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The efficiency of humoral immune transfer depends on both the graft and the immunosuppressive treatment

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Abstract The present study was designed to compare the efficiency of adoptive transfer of humoral immunity after liver, kidney, and heart transplantation in relation to the number of passenger lymphocytes, and to estimate the risk of a detrimental effect and the chance of a beneficial effect. Hepatitis B virus surface-antigen-vaccinated brown Norway rats (BNs) and AxC 9935 Irish (ACI rats) served as donors, and na Lewis (LEW) rats as recipients. The liver grafts contained 100 times more passenger lymphocytes than heart grafts, and the kidney grafts approximately ten times more, indicated by monoclonal CD45 antibody staining. Transient anti-HBs immunity did occur after transplantation of all three organ grafts. In all rejecting groups, the serum recipient-to-donor anti-HBs titer ratio (R/D ratio) was below 0.10%, with heart recipients showing half the level (0.05%) of liver recipients (0.09%). Under immunosuppression, R/D ratio doubled in liver or kidney recipients, but remained unaffected in heart recipients. Immune transfer was most efficient in immune-suppressed liver recipients in the spontaneously tolerant strain combination as indicated by a significantly higher R/D ratio (0.32%) and a longer titer persistence (up to 9 weeks) than in all other groups. Therefore, mainly liver and kidney graft recipients carry a risk, but also a chance of benefiting from the transfer of donor-derived immunity.

Keywords Adoptive immune transfer · Hepatitis B vaccine · Heart transplantation · Kidney transplantation · Liver transplantation · Rat

Introduction

Adoptive transfer of immunity by organ transplantation is clinically a rare phenomenon. It is normally observed due to its negative implications for the patients, which was first observed in the form of hemolytic anemia after kidney transplantation [21]. Since then, it has become a well-known complication of ABO-non-identical solid-organ transplantation (defined as a group-O organ transplanted to a non-group-O recipient or a group-A or B organ transplanted to a group-AB recipient) [20, 29, 30].

A beneficial effect of adoptive immune transfer through transplantation has so far been reported only in bone marrow recipients [10, 15, 34, 36, 44]. Virus clearance was observed in individual cases, and clinical studies have shown that bone marrow transplantation protected the recipients from hepatitis B virus (HBV) infection by transferred donor-derived anti-HBs immunity [11, 16]. Similar potentially protective effects, in terms of effective anti-HBs titer, were observed in experimental studies when the liver of a vaccinated donor was transplanted into a na recipient (U Dahmen [7b]).

The present study was designed to compare the efficiency of adoptive immune transfer after liver, kidney, and heart transplantation to estimate the risk of a detrimental effect and the chance of a beneficial effect after transplantation of various organs.

Material and methods

Experimental design

Standard orthotopic liver transplantation, orthotopic kidney transplantation, and heterotopic heart transplantations were performed in two allogeneic rat strain combinations: brown Norway rat (BN) to Lewis (LEW) and AxC 9935 Irish (ACI) to LEW rats. BN to LEW represents a spontaneously tolerant strain combination for liver transplantation, whereas kidney and hearts grafts are acutely rejected, as are all three organs in the strain combination ACI to LEW. Donor rats were vaccinated with 0.2°ml recombinant HBV vaccine (HBVac, Engerix-B, SmithKline Beecham Pharma, Munich, Germany) containing hepatitis B surface antigen (HBsAg) 20°μg/ml) 6 weeks before and boosted 2 weeks before organ donation.

Transplantations were performed from vaccinated donor rats to na recipient rats. According to our previous studies, a donor titer under 10,000°mIU/ml always failed to lead to a positive seroconversion in its recipient, irrespectively of the type of graft (liver, kidney, or heart). Thus, only donors with anti-HBs above 10,000°mIU/ml were chosen for analysis. Applying this criterion, we performed 27 liver transplantations, 25 kidney transplantations and 25 heart transplantations in this study. Some of the recipients underwent immunosuppressive treatment with cyclosporin A (Sandimmune, Novartis, Basel, Switzerland) at a dose of 5°mg/kg per day, which was administered by subcutaneous injection [26]. Anti-HBs titers were measured before the first vaccination in the donors, and weekly thereafter; prior to transplantation in the recipients, and at weekly intervals after transplantation.

