The role of human microglia for glioma progression

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The project aims to characterize interactions of human microglia with glioma cells. Due to the close interaction of the clinical partner, Michael Synowitz, with the basic researcher Helmut Kettenmann, we will develop paradigms to study the interaction of these two cell types from human material and determine the role of human microglia for glioma progression.
In our previous collaboration we have used mouse models to study microglia-glioma interactions. We demonstrated that mouse microglial cells strongly promote glioma growth and expansion. We have identified molecular mechanisms of this interaction. Versican released from glioma cells stimulate microglial Toll-like receptor-2, resulting in microglial MT1-MMP expression via the TLR downstream signaling molecules MyD88 and p38 MAPK. In turn, MT1-MMP expression and activity in these immune cells promotes glioma cell invasion and tumor expansion. Thus in the mouse model, we have identified the signaling cascade by which glioma cells reprogramme microglia into a pro-tumorigenic phenotype. Hence, glioma cells attenuate proinflammatory immune-responses by microglia and induce a unique microglial phenotype, the tumor-associated microglia. Glioma tissue contains up to 30% microglial cells/brain macrophages and we have developed a procedure to isolate these cells from mouse models of glioma, but also recently from human resection material. In this context it is also important that we compare different grades of brain tumors such as low grade astrocytomas and the high grad malignant glioblastoma multiforme.
The student will get the human resection material from Michael Synowitz, transport it to the MDC and then isolate the cells. Two isolation procedures have been implemented: 1. With antibodies specific for microglia attached to magnetic beads, we can isolate microglia by magnetic activated cell sorting (MACS). 2. An alternative approach is using fluorescence activated cell sorting (FACS) to separate microglia and glioma cells. We will analyze the genomic profile of the human microglia obtained from glioma tissue and compare them to microglia obtained from control tissue (approved by the ethical committee at the Charité). We already have the data set for the mouse model and have identified several interesting genes which are upregulated in glioma associated microglia. This new approach will help us to focus on genes which are relevant in the human context.
As a next step we will develop procedures to culture the human microglial cells either in isolation or combined with glioma cells. We have recently established protocols to culture microglial cells isolated from the adult mouse brain (the common methods is to culture them from early postnatal brain). We will adapt this model for human microglia. The cell culture of primary human glioma cells is already established. This will allow us to study the interactions of these cells and analyze whether the molecular interactions which we have identified in the mouse models are also relevant for humans. It will also enable us to perform functional studies such as measuring cytokine and chemokine release, determine the phagocytic capacity or measure the migratory activity of human microglia. We will also test which substances act as chemoattractants. We will test whether soluble factors from glioma change the human microglial phenotype and vice versa. We have already identified the antibiotic minocycline which interferes with this microglia-glioma interaction and has a strong impact on glioma growth in the mouse model. We will investigate whether this interaction is also applicable in the human system. Moreover, we will try to identify novel pathways of this interaction and

test for potential new drugs which might interfere and, thereby, may qualify as new candidates for glioma treatment.
This project will be only possible by a very close interaction between the basic research lab and the clinical partner. The PhD student will obtain human tissue directly from the neurosurgery and perform the cellular approaches at the MDC.