Report on study number 2008-007235-40 (Sponsor's protocol code number 2008-007235)

Development of Varicella-zoster virus (VZV)-specific CD4+ T cells on primary VZV infection or vaccination in renal transplant (RTX) recipients and healthy donors

Purpose: So far, only IgG-anti-VZV antibody concentrations were used to estimate immunity against VZV, but the antibody binding strength (avidity) together with VZV-specific cellular responses have not been evaluated in solid organ transplant (SOT) recipients.

Methods: Thus, we assessed the humoral and cellular immune responses to two doses of the VZV vaccine(vacc) and wild-type VZV infection (wt) in 23 kidney (KTx) and 19 liver transplant (LTx) recipients including children and adults compared to 48 healthy controls (HC) for measurement of IgG-anti-VZV relative avidity index (RAI) and frequency of VZV-specific peripheral blood mononuclear cells (PBMCs) in vaccinated individuals using an adapted ELISA and IFN-gamma ELISPOT, respectively.

Randomization and study groups: Sixty HC and 53 SOT patients were asked to participate in the study, of them 14 HC refused to donate blood for the study and 11 SOT patients were excluded for following reasons: 1 SOT patient refused to donate blood, 4 had lymphopenia, 2 IgG deficiency, 1 immunoglobulines in the last six months and 3 had proteinuria. Thus, forty-six HC (chronological age: 14.4 ± 7.0 years) and 42 SOT patients (chronological age: 15.2 ± 6.2 years) were included into the study. Patients and HC were separated into a wild-type VZV infected (wt) group with clinically and/or serologically confirmed varicella infection and into a vaccination group (vacc) if they had received two doses of VarivaxTM (Merck, Whitehouse Station, NJ) or VarilrixTM (Glaxo Smith Kline, Brentfort, UK).

Inclusion and exclusion criteria: Inclusion criteria were SOT (cadaver transplant in all cases) more than 12 months ago, with no rejection episodes in the last 12 months and low-dose immunosuppressive therapy with: cyclosporin A (base line serum levels 70–110 ng/mL), tacrolimus (base line serum levels at 4–7 ng/mL), mycophenolate mofetil (1.0–1.5 g/day),

prednisolon (0.1 mg/kg bodyweight/day or 5–7.5 mg/day), azathioprin (1–3 mg/kg/day), everolimus or sirolimus (1–4 mg/day). None of the patients had received anti- CD3 monoclonal antibodies (OKT3), anti-thymocyte globulin (ATG), anti-IL-2alpha chain receptor antibodies or anti-CD20 treatment in the past 12 months. Other exclusion criteria were significant proteinuria (protein >250 mg/dL in 24 h urine collection), administration of immunoglobulins or blood products in the last 6 months prior to evaluation, lymphopenia (<1500/µl) and IgG deficiency (<lower limit for age).

Study endpoints: The study was designed to investigate the humoral and cellular immune response to VZV vaccination or wild-type infection in kidney (KTx) and liver transplant (LTx) recipients by assessment of IgG-anti-VZV avidity and of VZV-specific lymphocytes.

Statistical analysis: After testing for distribution of variables (Shapiro–Wilks-test), the Mann–Whitney U test was applied for not normally distributed independent variables (SPSS for Windows, Version 19.0, Chicago, USA). Correlations were analyzed by Spearman's Rank correlation. Numbers of participants reaching arbitrary cut-off levels (IgG-anti- VZV concentration >100 mIU/mL, RAI >40% and RAI >60%) and frequency of immunosuppressive drugs were compared using Chi2 test.

Results: KTx(wt) (median RAI 72.3%) or LTx(wt) (79.2%) and KTx(vacc) (91.0%) or LTx(vacc) (72.5%) showed lower avidities compared to HC(wt) (84.5%) and HC(vacc) (94.0%), respectively, despite equally distributed IgG-anti-VZV concentrations. RAI > 60% (high avidity) was detected in all HC, but only in 69.0% of SOT patients. KTx(vacc) (median 64 spot forming units SFU/500,000 PBMCs) and LTx(vacc) (67 SFU) had significantly lower VZV-specific cellular responses compared to HC(vacc) (268 SFU).

Conclusions: In conclusion, IgG antibody avidity in SOT recipients may serve as a marker to evaluate long-term aspects of humoral immunity, although the clinical relevance and association with protection against VZV re-infection and re-activation has to be awaited. Cellular immunity against VZV is significantly diminished in SOT patients which has to be considered when evaluating immunity against VZV.