Effective Immune Transfer, along with Induction of Rejection, Are Consequences of Using Grafts from Highly Immunized Donors

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Adoptive transfer of immunity has recently been proposed as a new strategy to prevent or treat hepatitis B infection in bone marrow graft recipients. However, although immune transfer by clinical organ transplantation has been observed only occasionally, it has led to severe complications in the recipient. The authors aimed to investigate the potential risk and benefit of adoptive immune transfer from donor to recipient after rat liver transplantation in a spontaneously tolerant model (BN→LEW). Using liver grafts from highly immunized donors (sensitized by 4 repeated nonspecific skin grafts), lethal rejection was induced in 40% (6/15) of the recipients compared with a rejection rate of 50% (13/26) after single nonspecific sensitization of the recipients. Transfer of donor-derived cellular immunity was shown by the accelerated rejection of an AxC 99235 Irish rat (ACI) skin graft. LEW recipients of a liver graft from an ACI-skin-sensitized BN donor rejected the challenged skin graft in 10.4 ± 0.5 days (n = 5), compared with the control group (13.5 ± 2.1 days, n = 8, $P < 0.05$). Transfer of donor-derived humoral immunity was demonstrated in all animals in both models—donor sensitization as well as donor vaccination. In conclusion, adoptive transfer of immunity by liver transplantation seems to occur uniformly after liver transplantation, independent of the immunization protocol. Using organs from highly immunized donors potentially puts the recipients at the risk of enhancing ongoing rejection.

ABBREVIATIONS

Anti-HBs	Anti-hepatitis B surface antibodies
BMT	Bone marrow transplantation
BN	Brown Norway rat
CDC	Complement dependent cytotoxicity assay
DTH	Delayed type hypersensitivity
HBV	Hepatitis B virus
IHVC	Infrahepatic vena cava
LEW	Lewis rat
LTx	Liver transplantation
MEIA	Microparticle-enzyme-immunoassay
MHC	Major histocompatibility complex
POD	Postoperative day
PV	Portal vein
SHVC	Suprahepatic vena cava
SkTx	Skin transplantation

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Introduction

Bone marrow transplantation implies the transplantation of a functioning immune system to the recipient. Engraftment is identified by the development of stable macrochimerism, the levels of which may vary depending on the myeloablative protocols used and the composition of the bone marrow being transplanted.[1] In the past, several reports have described potentially immunologically beneficial effects of transplanting a functioning immune system to the recipient.[2-4] Some studies examined the effect of donor anti-hepatitis B virus (HBV) immunity on an HBV-positive recipient. Virus clearance has been reported in single cases.[5-7]

Having observed long-term microchimerism after organ transplantation, T. E. Starzl regarded organ grafts, especially liver grafts, as equivalent to mini bone marrow grafts.[8] This idea is supported by the observation of complete hematopoietic reconstitution after lethal irradiation and organ transplantation.[9] The survival rate of the animals was closely related to the engraftment rate, as indicated by the development of chimerism.[10] Most of the studies in the past[11] evaluated the relevance of microchimerism

for tolerance induction, whereas the functional aspects were not investigated.

However, single cases of successful immune transfer after organ transplantation have been reported and identified by the manifestation of unwanted, potentially harmful donor-derived immune function in the recipients.[12-14] Demonstration of positive effects, such as the protection from reinfection in HBsAg-positive liver recipients, is much harder to obtain but might be of clinical relevance.[15,16] Our own previous studies using a spontaneously tolerant allogeneic rat liver transplantation model showed that sensitization of the liver graft donor by an unrelated skin graft led to accelerated rejection of a test skin graft of the same origin in the liver graft recipient.[17] Furthermore, antigen-specific donor-derived antibodies were observed in the recipient after transplanting the organ of an immunized donor. On the other hand, repeated nonspecific sensitization led to lethal rejection in some of the recipients.[18] Co-transplantation of a highly activated immune system fragment within the donor graft apparently imposed a risk to the recipient.

Therefore, we intended to evaluate the potential benefit, but also the possible harmful effect of using organs from highly sensitized donors. Using the spontaneously tolerant allogeneic liver allograft model BN→LEW permitted focus on investigations regarding the effect of donor sensitization on liver graft rejection, on tolerance induction, and on adoptive transfer of immunity.

Material and Methods

Experimental Design

Two different clinically important models of donor immunization were chosen: sensitization by a 3rd-party skin graft and HBV vaccine (Fig. 1). Part of the donors were sensitized up to 4 times (1×, 2×, or 4×, 2 weeks apart) with a 3rd-party skin graft, mimicking the clinical situation of a patient sensitized by previous blood transfusions, transplants, or pregnancies. Recipient sensitization was chosen in some experiments for comparative reasons. In another group of animals, HBV vaccine was chosen as a clinically relevant antigen. Donors were vaccinated with recombinant HBsAg vaccine (6 weeks, boosted 2 weeks prior to donation) to allow detailed analysis of the efficiency of immune transfer by measuring antibody development in the recipient.

Animals

Male inbred rats ACI ($RT1^a$), Brown Norway (BN, $RT1^n$) and Lewis (LEW, $RT1^l$), were obtained from Harlan-Winkelmann. All operative procedures were carried out under methoxyflurane anesthesia (Metofane, Janssen GmbH, Neuss, Germany). Throughout the experiments, the animals were maintained behind barriers under controlled environmental conditions, and all animal housing and procedures were carried out in accordance with the German Animal Welfare Legislation. Animals were monitored daily (weight, jaundice) and sacrificed in the case of deteriorating condition or weight loss of more than 20% of body weight.

