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Department of Medicine Huddinge

Specific T- and B-cell Responses against *Human Cytomegalovirus* after Hematopoietic Stem Cell Transplantation

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ABSTRACT

Human cytomegalovirus (CMV) remains an important complication in allogeneic hematopoietic stem cell transplantation (HSCT). CMV-reactivation may lead to CMV-disease associated with high morbidity and mortality in patients after HSCT. In this PhD thesis I have investigated how specific T- and B-lymphocyte responses against CMV reconstitute after HSCT. In my thesis I will therefore explain both how our studies were preformed and what they have shown and furthermore I will give the reader some background in the fields of immunology, basic virology regarding CMV and the transplantation setting, which are all needed to understand the general aspect of the research.

In our 1st study we analyzed the effect of different pre-transplant related factors on the viral load (VL) and the effect of the VL and VL kinetics on the risk for CMV-disease in a series of consecutive allogeneic HSCT recipients. The VL influenced the risk for CMV-disease in univariate analysis but not when different factors were included in a multivariate analysis where only acute GVHD grades II-IV and the use of a CMV-negative donor to a CMV-positive recipient were significant risk factors. In patients, who required more than one course of preemptive therapy, acute GVHD and the rate of decrease in viral load during first preemptive therapy were significant risk factors for subsequent development of CMV-disease. The latter was a previously not described finding. Thus, the CMV VL kinetics is important after HSCT although we were unable to find a direct influence of the initial or peak VL on the risk for CMV disease.

In our 2nd study we evaluated the immune competence after HSCT by examining T-cell signaling and tested the phosphorylation of STAT5 in $CD4^+$ T cells, $CD8^+$ T cells and TCRγδ T cells in response to stimulation with IL-7 or IL-2 after HSCT in association to CMV clinical outcome. Reduced responses to IL-7, reflected by STAT-5P may represent a clinically relevant functional biomarker for individuals at increased risk for CMV reactivation. This finding may also aid to design better strategies to improve anti-CMV immune responses without increasing the risk to develop GVHD.

For our 3rd and 4th study we wanted to investigate the reconstitution of humoral immunity against CMV after HSCT. We screened the entire CMV proteome to visualize the humoral epitope-focus profile in serum with a peptide microarray technology before and after HSCT in serum from patients divided into groups depending on CMV-serological status of donor and recipient (D+R+, D+R-, D-R+, D-R-). Data were analyzed using MaSigPro, PAM and the 'exclusive recognition analysis (ERA)' to identify unique CMV epitope responses for each patient group. Strongly (IgG) recognized CMV targets showed also robust cytokine production in intracellular cytokine staining (IL-2, TNF-α, IFN-γ and IL-17). To enable the global visualization of the entire intensity of Ig responses against CMV-epitopes a 3D model was used reflecting both the breadth and profile prior to and after reconstitution of the humoral immune response at different time points. Two different types of 3D graphs were constructed: CMV antigen regression surfaces (3DPOS) and peptide bulkiness /polarity regression surfaces (3DBP). We believe that high-content peptide microarrays allow epitope profiling of entire viral proteomes useful for diagnostics and therapy and that they may also be used to visualize the breadth of B-cell immune reconstitution after HSCT.

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