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Antiviral Research 56 (2002) 29–37

Antiviral Research

www.elsevier.com/locate/antiviral

Adoptive transfer of HBV immunity by kidney transplantation and the effect of postoperative vaccination

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Received 22 October 2001; accepted 15 April 2002

Abstract

Transfer of hepatitis B immunity occurs upon the transfer of immunologically active cells from the donor to the recipient by means of an organ graft. This has been repeatedly demonstrated for bone marrow and liver transplantations. Evidence is now presented for the transfer of anti-hepatitis B surface antibodies (anti-HBs) after kidney transplantation in rats. Kidney donors from one syngeneic and two allogeneic rat strains were immunized twice with 4 μg of recombinant hepatitis B vaccine. In week 6 after the first vaccination, kidney grafts were transplanted into Lewis (LEW) rats. Half of the recipients underwent daily immunosuppressive treatment with cyclosporin A (CsA). All recipients were vaccinated either after 10 weeks or 1 week postoperatively. Anti-HBs titer was measured weekly. Effective anti-HBs titers (10–227 mIU/ml, lasting for 1–7 weeks) were detected in 86% (25/29) of recipient rats, whose corresponding donors all had a titer above 15,000 mIU/ml. Immunosuppression enhanced the donor-derived immunity in terms of recipient-to-donor titer ratio, maximal titer and titer persistence.

Keywords: Adoptive transfer; Hepatitis B; Rat kidney transplantation

Abbreviations: Anti-HBs, anti-hepatitis B surface antibody; ACI, AxC 9935 Irish; BN, brown Norway; CsA, cyclosporin A; GMT, geometric mean titer; HBV, hepatitis B virus; LEW, Lewis; MEIA, microparticle enzyme immune assay; MHC, major histocompatibility complex; POD, postoperative day; POW, postoperative week; R/D, recipient-to-donor titer ratio; SD, standard deviation; SVT, surviving time; WHV, woodchuck hepatitis virus.

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0166-3542/02/$ - see front matter
PII: S0166-3542(02)00068-2

1. Introduction

Adoptive immune transfer has been recently discovered as a new approach for inducing immunity to hepatitis B virus (HBV) infection in organ graft recipients. The first example of successfully transferring donor-derived HBV immunity after bone marrow transplantation was the observation of clearance of HBV infection from an immune donor to its HBV-positive recipient (Ilan et al., 1993). This was confirmed by similar observations from other groups (Lau et al., 1997), followed by clinical investigations showing that bone marrow transplantation from immunized donor resulted in a detectable donor-derived immunity for up to 4 years post-transplantation (Ilan et al., 2000).

These results formed the basis for further experimental studies investigating the effect of immune transfer after the transplantation of solid organs. Transfer of humoral immunity was documented, as donor-derived antibodies were detected in the recipients when using liver grafts from either skin-sensitized or HBV-vaccinated donors. The transfer of donor-derived cellular immunity was clearly demonstrated, as recipients of a liver graft from a third-party skin-sensitized donor rejected a test skin and heart graft of the same third-party origin in an accelerated fashion (Dahmen et al., 1997). Recently, liver transplantation was established in woodchuck (Dahmen, in press), which is an accepted animal model for HBV infection. In this model, reinfection occurred in 4/4 animals after transplanting a woodchuck hepatitis virus (WHV)-negative liver graft into a WHV-carrier animal. In contrast, when using a liver graft from a vaccinated donor, the severity of reinfection was dramatically reduced in 2/3 of the animals (in preparation).

The hepatitis B carrier status of the recipient is not only of special importance in liver transplantation but also in kidney transplantation. HBV carriers receiving a kidney graft seem to have a higher rate of liver-related complications than HBV-negative recipients, although the impact on long-term graft and patient survival remains questionable. Yagisawa et al. (1997) reported that hepatic dysfunction occurred in up to 80% of kidney-grafted hepatitis B carriers, and liver-related death occurred in nearly 30% of these patients within the first 2 years after the transplantation. Similar results were reported by Lee et al. (2001). They analyzed 477 patients and found chronic liver disease in more than 60% of HBsAg-positive patients. Cox regression analysis revealed that HBV infection is likely to be an independent risk factor for patient mortality, although the statistical significance was only borderline (Lee et al., 2001). A similar observation was reported by Huo et al. (2001). They described a higher rate of liver-related complications and deaths in HBsAg-positive kidney graft recipients compared to HBsAg-negative recipients, but the difference was not statistically significant (Lau et al., 1997).

