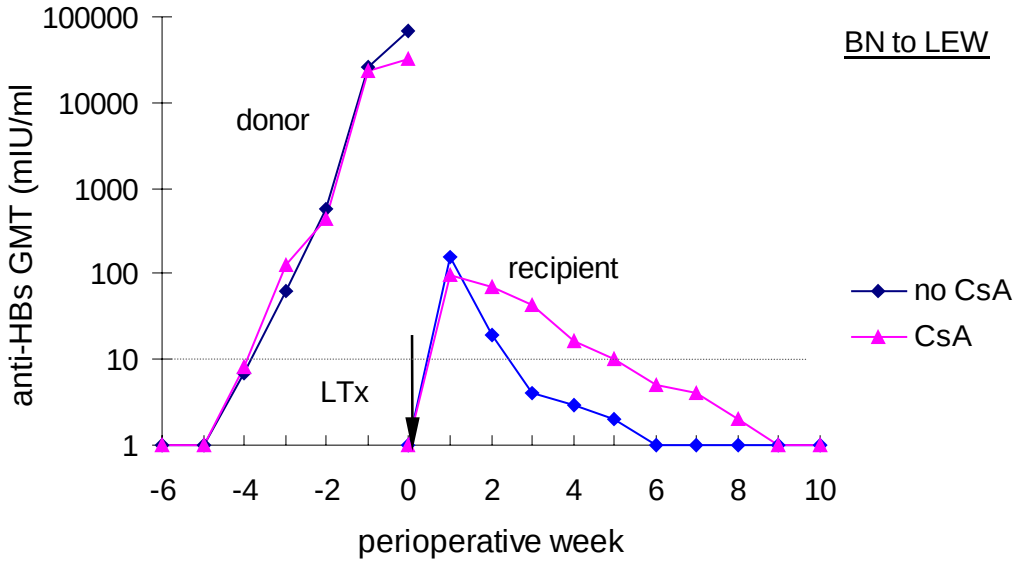

Figure 15. Anti-HBs titer development in BN to LEW liver transplantation (LTx), shown as geometric mean titer (GMT) of donors and recipients in both cyclosporine (CsA) treated (n=8) and untreated groups (n=6). The effective titer ($\geq$10 mIU/ml) lasted for about 2.8 or 5.5 weeks in each group of recipients respectively ($p < 0.01$).

ACI to LEW strain combination In this strain combination 12 liver transplantations were performed successfully with half of the recipients being treated with CsA (Table 4). The six recipients without CsA treatment seroconverted in the first postoperative week, showing a geometric mean anti-HBs titer of 71 mIU/ml (ranging from 32 to 334 mIU/ml). This represented 0.10% (0.06~ 0.25%) of the donor' s titer measured on the day of liver donation. All these recipients died from acute graft rejection at the mean time of 11 ± 6 (mean ± SD) days. The other 6 LEW recipients were protected from rejection by daily immunosuppressive treatment with CsA. Five of them seroconverted to anti-HBs with a GMT of 147 mIU/ml (55~ 250 mIU/ml), representing 0.13% (0.07~ 0.30%) of the donor' s titer on the day of liver donation. The effective titer lasted for about 5.3 weeks (Figure 16). In one recipient rat, the anti-HBs titer remained detectable until week 10 posttransplantation.

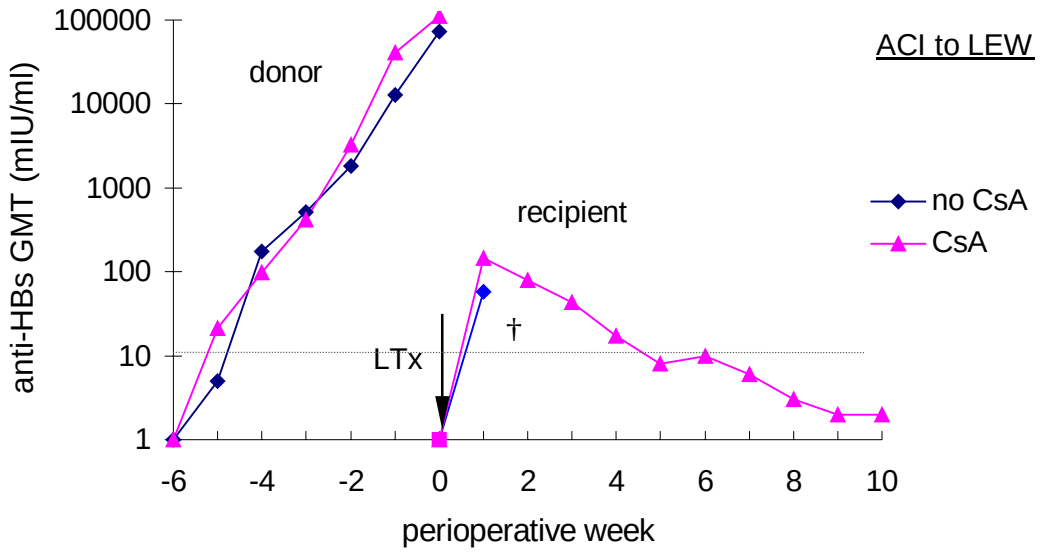

Figure 16. Anti-HBs titer development in ACI to LEW liver transplantation (LTx), shown as geometric mean titer (GMT) of donors and recipients in both cyclosporine A (CsA) treated (n=5) and untreated group (n=6)*. The effective titer (≥10 mIU/ml) lasted for about 5 weeks in CsA treated recipients.

†* Without immunosuppression, the liver recipients died from acute rejection at a mean time of 11 days.

3.3.2. Analysis of the efficacy of anti-HBs seroconversion in liver recipients

3.3.2.1. Influence of donor titer on anti-HBs seroconversion in liver recipients

Analysis of donor titers irrespectively of postoperative treatment revealed an association between the level of anti-HBs titer in donor rats and the corresponding recipient rats: all six recipients, which did not seroconvert to an effective anti-HBs titer at the first postoperative week (below 10 mIU/ml), had a donor anti-HBs titer below 15000 mIU/ml (GMT in these rats was 6726 mIU/ml). The other 34 donor rats, whose corresponding recipients had developed an effective anti-HBs titer, showed a GMT of 48889 mIU/ml (ranging from 11215 to 312809 mIU/ml) on the day of organ donation (Figure 17).

If anti-HBs titer of 15000 mIU/ml was chosen as a cut-off level for grouping donors, recipient seroconversion rate of 45% was observed in the group whose donor anti-HBs titer was below 15000 mIU/ml. The effective titer in those recipients lasted for about 2 weeks. Donor anti-HBs titer above 15000 mIU/ml was associated with 100% seroconversion rate in their liver recipients. The effective anti-HBs titer lasted for about 4 weeks in average (Table 5).

