ImmunoTools *special* Award 2014

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Dissecting the role of C5a/C5aR in the pathogenesis of primary Brain Neoplasia

Gliomas are highly aggressive brain tumors with dismal prognosis, despite advances in combination therapy and multi-modal patient treatment. Glioma cells remain notorious for their multiple immunosuppressive strategies, their highly hypoxic phenotype and inherent resistance to radiation therapy. Tumor hypoxia defines a major obstacle to conventional therapy as it triggers a robust pro-angiogenic transcriptional program and also promotes the development of tumor-initiating cancer stem cells (CD133+ glioma cells).

Brain neoplasia evokes a chronic inflammatory response that is associated with tissue injury and aberrant remodelling. It can also trigger **complement activation** and the local release of complement anaphylatoxins in the brain parenchyma. Complement (C') -a key sentinel of innate immunity- has been implicated in neuroinflammatory diseases and has also been shown to modulate anti-tumor immunity. C' proteins are expressed by several neural cell types. Brain astrocytes express C5aR and represent the main cellular source that gives rise to gliomas. To date the role of the complement anaphylatoxin C5a and its receptor C5aR1 in the pathogenesis of Glioblastoma remains elusive.

My research will focus on investigating the impact of C5a/C5aR1 signaling on the regulation of glial tumor hypoxia and oxidative responses following exposure to therapeutic doses of ionizing radiation. The long-term goal of this research is the development of novel glioma radiotherapeutics that will exploit pro-inflammatory stimuli (e.g. C5a) generated in the tumor microenvironment.

1a) In the first stage of my research thesis, C5aR1-bearing glioma lines will be subjected to hypoxia (cultured in low oxygen tension) and treated with C5a at optimum doses (1-100nM). RNA will be extracted from these cells and used for the screening of a human gene Atlas cDNA microarray and the construction of distinct gene expression signatures associated with tumor hypoxia. Exposure of glioma cells to hypoxic conditions similar to those applying to the solid tumor environment will allow us to correlate complement activation with the induction of pro- or anti-angiogenic genes (e.g. HIF-1a-responsive targets). To validate the expression profiles obtained from these microarray analysis we will perform Flow Cytometry and ELISA studies of hypoxia-regulated factors in various cell populations that infiltrate the glioma tumors.

To this end, the following ImmunoTools antibodies will be used to discriminate lymphocytic from myeloid cells: **FITC - conjugated anti-human CD14, CD11b, CD3/ CD4/CD8 (T-cell markers), CD19 and CD21 (B-cell markers), CD56 (NK- Cell marker), CD33.**

1b) Hypoxia is known to regulate the expression of various pro-angiogenic cytokines and growth factors, a panel of which will be analysed in glioma cell cultures, grown in hypoxic conditions. For this purpose we will collect supernatants from cell cultures exposed to hypoxia (1, 2, or 5 % O_2) in the presence or absence of 1-100 nM C5a. These supernatants will be assayed for the presence of VEGF, RANKL, SDF-1α and MIP-1. In order to establish a mechanistic link between C5aR simulation and the regulation of various cytokines involved in tumor hypoxic responses, we will treat glioma cells with a combination of ImmunoTools **human recombinant cytokines rh VEGF-A/VEGF-165, rh RANKL, rh SDF-1α / CXCL12a, rh MIP-1α/ CCL3, and rh RANTES / CCL5,** in the presence of a specific C5aR antagonist. In this way we will determine whether signaling through C5aR is essential for the expression of these key cytokines/growth factors.

2. Reactive oxygen species (ROS) measurements will be performed in irradiated glioma cultures treated with C5a and specific C5a receptor inhibitors to determine the effect of anaphylatoxin signalling on ROS-mediated cytotoxicity following irradiation.

In addition to measuring the ROS levels of glioma cells treated with C5a, we will also determine the combined impact of a panel of pro-inflammatory cytokines on oxidative

responses of glioma cells. In this respect we will measure ROS levels in glioma cells treated with C5a, in combination with various cytokines including ImmunoTools **rh IL-6, rh IL-1beta /IL-1F2, rh TNF-α,** and also in the presence of the **anti-inflammatory cytokines** ImmunoTools **rh IL-10 and rh TGF-beta3.**

Overall, the successful outcome of this research project will rely on the use of highly selective immunoTools reagents (both antibodies and recombinant cytokines) that will allow us to dissect the crosstalk of complement-driven inflammation with the hypoxic signature and cytokine milieu of a primary brain neoplasia (i.e. glioma) that is highly refractory to conventional therapy.

ImmunoTools *special* AWARD for **Antigoni Stavridou** includes 25 reagents

FITC - conjugated anti-human CD14, CD11b, CD21, CD3, CD4, CD8, CD19, CD56, IL-6, Control-IgG1,

PE - conjugated anti-human IL-8, TNF-α,

human IL-6 ELISA-set for 96 wells, (3 reagents),

recombinant human cytokines: rh VEGF-A/VEGF-165, rh RANKL, rh SDF-1α / CXCL12a, rh RANTES / CCL5, rh MIP-1α/ CCL3, rh IL-6, rh IL-1beta /IL-1F2, rh TNF-α, rh IL-10, rh TGF-beta3 DETAILS more AWARDS