Carrier status in kidney graft recipients is frequently due to blood transfusions during long-term hemodialysis (Mioli et al., 1992). This holds especially for Asian countries. An important proportion of kidney graft recipients in Asian populations, e.g. 34/143 of kidney graft recipients from Taiwan (Lankarani et al., 2001), were chronic hepatitis B surface antigen carriers. Response rates to active hepatitis B vaccination are much lower in patients with end-stage renal disease. According to the Centers for Disease Control Survey of Dialysis-Associated Diseases, a response rate of 62% was achieved under the specific conditions of a continuous quality-improvement-based approach (Brown and Peters, 2000), which is much lower compared to a response rate over 95% in a healthy population.

Therefore, this article is directed to evaluate the efficacy of adoptively transferring HBV immunity in rats from the kidney donor to its recipient as a potential additional strategy to prevent hepatitis B and to investigate the potentially augmenting effect of postoperative vaccination. Seroconversion rate, anti-hepatitis B surface antibodies (anti-HBs) titer in the recipient, titer duration and recipient-to-donor titer ratio (R/D) were used as parameters to judge the efficacy of the immune transfer. Analysis was directed to factors influencing the efficiency of the immune transfer, such as the donor anti-HBs titer, the alloimmune response and the immunosuppressive treatment. Furthermore, the effect of postoperative vaccination on

the donor-derived immune response was evaluated.

2. Materials and methods

2.1. Experimental design

In order to investigate the influence of the genetic background on the immune transfer, three fully major histocompatibility complex (MHC)-mismatched rat strains, Lewis (LEW, $RT1^l$), brown Norway (BN, $RT1^n$) and AxC 9935 Irish (ACI) ($RT1^a$) were used as donors and LEW rats as recipients ($n = 48$) (Table 1). Two allogeneic strain combinations were chosen to mimic the clinical situation of allo-transplantations where different HLA incompatibilities are present.

In the experimental groups, donors ($n = 35$) were immunized twice with 4 μg of recombinant hepatitis B vaccine (0.2 ml Engerix-B™, SmithKline Beecham Pharma GmbH, Munich, Germany) by intramuscular injection 6 weeks and 2 weeks prior to organ donation. Vaccinated donors were monitored weekly for the development of anti-HBs. As no experience with rat HBV immunization existed, the dose for the immunization protocol was extrapolated based on a weight adaptation from the dose used in a clinical protocol in neonates (Ilan et al., 1993) and by referring to a previously published study in the mouse (Shouval et al., 1993). Donor animals from control groups did not receive any vaccination ($n = 13$).

Kidney donation took place 6 weeks after the initiation of the vaccination protocol. Recipients were monitored every week for the presence of donor-derived anti-HBs. The influence of immunosuppressive therapy on the efficacy of the immune transfer was evaluated by treating half of the recipients with cyclosporin A (CsA) postoperatively (5 mg/kg/d, Sandimmune, Novartis, Basel, Switzerland), administered by intramuscular injection (Okamura et al., 1989).

Augmentation of adoptive transfer by postoperative vaccination was primarily evaluated using a late time point after the transplantation (postoperative week (POW) 10) to examine the response of memory cells. As weekly determination of the anti-HBs titer revealed that late vaccination did not evoke a strong response to vaccine in most of the animals, an additional experimental group was designed, receiving the postoperative vaccination on the seventh day after operation (POW 1). This so-called early vaccination (POW 1) was performed in one of the allogeneic strain combinations (BN–LEW). Control groups using non-vaccinated donors were employed to investigate the effect of postoperative vaccination on adoptive transfer. Weekly monitoring of the antibody response was continued after the postoperative vaccination.

Table 1
Design of groups according to experimental studies

Strain combination	Group	n	Donor vaccination	CsA	Time point of vaccination post-transplantation
LEW–LEW	1	5	+	–	POW 10
	2	5	+	+	POW 10
	Control 1	4	–	–	POW 10
ACI–LEW	3	5	+	–	POW 10
	4	5	+	+	POW 10
	Control 2	4	–	–	POW 10
BN–LEW	5	5	+	+	POW 10
	6	5	+	–	POW 1
	7	5	+	+	POW 1
	Control 3	5	–	–	POW 1
Total		48			

2.2. Animals

Male LEW and ACI inbred rats were purchased from Charles River GmbH (Sulzfeld, Germany) and BN rats from Harlan Winkelmann GmbH (Borden, Germany). Young rats weighing 80–100 g were chosen for vaccination and used 6 weeks later (body weight 200–300 g) as kidney donors. Rats weighing 200–300 g were used as recipients. 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.