Experimental Procedures

Orthotopic Liver Transplantation

Orthotopic liver transplantation was performed according to the technique of Kamada.[19] Cold ischemic time did not exceed 1 h, and anhepatic time was under 20 min. After the donor liver was mobilized by dividing its ligaments, the infrahepatic vena cava (IHVC) was separated from the right adrenal and the right renal vessels. The portal vein was divided from the pyloric, splenic, and inferior mesenteric vein. The bile duct was transected and cannulated with a tube stent (Klinika 22GA, KLINIKA Medical GmbH, Usingen, Germany) for biliary anastomosis. After injecting 1.0 ml of saline solution containing 200 units of heparin via the penile vein, the hepatic artery was dissected. The liver graft was perfused through the portal vein (PV) with chilled Ringer's solution and kept at 4 °C. Cuff attachment of PV and IHVC was performed immediately following the harvest of the donor liver. The suprahepatic vena cava (SHVC) was trimmed and attached with two 7-0 polypropylene sutures. After removing the recipient liver, the liver graft was placed orthotopically in the abdomen. The donor SHVC was anastomosed end-to-end with the recipient SHVC using continuous 7-0 polypropylene suture. The PV and IHVC anastomoses were performed by pulling the recipient's vein over

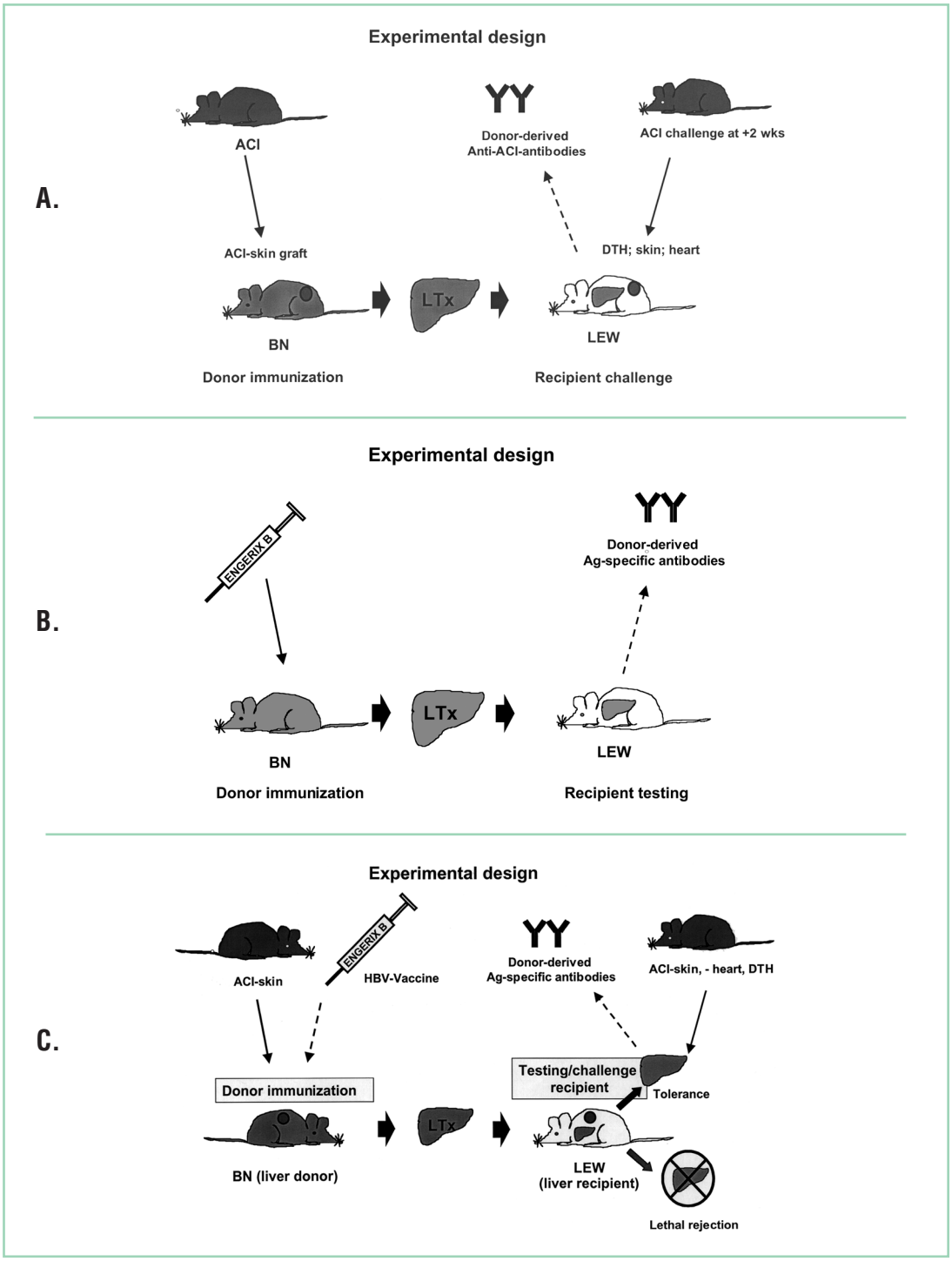

Figure 1. Experimental design. Liver donors (BN rats) were either sensitized by ACI rat skin grafting or vaccinated with recombinant HBs-Vaccine (Engerix B, Germany). Liver recipients (LEW rats) were challenged by a test skin or heart graft from ACI origin or tested weekly for the development of donor-derived antigen-specific antibodies (anti-ACI, anti-HBs).

the cuff and were secured with a circumferential 6-0 silk suture. Biliary continuity was restored by tying the duct over the stent. Finally, the abdominal incision was closed with continuous 3-0 polyester suture in 2 layers. All recipients received antibiotic treatment by a single-shot intramuscular injection of 100 mg/kg/d Mezlocillin (Baypen, Bayer AG, Leverkusen, Germany) after operation. Animals were sacrificed in the case of deteriorating general condition, indicated by severe weight loss, lack of spontaneous activity, or jaundice.