Animals

Male inbred BN ($RT1^n$), and ACI ($RT1^a$) rats (Charles River Wiga, Sulzfeld, Germany) aged 5–6 weeks (80–100°g/rat) were chosen for vaccination. Six weeks later, they were used as organ donors. Male inbred LEW ($RT1^l$) rats aged 10–11 weeks served as recipients. At the time of transplantation, the weight of each rat was within the range of 230–280°g. The animals were housed under standard animal-care conditions and fed with rat chow ad libitum before and after the operation. All procedures and housing were carried out according to German animal-welfare legislation.

Orthotopic liver transplantation

Orthotopic liver transplantation was performed according to the technique of Kamada and Calne [14]. The cold ischemia time did not exceed 1°h, and the anhepatic time was below 20°min. Briefly, after mobilization, the donor liver was perfused through the portal vein with chilled 0.9% NaCl solution until the effluent from the suprahepatic vena cava was clear. The organ was preserved at 4 °C until it was placed orthotopically in the recipient abdomen. The donor suprahepatic vena cava was anastomosed end-to-end with the recipient's with a continuous 7–0 polypropylene suture. We accomplished the portal vein anastomosis and the infrahepatic vena cava anastomosis by pulling the recipient' s vein over a cuff that was secured with a circumferential 6–0 silk suture. The tying of the bile duct over a stent restored biliary continuity.

Orthotopic kidney transplantation

Orthotopic kidney transplantation was performed according to the technique by Oesterwitz and Althaus [25]. Division of the suprarenal vein was followed by dissection of the renal artery and vein. The ureter was divided close to the ureteropelvic junction. After injection of 100 units of heparin via the penile vein, the renal vein and artery were transected, and the kidney was removed and placed in saline solution at 4 °C. In the recipient, the renal vessels were occluded and transected distally from the clamp. The ureter was dissected in the way same as in the donor. The kidney graft was placed on the posterior abdominal wall of the recipient. Renal veins were anastomosed end-to-end by running suture. The reconstruction of the artery and ureter were performed end-to-end with interrupted sutures.

Heterotopic heart transplantation

Heterotopic heart transplantation was performed according to Lee et al. [17]. After injection of 100°IU heparin via the penile vein, the anterior chest wall was opened, and the heart was exposed. The right superior vena cava was ligated near the atrium. The ascending aorta, as well as the main pulmonary artery, was mobilized and transected at the point of bifurcation. The left superior vena cava and pulmonary vein were ligated and divided distally. The heart was then excised and immersed in cold saline (4 °C). The recipient's abdominal aorta and the inferior vena cava were mobilized for a short segment from bifurcations of renal vessels to bifurcations of the common iliac vessels. The blood flow was temporarily blocked by the placing of vessel clamps at the two ends. The aorta of the heart graft was anastomosed end-to-side to the recipient abdominal aorta. The main pulmonary artery was anastomosed end-to-side to the recipient inferior vena cava.

Postoperative follow-up

All recipients received single-shot antibiotic treatment by intramuscular injection of 100°mg/kg per day of mezlocillin (Baypen, Bayer, Leverkusen, Germany) after operation. In cases of deteriorating general condition, as indicated by severe weight loss, lack of spontaneous activity, or jaundice, the animals were killed.

