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Case report

Anti-HBs re-seroconversion after liver transplantation in a patient with past HBV infection receiving a HBsAg positive graft☆

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***Background/Aims*: Orthotopic liver transplantation (OLT) is an important therapeutic option for HBV-related end-stage-liver disease, yet it is often hampered by a scarcity of organ availability. One option to increase organ availability is the use of virologically compromised organs from HBV-infected donors. Transplantation of anti-HBcore positive grafts has been associated with a low risk of HBV recurrence if adequately treated with nucleoside analogs, irrespective of concomitant HBV-specific immunoglobulin therapy. Experience using HBsAg positive grafts is, however, very limited.**

***Methods*: Here, the analysis of the cellular and humoral HBV-specific immunity of a subject with past HBV infection (anti-HBs and anti-HBc positive) receiving an HBsAg positive liver graft is reported.**

***Results*: Nine months post-OLT, the patient experienced a spontaneous anti-HBs re-seroconversion allowing the discontinuation of HBIG. The data show a concurrent increase in the cellular and humoral immunity at times of reduced viral antigenemia, demonstrating effective immune control of HBV post-OLT.**

***Conclusions*: These data support the use of marginal organs in this setting, providing a potential strategy to further alleviate organ shortage.**

***Keywords*: Orthotopic liver transplantation; HBsAg positive marginal graft; Humoral immunity; T cell immunity; Organ shortage**

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Abbreviations: OLT, orthotopic liver transplantation; ESLD, end-stage liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; HBIG, hepatitis B immunoglobulin; ALT, alanine aminotransferase; ELISpot, enzyme-linked immunospot.

1. Introduction

Orthotopic liver transplantation (OLT) is an effective therapeutic option for end-stage liver disease (ESLD) and hepatocellular carcinoma, but survival on the waiting list is hampered by the shortage of available organs [1]. To overcome this limitation, so-called "marginal grafts", including organs from aged donors or infected by hepatitis B (HBV) or C virus (HCV) in case of HBV- or HCV-related ESLD, are increasingly employed [1]. Liver grafts from HBV core antibody-positive (anti-HBc) but HBV surface antigen

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(HBsAg) negative donors are currently used, showing an overall low risk of HBV reactivation when treated with nucleos(t)ide analogues [2–6]. However, *de novo* infections, as defined as HBV transmission from donor to the recipient, can occur and rapid graft failure and patient death have been reported [7,8]. In contrast to livers from anti-HBc positive donors, the use of HBsAg positive grafts is more limited, and the few available studies or case reports yielded conflicting results [9–11]. HBV-specific cellular and humoral immunity plays a key role in the course and outcome of HBV infection in immunocompetent patients [12]. Recent analyses of the HBV-specific cellular immunity in OLT recipients have shown a profile similar to that of patients with chronic HBV infection and suppressed HBV-DNA, with a preferential targeting to the viral nucleocapsid antigen [13]. These data indicate that transplanted subjects can maintain or even develop new virus-specific cellular immunity, contributing to viral control after transplantation [13].

We report a case of a patient undergoing OLT for HCV-related ESLD and past HBV infection (anti-HBcore and anti-HBs positive) who received an HBsAg-positive graft. The patient first showed the serological appearance of HBsAg, with subsequent seroconversion to anti-HBs antibodies nine months after transplantation. Seroconversion was concomitant with the increase in cellular immunity, suggesting that the cooperation between the two arms of adaptive immunity may effectively control the virus.

2. Case report

A 64-year-old man with HCV-related ESLD complicated by several upper digestive bleedings and hepatorenal syndrome was listed for OLT in August 2004 at the Bologna Liver Transplantation Center (MELD score 24). The main concomitant pre-OLT pathologies included autoimmune hemolytic anemia requiring treatment with steroids.