Skin Transplantation

For each skin graft, a square of skin (0.5 × 1 cm) was obtained from the abdominal wall of the donor animal. After removing the subcutaneous fat, the graft was placed in antisense direction on the back of the recipient animal to facilitate the identification of the graft by the opposite direction of hair growth and was fixed with 6 single stitches.[20] Each animal received a bandage, which was removed on postoperative day 7. Animals were followed daily to judge the rejection time of the skin graft as indicated by dry necrosis of more than 50% of the skin flap.

Vaccination

Male inbred LEW, BN, or ACI rats 5 to 6 weeks old were chosen for vaccination. Each rat was first anesthetized by inhalation of methoxyflurane. 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 mg /ml) was injected intramuscularly into the vastus medialis muscle. Four weeks after the 1st vaccination, all rats were boosted with another dose of HBV vaccine. They served as organ donors 2 weeks later.

Sampling

Some of the animals were bled weekly (0.3-0.5 ml) for assessment of antibody titers. Serum from each rat was separated and stored at –20 °C until either skin-donor-specific antibodies were measured by complement-dependent cytotoxicity assay (CDC) or anti-HB antibodies were determined by microparticle-enzyme-immunoassay (MEIA). At the end of the observation period, animals were sacrificed. Transplanted organs were subjected to histological analysis.

Delayed Type Hypersensitivity Assay (DTH)

Animals were either sensitized by 1 skin graft 2 weeks prior to performing the assay or received a liver graft from a sensitized donor 2 weeks prior to the assay. On the day of the assay, sensitized rats and unsensitized controls were injected with 50 µl of ACI lymphocytes (at a concentration of 5×10^6/ml), which were isolated from cervical lymph nodes. Twenty-four hours later, the increase in diameter of the ear was measured using a caliper.

Complement-Dependent Cytotoxicity Assay (CDC)

Peripheral lymph nodes of ACI donor animals were excised from the neck prior to mechanical disruption in cold RPMI-Medium. A Ficoll gradient was used for isolation of lymphocytes (2300 rpm for 35 min), followed by 2 washes with RPMI-Medium. Cells were counted and adjusted to a concentration of 5×10^6/ml. A serial dilution of serum was prepared. One ml of the cell suspension was placed in each well of a Terasaki miniplate filled with 1 ml of the appropriate serum dilution followed by 30-min incubation at 37 °C. Then complement (Guinea pig complement, Cedar Lane Hornby, Ontario, Canada) was added and incubated for another 10 min. Dead cells were visualized with trypan-blue and counted. The test was regarded positive when more than 50% of the cells were stained.

Microparticle-Enzyme-Immunoassay (MEIA)

A fully automated microparticle enzyme immunoassay was used for detection and quantification of rat serum antibody against a hepatitis B surface antigen (anti-HBs), which is described by Abbott Laboratories.[21] In the 1st step of the assay, microparticles coated with rHBsAg are added to the specimen. An aliquot of the reaction mixture is transferred to the glass fiber matrix. The microparticles bind irreversibly to the matrix, while unbound material is washed through the matrix. Biotinylated rHBsAg is then added to the matrix to react with captured anti-HBs. Anti-biotin/alkaline phosphatase conjugate is added to the matrix to re-

Table 1 | **ACCELERATION OF SKIN GRAFT REJECTION BY REPEATED SKIN GRAFTING**

RECIPIENT	SKIN GRAFT	1ST GRAFT	2ND GRAFT	3RD GRAFT	4TH GRAFT
Naive Lew	ACI	12.4 + 1.2 *n* = 70	9.7 + 1.2 *n* = 36	9.2 + 1.0 *n* = 21	nd
	BN	11.9 + 1.1 *n* = 21	9.3 + 0.9 *n* = 14	8.0 ± 0.9 *n* = 17	nd
Naive BN	ACI	10.4 + 1.0 *n* = 35	9.0 + 21.5 *n* = 30	8.3 + 1.4 *n* = 18	8.0 + 0.6 *n* = 13
	Lew	11.2 + 1.4 *n* = 9	8.4 + 1.2 *n* = 8	7.0 ± 0 *n* = 5	nd