Immunohistochemistry

Organs (liver, kidney and heart) of three normal rats in each strain (BN and ACI) were embedded in optimal cryo-embedding compound (Microm Laborgeräte GmbH, Walldorf, Germany) prior to snap freezing in liquid nitrogen, and stored at 80 °C until use. We obtained 5-μm cryosections. We used CD45 expression on passenger cells to quantify the number of leucocytes in the organ grafts. CD45 was visualized by immunohistochemistry (indirect avidin-biotin method) using a mouse monoclonal antibody (OX-1, Mouse IgG, Pharmingen, Hamburg, Germany). Briefly, sections were incubated with the primary antibody (1:100 dilution) for 30°min at room temperature prior to addition of the biotinylated secondary antibody at a dilution of 1:300 (Biotin rabbit-anti-mouse, Dako, Hamburg, Germany). The antigen was visualized by alkaline phosphate conjugated streptavidin (Zymed Laboratories, California, USA) followed by the application of the substrate solution (Fast red, Boehringer Mannheim). Sections were counterstained with Mayer's hemalum solution (Merck KGaA, Darmstadt, Germany).

We quantified positive staining for CD45 by analyzing up to nine digital pictures per section. In the liver, separate pictures were taken of the portal tract, the central vein and lobular tissue; and in the kidney, pictures of glomeruli and tubuli were taken, as well as the areas in between. Identification of positive cells was based on

the staining result in combination with the typical lymphocyte morphology. We estimated the relative number of lymphocytes per organ by multiplying the organ weight by the number of CD45-positive cells/high-power field.

Measurement of anti-HBs titer

A fully automated microparticle enzyme immunoassay as described by the Abbott Laboratories [28] was used for detection and quantification of rat serum antibody against hepatitis B surface antigen (anti-HBs).

Statistical analysis

The antibody titers of the individual rats in each group were calculated as geometric mean titers (GMTs) in "mIU/ml" [13, 37]. The calculations of GMT and titer duration were based on the seroconverted animals. Analysis of variance and two-tailed Student's *t*-test were employed to assess the titer differences within each group with the help of SPSS computer software (SPSS, Chicago, Ill., USA). $P < 0.05$ was considered to be statistically significant.

Results

Anti-HBs seroconversion in recipients

The maximum donor titer after two immunizations with recombinant HBVac was 31,2809°mIU/ml. No significant difference in donor anti-HBs GMT was observed within the three groups of different organ donors ($P > 0.05$, Table 1).In the first postoperative week, 25 out of 27 (93%) liver recipients, 23 out of 25 (92%) kidney recipients, and 21 out of 25 (84%) heart recipients were identified as having efficient anti-HBs titer levels (≥10°mIU/ml) [24] (Table 2). Titer duration in all animals was mostly dependent on the anti-HBs titer level in the first postoperative week (POW 1). Anti-HBs antibodies declined in all animals over time, persisting for the longest in the animals under immunosuppression with a high titer at POW 1.

CD45 positive cells in liver, kidney and heart

The weight of the rat liver (8.34 ± 1.21°g) was roughly eight times higher than that of the rat kidney (0.94 ± 0.08°g) or the heart (1.01 ± 0.22°g); see Fig. 1a. The number of CD45+ cells/high-power field (HPF) varied between 5~10 in livers, 3~9 in kidneys, and 0.4~0.9 in hearts; see Figs. 1b and 2. We estimated the relative amount in passenger lymphocytes per organ by multiplying the number of positive cells/HPF by the organ weight, which led to large differences between the three organs, with a ratio of liver:kidney:heart of 100:10:1 (the relative number of passenger lymphocytes in the liver amounted to 41~83 cells, in the kidney 6.3~8.8 cells, and in the heart 0.09~0.51 cells; see Fig. 1c).