The serum HBV markers suggested a past exposure to HBV, with positive serology for anti-HBs (320 mUI/mL) and anti-HBc IgG, and undetectable HBV-DNA. In May 2005, because of significant worsening of liver function (MELD 27), after providing written informed consent, the patient underwent OLT receiving a partially HLA-matched HBsAg-positive liver graft (Table 2) from a deceased donor, as from experimental protocol launched by the Italian Health Authority on 2002 [14]. The HBV-DNA of the donor was positive (190 UI/mL). The immunosuppressive regimen consisted of tacrolimus and steroids (starting with a dosage of 50 mg daily and tapered down over eight months and stopped nine months post-OLT). To control post-OLT HBV infection, 10'000 IU HBIG were infused during the anhepatic phase, followed by 5'000 IU every other day for the first month, then 5'000 IU weekly for the second month and subsequently 5'000 IU every 3–4 weeks to maintain an anti-HBs titer above 250 IU/ml. In addition, lamivudine was started at day one post-OLT (100 mg/daily) and continued throughout. Monitoring of HBV serology and HBV-DNA was performed monthly in the first year post-OLT, and then every three months thereafter (Table 3). HBsAg and HBV DNA were undetectable and anti-HBs very high the first three

Table 1
Amino acid sequence and protein location of peptides targeted by the OLT recipient. A set of 208 overlapping peptides (OLP) was synthesized covering the entire HBV proteome including the nucleocapsid protein (24 peptides), envelope (53 OLP), X-protein (20 OLP) and polymerase protein (111 OLP) as described [13]. Peptides were adapted 18-mers overlapping by 10 amino acids and were based on the reported HBV genotype D reference strain ayw since HBV genotype D is predominant in Italy. In addition, a previously described set of defined HBV-derived CD8 T cell epitopes was included [15]. Only sequences of peptides targeted at least at one time point are shown (Table 2).

Peptide	Sequences	Protein
S17	LLVPFVQWF	Envelope
D16	GRETVIEYLVSFGVWI	Core
D17	YLVSFGVWIRTPPAY	Core
Pol23	FLLAQFTSA	Polymerase
Pol24	LLAQFTSAI	Polymerase
D165	PMGVGLSPFLLAQFTS	Polymerase
D166	FLLAQFTSAICSVVRR	Polymerase

Table 2
Human Leucocyte Antigen (HLA) Typing of organ donor and recipient performed by SS-PCR.

	HLA-A locus	HLA-B locus	HLA-DRB1 Locus	Race
Recipient	02/03	18/70	16	Caucasian
Donor	01/02	39/62	13	Caucasian

Table 3
Serologic, virologic and biochemical time course after OLT.

Post-transplant months	HBsAb mUI/mL	HBsAg UI/mL	HBV-DNA UI/mL	ALT U/L (0–40)
0	320	0.01	Not performed	19
2	6005	0.01	<3.3	42
3	2814	0.01	<3.3	87
3.5	54	7.59	<3.3	34
4	294	2.24	<3.3	33
5	503	1.63	<3.3	38
6	3500	2.24	<3.3	32
7	59	9.78	<3.3	32
8	375	3.09	<3.3	24
9	5237	0.25	<3.3	68
11	5901	0.42	<3.3	19
12	5236	0.55	<3.3	23
15	9145	0.15	<3.3	36
18	8290	0.04	<3.3	33

months post-OLT. Despite antiviral therapy and the passive immunoprophylaxis with HBIG, serum HBsAg appeared 14 weeks post-OLT (3.5 month), with a concomitant decrease of anti-HBs levels to a minimum of 54 IU/L but without appearance of HBV-DNA or increase of ALT (Fig. 1 and Table 3). Regardless of HBIG and lamivudine treatment, HBsAg and anti-HBs fluctuated reciprocally between month four and eight post-OLT, while serum HBV-DNA remained persistently undetectable. At month eight, the last administration of HBIG was performed since the anti-HBs levels increased spontaneously thereafter (Fig. 1 and Table 3). After month three, steroids were continuously tapered and stopped at month nine post-OLT. From the ninth month onwards, a progressive increase of anti-HBs was associated with a concomitant decrease of HBsAg levels until negativity at month 18 post-OLT. During persistence of HBs antigenemia ALT levels remained within normal ranges, except for only a slight increase at month nine. At the time of writing, despite HBIG interruption and lamivudine treatment, the patient still presents high anti-HBs levels, negative HBsAg and undetectable HBV-DNA. One-year post-OLT a biopsy was performed and documented, despite HCV recurrence, only mild inflammation (Knodell score 6). HBsAg was focally detectable in rare hepatocytes and HBV-DNA resulted positive.

