ImmunoTools *special* Award 2018

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Deciphering the mechanism(s) of antigen presentation impairment in the cancer microenvironment

Colorectal cancer (CRC) is the third most common cancer in the world and one of the leading causes of cancer death. The pathogenesis of CRC relies on a complex of interactions between developing cancer and surrounding tissue, including the immune system. In this context, one of the most abundant tumor-infiltrating cell population is represented by tumor-associated macrophages (TAMs).

Although the original postulates proposed that macrophages were involved in anti-tumor immunity, compelling evidence support the notion that in the majority of cases these cells interact with tumor cells to promote the initiation, growth and metastasis of tumors. Moreover, the remarkable plasticity of macrophages makes them very sensitive to environmental factors, including the extracellular matrix (ECM).

Referring to CRC, strong evidence was provided that demonstrate that TAM facilitate tumor progression, growth and aggressiveness, primarily via modulation of ECM remodeling, tumor metabolism, angiogenesis and the tumor microenvironment. Therefore, TAM could serve as a target for CRC therapeutic treatment. A productive anti-tumor response requires the contribution of an effective antigen presentation by APC cells (macrophages and dendritic cells), via MHC-II, to CD4 T lymphocytes and it has been shown that MHC-II antigens are not expressed in about two third of colorectal cancers and the loss of MHC class II expression is associated with a decrease of tumor-infiltrating T cells and an increase of metastatic potential of CRC; however, the mechanisms responsible for the down-regulation of MHC-II in CRC remain to be elucidated.

In a recent work, we have demonstrated that the bacterium *Helicobacter pylori* induces the expression of the immune receptor CD300e in macrophages. CD300e is an immune receptor belonging to a family of seven receptors that includes activating and inhibitory members, expressed on myeloid cells. Interestingly, we found that in *H. pylori*-infected macrophages, CD300e over-expression is accompanied by a decrease

in MHC-II exposure and to a consequent reduction of their presentation/activation capacity towards CD4 T lymphocytes. Most importantly, also the activation of CD300e by an agonistic monoclonal antibody leads to a decrease in MHC-II exposure and to a consequent reduction of the presentation of bacterial antigens on the surface of macrophages, resulting in an impaired activation capacity of macrophages towards CD4 T lymphocytes.

Based on the evidence we got from *H. pylori*-infected macrophages revealing that the activation of CD300e triggered the down-regulation of MHC-II molecules, we plan to assess whether the immune receptor could be involved in the loss of MHC-II also in macrophages exposed to the CRC microenvironment.

The objective of this project is to understand the mechanisms that regulate the antigen presentation process on macrophages in CRC; we plan to address the problem by evaluating the contribution of tumor cells in the impaired ability of macrophages to present tumor antigens to CD4 T lymphocytes.

To pursue our objective, we plan to analyze the axis CD300e/MHC-II and evaluate the functional profile in monocytes exposed to colon cancer cell line conditioned medium (CRC-CM); with this aim monocytes cultured in presence of CRC-CM will be evaluated in terms of CD300e and MHC-II expression. Moreover, the phenotype acquired by the immune cells will be evaluated in terms of surface markers typically expressed by pro-inflammatory M1-macrophages or anti-inflammatory M2-macrophages (CD86, MHC-II, CD163 and CD206), as well as genes' expression and cytokines/chemokines and metalloproteases (MMPs) release. Furthermore, in both conditions, the monocytes/macrophages antigen presentation capacity, will be assessed.

This study could add a piece of information about the pathway of antigen presentation and it could help the path for the development of systems aimed at increasing the immune response against CRC.

ImmunoTools *special* Award for **Sara Coletta** includes 25 reagents

FITC- conjugated anti-human: CD86

PE - conjugated anti-human: CD16, CD163, HLA-DR

PerCP - conjugated anti-human: HLA-DR

APC - conjugated anti-human: CD1a, CD8, CD86, HLA-DR

Human ELISA-set (for one 96 plate): human IL-1beta, human IL-6, human MIP-4, human TNF-a

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