Table 1 Immune response to recombinant HBVac in donorrats. Anti-HBs titers were measured 2 weeks after the second vaccination

Transplantation	Total number	Geometric mean anti-HBs titer (minimum~maximum)
Liver	$n = 27$	49,998 (11,894~31,2809) mIU/ml
Kidney	$n = 25$	45,432 (12,000~31,2809) mIU/ml
Heart	$n = 25$	52,613 (11,894~31,2809) mIU/ml

Table 2 Seroconversion rate to anti-HBs in recipient rats with liver, kidney, or heart grafts from HBVac immunized donors

Strain combination	Immunosuppression (daily CsA)	Liver Tx	Kidney Tx	Heart Tx
BN to LEW	No	6/8	5/6	4/6
BN to LEW	Yes	8/8	7/7	7/9
ACI to LEW	No	6/6	4/4	4/4
ACI to LEW	Yes	5/5	7/8	6/6
Summary		25/27	23/25	21/25

Influence of donor titer on the recipient's immune response

The anti-HBs level in the recipients was mainly determined by the donor's response to HBVac. Correlation was found between recipient and donor anti-HBs titer in POW 1 at the significance level of 0.01. The coefficient of correlation was 0.70, 0.66, and 0.63 for liver, kidney, and heart transplantation, respectively. Therefore, the recipient-to-donor titer ratio (R/D ratio) was used as the main parameter to estimate the efficiency of adoptive immune transfer after transplantation of various organs.

Principally, donor titers required for a 100% seroconversion rate and minimal donor titer necessary for seroconversion differed between the grafts. The lowest donor anti-HBs titer to establish seroconversion was as low as 11,929°mIU/ml in liver recipients, 14,000°mlU/ml in kidney recipients, and 22,255°mIU/ml in heart recipients. All liver graft recipients seroconverted, if the donor titer was above 14,834°mIU/ml, whereas in kidney and heart transplantation, higher donor titers were required (26,000°mIU/ml for kidney recipients, and over 72,579°mIU/ml for heart recipients; Table 3).

Influence of immunosuppression on immune transfer

Comparing recipients of different organ grafts, we found that donor titers were in a similar range (GMTs for liver, kidney, and heart donors were 49,998, 45,432 and 52,613°mIU/ml, respectively). Immunosuppressed liver