To investigate whether the recovered humoral immune response eight months post-OLT was accompanied by a similar boost in the cellular immunity, HBV-specific T cell responses were comprehensively assessed using an overlapping peptide set (208 peptides) spanning the entire HBV genome before OLT as well as three, nine and 12 months post-OLT (Fig. 2). Weak responses to three individual peptides were detected in the pre-OLT sample. These responses disappeared three months post-OLT, and were not replaced by other responses. However, nine months after OLT, when steroid treatment was withdrawn, and a sharp increase of the humoral response become evident, a robust T cell activity directed against both polymerase and envelope antigens was observed. Such a T cell response then contracted again over the next three months, together with a reduction in viral antigen burden. The peptide sequences of the peptides with positive responses are shown in Table 1.

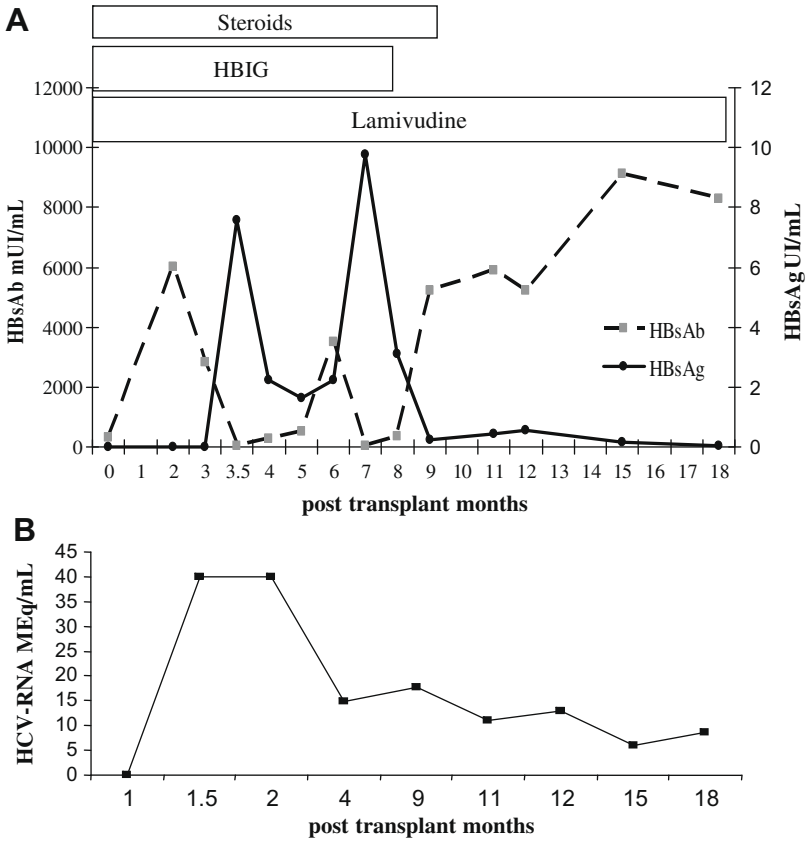

Fig. 1. Longitudinal time course of HBV virological and serological markers (1a) and HCV-RNA (1b). (A) Changes in the levels of HBV surface antigen (HBsAg; right Y axis and solid line) and fluctuation in the titer for anti-HBs antibodies (HBsAb, left *Y*-axis and hatched lines) are shown for the first 18 months post-transplantation. (B) HCV viral loads expressed as Meq per ml are shown for the same period of time as in A).

