ImmunoTools *special* Award 2014

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Influence of GM-CSF knockdown in glioma cells on infiltration and functional polarization of myeloid cells and T lymphocytes in experimental gliomas

Glioblastomas are considered to be one of the most difficult human malignancies to manage, due to frequent dysfunctions of tumor suppressors and oncogenes, diffusive growth and poor response to current therapies. Despite of development of new therapeutic modalities, the median survival is approximately 14 months among patients with the deadliest form of brain tumor, glioblastoma multiforme (GBM). Treatment of glioblastoma patients remains a paramount challenge for clinicians and researchers.

Microglia are myeloid, immune cells residing in the central nervous system (CNS), which respond to pathogen or injury. In response to pathological conditions they become activated and capable of phagocytosis, antigen presentation, and lymphocyte activation. Experimental and clinical studies show that microglia and peripheral macrophages in glioblastomas, are attracted by tumor-released molecules and re-programmed into anti-inflammatory cells supporting tumor progression. Glioblastomas are highly infiltrated with microglia and blood-derived macrophages that support glioma progression by secreting growth factors, pro-angiogenic molecules, extracellular matrix–degrading enzymes and immunosuppressive cytokines. Glioma-infiltrating microglia/macrophages in mice and human exhibit the immunosuppressive, M2-like phenotype (Gabrusiewicz et al. 2011; Sielska et al. 2013).

Signals for macrophage recruitment and polarization in glioblastomas

Signals that are responsible for macrophage recruitment to glioblastomas, heterogeneity of infiltrating populations and their contribution to tumor progression are poorly understood. Our studies demonstrate that gliomas attract not only local microglia but also blood- and bone marrow derived monocytes/macrophages. In general, macrophage proliferation, differentiation and chemotaxis could be regulated by several factors, including for example: macrophage colony-stimulating factor (M-CSF/CSF-1), granulocyte macrophage colony-stimulating factor (GM-CSF/CSF-2), interleukin 34, chemokine CCL-2. Using clones of GL261 glioma cells stably depleted of GM-CSF, we previously demonstrated that glioma-derived GM-CSF contributes to recruitment/activation of microglia/macrophages and tumor progression. GM-CSF knockdown in glioma cells strongly reduced activation and accumulation of Iba-1

positive cells in experimental gliomas that resulted in reduction of tumor growth and intratumoral angiogenesis (Sielska et al 2013).

Infiltration and functional polarization of myeloid cells and T lymphocytes in rat experimental gliomas

Glioblastoma infiltration by different cell populations was observed by the use of histopathology and flow cytometry studies in a model of experimental rat glioma. In this study we analyzed accumulation of microglia, macrophages and leucocytes, especially the T lymphocyte subpopulations. Adult Wistar rats were sham-operated or transplanted by rat C6 glioma cells. Animals were sacrificed 21 days after implantation. Brain tissue was processed by mechanical fragmentation and gentle tissue dissociation with a neural dissociation kit (Miltenyi Biotech.). Immune cellswere stained with cell-type specific antibodies, assessed by flow cytometry. The number of microglia ($CD11b+CD45^{low}$), blood-derived macrophages ($CD11b+CD45^{low}$), and leucocytes ($CD4^+$, $CD4^+FOXP3^+$, $CD8^+$) in glioma-bearing hemispheres at the 21^{st} day after implantation of C6 glioma cells wasdetermined. Detailed analysis of cell subpopulations indicated a significant increase of blood derived macrophages, T helper and T regulatory cells in gliomas. The percentage of T cytotoxic cells infiltrating glioma bearing brains was low. Accumulation of T helper and T regulatory cells in a tumor zone and successful blocking migration of T cytotoxic cells could be responsible for creating a highly immunosuppressive environment and lack of initiation of effective anti–tumor responses.

In present project we would like to analyse influence of GM-CSF gene silencing effect on infiltration process of different cell subpopulations coming from CNS and peripheral blood with the use of ImmunoTools anti-mouse antibodies for flow cytometry in a murine glioma model. The experiment will consist four groups: sham operated mice and mice implanted with parental GL261 cells, GL261 shControl cells, GL261 shGM-CSF cells . Until now, we obtained GL261 glioma cell lines expressing shGM-CSF and shControl, and established protocols for mechanical fragmentation of brain tissue,gentle tissue dissociation and flow cytometry. Antibodies from ImmunoTools Award will help us to realize our project.

ImmunoTools *special* AWARD for **Anna Gieryng** includes 25 reagents

FITC - conjugated anti- mouse CD3e, CD19, CD44, CD45R, CD62L, Gr-1, NK-cells, isotpye control IgG2b,

PE - conjugated anti-mouse CD4, CD8a, CD11b, CD19, CD62L, Gr-1, NK-cells, isotpye control IgG2b

APC -conjugated anti- mouse CD3e, CD4, CD11b, CD45, CD49d, CD62L, Gr-1, NK-cells, isotpye control IgG2b

DETAILS