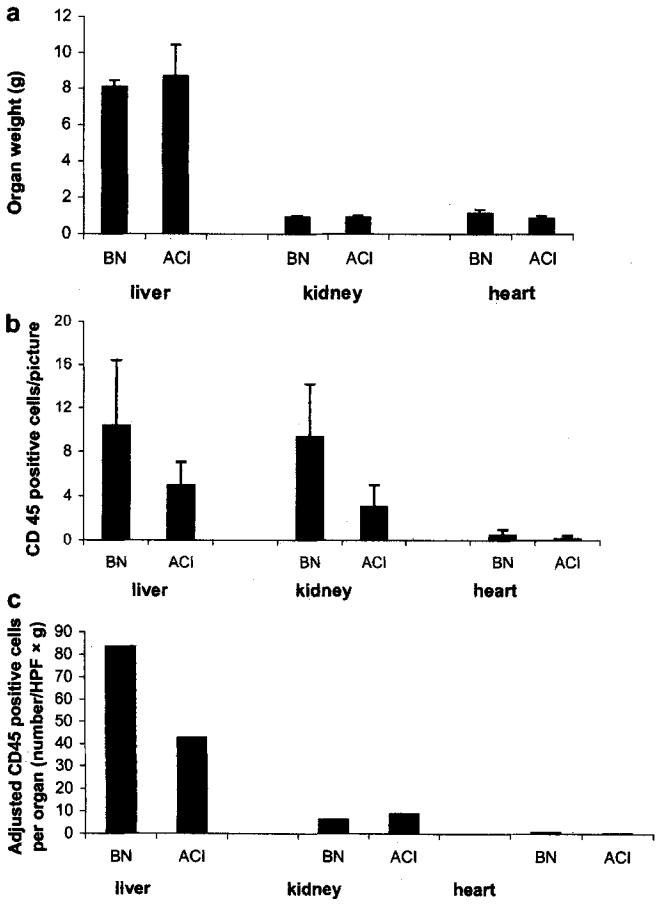

Fig. 1 Passenger lymphocytes indicated by monoclonal CD45 antibody staining in the normal rat liver, kidney and heart. **a** Organ weight in each strain of rats. **b** CD 45 positive cells within each organ according to the size of area measured ($2428 \pm 22°\mu m^2$ per picture). Independently of the rat strain used (BN or ACI, $n=3$ in each strain), the highest number of positively staining cells was identified in the liver, then in the kidney and the heart. **c** Relative number of CD45 positive cells per organ. The relative number of CD45 positively staining cells per organ was estimated by positive cell count per picture multiplied by the organ weight. Liver weight was eight times as high as the weight of the kidney and the heart and contained relatively more lymphocytes/given area, which resulted in a far higher relative number of passenger lymphocytes than in the two other organ grafts

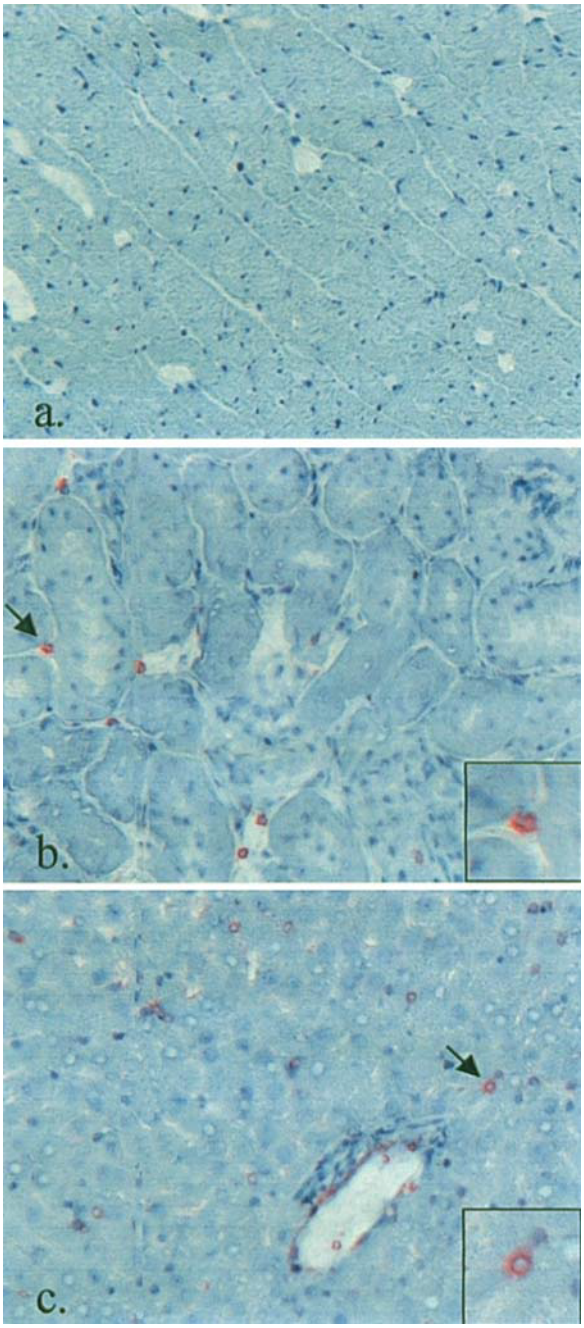

Fig. 2 CD45 (*red*) with hematoxylin counterstain in **a** the heart, **b** the kidney, **c** the liver of normal BN rats (× 200)

graft recipients had the highest titers at POW 1 and the longest titer durations (Fig. 3). The most efficient immune transfer was observed in the immunosuppressed liver graft recipients in the spontaneously tolerant strain combination. The highest R/D ratio was found in liver recipients, whereas the ratio was much lower in kidney grafts recipients, and reached a significantly low level ($P < 0.05$) in the heart transplant group (see Table 4). R/D ratio in these liver recipients ($0.32 \pm 0.10\%$) was ten times as high as in treated heart graft recipients ($0.03 \pm 0.01\%$), and twice as high as in treated kidney graft recipients ($0.14 \pm 0.07\%$).