Table 5. Influence of donor anti-HBs titer on seroconversion in liver recipients.

Donor titer	Recipient' s anti-HBs titer development		
	Seroconversion rate* (animal number)	Mean maximal titer mIU/ml (animal number)	Weeks with anti-HBs $\geq$ 10 mIU/ml (animal number)
< 15000 mIU/ml	45% (5/11)	32 (5)	2.6 (5)
> 15000 mIU/ml	100% (29/29)	109 (29)	4.7 (29)
overall	85% (34/40)	91 (34)	4.2 (34)

* anti-HBs ≥ 10 mIU/ml was considered as seroconversion

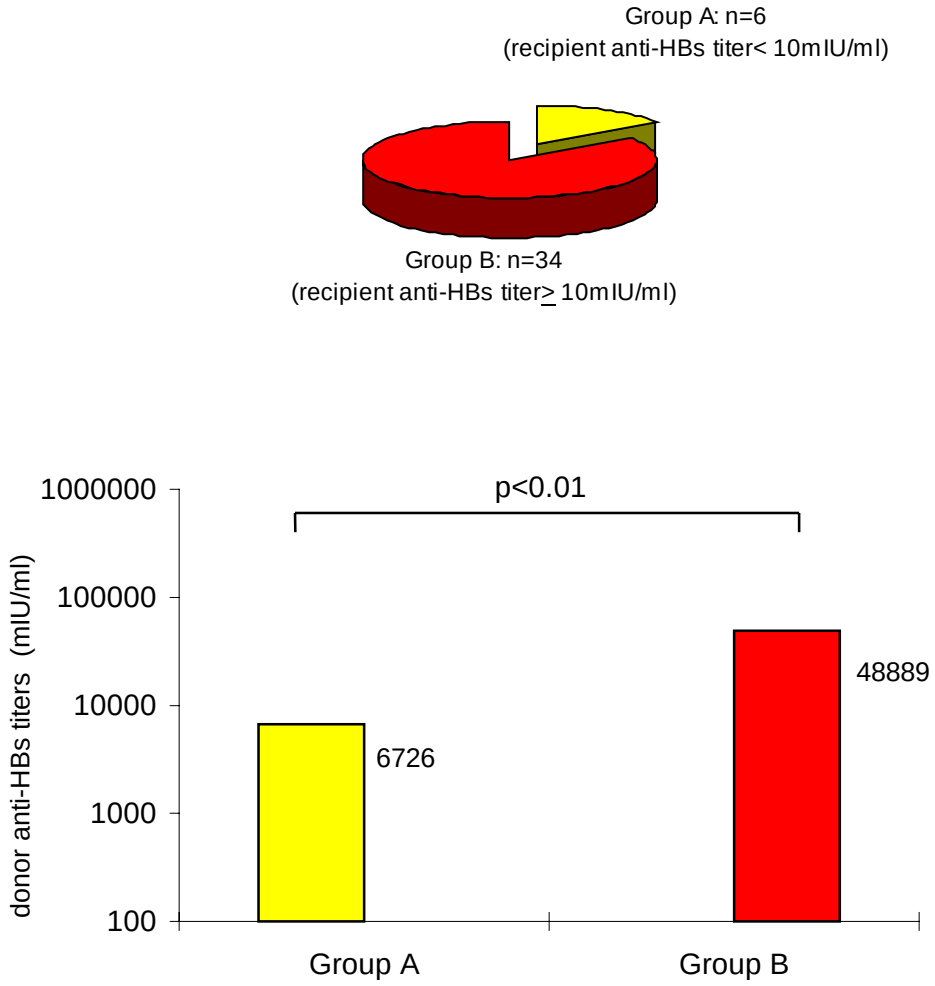

Figure 17. Comparison of donors' geometric mean anti-HBs titer in two groups that were divided according to with or without anti-HBs seroconversion in the liver recipients. Group A consisted of 6 transplantations, in which recipients did not have anti-HBs seroconversion (<10 mIU/ml) at the first postoperative week. Group B included 34 transplantations, in which the recipients had an anti-HBs titer over 10 mIU/ml at the first postoperative week. The anti-HBs GMT was higher in the donors of Group B (mean=48889 mIU/ml) than that of Group A (mean=6726 mIU/ml).

3.3.2.2. Influence of alloreactivity on anti-HBs development in liver recipients

Three different strain combinations were examined to analyze the influence of alloreactivity on the efficacy of the adoptively transferred immunity. A tendency towards a better transfer in the syngeneic strain combination was observed in recipients without immunosuppression (Table 6). Allogeneic liver recipients had a relative lower ratio of recipient to donor titer (0.10% in the rejecting model vs. 0.31% in the tolerant model vs. 0.32% in syngeneic model) and a shorter persistence of the effective anti-HBs titer (1 week in rejecting model vs. 2.8 weeks in tolerant model vs. 4 weeks in syngeneic model).

Table 6. Negative effect of alloreactivity on anti-HBs development in liver recipients*

Strain combination	Donor anti-HBs geometric mean titer mIU/ml (range)	Mean value of recipient- to- donor titer ratio %	Weeks with anti-HBs $\geq$ 10 mIU/ml (mean ± SD)
LEW -> LEW (syngeneic)	22851 (11215~80093)	0.32	4 ± 0
BN -> LEW (allogeneic tolerant)	50243 (11894~265380)	0.31	2.8 ± 1.2
ACI -> LEW (allogeneic rejecting)	70209 (28452~135039)	0.10	1 ± 0 **

* no immunosuppression was applied in any recipients

** anti-HBs titer decreased before rejection or follow-up was stopped due to animal death

3.3.2.3 Influence of immunosuppression on anti-HBs development in liver recipients

In the syngeneic liver transplantation model (LEW to LEW strain combination), immunosuppression had no visible influence on anti-HBs titer development in the recipients. Irrespective of the immunosuppressive treatment, the effective anti-HBs titer lasted three to four weeks (Figure 14).

After allogeneic liver transplantation, daily cyclosporine treatment prolonged the duration of the effective anti-HBs titer in both allogeneic strain combinations. In the case of BN to LEW liver transplantation, immunosuppressed recipients kept an effective anti-HBs titer for more than five weeks, whereas the period was less than three weeks in the untreated group ($p < 0.05$, Figure 15). In the rejecting strain combination (ACI->LEW), all untreated recipients died around 2 weeks posttransplantation from acute graft rejection. In the surviving immunosuppressed rats, effective titers were measured for at least 5 weeks, similar as in the spontaneous tolerant group with immunosuppression (BN-> LEW).