2.3. Kidney transplantation

Orthotopic kidney transplantation was performed according to the technique of Oesterwitz and Althaus (1982) under inhalation anesthesia with methoxyflurane (Metofane, Janssen GmbH, Neuss, Germany). Graft procurement was performed from heparinized donors (100 IU of Heparin–Natrium-250000-ratiopharm, Ratiopharm GmbH&Co., Ulm, Germany) using an atraumatic technique. Division of the suprarenal vein was followed by the freeing of renal artery and vein and by the mobilisation of the kidney and ureter from the retroperitoneal tissue. The ureter was divided approximately 0.5–1.0 cm below the ureteropelvic junction and cut obliquely. The renal vein and artery were transected, and the kidney was removed and placed in 0.9% NaCl solution at 0–4 °C until implantation.

In the recipient the renal vessels were occluded close to their origin using a microvascular clamp, and transected distally from the clamp. The ureter was dissected in the same way as in the donor. The donor kidney was placed on the posterior abdominal wall of the recipient. Arterial reconstruction was performed end-to-end using eight interrupted stitches with a 10-0 suture (Ethilon, Ethicon). Renal veins were also anatomized end-to-end, but in running suture technique. End-to-end anastomosis of the ureter was performed in an interrupted suture technique close to the renal pelvis.

The animals were followed daily and sacrificed 14 weeks after the transplantation or when general condition deteriorated. Histological analysis was performed on all kidney grafts to identify rejection (Solez et al., 1993—Banff-scheme) or complications.

2.4. Measurement of anti-HB titer

A fully automated microparticle enzyme immune assay (MEIA, IMx AUSAB, Abbott GmbH Wiesbaden, Germany) was used for the detection and quantification of anti-HBs in rat serum. Anti-HBs concentrations in specimens were calculated automatically by comparison of the specimen rate with values determined from a stored standard curve as described by Ostrow et al. (1991). IMx AUSAB sensitivity was 2–3 mIU/ml, equivalent in sensitivity to AUSAB RIA or EIA.

2.5. Statistical analysis

All measurements and calculations were analyzed using MICROSOFT EXCEL STATISTIC PROGRAM. The antibody titers of individual rat in each group were calculated as geometric mean titers (GMT) in 'mIU/ml' (Shouval et al., 1993).

The significance of differences in titer between the two groups was assessed using Mann–Whitney U-test. The significance of differences in R/D titer ratio between the two groups was assessed using χ^2-test. The result was considered significant when the P-value was below 0.05. A coefficient of correlation (r) was used to estimate the straight correlationship between the two groups.

3. Results

3.1. Donor vaccination

All donor rats were vaccinated twice (6 weeks and 2 weeks before donation) with recombinant HBV vaccine in order to obtain a high anti-HBs titer. A response was considered positive if the anti-HBs titer was above 10 mIU/ml (Center for Disease Control and Prevention, 1987).

Table 2
Anti-HBs titers in donors and recipients[a]

	Anti-HBs titer		
	GMT	Mean ± SD	Range
Donor titer (mIU/ml)[b]	47,256		15,000 ~ 312,809
Recipient titer (mIU/ml)[c]	46		10 ~ 227
Donor–recipient titer ratio (%)	0.084%		0.029 ~ 0.22%
Persistence of the recipient titer (weeks)		3.7 ± 1.6	1–7

[a] Total $n = 29$, effective response in recipients $n = 25$.
[b] At the time point of donation.
[c] In POW 1.

After two vaccinations, all LEW and ACI rats had responded to the vaccine (response rate 100%, $n = 14$ in both groups), whereas one non-responder was identified within the BN animals (response rate 93%, $n = 15$), resulting in an overall response rate of 97.1% (34/35).

3.2. Development of anti-HBs titer in recipients

Totally six out of 48 animals died within the first week after operation due to surgical complications, leading to a 1 week survival rate of 88% (42/48). Altogether, 29 out of the 42 surviving animals (experimental group) were subjected to serological analysis. 25 animals developed effective anti-HBs titers postoperatively, resulting in a seroconversion rate of 86%.

After two vaccinations GMT of these 25 donors at the time point of donation was 47,257 mIU/ml (15,000 ~ 312,809 mIU/ml). Recipients developed GMT of 46 mIU/ml (10–227 mIU/ml) at POW 1. All recipients remained seropositive for 1–7 weeks (mean 3.7 ± 1.6 weeks, Table 2), as determined by weekly measurements.