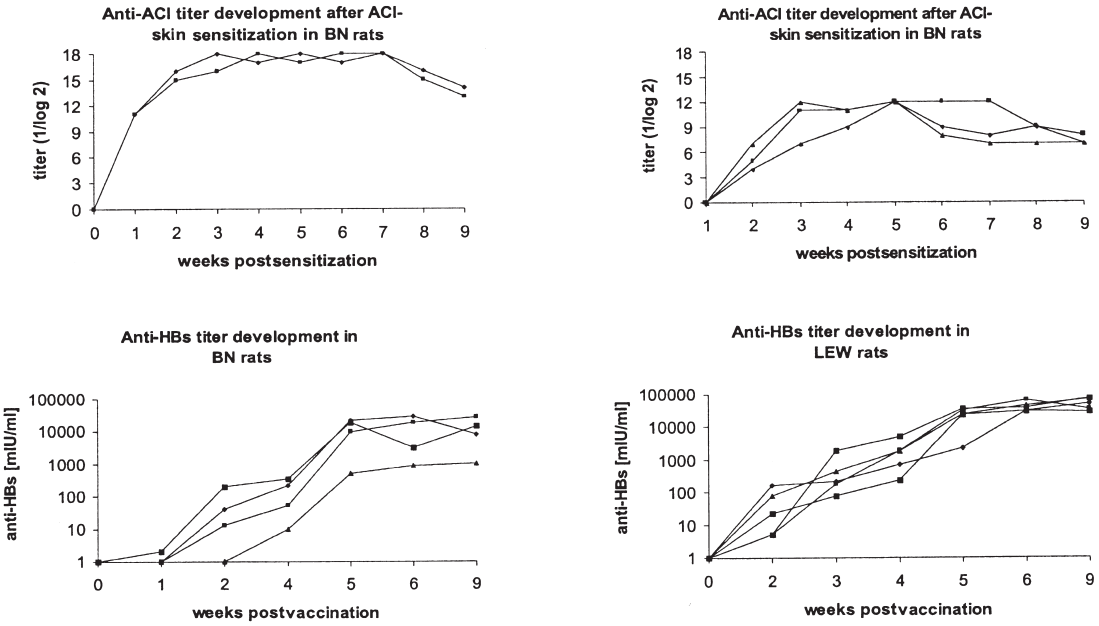

Figure 2. BN and Lew rats had an equally effective humoral immune response to the ACI-skin graft (development of anti-ACI antibodies) and also to the recombinant HBs-Vaccine (anti-HBs antibodies).

act with the biotinylated rHBsAg/captured latex microparticle complex. Unbound conjugate is removed by washing the matrix. The bound conjugate complex is detected by incubation with the fluorogenic substrate 4-methylumbeliferyl phosphate. The rate of fluorescence signal generation is proportional to the amount of anti-HB bound to the microparticle/matrix solid phase. Anti-HB concentration in specimens is calculated automatically by comparison of the specimen rate to the values determined by a standard curve.

Results

Establishment of the Experimental Models

Donor and recipient strains of rat had an equally effective immune response to skin sensitization and vaccination (Table 1). Sensitization with ACI skin grafts led to rejection within 12.4 ± 1.2 days in LEW rats and 10.4 ± 1.0 days in BN rats. Repeated skin grafting resulted in an increasingly accelerated rejection response in both strains, with a slightly higher response in BN (3rd ACI skin graft was rejected in 9.2 ± 1.0 days by LEW rats and in 8.3 ± 1.4 days by BN rats). Antibody titers, as measured in the CDC assay, showed a fast titer increase after the 1st skin graft, reaching its maximum within 3 weeks persisting for a minimum of 7 weeks at this level (Fig. 2).

Immunizing donors and recipients with recombinant HBV vaccine showed a strong antibody response with a maximal titer of 100-1000 mIU/ml in LEW rats and a lower titer (10-100 mIU/ml) in BN rats after the first vaccination. This was fol-

Table 2 | **Induction of Liver Graft Rejection by Repeated Nonspecific Donor Sensitization, but Preservation of Tolerance Induction and Immune Transfer in Surviving Animals**

D versus R Sensitization	Spontaneously Tolerant Liver Allograft Model BN LEW		Sensitization-Inducing Rejection		BN-Test Graft: Tolerance Induction in Animals Surviving Ltx		BN-Test Graft: ACI-Challenge Immune Transfer in Tolerant Animals		
Experimental Group	Donor	Recipient	% R	SVT BN Liver Graft	SVT BN Skin Grafts	SVT BN Heart Graft	SVT ACI-Skin Graft	SVT of ACI Heart Graft	DTH-Assay ACI-Cells
Control I Naive LEW	na	na	na	na	11.9 + 1.1 *n* = 21	6 × 2, 7 × 6, 9 7.1 + 0.9 (*n* = 9)	12.3 + 1.3 *n* = 89	6 × 4, 7 × 3 6.5 + 0.5 (*n* = 7)	30 + 4.2 *n* = 8
Control II Sens LEW	na	$^{1\times ACI}$LEW *n* = 7	na	na	na	na	na	na	41 + 6.0 *n* = 7
	na	$^{4\times ACI}$LEW *n* = 5	na	na	na	na	na	6 min, 8 h, 10 h, 3 d, 4 d *n* = 5	na
Control III BN-LEW Ltx	BN	LEW *n* = 8	0% 0/16	> 30 × 8, > 100 × 8	17, 18, 19, 19, 19, 28 > 100, > 100	> 150 *n* = 1	13.5 + 2.1 *n* = 8	6 × 4, 7, 8, 10 7 + 1.6 (*n* = 6)	na
Donor-specific recipient sens BN-^{BN}LEW	BN	$^{1\times BN}$LEW *n* = 4	100% 4/4	14, 14, 15, 16	na	na	na	na	na
	$^{1\times ACI}$BN	LEW *n* = 8	13% 1/8	25* 62, > 70 × 3, 100 × 3	18, 19, 20, 100 *n* = 4	na	10.8 + 1* *n* = 4	na	24 + 5.2 *n* = 4
Sens donor $^{AC\,I}$BN-LEW Ltx	$^{2\times ACI}$BN	LEW *n* = 8	0% 0/8	>100 × 8	19, 20, > 100 × 6 *n* = 8	na	11.3 + 2.6* *n* = 8	na	na
	$^{4\times ACI}$BN	LEW *n* = 14	40% 6/15	11, 12, 13, 19, 21, 25, > 40 × 6, ≥ 100 × 3	na	> 104, > 63, > 75 *n* = 3	na	3, 5, 5, 5, 6, 6 5 + 1.0 (*n* = 6)	na
Sens recipient Bn-^{ACI}LEW Ltx	BN	$^{1\times AC}$LEW *n* = 26	50% 13/26	9, 10 × 2, 13 × 3, 14 × 2, 15, 16, 18 × 2, 25* > 50 × 7, > 80 × 3, < 100 × 2	16, 24, 25, 26, 35 *n* = 5	na	10.4 + 0.5 *n* = 5	na	na
	BN	$^{2\times ACI}$LEW *n* = 4	50% 2/4	15, 30, 40, > 95	20, > 81 *n* = 2	na	9.5 + 0.7 *n* = 2	na	na
	BN	$^{4\times ACI}$LEW *n* = 8	63% 5/8	14, 18, 21, 29*, 34*, > 60, 116, > 106	na	> 106, > 62 *n* = 2	na	2 d, 3 d, 4 d, 3 + 1 (*n* = 3)	na

lowed by an exponential increase after boosting, leading to titers of more than 50,000 mIU/ml in both strains (Fig. 2).

