Summary

HIV associated neurocognitive disorders (HAND) are a common comorbidity in HIV positive patients. Since introduction of combined antiretroviral therapy (cART), the phenotypic picture of HAND changed. Although treatment with cART leads to effective suppression of the viral load, the prevalence of HAND remained stable, which argues for virus independent mechanisms responsible for neurodegeneration. It is commonly proposed and postulated in the bystander hypothesis that mainly HIV infected monocytes enter the central nervous system (CNS) and activate microglia, the resident immune cells of the CNS, which causes a persistent inflammation and consequently neurodegeneration. In previous studies of our group, we developed a cell-culture model, mimicking the interactions of microglia with HIV transduced monocytoid cells and resulting inflammatory processes.

This thesis aimed at modifying the established co-culture model by introducing a microglial cell line. In experiments with the modified cell culture model, presence of HIV-transduced monocytoid cells was crucial for full co-culture activation. Because the molecular processes occurring during the infection of monocytoid cells by HIV are not well understood, those processes should be further investigated by detecting changes in the proteome of the cells. Finally, taking advantage of two different pharmacological compounds, namely teriflunomide and monomethylfumarate, the release of proinflammatory chemokines and cytokines by co-culture was targeted with the aim to reduce neurotoxicity.

The developed cell-culture model was further modified by introducing the human microglial cell line HMC3. This resulted in a better availability of cells and a more homogeneous cell response to HIV transduced cells compared to human embryonic microglia. HMC3 in contact with HIV-vector transduced monocytoid cells showed a specific upregulation of the cytokines CXCL10 (p < 0.001), CCL5 (p < 0.01), CCL2 (p < 0.001) and IL-6 (p < 0.001) compared to control conditions. Furthermore, HMC3 showed a significant higher secretion in co-culture of HIV-vector transduced monocytoid cells (CXCL10, CCL5 and CCL2: p < 0.001; IL-6: p < 0.01) compared to HMC3 in contact with HIV-vector particles without monocytoid cells. To further validate these obtained results, experiments with ex vivo primary adult microglia were performed. These experiments revealed similar response of HMC3 and adult microglia in contact with HIV transduced monocytoid cells.

Furthermore, effects of HIV transduction on monocytoid cells were examined by a label-free proteome approach, in which the expression of proteins in HIV-transduced and non-transduced monocytoid cells was compared. Here, transduction with HIV-vector leads to an upregulation of proteins mainly involved in disease associated pathways (FDR (false discovery rate): 8.76 * 10^-4). Highest expression was found for the proteins superoxide dismutase (ratio:
3.24; \( p = 8.3 \times 10^{-14} \) and transgelin-2 (ratio: 2.98; \( p = 3.7 \times 10^{-6} \)). In contrast, downregulated proteins were mainly associated with DNA-replication (FDR: 8.83 \( \times 10^{-4} \)) and gene expression (FDR: 4.21 \( \times 10^{-3} \)) and especially connected to ribosomal activity. In further experiments it could be demonstrated that direct cell-cell contact of microglia and monocyloid cells was not crucial for full activation.

Finally, inflammatory processes in co-culture settings could be attenuated using immunomodulatory drugs known from multiple sclerosis treatment, teriflunomide and monomethylfumarate. Treatment of HIV transduced co-culture with either 30 \( \mu \)M teriflunomide or 100 \( \mu \)M monomethylfumarate caused a decreased secretion of CXCL10 (teriflunomide: \( p < 0.001 \); monomethylfumarate: \( p < 0.001 \)), CCL2 (teriflunomide: \( p < 0.001 \)) and IL-6 (teriflunomide: \( p < 0.001 \)) compared to untreated HIV transduced co-culture. Supernatant of HIV transduced co-culture treated with different concentrations of teriflunomide or monomethylfumarate caused 20% less neuronal cell death in a culture of human fetal neurons compared to untreated HIV transduced co-culture supernatants (\( p < 0.05 \)).

These data help to further understand molecular and proteomic mechanisms in HAND. Interaction of HIV-transduced monocyloid cells and microglial cells lead to inflammatory processes which are potentially neurotoxic. Treatment with either teriflunomide or monomethylfumarate ameliorates inflammatory processes and occurring neurotoxicity. This argues for a potential treatment option of these immunomodulatory drugs additionally to cART, to obtain beneficial effects in the context of HAND.