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Human cytomegalovirus infection of M1- and M2-macrophages: analysis of antigen presentation and T cell stimulation

Human cytomegalovirus (HCMV) can cause life-threatening infection syndromes in fetuses, newborns, or immunosuppressed individuals such as transplant recipients or AIDS-patients whereas the infection remains mainly asymptomatic in immunocompetent persons. After primary infection, HCMV-specific antibodies and T cells can be detected in the host's blood throughout life. Important targets of the virus are antigen-presenting cells like monocytes, macrophages and dendritic cells, which should, as a first line of defense, eliminate the pathogen. But HCMV has developed several mechanisms to manipulate the host cells and circumvent immune response, mainly investigated in fibroblasts. In detail, viral gene products US2 to US11 have been identified to reduce human leukocyte antigen (HLA) expression on the cell surface of fibroblasts as well as dendritic cells and, consequently, impaired T cell response was observed. Until now, expression and resulting effects of those immune-evasive genes on the immunostimulatory capability of macrophages remains unclear.

We are working with two extreme polarities of macrophages (M1- and M2-Mφ), differentiated *ex vivo* from freshly isolated human monocytes. My PhD-project focuses on the capability of HCMV-infected M1- and M2-Mφ to present viral antigens and stimulate T cells. We could already show that HCMV is able to induce down-regulation of HLA-A,B,C (M1+M2) and –DR (M2) in infected Mφ. For further experiments we will use viral mutants lacking the known immune-evasive genes and determine changes in the immunophenotype of Mφ by analyzing expression of CD14, HLA-ABC, -DR and the co-stimulatory molecules CD80 and CD86 by flow cytometry. Furthermore we could show proliferation of autologous lymphocytes after co-culture with HCMV-infected M1- and M2- Mφ, but only if the cells were obtained from HCMV-seropositive but not from -seronegative individuals, indicating that Mφ are only able to stimulate memory T cells. In order to clarify the identity of the proliferating T cell subpopulations we will label peripheral blood lymphocytes with carboxyfluorescein succinimidyl ester (CFSE) and assess the amount of responsive $CD4^+$ and $CD8^+$ T cells by flow cytometry. After this first step we will further distinguish between naïve and memory T cells by using the markers CD45RA/B and CD45R0, respectively. The memory T cells themselves can be classified into central memory T cells which constitutively express CCR7 and CD62L and effector memory

T cells with no constitutive expression of CCR7 and heterogeneity for CD62L. Therefore we will analyze proliferated $CFSE^{low}$ T cells in respect for those surface markers.

Our aim is to fully understand the impact of HCMV on the ability of Mφ to present viral antigens and stimulate effective T cell response and elucidate the mechanisms lying behind.