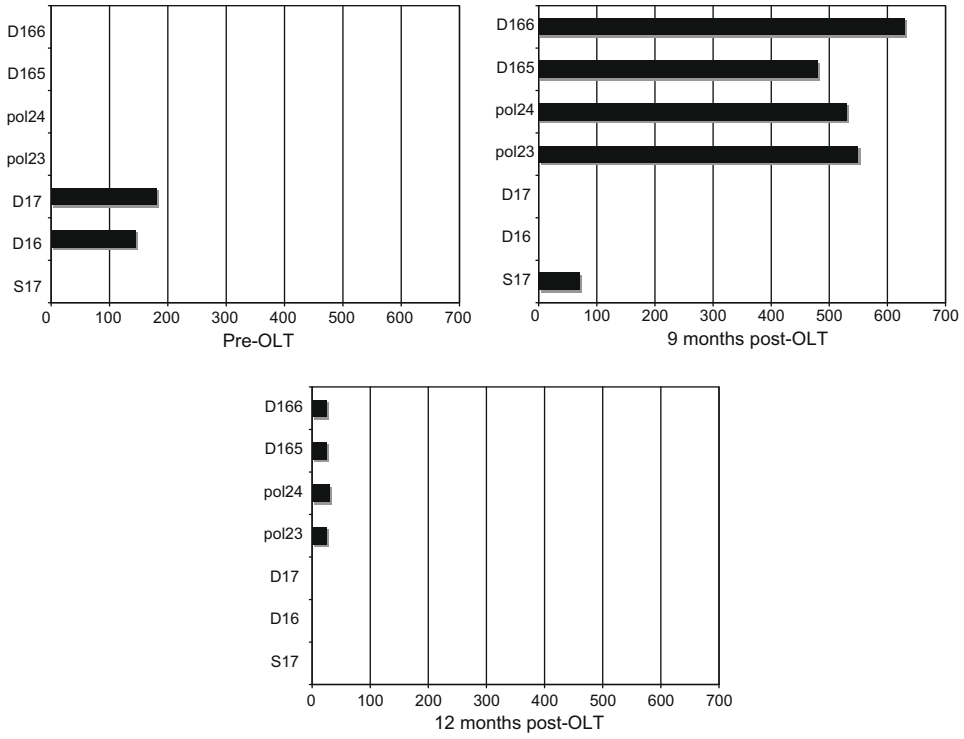

Fig. 2. Cellular HBV-specific immune responses over time: The breath and magnitude of HBV-specific T cell response to overlapping peptides (D165, D16 and D17) and optimally defined, HLA-A2 restricted CD8 T cell epitopes (s17, pol 23 and pol24) assessed by ELISpot assay at baseline and 9 and 12 months post-OLT are shown. At three months post-OLT no responses were detected (data not shown). The results are expressed as spot forming cells (SCF) per million input cells after antigen-specific *in vitro* stimulation.

3. Material and methods

3.1. Lymphocyte isolation and ELISpot assay

Peripheral blood mononuclear cells (PBMC) were isolated as described [13], stimulated as outlined below and used in an interferon gamma (IFN γ) based enzyme-linked immunospot (ELISpot) assay at 100,000 PBMC/well. ELISpot assays for all time points were performed at the same time and peptides were tested in 59 peptide matrix pools as described previously [15]. Each test included 4 negative wells (PBMC without peptides) and 1 positive well (PBMC stimulated with phytoemoagglutinin). Results are expressed as spot forming cells (SFC) per million cells and responses were considered positive when exceeding all of the following criteria: (a) a minimum of 5 spots per well; (b) mean of negative wells plus three times the standard deviation of the negative wells and (c) three times the mean of the negative wells.

3.2. Antigen-specific T-cell expansion

$5 - 10 \times 10^6$ cryopreserved PBMC were thawed and pulsed with five different peptide pools containing overlapping peptides spanning either the nucleocapsid, envelope, X protein or polymerase protein sequences or a set of previously described CD8 T cell epitopes. All peptides were added at a final concentration of 4 μg/ml per peptide. Recombinant interleukin-2 (50 IU/ml) was added on day 3 and twice a week thereafter. After 14 days of culture, cells were washed and starved overnight in IL-2 free medium before using them in a IFNγ-based ELISpot assay [15].