As immune responses in both strains were similar, the effect of donor and recipient immunization could be compared.

Effect of Donor Sensitization on Liver Graft Rejection

Liver transplantation in the spontaneously tolerant strain combination BN→LEW leads to permanent survival of the recipient without the need for immunosuppression.[22]

Lethal rejection can be induced in such a model by donor-specific recipient sensitization, as previously reported by Kamada in the strain combination DA→PVG.[23,24] Our results confirmed this observation (Table 2), since 1-time donor-specific recipient sensitization (using a BN skin graft) caused lethal rejection of BN-liver grafts in all LEW recipients (*n* = 4, rejection time 14-16 days).

Interestingly, 1-time nonspecific recipient (using an ACI skin graft) sensitization could also induce rejection, but to a lesser extent. Death due to rejection occurred in 50% (13/26) of sensitized recipients of a BN-liver graft. Repeated nonspecific sen-

sitization did not increase the rejection rate. Nonspecific donor sensitization with 1 or 2 unrelated (ACI) skin grafts did not interfere with spontaneous graft acceptance, whereas repeated nonspecific skin sensitization of the donor (4×) caused lethal rejection in 40% (6/15) of recipients.

The cotransplantation of a fragment of a highly activated immune system within the organ graft, therefore, represented a similar risk (40%-50%) to the activation of one's own immune system by a single sensitization for the recipient to die from rejection.

Activation of the donor immune system by 2 vaccinations resulted in a very efficient humoral immune response, as indicated by the titer height (> 50,000 mIU/ml), but it did not interfere with tolerance induction. No animal experienced lethal rejection after receiving a liver from a vaccinated donor.

Effect of Donor Sensitization on Tolerance Induction in Animals Surviving Liver Transplantation

Liver transplantation in the spontaneously tolerant strain combination leads to tolerance induction, not only to the liver graft itself but also to subsequent donor-specific organ grafts (see Table 2). BN-skin (n = 8) or heart grafts (n = 1) transplanted subsequently to the BN-liver graft into naive LEW rats were either prolonged or fully tolerated (survival over 150 days).

Although repeated, nonspecific donor sensitization induced lethal rejection in 40% of the animals. Once a liver graft was permanently accepted, donor sensitization did not interfere with tolerance induction for subsequent organ grafts, that is, 3 out of 3 BN test heart grafts survived for more than 60 days. The same phenomenon was observed in recipients undergoing preoperative sensitization: 2 out of 2 BN test heart grafts were prolonged for more than 60 days. Clinical examination revealed strong beating of the transplanted heart, and histological analysis of the heart grafts revealed normal morphology.

Despite induction of lethal rejection in about half of the rats undergoing either multiple nonspecific donor sensitization or single-recipient sensitization, the remaining animals accepted their liver graft and developed tolerance to a subsequent test graft.

Effect of Donor Sensitization on Transfer of Cellular Immunity in Tolerant Animals

Performing a DTH assay in naive LEW rats resulted in an increase in ear thickness of 30 ± 4.2 mm (n = 8). Previous sensitization of the LEW rat with 1 ACI skin graft induced a delayed type hypersensitivity response, as indicated by the significant increase in ear thickness, in comparison to the naive rats undergoing this assay (41.0 ± 6.0 mm in comparison to 30 ± 4.2 mm, n = 7, P < 0.01). LEW rats receiving a liver graft from a sensitized donor did not show a significant increase in diameter as compared with the control group (24 ± 5.2 mm, n = 4, compared with 30 ± 4.2 mm, n = 7, P > 0.05) (see Table 2). Adoptive transfer of donor-derived cell-mediated immune function could not be demonstrated using this assay, possibly due to the nonspecific immunosuppressive effect of the liver transplant itself.

Therefore, rejection of a challenged skin graft was the second strategy to test the recipient for donor-derived cellular immune function (see Table 2). The control group consisted of LEW recipients of BN-liver grafts from a nonvaccinated naive donor. These rats rejected a 3rd-party skin graft of ACI origin as well as an ACI heart graft in a slightly delayed fashion compared with naive LEW animals (skin graft: 13.5 ± 2.1 days in transplanted rats, n = 8, compared with 12.3 ± 1.3 days in naive rats, n = 89, P < 0.05; heart graft: 7 ± 1.6 days in transplanted rats, n = 6, compared with 6.5 ± 0.5 days in naive rats, n = 7, P < 0.05).

Nonspecific recipient skin-sensitization caused an accelerated rejection of the subsequent ACI-test skin graft (no sensitization: 13.5 ± 2.1 days [n = 8] compared with single sensitization: 10.4 ± 0.5, P < 0.01).

Nonspecific donor-sensitization had a comparable effect on the accelerated rejection of the test skin graft. (no sensitization: 13.5 ± 2.1 days [n = 8] compared with single sensitization: 10.8 ± 1 days [n = 4, P < 0.05).