3.3. Influence of donor anti-HBs titer on seroconversion rate and titer level in recipients

The seroconversion rate and the titer of the recipient were influenced directly by the donor titer. R/D varied between 0.029 and 0.22% with a mean of 0.084%. Seroconversion in recipients did not occur when transplanting a kidney from a donor with a titer below 15,000 mIU/ml ($n = 4$).

Statistical analysis between donor titer on the day of donation and recipient titer on the seventh postoperative day (POD) (POW 1) revealed a correlation coefficient (r) of 0.730 (Fig. 1). Further grouping according to the treatment resulted in an even closer correlation ($r = 0.843$ in CsA-treated animals and $r = 0.862$ in untreated animals).

3.4. Influence of immunosuppression on the immune transfer

GMT of the treated recipients in POW 1 was significantly higher (two-fold higher than in the non-immunosuppressed group—70 mIU/ml in CsA-treated group ($n = 15$) and 27 mIU/ml in untreated rats ($n = 14$), $P = 0.024$, respectively), although the corresponding donor titer in the two groups did not show a statistically significant

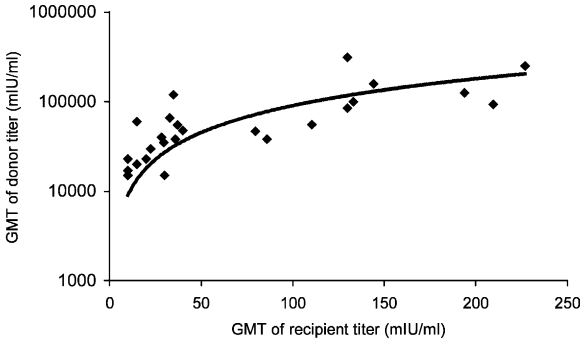

Fig. 1. Regression analysis between donor and recipient anti-HBs titer. Analysis was based on all seroconverted animals and showed a strong correlation between the donors' anti-HBs titers on the day of donation and the recipients' titers in POW 1 ($r = 0.730$).

Table 3
Influence of CsA treatment on adoptive immune transfer

	n_1[a]	n_2[b]	GMT of anti-HBs (mIU/ml)		R/D	Persistence (weeks)
			Donor[c]	Recipient[d]		(Mean ± SD)
CsA(+)	15	14	47,515 (15,000 ~ 250,000)[e]	69.7 (10 ~ 227)[f]	0.113% (0.026 ~ 0.23)[f]	4.8±1.7
CsA(−)	14	11	40,876 (15,000 ~ 312,809)[e]	27.0 (10 ~ 144)[f]	0.054% (0.025 ~ 0.09)[f]	3.2±1.3

[a] Total number of recipients.
[b] Number of seroconverted recipients with effective titer (titer > 10 mIU/ml).
[c] GMT and range in donors on the day of donation.
[d] GMT and range in recipients in POW 1.
[e] $P > 0.05$.
[f] $P < 0.05$.

difference (47,515 mIU/ml in the treated group and 40,876 mIU/ml in the untreated group). Furthermore, R/D in the treated animals was also more than two-fold higher compared to untreated animals (0.113% in treatment group compared to 0.054% in untreated animals, $P < 0.05$, see Table 3). Separate analysis of the two allogeneic strain combinations showed similar results.

The effect of the immunosuppressive treatment on the antibody persistence was even better demonstrated in a matched pair analysis. Recipients were matched for strain combination and recipient titer in POW 1 and differed in treatment condition (with or without immunosuppressive treatment). Due to the high variation in recipient titer, only one pair was identified that fulfilled these criteria. Comparing those two individual recipients, the effective titer persisted longer in the CsA-treated recipient than in the untreated one (42 days, compared to 28 days—Fig. 2).

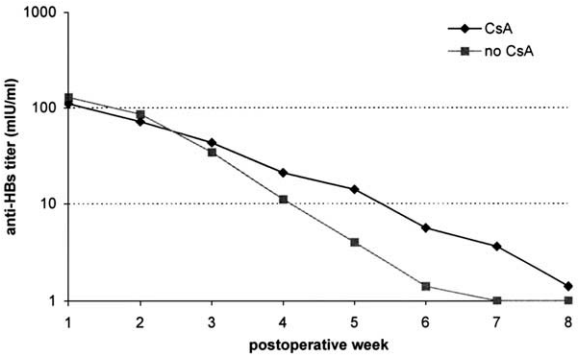

Fig. 2. Titer influence of CsA on two recipients with similar titer on POW 1. The effective titer persisted longer in the CsA-treated recipient than in the untreated one (42 days as compared with 28 days).