3.3. Viral serology

HBsAg and anti-HBs titers were assessed by Chemiluminescent Microparticle Immunoassay (CMIA) technology (Abbott Diagnostic, Italy). For HBsAg, values above 0.05 UI/mL are considered positive; for anti-HBs values above 10 mUI/mL were considered protective. HBV-DNA viral load was tested by real time quantitative PCR (Affigene, Alfa Wassermann, Italy) with a detection range of 3.3 IU/mL to 1.72×10^8 IU/mL. Quantitative assessment of HCV-RNA was performed by branched DNA technology (bDNA) Versant® HCV RNA 3.0 assay (Siemens, Italy), detection range from 3.200 to 4.0×10^7. copies/mL.

4. Discussion

The use of HBsAg positive liver grafts is currently precluded in most countries, because of the high risk of *de novo* HBV infection post-OLT. However, this risk might change in transplant recipients with a pre-OLT full immunity against HBV i.e. those patients with positive serology for anti-HBs and anti-HBc antibodies. While in the era prior to antiviral prophylaxis the use of HBV infected grafts was not a feasible option, the introduction of nucleos(t)ide analogues together with HBIG substantially improved the OLT outcome using anti-HBc grafts [2,16–19]. However, the few available reports using HBsAg positive grafts for OLT indicate that their use requires a lot of caution and restriction, particularly in recipients who are co-infected with HDV [10]. So far no data are available on the outcome of this approach in patients immunized against HBV pre-OLT, namely with anti-HBs and anti-core positivity. Patients with past HBV infection maintain not only the humoral immunity against HBV but broad and multi-specific cellular

immune responses can been detected even years after recovery from infection [20]. The case described here showed a spontaneous anti-HBs re-seroconversion and the capacity to produce anti-HBs goes together with a boost of cellular immunity. Some hypotheses can be offered to explain this outcome: first, the patient was naturally immunized against HBV (past HBV infection) before OLT; thus despite immune suppression he was able to re-evoke an efficient antiviral immune response of both arms, humoral and cellular immunity against HBV. Second, the presence of HCV co-infection might have played a role in controlling HBV, as HCV can exert an inhibitory effect on HBV replication [21]. Finally and importantly, the withdrawal of steroids may have contributed to restoring the anti-HBV immunity since the genome of HBV contains a glucocorticoid-responsive element, that is responsible for an increase in serum HBV-DNA levels and enhanced production of viral transcripts including antigens on exposure to CCS [22].

OLT is generally performed across HLA mismatches between organ donor and recipient. As a result, the pre-existing virus-specific, recipient-HLA restricted T cells responses might no longer exert an effective immune control on HBV-infected hepatocytes. Instead, donor-HLA restricted responses may emerge, as described by Rosen and colleagues [23]. In the present study, donor and recipient were only matched for one class I allele (i.e. HLA A2, Table 2). Thus, it is conceivable that donor-HLA restricted, virus-specific T cell responses could crucially contribute to viral control post-OLT. Although more detailed analyses and large cohorts of patients would be required to clarify this issue, we can only speculate that a certain level of HLA match could allow the recipient to use his previously established cellular immunity to control HBV replication again. This is in line with the reported higher risk for post-OLT HBV recurrence in a context of complete HLA mismatch [24].

In summary, although limited to just one case, the report suggests that on selected occasions liver transplantation using HBsAg positive liver grafts is a possible option for recipients fully immunized against HBV, in other words with past HBV infection. We showed in this case how the memory T and B cell immune response helped to control the virus again. Considering the previous reports of OLT using HBsAg-positive grafts, to our knowledge, this is the first report of an anti-HBs and anti-HBc positive transplant recipient, who, after receiving a HBsAg liver graft, developed a transient HBs antigenemia followed by a rapid restoration of T and B cell immunity with spontaneous anti-HBs re-seroconversion consistent with his pre-OLT anti-HBV immunity.

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