In the rejecting strain combination (ACI–LEW), the R/D ratio was under 0.10% in all three organ transplant groups. The R/D ratio in immunosuppressed liver and kidney recipients was almost twice as high as untreated liver and kidney graft recipients (0.09% vs 0.15% in liver recipients and 0.06% vs 0.11% in kidney recipients). No effect of immunosuppression was seen in heart graft recipients (0.05% vs 0.04%).

Table 3 Minimal donor anti-HBs titer (mIU/ml) for seroconversion in recipient is different in three types of organ recipients

Parameter	Liver Tx	Kidney Tx	Heart Tx
Minimal donor titer for seroconversion	> 11,929	> 14,000	> 22,255
Requirement of donor titer to achieve 100%seroconversion	> 14,834	> 26,000	> 72,579

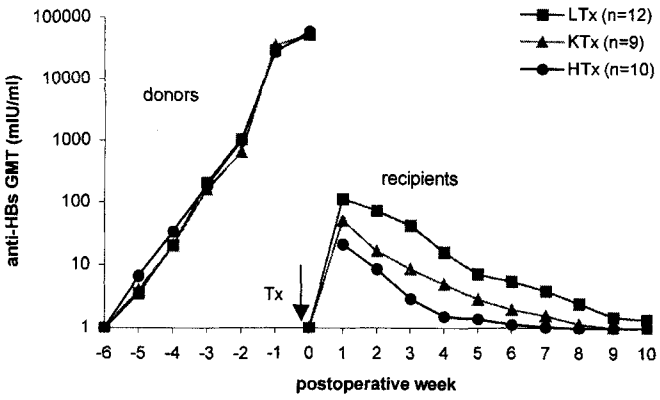

Fig. 3 Ten heart transplant (*HTx*) recipients, nine kidney transplant (*KTx*) recipients, and 12 liver transplant (*LTx*) recipients, with a follow-up of more than 10 weeks, were compared for effective titer duration. Similar donor anti-HBs titer led to different antibody development in the recipients with heart, kidney, or liver graft

Discussion

Transient immune transfer did occur after transplantation of all three organs, confirming clinical observations. Hemolytic anemia after ABO-incompatible organ transplantation has been reported most often in liver recipients, but also in kidney, heart-lung, lung, spleen, and pancreas recipients [3, 4, 5,7a, 12, 29, 31, 33, 38, 40]. Detailed analysis of clinical cases by Ramsey showed that the frequencies of alloantibodies and hemolysis were higher in liver transplant patients (40% and 29%, respectively) than in kidney recipients (17% and 9%).

In addition to antibodies directed against ABO blood group antigens, other donor-derived humoral immune responses can cause the so-called humoral graft-versus-host immune response, which is also most frequently observed in liver and kidney transplantation. Examples of these responses include hemolytic anemia due to transfer of antibodies against erythrocytes, platelet deficiency due to transfer of antibodies directed against platelets (anti-HPA-2b) [9, 39], and peanut allergy due to transfer of IgE [18].

Donor passenger B lymphocytes are the most likely culprits. Early reports of hemolytic anemia already stated that the incidence was much higher in patients undergoing immunosuppressive treatment with CsA than those with additionally irradiated kidney grafts, which potentially destroyed the donor-derived lymphocytes [22] The observation of anti-A antibodies in a patient with blood group A, who received a kidney graft from a donor with blood group O, led to the suspicion that donor-derived antibodies were the underlying cause of disease [3]. Donor origin of antibodies was identified by gamma globulin-marker (Gm) allotyping and was confirmed thereafter [1, 27, 29, 38].