In summary, CsA treatment was associated with a higher GMT in POW 1, a higher R/D and a longer titer persistence. It is very unlikely that immunosuppressive treatment with cyclosporin influenced the antibody half-life. There was no evidence for a prolonged persistence of antibodies and an inhibition of degradation of antibodies in immunosuppressed individuals. Therefore, the most likely explanation is the protection of antibody-secreting cells from rejection.

3.5. *Effect of the postoperative vaccination on donor-derived immunity*

3.5.1. *Normal control*

In naive LEW rats a 100% seroconversion rate was achieved within 2 weeks after vaccination. In the fourth week after vaccination a GMT of 754 mIU/ml was reached with individual titers ranging from 34 to 9933 mIU/ml.

3.5.2. *Late vaccination*

Response rate was lower and seroconversion occurred later, when vaccination was performed in POW 10 after the kidney transplantation from non-vaccinated donors. Seven out of eight recipients responded to late vaccination within 3 weeks. Furthermore, titer levels in the fourth week after immunization differed in these two groups; they were significantly lower (GMT 78 mIU/ml, range

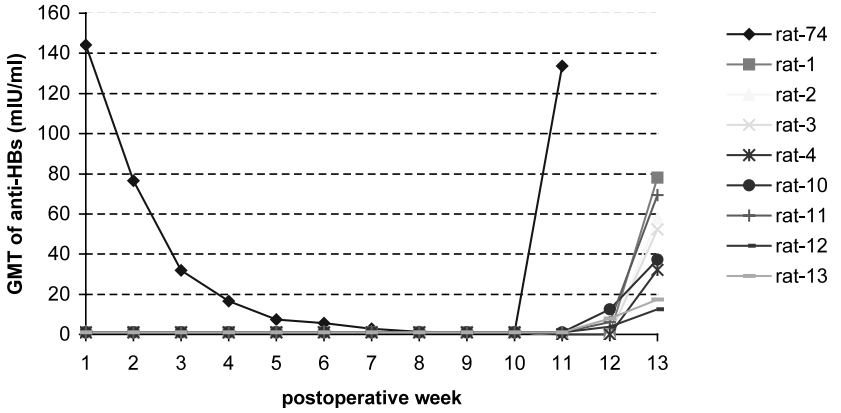

Fig. 3. Effect of late vaccination on one exceptional recipient. This animal developed a higher titer earlier (1 week after vaccination, 134 mIU/ml) than naive rats at 1 week after vaccination.

10–264 mIU/m, $P < 0.05$) in the kidney-transplanted animals.

The vaccination response after the kidney transplantation was not improved when using organs of previously vaccinated donors. Six out of nine experimental animals receiving a kidney graft from a vaccinated donor responded to late vaccination (POW 10). Four out of these animals started to respond between 2 and 3 weeks after vaccination, reaching a GMT of 80 mIU/ml at 4 weeks after vaccination with maximal titers between 10 and 300 mIU/ml.

However, one individual animal (Rat 74) did not only respond earlier (first week after vaccination), but also developed a high titer in the first week after immunization (134 mIU/ml), which was higher than that of all animals from the control group 1 week after vaccination (Fig. 3). Interestingly, this animal received an organ from a highly responding donor (anti-HB titer at the time of donation was 158,510 mIU/ml). The R/D ratio was 0.09%, and the rat developed a rather high titer in the first POW (140 IU/ml), which lasted for 4 weeks. Interpretation of this observation as a secondary response to vaccination would suggest an engraftment of donor-derived cells.

3.5.3. *Early vaccination*

As the response to postoperative vaccination was not clearly enhanced in animals with transferred immunity, an earlier time point (POW 1) was chosen for postoperative vaccination. All rats receiving a kidney graft from non-vaccinated donors (control group, $n = 5$) and being vaccinated in POW 1 seroconverted with anti-HBs titers between 26 and 214 mIU/ml within 4 weeks after vaccination, which was later and lower than the response of naive LEW rats. Apparently, the surgical intervention itself delayed the development of the humoral immune response. This delayed immune response was not augmented by the adoptively transferred immunity. Only 3/5 recipients of grafts from vaccinated donors seroconverted, reaching maximal titers not higher than 110 mIU/ml within 4 weeks. One animal responded only after 6 weeks.