Donor sensitization had a similar effect as recipient sensitization on acceleration of the test skin graft.

Repeated donor-specific recipient sensitization by skin grafting reportedly led to the induction of hyperacute rejection in the strain combination ACI→LEW 4-times.[25] Therefore, this protocol was chosen as another strategy to evaluate the efficiency

of immune transfer (see Table 2). After applying this sensitization protocol to naive LEW rats, they rejected a subsequent ACI heart graft hyperacutely to acutely (rejection time between 6 min and 4 days, $n = 5$).

Repeated donor sensitization prior to liver donation accelerated rejection of an ACI-test heart graft significantly (rejection time 5 ± 1 days, $n = 6$, compared with 7 ± 1.6 days in nonsensitized LEW liver graft recipients, $n = 6$, $P < 0.01$).

Repeated recipient sensitization prior to liver transplantation led to the rejection of the ACI-test heart grafts within 3 days, which was further accelerated when compared to donor sensitization (3 ± 1 days compared with 5 ± 1 days, $n = 6$, $P < 0.05$).

Using an enhanced sensitization protocol, the effect of donor sensitization on accelerating the rejection of the test graft was very pronounced, although slightly milder when compared with the influence of recipient sensitization.

Effect of Donor Vaccination on Transfer of Humoral Immunity Transfer in Tolerant Animals

LEW recipients of BN allografts from either sensitized or vaccinated donors underwent weekly blood sampling for determination of donor-derived antibodies. Antibody titer, measured by CDC (sensitization protocol) or MEIA (vaccination protocol), was negative at the time of liver transplantation. One week later, all animals showed positive titers (see Fig. 3), which ranged between 0.024% and 0.195% of the donor's titer (0.024%-0.488% in sensitization group and 0.075%-0.195% in vaccination group).

Maximal titers were measured at either the 1st or 2nd week posttransplantation (60-694 mIU/ml). An anti-HBs titer of >10 mIU/ml is regarded as protective against de-novo infection.[26] All recipients of a liver graft from a vaccinated donor maintained a titer around this level throughout the observation period (8-101 mIU/ml).

Prolongation of Titer Persistence in the Recipient Compared with Calculated Antibody Degradation

Although antibody titer was decreasing continuously, it remained higher than estimated, according to the half-life of antibodies (Fig. 4). Half-life of IgG is supposed to be 5 days, whereas half-life for IgM is as short as 2.5 days.[27] Taking a recipient antibody titer of 60 mIU/ml on POD 7 as a starting point, IgG would have reached levels below 5 mIU/ml within 5 weeks and IgM would have dropped below 5 mIU/ml within 2.5 weeks (Fig. 4). The observed titer was as high as 29 mIU/ml at the end of the observation period of 5 weeks.

The observation of continuous antibody degradation may lead to the assumption that the titer in the recipient is exclusively due to passively transferred antibodies, which remained in the organ graft despite perfusion during the harvesting procedure, and which were washed out in the recipient's circulation after transplantation. Hence, antibody titer persisted in height and duration clearly above the calculated levels, which suggests that not only antibodies but also antibody-secreting cells were transferred and contributed to the higher antibody levels in the recipient. Longer observation periods are necessary to determine whether these antibody-secreting cells die off and the antibody titer eventually becomes undetectable.

Discussion

Using Organs from Highly Immunized Donors Represented a Risk, Albeit Small, to Induce Rejection

In clinical transplantation, the risk of experiencing rejection is enhanced in sensitized recipients. Crossmatch-positive recipients experienced significantly ($P < 0.05$) more rejections and more steroid-resistant rejections ($P < 0.05$) than crossmatch-negative recipients.[28-30]

This is even more obvious in experimental transplantation when using a spontaneously tolerant liver allograft model.[22] Sensitizing the recipient in this model with donor-specific antigen inhibits tolerance induction. Instead, lethal rejection is induced, which is otherwise difficult to achieve in this model. The rejection-inducing effect of donor-specific antigen pretreatment prior to organ transplantation was demonstrated in rats by Kamada[24] and in mice by Qian and Thai.[31,32]

Interestingly, nonspecific activation of the recipient's immune system by 3rd-party skin grafting apparently also had an immunostimulatory effect on the recipient, which was sufficient to induce rejec-

