INTRODUCTION: The treatment of glioblastoma multiforme (GBM) is a major challenge of neuro-oncology. Despite aggressive therapeutic strategies including surgery and radio-chemotherapy, median survival remains extremely low. This points to the urgent need for alternative treatment strategies such as immunotherapy. Immune escape mechanisms, involving the Natural Killer Group 2 member D (NKG2D) system, play a major role in tumor progression. Cell-bound NKG2D ligands such as MHC class I related molecule A and B (MICA and MICB), and the UL-16 binding protein family (ULBP1-6) are recognized by the NKG2D receptor (NKG2D) and trigger cytotoxic effector functions in NK-cells and T-cell subsets. By releasing soluble NKG2D ligands (sNKG2DL), which then bind to NKG2D, tumor cells inhibit the killing potential of the effector cells. Numerous studies documented the importance of NKG2D system in vitro GBM-model systems. Here, we aimed to analyze NKG2D system in GBM-patients ex vivo.

MATERIALS AND METHODS: Until now, 37 GBM patients and 19 healthy controls (HCs) have been included in the study. A total of 24 GBM patients were on medication with dexamethasone prior to surgery. We analyzed serial levels of sNKG2DL in HCs and GBM patients before and 3 months after surgery and radio-chemotherapy via Luminex-based multiplex assay. Absolute cell numbers of distinct immune cell subsets and the expression of NKG2Dr were analyzed by flow cytometry. NKG2DL-expression on GBM primary cell cultures was studied via flow cytometry and western-blotting.

RESULTS: Our data show that in comparison to healthy controls GBM patients show reduced numbers of leukocytes and cytotoxic effector cells in peripheral blood, but elevated numbers of immunosuppressive cells. Whereas NKG2Dr is not detectable in primary cell cultures, the tumor cells moderately express NKG2DL and release sNKG2DL into the culture medium. Serum levels of sNKG2DL are differentially modulated in GBM patients when comparing pre- to 3 months postop. This effect is independent of medication with dexamethasone.

CONCLUSION: GBM patients seem to have impaired cytotoxic immune response compared to HCs. As NKG2DL are released by the tumor cells, this effect might also be generated via sNKG2DL. Nevertheless, serum levels of sNKG2DL do not decline after treatment in most patients. A better understanding of the mechanisms regulating the NKG2D system in GBM patients will be crucial for the development of new therapeutic strategies targeting the NKG2D system.

P04.04 EX VIVO EXPANSION OF HUMAN GLIOMA-INFLTRATING LYMPHOCYTES ALTERS THE EXHAUSTION PHENOTYPE OF T CELLS


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Immunotherapy for brain tumors is entering the clinical arena with novel immunotherapeutic strategies that enhance the immune system's ability to target tumor antigens (TA). Consequently, there is increasing interest in identifying and characterizing tumor antigen-specific tumor-infiltrating T lymphocytes (TILs) reactive to tumor antigens for further tumor immunotherapy such as adoptive T cell therapy. T cells typically upregulate exhaustion markers such as PD-1 after antigen-experience. Protocols for selecting TA-specific T cells from the periphery help make use of these exhaustion markers to select for relevant antigen-experienced T cells. Commonly used protocols for the isolation of TILs make use of an ex vivo expansion of TILs in order to achieve reasonable T cell numbers for further analysis. Whether this expansion affects the phenotype of TILs, however, remains unclear. In this study, we investigated the influence of a commonly used ex vivo expansion protocol for the isolation of human glioblastoma (GBM)-infiltrating T lymphocytes on their phenotype. This protocol involved ex vivo expansion of TILs by culturing tumor fragments with complete medium supplemented with IL-2 and CD3 ligand, whereby TILs were allowed to migrate out of the tumor tissue and further expanded using IL-2 for two weeks. We demonstrate that the CD8/CD4 T cell ratio shifts dramatically after an ex vivo expansion of GBM TILs towards a CD4 dominance, when compared to freshly isolated unperturbed TILs. Furthermore, ex vivo expansion of GBM TILs resulted in an increase of LAG-3+ and TIM-3+ CD8 and CD4 T cells, and the frequency of PD-1+ CD8 T cells decreased considerably after ex vivo expansion of patient TILs resulting in a profound skewing of the TIL phenotype, particularly markers of exhaustion and reinvigoration. Hence, direct analysis of freshly isolated unperturbed TILs may be necessary to allow a faithful assessment of the relevant TIL profile for further identification of TA-specific TILs.

P04.05 THE CAR2BRAIN STUDY: A MONOCENTRIC PHASE III TRIAL WITH ERBB2-SPECIFIC NK-92/5.28.Z CELLS IN RECURRENT GLIOBLASTOMA


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Recent preclinical studies from our institutions and other researchers suggest that natural killer (NK) cells have the potential for adoptive immunotherapy.