3.4. Augmentation of Adoptive Immune Transfer in Liver Graft Recipients

3.4.1. Augmentation by simultaneous bone marrow cells infusion

3.4.1.1. Development of anti-HBs titer development in bone marrow augmented liver recipients

LEW to LEW strain combination Six LEW to LEW rats underwent simultaneous liver transplantation and bone marrow transplantation (Table 7). No immunosuppressive treatment was applied to those recipients. Four of them had an effective anti-HBs titer in the first postoperative week, with a geometric mean anti-HBs titer of 168 mIU/ml (ranging from 83 to 238 mIU/ml). This titer represented in average 0.34% (0.21~ 0.68%) of the donor' s titer on the day of organ donation. The effective anti-HBs titer lasted in average for 3.7 weeks posttransplantation (Figure 18). The other two recipient rats had an anti-HBs titer below 10 mIU/ml in the first postoperative week, corresponding to the weak response of their donors to the vaccine (both were below 1000 mIU/ml on the day of organ donation).

Table 7. Seroconversion to anti-HBs in bone marrow augmented rat liver recipients from three strain combinations.

Strain combination	Postoperative treatment	Seroconversion rate	Mean value of recipient- to-donor titer ratio* (range)	Weeks with anti-HBs $\geq$ 10 mIU/ml (range)
LEW-> LEW	Without CsA	4/6	0.34% (0.21~0.68%)	3.7 (3~5)
BN-> LEW	Without CsA	7/7	0.21% (0.03~0.99%)	2.6 (2~4)
	With CsA	4/4	0.27% (0.16~0.37%)	5.3 (5~10)
ACI-> LEW	With CsA	4/4	0.26% (0.13~0.73%)	6.1 (4~12)

* recipients, which did not seroconvert to anti-HBs titer over 10 mIU/ml, have not been included in the calculation.

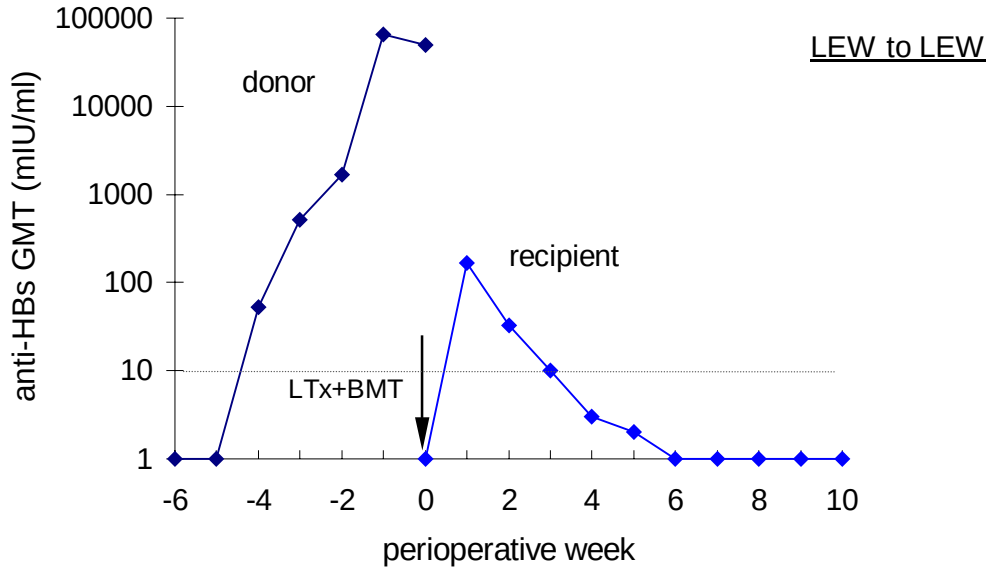

Figure 18. Anti-HBs titer development in untreated LEW to LEW bone marrow augmented liver transplant recipients (LTx+ BMT), showing the geometric mean titer (GMT) of donors and recipients (n=4).

BN to LEW strain combination Eleven LEW rats with BN liver graft received bone marrow augmented transplantation (Table 7). Seven recipients did not receive immunosuppressive treatment, whereas the remaining four rats were under daily cyclosporine therapy. In the group without immunosuppression, all recipients seroconverted and developed effective anti-HBs titers at the first postoperative week with an anti-HBs GMT of 81 mIU/ml (ranging from 12 to 889 mIU/ml). This titer represented in average 0.21% (0.03%~ 0.99%) of the donor' s titer on the day of organ donation. The effective anti-HBs titer lasted for about 2.6 weeks posttransplantation. The other four recipients receiving immunosuppressive treatment had an anti-HBs GMT of 54 mIU/ml (35~ 95 mIU/ml) at the first postoperative week that represented in average 0.27% (0.16~ 0.37%) of donor' s titer on the day of organ donation. The effective anti-HBs titer lasted for 5.3 weeks in average. Individual recipients, however, kept an effective anti-HBs titer for more than 10 weeks, which was not observed in rats with standard liver transplantation without augmentation (Figure 19).

ACI to LEW strain combination Four rats in the strain combination ACI to LEW received bone marrow augmented liver transplantation (Table 7). All animals were treated daily with CsA. All of them seroconverted and developed effective anti-HBs titer in the first postoperative week. The geometric mean anti-HBs titer of 143 mIU/ml (49~ 220 mIU/ml) represented 0.26% (0.13~ 0.73%) of the donor' s titer on the day of organ donation. The effective anti-HBs titer lasted in average 6.1 weeks, but again persisted for more than 10 weeks in individual recipients (Figure 20).

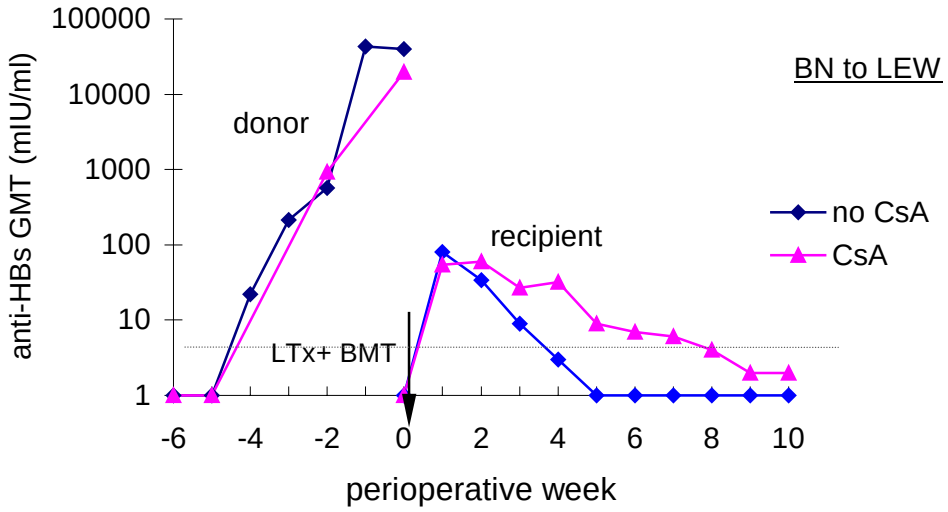

Figure 19. Anti-HBs titer development in BN to LEW bone marrow augmented liver transplant recipients (LTx+ BMT), showing the geometric mean titer (GMT) of donors and recipients in both cyclosporine A (CsA) treated (n=4) and untreated groups (n=7). The effective titer (≥10 mIU/ml) lasted about 2.6 or 5.0 weeks in each group respectively.