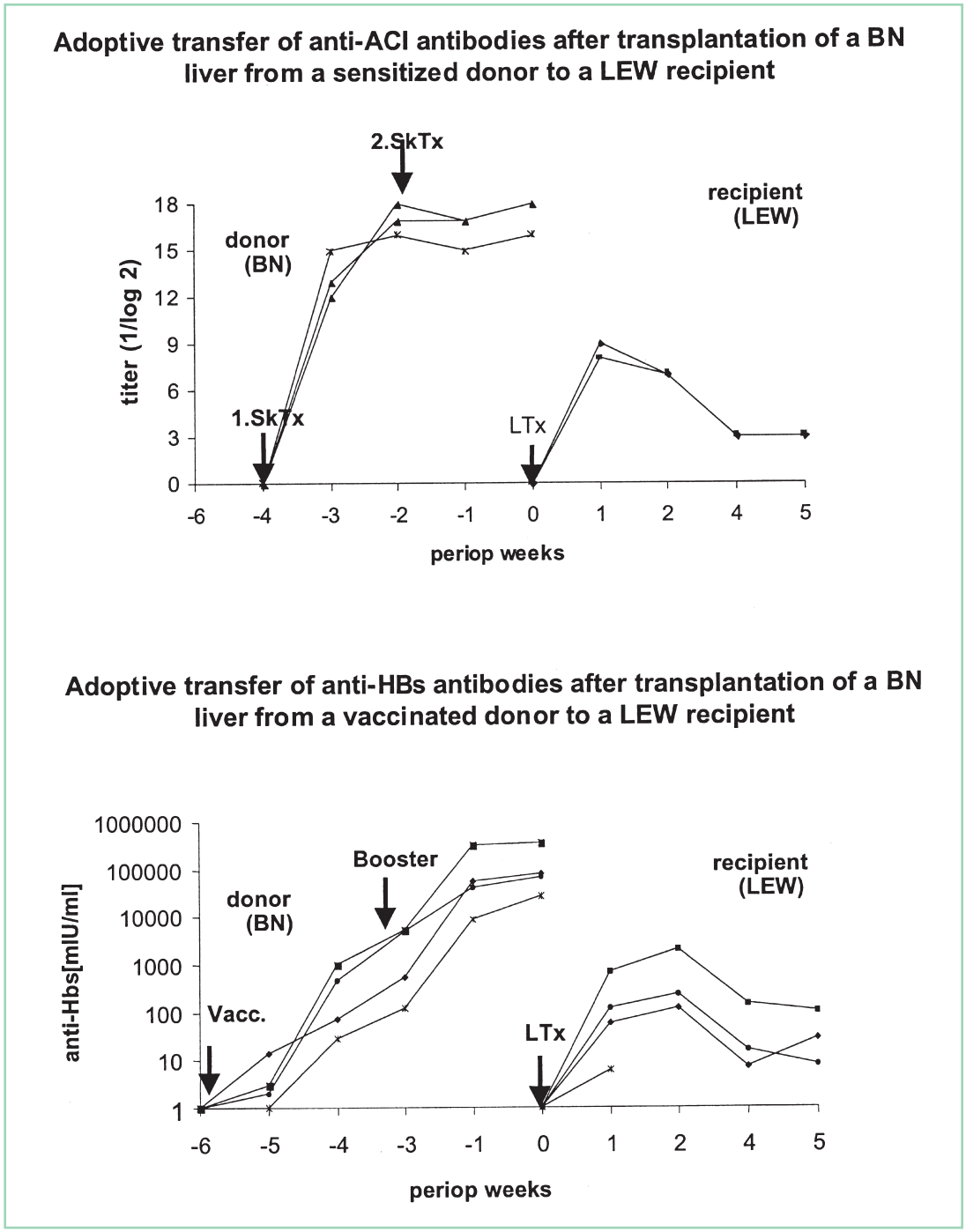

Figure 3. Development of donor-derived antigen-specific antibodies after liver transplantation from an immunized donor. Upper panel: skin-sensitized donor; lower panel: HBs-vaccinated donor. Titer persisted in both cases throughout the observation period of 5 weeks, although slowly decreasing. LTx = liver transplantation; SkTx = skin transplantation; Vacc. = vaccination.

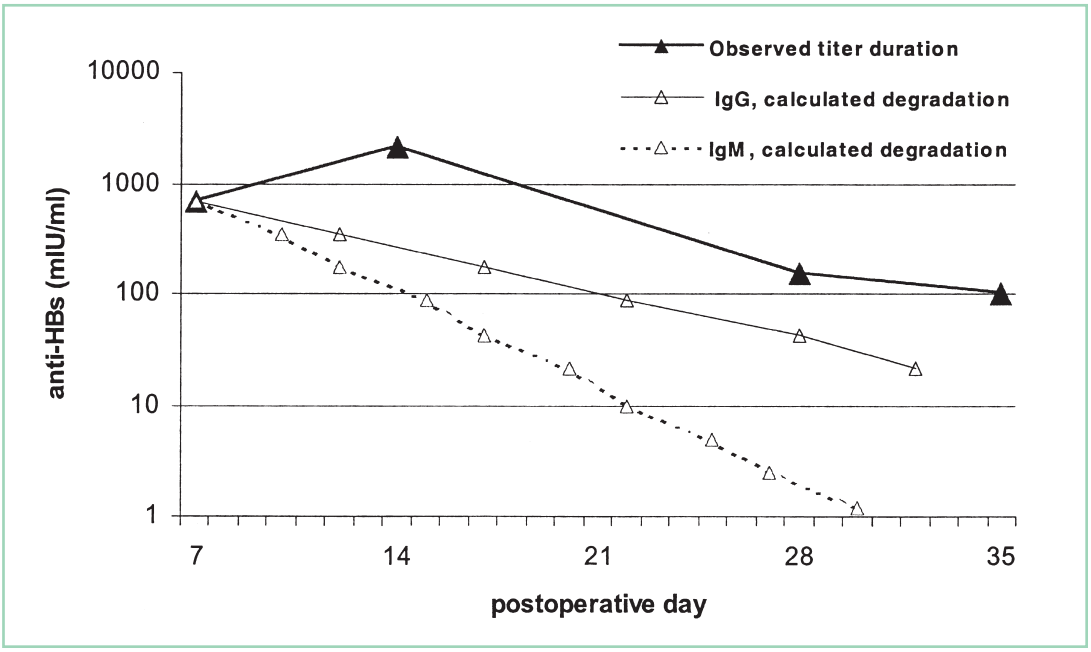

Figure 4. Observed titer duration in comparison to calculated antibody degradation. Donor-derived antibodies persisted longer than expected in the case of passive transfer and subsequent antibody degradation.

tion in half of the animals. A similar phenomenon was observed by Bathgate. Enhanced immunoreactivity was identified prior to liver transplantation by a positive reaction to a contact neoantigen and was successfully used to predict rejection.[33]

However, it was more striking that using organ grafts from highly immunized donors, for example, achieved by multiple, nonspecific donor sensitization, also induced lethal rejection in 40% of the animals. Donor immunization apparently imposed a small, but not negligible, risk on the recipient to experience rejection. The risk of inducing rejection seemed to be related to the intensity of the immunization procedure. Although 1-time recipient sensitization represented a sufficient stimulus to induce liver graft rejection, donor immunization required repeated antigen contact.

