ImmunoTools IT-Box-Cy55M-Award 2013

Alfonso Grimaldi

PhD Supervisor: Prof. Dr. Cristina Limatola

Department of Physiology and Pharmacology
"Sapienza" University of Rome
Piazzale Aldo Moro, 5 00185 Rome

"Glioma-infiltrating microglia as target of immunotherapy"

The project will consider the varied population of tumor associated microglia/macrophages (TAM); in particular, those infiltrating glioma. This is the most aggressive tumor of the brain, characterized by an unresponsive behavior to chemotherapy and a very low survival rate in patients; it is known to be highly microglia-infiltrated, up to 30% of the entire tumor mass, and it is clear that the more is the presence of microglia, the worst will be the outcome of therapy and the grade of invasiveness and malignancy of the tumor.

We know there are at least two different populations of TAM: the classical activated ones (M1), that show a proinflammatory and tumoricide effects, and the alternatively activated ones (M2), whose role is favoring tumor growth and immunosuppression.
Recent studies have demonstrated that in a first time microglia try to fight against glioma, producing tumoricide cytokines and activating immune response (M1); after, when the presence of glioma becomes a cronic stimulus, it is created a microenvironment that stimulate resident and incoming microglia to assume an M2 phenotype, secreting antiinflammatory cytokines and promoting tumor growth.

It has been recently demonstrated that a pivotal role in glioma biology is played by the intermediate conductance Ca^{+2}-activated potassium channels (Kca3.1), involved in glioma cells migration, cytokines secretion, and immune cells activation.

The expression of this protein has been shown in glioma cells as well as in microglia.
Our goal is first of all better define glioma microenvironment in different stages of tumor mass development, using mice injected with a murine glioma cell line and analyzing presence of specific cytokines in their brain . After we could examine how this microenvironment is changed after *in vivo* tretament with a selective blocker of Kca3.1 and underline how many and which cytokines are differentially expressed and secreted.

From preliminary experiments we noticed that modulation of Kca3.1 is correlated with microglia activation level. We therefore could isolate microglia from mice brains treated or no with this drug and evaluate if specific cytokines can still activate or

generate responses in the two different microglia populations. So we will treat microglia with M1-polaryzing cytokines (CCL2, CCL5, CXCL10, CXCL12, TNFα) or M2 ones (IL-4, IL-10, IL-13, VEGF) to evaluate *in vitro* microglia alteration of migration, phagocytosis, cytokines production, glioma cells apoptosis induction and electrophysiological changes.

Therefore, we could treat *in vitro* TAM with cytokines targeting specific pathway tested in tumor therapy, trying to switch them towards an M1 phenotype and finally associate *in vivo* treatments to evaluate possible improve of mice survival rate.