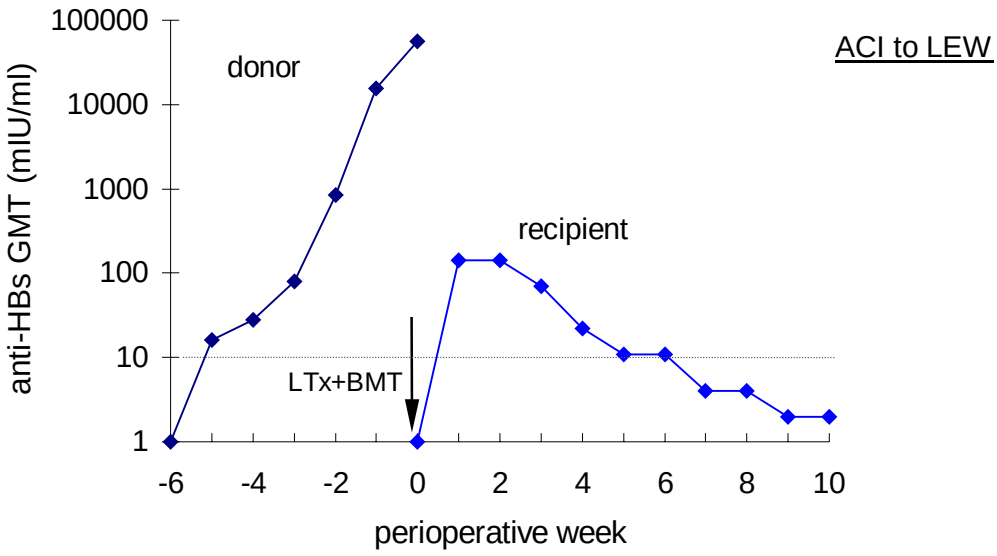

Figure 20. Anti-HBs titer development in cyclosporine A treated ACI to LEW bone marrow augmented liver transplant recipients (LTx+ BMT), showing the geometric mean titer (GMT) of donors and recipients (n=4).

3.4.1.2. Comparison of bone marrow augmented to standard liver transplantation on anti-HBs development

3.4.1.2.1. Effect on anti-HBs seroconversion rate

Forty standard liver transplantations and 21 bone marrow augmented liver transplantations were compared. Irrespective of strain combination effective anti-HBs titers were measured in 85% of the 40 liver recipients (34/40) and in 90% of the 21 liver recipients with bone marrow augmentation (19/21). In bone marrow augmented liver recipients, 100% seroconversion occurred when the donor's anti-HBs titer was above 1000 mIU/ml (GMT= 717 mIU/ml). Without augmentation, the critical anti-HBs titer was as high as 15000 mIU/ml (GMT= 6726 mIU/ml) (Figure 21). In other words, an anti-HBs titer of above 15000 mIU/ml in the donor was the minimal titer for a 100% seroconversion rate in liver recipients without infusion of bone marrow cells. This minimal level of donor anti-HBs titer was reduced to 1000 mIU/ml when augmentation by simultaneous bone marrow transplantation was performed (Table 8).

Table 8. Seroconversion rate of anti-HBs after liver transplantation (LTx) with or without bone marrow augmentation in rats.

Donor anti-HBs titer (mIU/ml)	Seroconversion rate of anti-HBs	
	LTx (animal number)	Bone marrow augmented LTx (animal number)
<1000	0% (0/3)	33% (1/3)
1000 - 15000	63% (5/8)	100% (3/3)
> 15000	100% (29/29)	100% (15/15)