Vaccination did not lead to liver graft rejection in any case, which might be due to the relatively mild immunization protocol. However, donor rats were only vaccinated twice, but using a 50-fold higher dose as compared with human protocol. Using this vaccination protocol led to a very efficient antibody response but was apparently not harmful in term by increasing the alloimmmune response toward the graft.

Nevertheless, this potential, although rather small, risk of enhancing ongoing rejection by increasing the alloimmune response in the recipient must be taken into consideration when using organs from such highly immunized donors.

Induction of Rejection Did Not Interfere with Tolerance Induction

Heart grafts of BN origin were tolerated by all animals surviving the liver transplantation, being either naive rats, sensitized recipients, or recipients receiving a liver graft from a highly immunized donor. Similar findings have been reported in other models.[23] Despite strong immune activation by sensitization, tolerance induction to donor-specific heart grafts was not affected in animals surviving the liver transplantation, although liver graft acceptance is not necessarily associated with tolerance induction.[34]

Cellular As Well As Humoral Immune Functions Were Transferred Adoptively

As described in case reports, donor immune functions can be accidentally transferred to the recipients of an organ graft. In general, immune transfer

was only identified because of its unwanted and potentially harmful symptoms in the recipient. The well-known cases include the demonstration of isohemagglutinins of graft origin after ABO-unmatched liver transplantation,[14] 1 transmission of idiopathic (autoimmune) thrombocytopenic purpura by liver transplantation,[12] and the transfer of symptomatic peanut allergy to the recipient of a combined liver-and-kidney transplant.[13] Despite the qualitative demonstration that transfer of immune function occurred not only after bone marrow transplantation but also after organ transplantation, no systematic study has been undertaken until now. The reported incidence is restricted to a few case reports, which might also be related to the fact that transfer of potentially protective immune functions is not obvious and requires an active search. Only cases where the transferred immune function put the recipient at risk have been identified to this point. However, as demonstrated in this study, immune transfer seems to be of uniform consequence in organ transplantation, obviously paralleling the currently accepted phenomenon of microchimerism. Although the functional relevance of microchimerism, as well as the frequency of donor-derived cells, is still discussed controversially, the phenomenon itself is no longer questioned. The reported frequency of donor hematopoietic cells depends on the transplanted organ and possibly the immunosuppressive therapy, but more important, on the system used to identify those cells.

It has long been known that major abdominal surgery or trauma exerts a nonspecific immunosuppressive effect on the recipient.[35,36] This effect could also be attributed to the transplantation procedure itself. Skin graft rejection was slightly delayed after liver transplantation, compared to naive animals. However, acceleration of skin graft and heart graft rejection using the organ of an immunized donor indicated the transfer of cellular immune functions after donor sensitization.

Interestingly, donor sensitization was as equally effective as recipient sensitization. Increasing the sensitizing stimulus did not lead to a quantifiable difference of the immune response in the recipient.

Transfer of humoral immune function was clearly demonstrated by the appearance of donor-derived, antigen-specific antibodies in the recipient. Although the liver graft was perfused to minimize contamination of donor-derived blood, blood as the source of donor-derived cells could not be distinguished from liver parenchyma as the reservoir of passenger lymphocytes. Furthermore, it could not be proven at this point that these antibodies were produced in the recipient, but calculation of the expected titer duration based on the known half-life of passively transferred antibodies[27] suggests the transfer of antibody-producing cells from donor to recipient.

Conclusion

Purposely taking advantage of donor-derived immunity is an attractive and interesting concept in the era of living organ donation. Living donation allows and requires an exact timing of the operative procedure.[37] This allows for the first time the possibility of immunological conditioning of the donor before the organ is removed. Promising results have been achieved with this strategy in bone marrow transplantation. The group of Shouval and Ilan has dedicated a large effort to investigating this approach in animal models and in implementing this strategy successfully in clinical practice.[5,38,39] They even found evidence for the maintenance of immune memory to the hepatitis B envelope protein following adoptive transfer of immunity in bone marrow transplant recipients.[40]

Based on the results of our systematic study, immune transfer is occurring uniformly after liver transplantation, which makes this concept interesting for further investigation. Donor pretreatment by a simple vaccination protocol, for example, against hepatitis B, is of possible benefit for the donors themselves and may be of benefit for the recipients. HBV vaccination of the living donor should protect one from acquiring an active infection perioperatively. Liver transplant recipients possibly benefit by the prevention of a de novo infection, but potentially to a much higher extent is preventing reinfection in the case of liver transplantation due to HBV-related cirrhosis. One can envisage for the recipient that actively maintaining a high anti-HBs titer may contribute to a lower probability of reinfection.

Whatever the source of donor-derived antibodies might be, passively transferred antibodies being

washed from the graft, contaminating blood-derived lymphocytes, or active migration of passenger lymphocytes (including antibody-secreting plasma cells), remains irrelevant from the practical point of view. If a simple procedure such as the active HBV-vaccination of a living donor could contribute to a higher antibody titer in the recipient with hepatitis B cirrhosis, without exposing one to an additional risk, it is worthwhile to pursue this strategy. Further work needs to be dedicated to exploring the effect of immunosuppressive treatment on adoptive transfer and developing strategies to potentially augment the efficacy of the immune transfer.

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