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BRIEF COMMUNICATIONS

RAPID DECLINE OF ANTIBODIES AFTER HEPATITIS A IMMUNIZATION IN LIVER AND RENAL TRANSPLANT RECIPIENTS

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Background. **Hepatitis A vaccine is safe and achieves good seroconversion rates in liver (LTX) and renal (RTX) transplant recipients.**

Methods. **A study was performed to determine the anti-hepatitis A virus (HAV) antibody decline in LTX and RTX patients, and in healthy controls who have been immunized with two doses of hepatitis A vaccine.**

Results. **LTX and RTX patients had a satisfactory seroconversion rate after complete immunisation. However, 2 years later they had experienced a much more rapid antibody decline than controls, and only 59% of LTX and 26% of RTX seroconverters showed titres above the cut-off level defined as protective.**

Conclusions. **Patients on immunosuppressive therapy may not be adequately protected against hepatitis A a few years after vaccination and alternative vaccination schemes may have to be considered.**

Liver transplant (LTX) recipients should avoid any liver injury and therefore be immunized against hepatitis A even if the risk of exposure is low. Other nonimmune transplant recipients should also receive the vaccine if they are highly exposed to hepatitis A (e.g., travelers to endemic areas) or if they have chronic hepatitis B or C associated with an increased risk of fulminant hepatitis A (*1*). In LTX and renal transplant (RTX) recipients a satisfactory anti-hepatitis A virus (HAV) immune response after two vaccinations was observed, although geometric mean titres (GMT) were lower than in healthy controls (*2*). Follow-up data and mathematical models suggest that in healthy individuals protective HAV antibodies after complete vaccination may persist for more than 20 years (*3*, *4*). However, no data are available on the antibody decline in vaccinated individuals on immunosuppressive therapy. Such data are needed when planning vaccination schemes (vaccine dose, timing of booster injections) in organ transplant recipients.

Thirty-eight LTX patients, 39 RTX patients, and 27 controls who had no detectable HAV antibodies received two doses of hepatitis A vaccine (Havrix 1440; SmithKline Beecham, Rixensart, Belgium) 6 months apart. Anti-HAV titres were assessed 4 weeks after the first and second vaccine dose, respectively (*2*). Two years after the second vaccine dose participants were again tested for anti-HAV antibodies by the same commercial ELISA (Boehringer Enzymun kit; Boehringer Mannheim, Mannheim, Germany), which was calibrated by use of World Health Organization international standard reference serum. Anti-HAV titers ≥33 mIU/ml were considered positive according to the manufacturer and previous studies (*2*, *5*). Thus, patients who showed postimmunisation titres of ≥33 mIU/ml were defined as seroconverters. Only patients who had seroconverted after the second vaccine dose were included in the 2-yr follow-up analysis. Chi-square test and Fisher's exact test were used to compare proportions between groups. Geometric mean anti-HAV titers were calculated and comparisons of the titres between groups were done by nonparametric tests (Mann-Whitney *U* test, Kruskal-Wallis test). The seroconverters followed-up for 2 years did not differ significantly from those who were lost to follow-up with respect to age, gender, immunosuppressive therapy, duration of posttransplantation period, and anti-HAV titers after the first and second vaccine dose (Table 1).

The seroconversion rates after complete immunization were 97% (37/38) in LTX patients, 72% (28/39) in RTX patients, and 100% (27/27) in controls. RTX had significantly lower GMTs than LTX and controls. Follow-up sera were available from 70 individuals (27 LTX, 23 RTX, 20 controls) who had seroconverted. The mean age was 47.6 years (SD 10.4) in LTX, 42.7 (SD 10.7) years in RTX, and 39.4 years (SD 7.6) in controls (LTX versus controls $P<0.05$, *t* test).