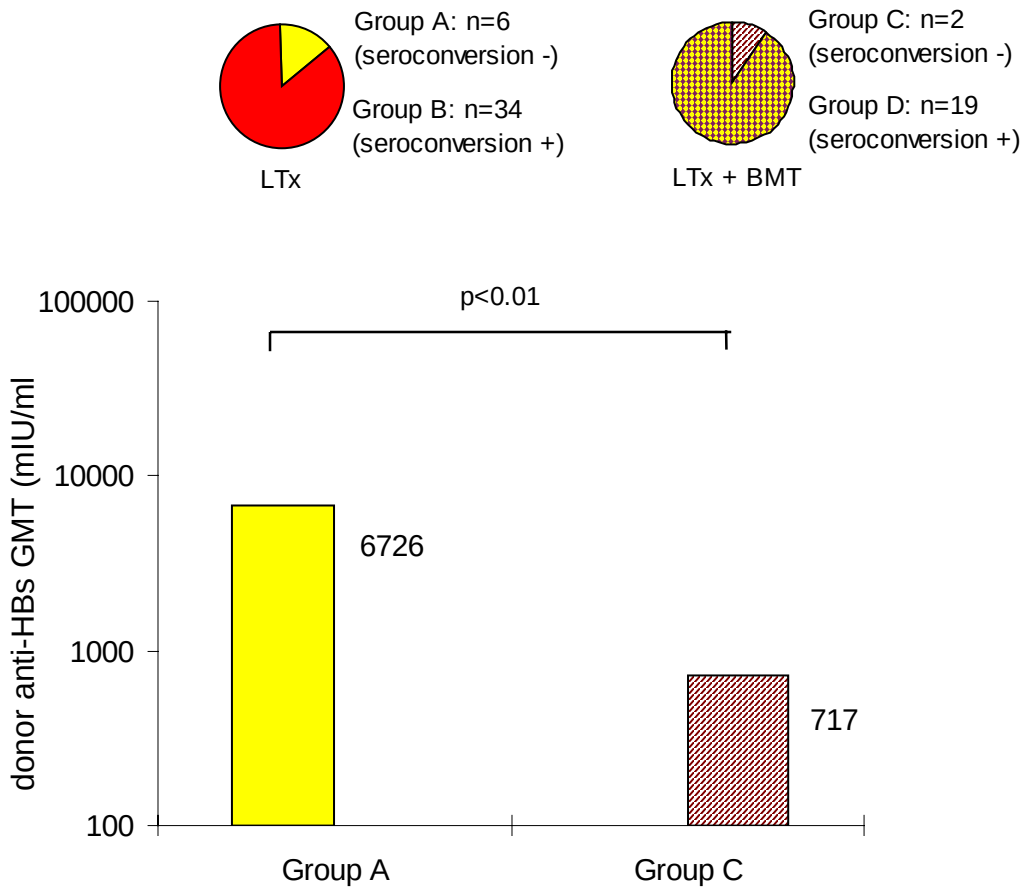

Figure 21. Minimal donor anti-HBs titer to induce seroconversion in recipient, comparing liver transplantation (LTx) with bone marrow augmented liver transplantation (LTx+ BMT). Six out of 40 liver recipients (Group A) had no seroconversion to anti-HBs. The geometric mean titer of their donors was 6726 mIU/ml. Two out of 21 bone marrow augmented liver recipients (Group C) had no seroconversion of anti-HBs. The geometric mean titer of their donors was 717 mIU/ml, which was significantly lower than in Group A ($p<0.01$).

3.4.1.2.2. Effect on the maximal anti-HBs titer in recipients

Fifteen bone marrow augmented liver recipients developed their maximal anti-HBs titer in the first week post-operation, whereas 4 recipients showed the maximal titer at 2 to 3 weeks post-transplantation (Figure 22). Such a persisting high anti-HBs titer plateau was never observed in liver recipients without bone marrow augmentation. All these animals (34/34) had their maximal titer at the first week post-transplantation.

3.4.1.2.3. Effect on the duration of effective anti-HBs titer in recipients

Duration of effective anti-HBs titer in recipients of liver transplantations was compared in animals with and without bone marrow augmentation. In all three rat strain combinations no significant difference was recognized ($p > 0.05$): after syngeneic liver transplantation without immunosuppression, an effective anti-HBs titer was detected for 4 and 3.7 weeks in standard liver transplantation group and bone marrow augmented group respectively; in BN to LEW strain combination with immunosuppressive treatment, effective anti-HBs titers lasted for 5.5 and 4.3 weeks, respectively. The effective anti-HBs titer of recipients in this strain combination lasted 2.6 and 2.8 weeks, respectively, when they were left untreated; in ACI to LEW strain combination with CsA treatment effective anti-HBs titers were measured for 5.3 weeks and 6.4 weeks, respectively.

In the groups whose donor titer was over 15000 mIU/ml and recipient seroconversion rate was 100%, the effective anti-HBs titer duration in allogeneic bone marrow augmented liver recipients under immunosuppression was 6.7 weeks, which was slightly longer than that of liver recipients without bone marrow

augmentation (4.6 weeks), although the difference was not statistically significant (P>0.05).

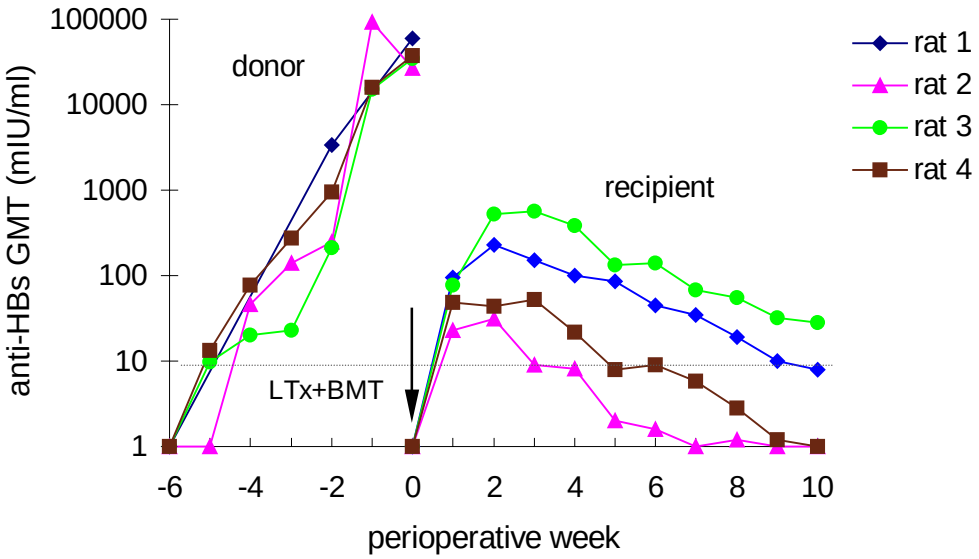

Figure 22. Anti-HBs titer development in bone marrow augmented liver transplant recipients (LTx+ BMT). Rat 1 & 2: BN to LEW strain combination. Rat 3 & 4: ACI to LEW strain combination. Maximal anti-HBs titer appeared at 2 to 3 weeks postoperatively in these recipients.

3.4.2. Augmentation by posttransplant vaccination

3.4.2.1. Efficacy of vaccination in liver recipients

Control groups:

1) vaccination in naive LEW rats All 28 LEW rats which were vaccinated prior to organ donation were assessed for seroconversion rate at four weeks after the first vaccination. 96% (27/28) of them had anti-HBs titer ranging from 34 to 9564 mIU/ml. The GMT in this group was 411 mIU/ml.

2) vaccination in liver transplanted LEW rats Six liver recipients in this group had not been vaccinated before transplantation, neither had their liver donors. Ten weeks after liver transplantation, they received a single dose of rHBVac. Only two of them (2/6=33%) seroconverted within 4 weeks with anti-HBs titer of 34 and 79 mIU/ml (Table 9).

Experimental groups:

1) vaccination in liver recipients with adoptively transferred anti-HBs immunity to HBV The organ donors in this group were all immunized with HBVac. After liver transplantation, every liver recipient seroconverted with effective anti-HBs titers, which persisted for 2 to 8 weeks. At the 10th postoperative week, when the antibody titers were undetectable in all animals, they were vaccinated with HBVac. Four weeks later an effective anti-HBs titer was detected in 1 out of 2 in the syngeneic group (LEW-> LEW strain combination; anti-HBs titer of 1035 mIU/ml), and 0 out of 7 in the allogeneic groups (4 from BN-> LEW strain combination; 3 from ACI-> LEW strain combination). All these 9 recipient rats were treated with CsA. Liver recipients without immunosuppressive treatment were followed for more than twenty

weeks to observe the long-term development of anti-HBs titer. Therefore, they were not included into the study of post-transplant vaccination.

2) vaccination in bone marrow augmented liver recipients with adoptively transferred HBV immunity 12 recipients were vaccinated at the 10th postoperative week. Six of them received CsA after transplantation, while the other six did not. At the time of vaccination only one recipient still had an anti-HBs titer over 10 mIU/ml. Four weeks later, effective anti-HBs titers were detected in 4 out of 6 animals without immunosuppression (67%). Among the recipients with immunosuppression, one animal seroconverted to anti-HBs despite of CsA treatment (17%) (Table 9).

3.4.2.2. Analysis of the effect of posttransplantation vaccination

Liver transplantation reduced the immune response to a single HBV vaccine dramatically in rats: the seroconversion rate was 96% (27/28) in naive LEW, whereas it was only 33% (2/6) in four LEW liver recipients.

This low response rate was increased by donor vaccination: rats receiving a liver graft from a HBV vaccinated donor with bone marrow augmentation showed a seroconversion rate of 67% (4/6), whereas only 33% (2/6) of rats having a liver graft from a non-vaccinated donor seroconverted. The higher seroconversion rate must be attributed to the adoptive immune transfer by liver transplantation, which was possibly enhanced by the simultaneous bone marrow transplantation.

This enhancement of the immune response to HBVac by adoptive transfer was also observed in the immunosuppression group. Although immunosuppression should abrogate the immune response completely, seroconversion to post-transplant vaccination was observed in 13% (2/15) treated animals, which received liver grafts

from HBV vaccinated donors. This observation can be possibly attributed to the adoptive transfer.

Table 9. Response to single dose HBV vaccination in LEW rats*

Groups	CsA treatment	Response rate % (animal number)	Anti-HBs GMT mIU/ml (range)
-Naive rats	no	96% (27/28)	411 (34~9564)
-Liver recipients (donor without immunization)	no	33% (2/6)	52 (34~79)
-Bone marrow augmented liver recipients (donor HBV-immunized)	no	67% (4/6)	125 (47~333)
-Liver recipients (donor HBV-immunized)	yes	11% (1/9)	1035
-Bone marrow augmented liver recipients (donor HBV-immunized)	yes	17% (1/6)	41

* the serum anti-HBs titer of each animal was measured in the 4th week after vaccination

4. DISCUSSION

4.1. Study design

A rat model was used to study adoptive immune transfer by liver transplantation. Generally, in clinical situation of transplantation the recipient's immune response to alloantigen is influenced by the genetic difference between donors and recipients, and immunosuppressive treatment. In this study, those two factors were investigated on the basis of three rat strain combinations:

1) LEW to LEW syngeneic liver transplantation, allowing the analysis of the natural course of immune transfer in liver recipients;
2) BN to LEW spontaneously tolerant allogeneic liver transplantation, in which BN liver allografts are spontaneously accepted by LEW recipients. Using this model enabled the investigation of the long-term development of donor specific immunity in allogeneic recipients without the influence of immunosuppressive treatment;
3) ACI to LEW strain combination, representing the clinical situation, as it is a model of acute rejection in allogeneic liver transplantation. Long term survival of the liver recipients can only be achieved under immunosuppressive treatment.

For theoretical as well as for technical reasons, the animals' humoral response to HBsAg was chosen as the parameter to judge the efficacy of the immune transfer: it has a potential clinical application, namely, reducing HBV reinfection in HBV related liver recipients. Moreover, recombinant HBV vaccine is easily commercially available and the quantitative assessment of the immune response to this vaccine is standardized.

4.2. Adoptive transfer of HBV Immunity after Liver Transplantation

Using this animal model the donor derived humoral response against HBsAg was investigated in liver recipients. After liver transplantation from HBsAg immunized donors, anti-HBs titers ($\geq$ 10mIU/ml) were detected in most of the recipients with an overall seroconversion rate of 85%, irrespective of strain combination and immunosuppressive treatment. The effective antibody titer lasted for up to 10 weeks after transplantation, with a mean value of 3 to 6 weeks in the different groups.

Working with different rat strain combinations revealed that the immune response evoked by alloantigen had a negative effect on anti-HBs antibody development in liver recipients: effective anti-HBs titer persisted longest (4 weeks) in the syngeneic liver recipients (LEW->LEW strain combination), whereas it lasted for 2.8 weeks in BN->LEW allogeneic recipients and for only 1 week in ACI->LEW allogeneic recipients. This negative effect of allo-reactivity on adoptive immune transfer in liver recipients could be reversed by cyclosporine treatment: the anti-HBs titer persisted for over 5 weeks in both BN-> LEW and ACI-> LEW liver recipients, which was significantly longer than in untreated recipients.

Considering the possible mechanism of the anti-HBs development in the liver recipients, the following questions need to be addressed:

1) Is adoptive immune transfer after liver transplantation due to passive anti-HBs antibody transfer?

Although the liver graft has been perfused with physiological saline solution during the donation procedure, anti-HBs antibodies still can remain in the liver grafts of immunized donors. It cannot be excluded, that antibodies in the liver graft were washed out into the recipient' s blood circulation after reperfusion, and contributed to

the positive anti-HBs titer after transplantation. In our study, each liver graft was perfused by 10 to 15 ml saline solution until the venous effluent turned to be clear. Quantification of the remaining anti-HBs antibody in the liver would help to know the role of passive antibody transfer in this model. Additionally, it seems to be worthwhile to investigate the catabolism of extrinsic anti-HBs antibody in liver recipients.

2) Is adoptive immune transfer by liver transplantation due to transfer of immune cells (e.g. anti-HBs antibody-secreting cells)?

Although passive antibody transfer has to be taken into consideration, it is likely that donor derived lymphocytes also play an important role in the anti-HBs titer development in the recipients.

In a recent study, Hata detected large numbers of lymphocytes in the liver. These lymphocytes were mainly located in the portal tract and the perisinusoidal area, and consisted of three major lymphoid populations: T, NK and B cells (Hata K 1990). In human being, the overall number of liver associated lymphocytes was reported to be in the range of peripheral blood content (2~ 9×10^{9} cells per liver or 0.8~ 6×10^{6} cells/ g liver tissue: Schlitt HJ 1993; Bioulac-Sage P 1996). They were located predominantly in the portal field outside of the vessels and could not be removed from the liver tissue by perfusion during harvesting procedure (Schlitt HJ 1993; Pruvot FR 1995). Since 1992, it is known that after liver transplantation host organs are invaded by small numbers of lymphoid and dendritic cells of donor origin. This state of systemic mixed allogeneic microchimerism was first demonstrated by the presence of peripherally located donor cells many years after liver replacement (Starzl TE 1992). Despite the low frequency of these migrating donor cells, metabolic benefits have been shown, for example, in liver graft recipients with type IV glycogen storage disease and type 1 Gaucher' s disease (Starzl TE 1993). Therefore, it seems

justified to assume that the transfer of functionally active donor- type B cells might contribute to the anti-HBs seroconversion of liver recipients, although B cells and plasma cells only compose a limited portion of the liver-associated lymphocytes (Bioulac-Sage P 1996).

This hypothesis is supported indirectly by the following observations in this study:

1) Recipient' s alloreactivity reduced the immune transfer caused by liver transplantation. Recipient-to-donor titer ratio and anti-HBs titer duration were compared in the three rat strain combinations, which represented different levels of alloreactivity. As shown, the anti-HBs titers in ACI->LEW liver recipients (allogeneic acute rejecting model) represented 0.10% of the donor titers at the first post-transplant week. This value was lower than in the syngeneic (0.32% in LEW->LEW strain combination) or in the allogeneic spontaneous tolerant model (0.31% in BN->LEW strain combination). In respect of titer duration, effective anti-HBs titer persisted longest in the syngeneic liver recipients, for about 4 weeks, whereas it lasted for 2.8 weeks in BN->LEW allogeneic recipients and only for 1 week in ACI->LEW allogeneic recipients. Therefore, the reduction of titer persistence in allogeneic liver recipients was suspected to be due to either transient acute rejection (in BN->LEW liver recipients) or lethal acute rejection (in ACI->LEW liver recipients), which possibly caused a reduction of donor-derived ASCs in those two strain combinations.

2) As further shown, this negative effect of alloreactivity on adoptive transfer of immunity in liver transplantation was reversed by cyclosporine treatment. Cyclosporine blocks signal transduction necessary for the transcription of several early T-cell activation genes, including IL-2 and IFN-γ. Thereby synthesis and release of these cytokines are inhibited, activation of helper and cytolytic T cells is effectively blocked, and the graft is protected from acute rejection. In this study CsA did not

influence the adoptively transferred immunity in the syngeneic liver recipients. The maximal titer and the effective titer duration were similar in both groups, demonstrating clearly that CsA treatment did not affect ongoing antibody secretion. However, as expected, CsA did block effectively the activation of humoral response to HBsAg, since the antibody response was almost completely suppressed after posttransplant vaccination in the treatment groups. In contrast to syngeneic transplantation a prolonged titer duration was observed after allogeneic liver transplantation under CsA treatment: the anti-HBs titer persisted for over 5 weeks in immunosuppressed BN->LEW liver recipients. The same phenomenon was found in the allogeneic rejecting model (ACI->LEW strain combination), in which anti-HBs titer lasted for more than 5 weeks postoperatively.

In a study of B lymphocyte chimerism in liver allografts, donor B cells were found to migrate rapidly into recipient' s systemic circulation in both a spontaneous tolerant rat liver transplantation model and an acute rejecting model. Systemic chimerism was still detectable in the tolerant recipient by day 14, whereas it was undetectable in the rejecting combination by day 7 (Yokoi Y 1999). This observation parallels the results of our study, although antibody titer was chosen as read-out parameter instead of the frequency of antibody secreting cells.

One potential approach to obtain direct evidence of the transfer and function of donor specific immune cells is by using an MHC incompatible strain combination in which donors and recipients are different in IgG allotype. Immunoglobulin, generated in response to a vaccination of the recipient possibly by recipient B cells as well as donor derived immune cells, can be differentiated by an Ig-allotype specific enzyme-linked immunosorbent assay, taking advantage of Ig-allotype markers specific for donor and recipient strain (Gray D 1988; Shepherd DM 1991). This approach might help to answer one of the key questions regarding the adoptive transfer of immunity,

namely whether donor-derived ASCs contribute predominantly to the anti-HBs titer development in recipients of liver grafts from vaccinated donors. However, it is technically rather difficult as it based on the quantification of a low amount of antibodies, which is in a range close to the detection limit of the assay.

In the second step, it was attempted to increase the number of donor-derived lymphocytes by simultaneous bone marrow transplantation and to restimulate them by vaccination to achieve better humoral response to HBsAg.

4.3. Augmentation of Adoptive Immune Transfer in Liver Recipients

4.3.1. Augmentation by simultaneous bone marrow cells infusion

Bone marrow is both a primary and a secondary lymphoid organ. Being the site of maturation for B cells, it is abundant of lymphoblastoid B cells, which can both transform in antibody secreting plasma cells as well as develop to memory cells. (Lydyard P 1998). Recent studies using the mouse model for infection with lymphocytic choriomeningitis virus (LCMV) documented that the initial virus-specific plasma cell response occurred in the spleen and then migrated to bone marrow. From thirty days after infection and for the life of the mouse, bone marrow became the predominant site of antiviral antibody production, with nearly 90% of virus-specific plasma cells residing in this compartment (Slifka MK 1995; Slifka MK 1998). Moreover, in in-vitro studies with immune bone marrow cells a spontaneous antigen-specific IgG production has been observed (Benner R 1981; Shepherd DM 1991). These results led to our hypothesis that additional bone marrow transplantation could

possibly increase the number of donor-derived lymphocytes in liver recipients, and subsequently the antibody titers.

Two observations are pointing to the augmenting effect of simultaneous bone marrow transplantation.

First: Bone marrow augmentation increased the seroconversion rate in recipients receiving an organ from donors with a titer of <15.000mIU/ml from 45% (standard liver transplantation) to 67% (bone marrow augmentation). The minimal donor anti-HBs titer to induce a 100% seroconversion rate dropped from 15000 mIU/ml in recipients of a standard liver graft to 1000 mIU/ml in the bone marrow augmented liver transplanted group.

Second: 4 from 21 liver recipients demonstrated an unusual development of anti-HBs titer after simultaneous bone marrow cells infusion: in these liver recipients the anti-HBs titer did not decrease after the first post-transplant week, but kept rising until the second or third week. Delayed peaking of anti-HBs titer in the serum may be caused by the different life span between liver graft derived ASCs and bone marrow derived ASCs. Recently, it was shown that the bone marrow contains long-living ASCs while memory B cells mainly stay in spleen and lymph nodes. During the acute phase of the humoral response (the first 2 weeks after booster vaccination), B cells expand rapidly, differentiate into ASCs, and are depleted during clonal selection and affinity maturation (Kelsoe G 1996). The ASCs generated in this phase have a shorter life span compared with those in bone marrow compartment (Manz RA 1997; Slifka MK 1998). Possibly, ASCs originating from different sources have different life spans leading to the maximal anti-HBs titer appearing in the second or third week post-transplant in bone marrow augmented liver recipients: the passenger lymphocytes within the liver graft could presumably be a part of these short-lived ASCs according to the booster vaccination and liver donation schedule in this study;

the persisting antibody titer plateau might be due to transferred ASCs from the bone marrow which continuously secreted antibodies until they died off. This observation paralleled the one of Slifka, showing that a LCMV-specific IgG equilibrium was reached on the 15th day after adoptive transfer of bone marrow in mice containing LCMV-specific plasma (Slifka MK 1998).

