GESINAS - ImmunoTools Award 2016

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Targeting colorectal cancer: a macrophage immunomodulatory-delivery system

Tumour-associated macrophages (TAMs), key regulators of the immune response, have been reported to promote tumour progression and negatively impact responses to anti-cancer therapies. The macrophage functional plasticity to microenvironmental signals makes them susceptible to the influence of cancer cells, but also points them as promising therapeutic targets.

Macrophages can be differentiated in distinct phenotypes. M1-like macrophages are mainly induced by interferon-gamma (IFN-γ), microbial products and other cytokines. They are associated with pro-inflammatory/microbicidal/tumoricidal activities, antigen presenting capacity, secretion of pro-inflammatory cytokines, nitric oxide, reactive oxygen intermediates. On their turn, M2-like macrophages are induced by IL-4, IL-13, IL-10 or glucocorticoid hormones. The most extreme M2 subpopulation (IL-10-induced) presents low IL-12/IL-6 levels, high IL-10 secretion, ability to scavenge and tissue-repair, and they promote proteolysis, angiogenesis and tumour progression. Recently, our group demonstrated that IL-10-induced-macrophages are the most efficient in stimulating cancer cell invasion, motility/migration and proteolysis *(AP Cardoso et al., BMC Cancer,2015)*. Due to their plasticity, macrophages can be reverted upon a strong polarization stimulus. Our group demonstrated that chitosan (Ch) films itself elicits dendritic cells (DCs) towards a pro-inflammatory while inducing macrophage differentiation to an anti-inflammatory profile *(MI Oliveira et al., Eur Cells Mat,2012)* and that Ch and poly-γ-glutamic acid (γ-PGA) nanoparticles incorporating an anti-inflammatory drug decrease macrophage activation *in vitro (R. Gonçalves et al., J Mat Sci: Mat Med,2015).*

Thus, the major goal of this project is to develop a more complex delivery system based on the incorporation of an immunomodulatory cytokine on Ch/γ-PGA

nanocapsules to modulate the resident and recruited macrophages towards a pro-inflammatory/anti-tumour profile, opening new perspectives for immunotherapies.
To test this hypothesis, we first differentiated macrophages derived from healthy blood donors, will be subjected to Ch/PGA nanocapsules stimulation, incorporating or not the immunomodulatory agent. Macrophage populations will be analyzed by flow cytometry for the expression of lineage and activation markers (CD45, CD68, CD14, CD80, CD86) and M1/M2 phenotype markers (HLA-DR, CD163, CD206) and will be also evaluated the capacity of macrophage production of cytokines/chemokines (IL-4, IL-6, IL-8, IL-10, IL-12, IL-23, TNF-α) by flow cytometry and ELISA. The impact of nanocapsules will be also evaluated on DCs and T lymphocytes, and the surface markers (CD1a, CD11c, CD11b, CD3, CD4, CD8) will be analyzed by flow cytometry. As efficient controls of M1 and M2 macrophage polarization, human macrophages will be stimulated with LPS and IL-10 (see article from our team published in *Oncogene*, 2013).

The biological relevance of our *in vitro* findings will be assessed, taking the advantage from our acess to human colorectal cancer (CRC) samples from the Tumour Bank of Hospital S. João (Porto), when besides the tumour samples, we have acess to patients' clinicopathological information. Thus, the impact of Ch/PGA nanocapsules on macrophage populations and their relation with other immune cells and cancer cells will be evaluated by flow cytometry and immunohistochemistry on human CRC tumour specimens.

The ImmunoTools collection of reagents, recombinant cytokines and flow cytometry antibodies selected will be essential for an comprehensive evaluation of changes induced by nanocapsules on macrophage and other leukocytes surface receptors and secreted cytokines involved on macrophage polarization and migration. With the support of ImmunoTools our work will bring new vision on how immunotherapy modulates macrophage behaviour and how this affects macrophage/cancer cell communication, opening new perspectives for immunotherapeutic intervention.

GESINAS-Award application:

During the last years, I worked as volunteer of RED CROSS, at Guimarães, Portugal, participating in several social projects with children, in the area of health, education and child prevention from traffic accidents. I also worked as volunteer at *"Child care association"*, at Guimarães.
More recently, I participated in several scientific divulgation sessions to the non-scientific community at Life and Health Research Institute (ICVS), at Braga, Portugal. Now, at Institute for Biomedical Engineering (INEB)/i3S, I am also involved in scientific sessions directed to children, about bone formation.

GESINAS ImmunoTools AWARD for **Flávia Castro** includes 49 reagents

FITC - conjugated anti-human: CD1a, CD3, CD11c, CD14, CD33, CD35, CD47, CD80, CD86, CD105, CD147, HLA-DR, Control-IgG1, Control-IgG2a, Annexin V

PE - conjugated anti-human: CD1a, CD3, CD4, CD8, CD14, CD95, IFN-gamma, IL-8, TNFa, Control-IgG1, Control-IgG2a

PerCP - conjugated anti-human: CD3, CD4, CD8, Control-IgG1, Control-IgG2a,

APC - conjugated anti-human: CD1a, CD3, CD4, CD8, CD11b, CD14, CD33, CD56, CD80, CD147, IL-6, Control-IgG1, Control-IgG2a

recombinant human cytokines: rh GM-CSF, rh IFN-gamma, rh IL-4, rh IL-10

soluble human receptors: rh CTLA-4 / CD152

DETAILS more AWARDS