Two years after vaccination, protective antibody titres were detectable in 59% of LTX, 26% of RTX, and 100% of the controls (Table 1). Both LTX and RTX patients showed a significantly larger decrease in GMTs than controls. It is noteworthy that LTX had not differed significantly from controls in their GMTs after the second vaccine dose but had much lower GMTs 2 years later. All these results did not change notably if a lower anti-HAV cut-off level of 20 mIU/ml

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TABLE 1. HAV antibody titers after hepatitis A vaccination in LTX and RTX patients, and in healthy controls

Initial study population	LTX (n=39)	RTX (n=39)	Controls (n=29)	P
Seroconverters 4 wk after 1st vaccine dose	n=16/39 (41.0%)	n=9/38 (23.7%)	n=26/29 (89.7%)	<0.0001
GMT[a] (95% CI)	275 (101–532)	74 (19–288)	231 (133–371)	L/R <0.05 L/C 0.6 R/C <0.01
Seroconverters 4 wk after 2nd vaccine dose	n=37/38 (97.4%)	n=28/39 (71.8%)	n=27/27 (100%)	<0.001
GMT[a] (95% CI)	1452 (587–3458)	169 (97–314)	1596 (1093–2652)	L/R <0.0001 L/C 0.5 R/C <0.0001

Cohort followed up for 2 yr	LTX (n=27)	RTX (n=23)	Controls (n=20)	P
Seroconverters 4 wk after 1st vaccine dose	8/27 (29.6%)	6/23 (26.1%)	20/20 (100%)	<0.0001
GMT[a] (95% CI)	226 (52–1354)	94 (10–937)	222 (117–422)	L/R 0.1 L/C 0.5 R/C <0.05
Seroconverters 4 wk after 2nd vaccine dose[b]	27/27	23/23	20/20	
GMT[a] (95% CI)	1675 (620–4476)	153 (83–302)	1738 (836–2803)	L/R <0.001 L/C 0.7 R/C <0.0001
Proportion of patients still seropositive after 2 yr of follow-up	16/27 (59.3%)	6/23 (26.1%)	20/20 (100%)	<0.0001
GMT (95% CI) after 2 yr of follow-up[a]	171 (52–558)	167 (14–3018)	420 (242–724)	L/R 0.8 L/C <0.05 R/C 0.06
GMT (95% CI) after 2 yr of follow-up (all patients)	65 (25–170)	22 (10–44)	420 (183–612)	L/R <0.05 L/C <0.001 R/C <0.0001

[a] Seroconverters only.
[b] Inclusion criterion for 2-yr follow-up cohort.
GMT, geometric mean titer, in mIU/ml; CI, confidence interval. L/R, LTX versus RTX. L/C, LTX versus controls. R/C, RTX versus controls.

was chosen. Neither the seroconversion rates nor the GMTs 4 weeks after complete immunization and 2 years later were associated with gender, age, time interval since transplantation, or HBV or HCV serostatus. In three LTX patients, an untypical course of antibody titers was observed. They developed high titers, exceeding even the titers of most of the controls, and lost their antibodies at a much lower rate than the other transplant recipients.

The majority of LTX patients received either tacrolimus (44%) or cyclosporin A (44%) for immunosuppressive treatment, whereas all RTX patients were on combinations of at least two drugs including cyclosporin A, azathioprine, and prednisolon. None of the patients received mycophenolate mofetil. No difference in the seroconversion rate and GMTs 4 weeks after vaccination was found between LTX patients on cyclosporin A and those on tacrolimus. However, after 2 years protective anti-HAV titers were detected in 79% of LTX on tacrolimus, but only in 39% of LTX on cyclosporin A ($P<0.05$, χ^2 test), and GMTs were 115 and 35 mIU/ml, respectively ($P=0.05$, Mann-Whitney test). These differences remained after adjusting for posttransplantation time interval which was somewhat but not significantly shorter in the tacrolimus group. In RTX, the antibody decline was not associated with the type of immunosuppressive treatment.