4.3.2. Augmentation by post-transplant vaccination

Current immunological dogma holds that antigen stimulation drives B cells either to develop into memory cells or mature into plasma cells. Memory cells are ready to respond, if the same antigen is encountered again (Feldmann M 1998). Theoretically after BMT from a HBV immunized donor, the recipient does not only receive plasma cells with a limited life span to produce anti-HBs antibody, but also B memory cells, which could proliferate and differentiate into new antibody secreting cells upon antigen contact. This hypothesis is strongly supported by observations in BMT patients, who developed anti-HBs antibodies after transplantation from a vaccinated donor and showed a secondary response to the challenge with a booster vaccine (Ilan Y 1993; Shouval D 1995). Supposing that memory B cells are also transferred together with the liver graft, a primary vaccination of the recipient rats would elicit both: a primary response of the recipient own lymphocytes and a secondary response of the derived cells, probably could induce and maintain a higher level of anti-HBs titer compared to naïve rats.

On the other hand the immunosuppressive effect of an operative procedure itself is known (Nohr CW 1984). In transplant patients, the response rate to HBVac prior to operation was between 16% and 54% (Van Thiel DH 1992; Chalasani N 1998), but only 6.7%-23% when performed afterwards (Carey W 1990; Loinaz C

1997; Chalasani N 1998). However, the low response rate to vaccination performed after clinical transplantation was explained by the negative effect of the immunosuppressive medication.

After liver transplantation, immune response to HBVac was reduced from 96% (27/28) in naïve LEW rats to 33% (2/6) in syngeneic liver graft recipients. Besides the surgical procedure, increased age of the liver recipient rats (20 weeks vs. 5 weeks), can be another important contributor to the decreased humoral response to vaccine, which was found to be associated with decreased numbers of antibody secreting B cells and a decrease in the mean amount of antibody produce per B cells in the elderly population (Burns EA 1993; Jilg W 1989). These two negative effects on the humoral response to HBVac, however, were not noticeable when the same vaccination procedure was performed in bone marrow augmented liver recipients with adoptively transferred immunity. The seroconversion rate was 67% (4/6). Even in recipients with cyclosporine treatment, a seroconversion rate of 13% (2/15) was observed. This humoral response is presumably due to the restimulation of the anti-HBs specific lymphocytes (e.g. memory B cells) transferred to the recipient rats together with the liver graft. This result is important for two reasons. First it supports the idea of engraftment of donor immune cells. Second, recipient vaccination following transplantation of a graft from an immunized donor is a potential practical approach to reduce the HBV reinfection in the clinical situation.

4.4. Perspective and clinical relevance

Using liver grafts from HBV immunized donors with high anti-HBs titer resulted in an effective antibody response in the recipients, which lasted for up to 10 weeks. If

this result could be confirmed in the clinical situation, taking advantage of HBV vaccinated donor, this strategy might contribute to protecting recipients with high risk of HBV reinfection, regardless of the underlying mechanism. Clinical implementation in cadaveric liver transplantation would require a novel allocation strategy using vaccinated donors with high antibody titers for Hepatitis B cirrhotic recipients, but can be easily achieved in living donation. Simple HBV vaccination in donors not only provides a possibility to transfer an effective anti-HBs response to liver recipients, but also protects the donors from HBV infection. Very high anti-HBs antibody titer must be achieved in the donors in order to reach a high seroconversion rate with long lasting titers in the recipients. Thus accelerated or enhanced vaccination schedules, possibly using the currently developed DNA-vaccines, must be developed.

5. ABBREVIATIONS

anti-HBs- hepatitis B surface antibody
ASC- antibody secreting cell
BMT- bone marrow transplantation
CsA- cyclosporine A
GMT- geometric mean titer
HBcAg- hepatitis B core antigen
HBeAg- hepatitis B e antigen
HBIg- hepatitis B immune globulin
HBsAg- hepatitis B surface antigen
HBV- hepatitis B virus
HBVac- hepatitis B vaccine
HLA- human leukocyte antigen
IFN-α- Interferon-α
IHVC- infrahepatic vena cava
LAL- liver-associated lymphocytes
LCMV- lymphocytic choriomeningitis virus
LTx- liver transplantation
MEIA- microparticle enzyme immune assay
MHC- major histocompatibility complex
OLT- orthotopic liver transplantation
PV- portal vein
RAI- rejection activity index
rHBVac- recombinant hepatitis B vaccine
rpm- round per minute
SD- standard deviation
SHVC- suprahepatic vena cava

6. SUMMARY

We used a rat model to study adoptive immune transfer by liver transplantation. Animals' humoral response to HBsAg was chosen as the parameter to judge the efficacy of the immune transfer. Three strains of donor rats (LEW, BN and ACI) were vaccinated with recombinant HBV vaccine twice. After seroconversion to HBsAg 6 weeks later, they donated their livers to naïve LEW rats. Anti-HBs antibody titers were detected in 85% (34/40) of liver recipients, irrespective of strain combination and immunosuppressive treatment. Effective antibody titer was measured for up to 10 weeks after transplantation, with a mean duration of 3 to 6 weeks in the different groups. Alloreactivity limited the antibody development in the liver recipients, whereas Immunosuppression by cyclosporine A enhanced this immune transfer, probably by protection of donor lymphocytes from rejection. Simultaneous bone marrow transplantation, possibly by increasing the number of antibody secreting cells transferred, raised the seroconversion rate of anti-HBs in liver recipients, especially when the donor titer was relatively low (from 1000 to 15000 mIU/ml). Restimulation by post-transplant vaccination led to a better response rate in bone marrow augmented liver recipients, which could be taken as argument for cell engraftment. Two potential mechanisms may contribute to this antibody transfer. One is passive antibody transfer due to the antibodies washed out from the liver graft, another is the migration of donor derived immune cells into the recipients, which can keep on secreting antibodies. Although donor derived immunity was only transiently detectable, adoptive immune transfer could be a potential strategy to reduce hepatitis B reinfection rate after liver transplantation and therefore should be evaluated clinically.

7. LITERATURE

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Transplantation, 68:118-123.

8. ACKNOWLEDGMENT

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With my lots of thanks!

9. RESUME

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