ImmunoTools IT-Box-Cy55M-Award 2013

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Role of the complement system and TAMs in tumor development and progression

The complement (C) system is part of the local microenvironment able to exert an active and beneficial role in the fight against malignant cells, based on the ability to promote inflammation and to cause direct cell killing (Ricklin, *Nat Immunol*, 2010). The advantage of this defence system is to be made up of several components readily available at the tissue site, where they are secreted mainly by macrophages surrounding the tumor mass (Lu, *Immunobiology*, 2007) and also by tumor cells or recruited from the circulation as a result of an inflammatory process (Macor, *Immunol Lett*, 2007). Circumstantial evidence obtained from the analysis of tumor tissue from patients with breast, colorectal, ovarian and papillary thyroid carcinoma showing deposition of C activation products on cancer cells has suggested a role for C in the immune surveillance of cancer (Markiewski, *Trends Immunol* 2009). The recent finding that C5a promotes malignant growth in a mouse model of cervical cancer has raised the intriguing question whether complement activation at tumor site is in favour or not for cancer development (Markiewski, *Nat Immunol*, 2008).

The aim of my PhD research project is to investigate the contribution of the first complement component C1q in tumor development and progression. This idea comes from a recent finding in which locally secreted C1q was shown to be involved in trophoblast invasion of maternal decidual stroma and blood vessels during pregnancy, resembling to some extent tumor progression, except that this process is physiologic and is tightly regulated in time and space (Agostinis, *J Immunol*, 2010; Girardi, *Mol Immunol*, 2011). Preliminary observations obtained in our laboratory suggest that C1q may have a function similar to that made by Markiewski et al. about the role of C5a in promoting tumor development and progression, through a mechanism independent of complement activation. To reach this goal both *in vitro* and *in vivo* approaches will be developed to evaluate the role of C1q in melanoma, colon and breast carcinoma development and progression, as examples of solid tumors which share a poor prognosis, trying also to unravel the mechanisms by which C1q acts in the tumor microenvironment as well as its interactions with the local immune cells, particularly with the Tumor-Associated Macrophages (TAMs).

The ImmunoTools *IT-Box-Cy55M* would be of great help to me as it would be used to get pure and specific subsets of TAMs *in vitro* to evaluate their contribution on the C1q expression and production. Tumor-derived soluble factors, mainly cytokines, chemokines and growth factors, and macrophage conditioned medium (MCM) will be used in turn to stimulate murine Bone Marrow Derived-Macrophages BMDMs and several tumor cell lines (Solinas, *J Immunol*, 2010) for evaluating the expression of the three chain of C1q by qPCR and to assess leukocytes involvement in C1q deposition in the tumor microenvironment. Classically activated pro-inflammatory/anti-angiogenic and anti-tumoral (M1) macrophages will be obtained by using mGM-CSF and then polarized by stimulating fully differentiated mononuclear phagocytes with the proinflammatory cytokines mINFγ and mTNFα alone or in concert with microbial stimuli (LPS); conversely, anti-inflammatory/pro-angiogenic and pro-tumoral (M2) macrophages will be generated by the action of mM-CSF and subsequently polarized with the anti-inflammatory cytokines mIL-4, mIL-10 and mIL-13 to obtain at least three different forms of alternatively macrophage activation.

ImmunoTools *IT-Box-Cy55M* for **Damiano Rami**
includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS