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Objective: Haematopoietic stem cell transplantation (HSCT) is frequently complicated by early Human herpesvirus type 6 (HHV6) reactivation and is associated with poor survival and severe acute Graft-versus-host-disease (aGvHD). We hypothesized that HHV6 may be a trigger for immunedysregulation, resulting in alloreactivity. We investigated total T-cell numbers and HHV6-specific Interferon-γ (IFNγ) producing T-cells in children with or without HHV6-reactivation after HSCT using a newly developed enzyme-linked immunospot (ELISPOT) technique.

Methods: Prospectively, HHV6, Cytomegalovirus, Adenovirus and Epstein Barr virus DNA-loads were weekly monitored by quantitative realtime-PCR and clinical data were collected. HHV6 reactivation was defined as HHV6 DNA-load >250cp/mL. T-cell reconstitution was prospectively measured every other week by immunophenotyping (markers CD3, CD4 and CD8). Numbers of IFNγ-producing T-cells in PBMCs were retrospectively determined by ELISPOT after overnight stimulation with HHV6-virus lysate (ABI, Columbia, Maryland, USA) 2 months after HSCT.

Results: Twenty-one HSCT patients were analyzed (median age 4.4 years; range 1–16.5yrs). Within the first two months, 13/21 (62%) patients developed HHV6 reactivation; median time of reactivation was 14 (range 1–41) days. The development of other virusreactivations did not differ between the two groups; 4/13 versus 3/8 respectively. The median number of IFNγ-producing specific HHV6 T-cells 2 months after HSCT was significantly increased in the patients with HHV6-reactivation; 40 (0–362) versus 0 (0–25) specific T-cells per million PBMCs ($p = 0.006$). Additionally, the median CD3+ T-cell numbers were significantly increased in these patients; 393 (32–5514) versus 93 (0–641) T-cells/uL ($p = 0.03$), including median CD8+ T-cells; 79% (6–87 %) versus 33% (0–83%) ($p = 0.03$).

Conclusions: Patients with HHV6 reactivation had significantly higher numbers of IFNγ-producing HHV6-specific T-cells and more CD3+T-cells/uL, among which mainly CD8+T-cells. Given the association of HHV6 reactivation and aGvHD, these T-cells may be alloreactive.

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HIGH PERCENTAGE OF PERFORIN-EXPRESSING T-CELLS IS ASSOCIATED WITH HERPESVIRUS REACTIVATION CLEARANCE AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN

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Background: Haematopoietic stem cell transplantation (HSCT) is frequently complicated by human herpesvirus 6 (HHV6), cytomegalovirus (CMV) or Epstein Barr virus (EBV) reactivations. Herpesvirusreactivations are associated with severe morbidity and mortality after HSCT. Although T-lymphocytes play an important role in the clearance of viral reactivations after HSCT, an easy measure of potent T-cell responses for predicting outcome of viral reactivations is lacking. We analyzed expression of perforin (a cytolytic pore-forming protein) as a marker for cytotoxic potential in CD8+T-cells in children and related this to the clearance of viral reactivations after HSCT.

Methods: In a prospective cohortstudy (2007–2008), we weekly monitored HHV6, CMV and EBV DNA loads in plasma after HSCT by quantitative realtime PCR. Herpesvirus reactivation was defined as DNA load >1000 cp/mL. CMV- and EBVreactivation were treated according to local guidelines. HHV6reactivation was treated when there was clinical suspicion of disease. We have weekly analyzed Perforin expression in CD8+T-cells by whole blood FACSanalysis, until 4 months after HSCT.

Results: A total of 27 patients were included with a median age of 4.3 (range 0.3–20.1) years. 15/27 (56%) recipients received bonemarrow (7/15 from matched family donors) or unrelated peripheral blood stemcells while 12/27 (44%) recipients received unrelated cordblood stemcells. All patients received myeloablative conditioning and standardized Graft-versus-host disease prophylaxis. During the first 4 months after HSCT, 16 patients developed HHV6reactivation, 2 patients developed CMVreactivation and 1 patient developed EBVreactivation. The median time of herpesvirusreactivation peak was 2.4 weeks (range 1.3–15.6) after HSCT. Viral clearance was marked by a perforin peak in CD8+ T-cells; median time of maximum perforin expression in CD8+T-cells was 4 weeks (range 1.4–15.9) after HSCT. Patients with herpesvirusreactivation (HHV6, CMV or EBV) showed significantly higher perforin-expression in CD8+T-cells during viral load clearance than patients without herpesvirusreactivation (17.2% (range 0–63%) versus 6.8% (range 0–16%); $p = 0.003$).

Conclusion: Herpesvirus reactivation clearance was significantly associated with a peak in perforin-expressing CD8+T-cells. Although virus-specific immune responses after HSCT have to be elucidated, perforin expression in T-cells is a useful marker for antiviral T-cell responses and viral load clearance after HSCT.

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ENHANCED IN VITRO EXPRESSION OF NKG2D LIGANDS MIC A AND MIC B WITH HISTONE DEACETYLASE INHIBITOR (HDACI) ROMIDEPSIN IN PEDIATRIC LEUKEMIA AND LYMPHOMAS: POTENTIAL FOR NATURAL KILLER CELL MEDIATED TARGETED ADOPTIVE CELLULAR IMMUNOTHERAPY (ACI)

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NK cells play a significant role in tumor cell recognition and cytotoxicity (Trinchieri et al Adv Immunol, 1990). NKG2D-mediated recognition of malignant cells by NK cells is enabled through the tumor-associated expression of NKG2DL. Among these NKG2DL, MIC A and B are significantly expressed on epithelial compared to hematological tumors (Bauer et al Science, 1999). Skov et al (Cancer Res, 2005) demonstrated a significant increase in surface expression of MIC A and B in various epithelial cancer cell lines after exposure to HDACi. We compared the expression of NKG2DL MIC A and B in common pediatric hematological tumor cell lines and the impact of enhanced expression of NKG2DL on NK cell mediated cytotoxicity. Cell lines used were: ALL (SUP-B15, RS 4:11, REH, Jurkat), NHL (Toledo, Ramos). Cells were co-cultured for 24hrs with 0, 5 and 10ng/ml of Romidepsin. MICA/B PE antibody was used for flow cytometry staining. Purified adult peripheral blood NK ($CD56^+/3^-$) cells and IL-2 activated NK cells (12hr incubation) were added to target cells (cultured with 5–10ng/ml of Romidepsin x 24hrs) at effector to target ratio of 5:1 and 10:1 and incubated for 2hrs. NK cell cytotoxicity was determined by a standard europium assay. There was a significant increase in expression of MICA/B in ALL cell lines when co-cultured with Romidepsin at 10ng/ml (RS 4:11 0.2% vs 19.2% $p < 0.0001$, REH 0.2% vs 46% $p = 0.0003$, Jurkat 1.12% vs 44.7% $p < 0.0001$). We also noted significant increase in MICA/B in some NHL cell lines when co-cultured with Romidepsin at 10ng/ml (Toledo [DLBCL] 0.5% vs 15.8% $p = 0.0001$, Ramos [BL] 0.57% vs 33.6% $p = 0.0003$). There was significant increase in vitro cytotoxicty in RS 4:11 cells at E:T ratio of 5:1 and 10:1 (5:1 specific release: RS 4:11 cells+NK cells vs. RS 4:11 cells+IL-2 activated NK cells vs. RS 4:11 cells+NK cells with 10ng/ml of Romidepsin vs. RS 4:11 cells+IL-2 activated NK cells with 10ng/ml of Romidepsin was 3.3% vs 6% vs 45% vs 61.6% $p<0.01$, and at 10:1 ratio 9.3% vs 10.6% vs 71.6% vs 86.3% $p<0.05$, respectively). In summary, these results suggest that Romidepsin induces significant expression of MICA/B ligands, enhancing their susceptibility for NKG2D-mediated cytotoxicity by NK cells. The combination of Romidepsin and NK cells presents a potential for ACI. Future studies will be conducted with MIC A and B antibodies to determine the specificity of the Romidepsin effect and the invivo cytotoxicity in a human/mouse xenograft SCID model.

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ABSOLUTE LYMPHOCYTE COUNT AT DAY 30 PREDICTS SURVIVAL IN RECIPIENTS OF A T CELL DEPLETED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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