Hepatitis A vaccination should be recommended to all anti-HAV negative organ transplant recipients if their risk of HAV exposure is not negligible. In contrast to healthy controls, however, many of these patients experience a rapid decline of antibody titers after vaccination. At present the protective anti-HAV antibody level after vaccination is not exactly known and the role of cellular immunity in protection against hepatitis A in the case of low anti-HAV antibody titers in immunosuppressed patients is unclear and needs further investigation (*6*). However, it is possible that in some immunosuppressed vaccines protection against hepatitis A is seriously impaired or lost already after 2 years. The type of immunosuppressive treatment may have an effect on the antibody decline. In LTX patients, the larger antibody decrease in the cyclosporin A group compared with the tacrolimus group may result from differences in the immunosuppressive processes induced by the two drugs (*7*). In RTX patients, the different drug combinations had no impact on the anti-HAV status 2 years after immunization, although Huzly et al.(*8*) reported that in tetanus, diphtheria, and polio vaccination the antibody response was better in RTX on double than on triple therapy.

The relatively high antibody titers in a few LTX patients may be due to a normal immune response despite immunosuppressive treatment. However, we cannot rule out the possibility that these patients had acquired HAV infection before transplantation but lost their antibodies before vaccination. The booster effect by the vaccine would indicate

that the immunological memory resulting from previous natural infection may work even if the antibody titer is well below the cut-off level.

Further studies are needed including different vaccination schemes and long-term follow-up to identify the optimum vaccination strategy for transplant recipients.

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NON-TYPHOID *Salmonella* SEPTICEMIA AND VISCERAL LEISHMANIASIS IN A RENAL TRANSPLANT PATIENT

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Background. **We report on a renal transplant patient with recurrent attacks of fever, in which *Salmonella* septicemia as well as visceral leishmaniasis were diagnosed.**

Patient. **The patient was a 62-year-old man with diabetic nephropathy and a living related kidney transplantation.**

Results. **Nearly 2 years after the transplantation, the patient developed recurrent attacks of fever, which were initially diagnosed as non-typhoid salmonellosis and improved after treatment. Three months later, he had relapses of fever. As the patient developed pancytopenia, a bone marrow aspiration was done, showing *Leishmania* parasites. The patient responded well to treatment with sodium stibogluconate.**

Conclusions. **A high index of suspicion, together with better diagnostic assays to detect visceral leishmaniasis, is warranted in the diagnostic work-up of any fever of unknown origin in immunocompromised patients, especially in endemic areas.**

Visceral leishmaniasis has been reported as an unusual cause of fever in renal transplant recipients (*1, 2*). The disease often presents with prolonged subclinical and/or atypical forms. The differential diagnosis includes other infectious diseases that cause fever, such as brucellosis, tuberculosis, malaria, and salmonellosis, which are also common in the areas where leishmaniasis is endemic (*3*). One of our patients presented with recurrent attacks of fever, in which eventually more than one diagnosis had to be made.

CASE REPORT

A 62-year-old man with end-stage renal disease resulting from diabetic nephropathy received a living related kidney transplant in our hospital in December 1996. His immunosuppression consisted of cyclosporine, azathioprine, and prednisone. After transplantation, his renal function became normal and remained stable with a serum creatinine around 1.1 mg/100 ml. He had antibodies against hepatitis C virus, but his liver function tests were normal.

In November 1998, nearly 2 years after the patient received a transplant, he developed chills with fever up to 39°C. Investigations revealed a white blood cell (WBC) count of 14,600/mm^3, hemoglobin (Hgb) level of 13.2 g/dl and platelets count of 142,000/mm^3. The renal function was stable. Urine microscopy showed leukocyturia with WBC in urine of more than 100/high power field (hpf). Both urine and blood culture grew *Salmonella* group D. Stools culture showed no pathogens isolated. Serum total bilirubin was elevated (7.5 mg/dl), as were serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels (112 and 70 U/L, respectively). Febrile agglutinins were as follows: typhoid O, 1:80; typhoid H, 1:80. *Brucella* titers (abortus and melitensis) were negative. Blood culture for *Brucella* did not show growth. The cytomegalovirus-IgG titer was positive, and had not risen compared to previous values, and the cytomegalovirus-IgM titer was negative. The malaria smear repeatedly did not show parasites. The human immunodeficiency virus test was also negative. The chest x-ray was clear, and the ultrasound of abdomen showed a large

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