Immunosuppressive treatment abrogated the response to postoperative vaccination completely. No animals with adoptively transferred immunity seroconverted when undergoing immunosuppressive treatment, irrespectively of the time point of postoperative vaccination (no response in 10/10 animals after late vaccination and 4/4 animals after early vaccination).

4. Discussion

In this study, transfer of humoral immunity was observed in 86% of all rats receiving an organ from a vaccinated donor and was strongly related to the donor titer, confirming the observations made in

bone marrow transplantations. Shouval et al. (1993) demonstrated a strong relationship between the number of vaccinations in the bone marrow donor, which obviously resulted in higher donor titers, and the efficacy of the immune transfer to the recipient.

Besides the donor titer as a major influence on the immune transfer, donor-derived immunity was also affected by the immunosuppressive treatment. R/D, GMT in the first POW and titer persistence were enhanced in immunosuppressed animals compared to untreated rats, although donor titers in the two groups were not significantly different. Similar findings were obtained in a separate study when transplanting a liver from a vaccinated donor to a naïve recipient (Dahmen et al., submitted for publication).

The presence of donor-derived antibodies in the recipient can be due to a passive transfer of antibodies remaining in the graft or due to the transfer of graft-derived antibody-producing cells, which continue to secrete anti-HBs in the recipient. As antibody degradation is not influenced by cyclosporin treatment, these results suggest, but do not prove, a protection of the antibody-producing cells by the continuous immunosuppressive treatment. However, immunosuppressive treatment did not protect the cells from dying off within a period of weeks, which led to a constant decrease in antibody titer.

As anti-HBs titers were only transiently detectable in the recipient, augmentation of the anti-HBs immune response in the recipient by postoperative vaccination was pursued. As immunological memory leads to a fast and strong antibody response after stimulation with the same antigen (Feldman, 1998), a second set response was expected in response to the postoperative vaccination in experimental animals. However, response to the postoperative vaccination was lower than in naive animals in terms of response rate and kinetics of titer development within the observation period of 4 weeks post-vaccination.

Two reasons might be responsible for this observation. On the one hand, the very limited effect of the booster vaccination, especially the low response rate after late vaccination, could be explained by the minute amount of memory cells being possibly transferred via the kidney graft. Only part of the passenger cells within the graft are responding to the immune stimulus, but not all of them act as memory cells. The frequency of memory cells being transferred is potentially not sufficient to elicit a typical secondary response.

On the other hand, the low response after early vaccination might be attributed to a nonspecific immunosuppressive effect of surgical intervention, as already reported by Nohr et al. (1984), which was also observed in the control group.

Despite the clear evidence of achieving reproducibly effective antibody titers by adoptive immune transfer, clinical application might be hampered by the short duration of the donor-derived immune response, which is only achieved at the expense of extremely high antibody titers in the potential donor. The range of required titer levels can be estimated based on the results in this study. Seroconversion with titers clearly above 10 mIU/ml in the rat recipients was only observed, when donor titers were higher than 15,000 mIU/ml with R/D varying between 0.029 and 0.22%.

When assuming a similar R/D of 0.2%, donor titers should be in the range of 5000–50,000 mIU/ml to potentially result in a high seroconversion rate and effective titers in the recipient (10–100 mIU/ml). Antibody levels obtained after standard clinical vaccination protocols tend to be lower (far below 10,000 mIU/ml—Leroux-Roels et al., 2000). In the case of living-kidney donation, immunologic conditioning of the potential donor can be envisioned, but would call for an optimization of currently available vaccination protocols. Donor preparation would require a prolonged period of time to achieve the necessary high antibody titer in the potential donor.

In conclusion, adoptive immune transfer to hepatitis B, leading to efficient titers in the recipient, occurred transiently after the kidney transplantation. Immunosuppression enhanced the donor-derived immune response, pointing to the transfer of functionally active cells in addition to a potential transfer of antibodies remaining in the graft. Augmentation of anti-HBs response was not achieved by the postoperative vaccination at the selected time points. Potential application of this theoretically useful strategy in clinical kidney

transplantation would require the optimization of the peri-operative vaccination protocol regarding dose and timing to ensure potent donor and recipient conditioning.

Acknowledgements

YanLi Gu was supported by a grant from Else Kröner-Fresenius Stiftung. Jun Li was supported by a stipend from Gottlieb Daimler- and Karl-Benz-Stiftung. Lothar Doebel was supported by a grant from Else Kröner-Fresenius Stiftung.

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