Summary

Liver transplantation is often the ultimate option of therapy for chronically hepatitis B virus (HBV) infected patients. Prevention of reinfection by HBIg is long-lasting and cost-intensive. Adoptive transfer of HBV immunity after transplantation of the liver from an immune living liver donor (LLD) could be a new approach to prevent reinfection in the recipients.

A short time immunization protocol (four injections in 2 weeks intervals) was established to achieve HBV immunity in LLD within 2 months. Using this protocol, the humoral and cellular immune response of Sci-B-Vac, a recombinant vaccine that contains HBV L, M and S surface proteins was compared with a standard HBV vaccine (HBVAXPRO®) that contains only the S surface protein. The humoral immune response could already be detected after the first immunization in nine out of fifteen Sci-B-Vac vaccinated individuals while it was only observed in one out of fifteen volunteers of the group immunized with HBVAXPRO®. Anti-HBs titers were significantly higher in Sci-B-Vac volunteers ($P < 0.01$) following all four vaccinations. HBV-specific T-cell immune response was significantly higher in Sci-B-Vac volunteers ($P < 0.001$) after the third vaccination. Proliferative response was also significantly ($P < 0.01$) higher in the Sci-B-Vac group after second to fourth vaccination.

With this good experience, Sci-B-Vac was subsequently used for vaccination of forty-six potential LLD using the short time immunization protocol as described above. Humoral and cellular immune responses were examined in donors after immunization and in recipients before and after transplantation. Anti-HBs-titers of up to 50,000 IU/l were detected in LLD. Fourteen patients received livers from these immunized donors. We detected humoral immunity in one HBV-naïve recipient and in one chronically HBV-infected recipient. A transfer of cellular immunity ($SI > 3$) was seen in three recipients. These three patients received livers from donors with high anti-HBs-titers of more than 9,000 IU/l. Cellular immunity was also detected in the corresponding donors ($SI > 3$ and spots > 22). Our study demonstrates that HBV-specific humoral and cellular immunity can be transferred by liver transplantation after vaccination of the donors. The transfer of B- and T-cell immunity in transplanted patients correlates with the magnitude of immune responses in the donor.

Further, using the woodchuck model we successfully expressed six chimeric PreS1-core particles (WHcPep1, WHcPep2, WHcPep3, WHcPep4, WHcPep5, WHcPep6) in *E. coli*. We successfully purified three out of six constructs (WHcPep3, WHcPep4, WHcPep6). Immunization of mice with chimeric particles purified in this study induced a better immune response compared to immunization with an n-terminal PreS1-fragment aa 1-81. Already, after the first immunization all groups of mice immunized with virus like particles (VLPs)

showed PreS1 specific humoral immune response; whereas mice immunized with the PreS1 fragment aa 1-81 showed no immune response at this time point. Particles created in this study can be used in the future in woodchucks to confirm the protection ability and safety of this vaccine for application of HBV VLPs in humans.