Immunosuppressive treatment enhanced the transfer of anti-HBs immunity in liver, kidney and heart recipients. The R/D ratio was twice as high as in treated liver and kidney recipients compared with that of untreated rats, and the titer duration was prolonged. CsA-effected protection of immune cells, including antibody-secreting plasma cells from rejection, might explain the higher R/D ratio and the prolonged persistence. No influence of immunosuppression was seen after heart transplantation, possibly related to the very small number of passenger lymphocytes in the graft, leading to the suspicion of a predominantly passive transmission after heart transplantation. However, one might add further weight to this hypothesis by performing additional experiments using irradiated passenger leucocyte-depleted grafts.

Reported levels of microchimerism after cardiac transplantation are lower than after other solid organs and never exceeded a level of 10^4 [2, 8, 23, 43]. Therefore, the relative risk of a harmful effect due to an incidental transfer of donor-derived immune function from donor to recipient following heart transplantation seems to be rather low. Mainly liver, but also kidney graft recipients carry a risk, but they also have the chance of benefiting from the transfer of donor-derived immunity. Graft recipients are at high risk of acquiring

Table 4 Recipient-to-donor anti-HBs titer ratio (R/D ratio, mean±SD) in three different organ recipients

Strain combination	Immunosuppression (daily CsA)	Liver Tx	Kidney Tx	Heart Tx
BN to LEW	No	0.23±0.10%*	0.06±0.02%	0.04±0.01%*
BN to LEW	Yes	0.32±0.10%*	0.14±0.07%	0.03±0.01%*
ACI to LEW	No	0.09±0.03%*	0.06±0.02%	0.05±0.03%*
ACI to LEW	Yes	0.15±0.29%*	0.11±0.06%	0.04±0.02%*

*$P < 0.05$ in liver Tx versus heart Tx for all groups

infections such as those derived from the cytomegalovirus, Epstein-Barr virus, and hepatitis B or C virus [6, 32, 41, 42]. Transfer of donor-derived immunity to HBV, although apparently of transient persistence, might help to reduce HBV reactivation, re-infection or de novo infection, after solid-organ transplantation. In liver grafted animals, the minimal donor titer for a successful seroconversion at a 100% seroconversion rate was lower than in animals with a kidney graft, and much lower than in animals with a heart graft. A beneficial effect would be more likely in liver transplantation than in kidney transplantation and presumably unlikely after heart transplantation.

Clinical application may be envisioned in the context of living donation, which allows immunological preconditioning of the potential donor. However, optimization of vaccination protocol would be necessary for the required high donor titer to be achieved. Antibody levels obtained after standard clinical vaccination protocols tend to result in titer levels below 10,000°mIU/ml [19]. The time for donor preconditioning may be limited, depending on the progress of the liver failure in the future recipient. In kidney transplantation, even higher donor titers are necessary to achieve an efficient immune response in the recipient. The time required for a sufficiently high titer to be attained in the potential living kidney donor is presumably longer than that for liver donors. However, as kidney graft recipients are on maintenance dialysis, the period of time required for preconditioning is not as incommoding as in liver donors. Bone marrow augmentation, currently practiced in selected centers due to its controversially discussed tolerance-promoting effect [7a, 35], could be used as a strategy to enhance donor-derived immunity in the recipient, since more antibody-secreting cells would be transferred, and the likelihood for engraftment of donor cells rises.

In conclusion, donor-derived anti-HBs immunity was transiently transferred to liver, kidney and heart recipients. The potentially beneficial effect, but also the risk of a detrimental effect, is highest for liver recipients, followed by kidney recipients, and lowest for heart recipients. Clinical application is limited by the transient persistence of the donor-derived immune response in the recipient. Further studies are needed, that focus on donor conditioning for immunity against other pathogens, and develop screening strategies to avoid the complications caused by the transfer of potentially harmful donor-derived immune functions.

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