ImmunoTools *special* Award 2014

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Exploiting proinflammatory pathways of innate immunity for the design of novel therapeutic agents for aggressive brain tumours

Glioblastoma represents the most devastating primary brain tumor in adults, with a dismal prognosis of 12 - 16 months post diagnosis. It appears refractory to therapy due to its hypoxic nature, pronounced radioresistance, infiltrative phenotype, and multiple immunosuppressive strategies. Despite advances in glioma treatment, patient responses remain disappointing and more selective therapeutic avenues are warranted to improve patient management. The development of neoplasia is accompanied by a distinct and chronic inflammatory response that is thought to act as a 'double-edged' sword, either by promoting tumorigenesis or destructing tumor cells by antibody-dependent cytotoxicity. **Complement (C')** is such an inflammatory pathway that exerts multiple immunomodulatory functions in the tumor microenvironment. Interestingly, C' proteins are expressed by various brain cell types, including neurons, microglia and astrocytes. Both primary astrocytes and astrocytic (glioblastoma) cell lines have been shown to express C' anaphylatoxin receptors C3aR and C5aR; however their function in astrocyte-driven pathology and glioma pathogenesis remains largely elusive.

The goal of my research is to define the involvement of C' anaphylatoxins in glioma pathogenesis employing both *in vitro* and *in vivo* models of astroglial tumor pathology. To this end, our studies will address th**e effect of' anaphylatoxin signaling on critical responses that drive the inherent resistance of glioma cells to therapy, such as glioma cell survival and invasiveness *in vitro*.**

First, human glioma cell lines will be phenotyped by flow cytometry for expression of C' anaphylatoxin receptors C3aR and C5aR/CD88. Next, i*n vitro* radiosensitivity studies will be pursued in receptor-bearing glioma cells to dissect the role of complement in modulating tumor cell radioresistance. The combined effect of anaphylatoxins C3a/C5a and proinflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α, and rh SDF-1-α on tumor radiosensitivity will be explored using clonogenic survival assays. Further studies will also address the impact of C' anaphylatoxin signaling on SDF-1α-dependent migration of glioma cells and the rhVEGF-A-mediated invasion of T98G gliomas in matrigel plug assays. The proprietary ImmunoTools Human IL-6 ELISA set will be an essential tool for probing the inflammatory activation of glioma cells in response to C5a or C3a stimulation.

To study the effect of anaphylatoxin stimulation on glioma cell apoptosis we will treat tumor cell lines U87MG/ and T98G with a range of doses of human C5a and assess the binding of FITC- conjugated anti-human Annexin V on the surface of these cells as an early marker of apoptosis.

Furthermore the role of C3aR /C5aR activation on glioma-driven neoangiogenesis will be investigated using glioma culture supernatants treated with anaphylatoxins and rh VEGF-A/ VEGF-165, and also rh IL-8 as stimulants of HUVEC cell proliferation and vessel tube formation.

In the second phase of the project, we will evaluate the *in vivo* role of complement anaphylatoxins in modulating glioma growth by applying preclinical models of glioblastoma development. To determine the role of C3a and C5a in glioma growth, we will establish a U87MG human glioma xenograft model in athymic mice. To assess the effect of local C3a or C5a administration on the growth of U87MG glioma cells we will inject mice with a panel of C3a and C5a analogs and monitor tumor growth by volumetric measurements.

To define the contribution of anaphylatoxin signaling to the recruitment of bone marrow-derived myelomonocytic cells and stem cells to the tumor site we will collect tumor homogenates from C3a/C5a-injected mice and isolate the $CD133^{+}/CD11b^{+}$ stem cell compartment by means of flow cytometry. We will gate myeloid cell populations by the combined use of Gr-1, CD11b and CD45 markers and filter out lymphoid populations by the use of CD19, CD45 markers for immune cells and also CD3/CD4/CD8 as T-cell markers. This part of our research will rely on the use of ImmunoTools anti-mouse antibodies against these selected myeloid and immune cell markers in glioma cell infiltrates. Overall, the requested Immunotools reagents will help us dissect crucial interactions between the innate immune compartment and key cell populations regulating glioma growth, tumor cell survival and therapy response.

ImmunoTools *special* AWARD for **Maria Georgoutsou** includes 23 reagents

FITC - conjugated Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V,

PE - conjugated Control-IgG1, Control-IgG2a, Control-IgG2b,

recombinant human cytokines: rh IL-1alpha, rh IL-6, rh IL-8, rh VEGF-A/VEGF-165, rh SDF-1α, rh TNFα,

human IL-6 ELISA-set (3 reagents)

FITC - conjugated anti-mouse CD3e, CD4, CD11b, CD19, CD45

PE - conjugated anti-mouse CD8a, Gr-1, DETAILS more AWARDS