ImmunoTools *special* Award 2019

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Crosstalk between Mast cells and epithelial cells in colon organoid development

Background

Mast cells (MCs) are long living innate immune cells widely distributed in mucosal and connective tissues, in close contact with blood vessels and the external environment. MC can be found in almost any tissue, but they are more present at tissues' barriers where they act as immune sentinels, both in homeostatic and in infectious conditions. Since they reside at the interface with the external environment, they are prone to rapidly respond to pathogen interaction[1]. When MC are activated, they start a process defined degranulation, that is the massive exocytosis of all the compounds stored in their granules, and which is the basis of the rapidity of their response[2].

The intestinal mucosa is a complex system in which several elements, namely the nervous system, the microbiota and the immune system, coexist. All these elements are closely connected to each other, so the variation of anyone of them can lead to strong alterations of the whole system.[4] Mast cells, which represent about 2-3% of the resident immune cells at these sites, reach the intestine by the interaction of α4β7 integrin with several molecules expressed from the endothelial cells.[5]

Although several works[6,7] reported that there is an increase in mast cell numbers in the colon of patients suffering from Chron Disease (CD) and Ulcerative Colitis (UC), where they can be activated and release all the mediators which can in turn affect the integrity of the barriers, an evidence of their direct effect is still missing.

Objectives and Methods

In this scenario, the aim of my project is to study the role of terminally differentiated mast cells on colon organoids architecture. To answer this question, I used 3D culture of colon organoids. To this end, organoids were obtained from Lieberkühn crypts, as described

before[8], and co-cultured in presence of CFSE-labeled differentiated bone marrow derived mast cells (BMMCs) for up to one week. We observed that the mast cells are chemo-attracted by the organoid, and that they localize on the surface of the same, at the level of the lamina propria, site where they can be observed physiologically *in vivo*. The differentiation rate of the organoids was found to be increased in the presence of mast cells as demonstrated by decrease in the mRNA levels of Lgr5, one of the most important marker of stemness in the colon crypt, in the co-cultures. Moreover, by means of immunofluorescence (IF) staining, the expression of two structural markers of colon organoids such as Ezrin (an apical protein that delimits organoids lumen) and Claudin-4 (a tight junction that is expressed throughout all gastrointestinal tract, but especially in the colon) were found to be influenced by the presence of the mast cell. Preliminary IF staining reported that there is a general reorganization of both Ezrin and Claudin-4 in presence of IgE-Ag activated mast cells, underlying a deep involvement of the mast cells mediators in the polarization and permeability of the colon organoid.

Given these preliminary data, we would like to further evaluate the role of the mast cells on the architecture of colon organoids in different microenvironments. Our idea is to mimic diffident in vivo inflammatory microenvironments in our in vitro 3D culture organoids-mast cell co-cultures through the addition of several cytokines, known to have an effect on colon cells both in homeostatic and inflammatory conditions, in the co-cultures medium. For this reason, we kindly ask **Immunotools** to provide us with the following products:

The mouse cytokines mIL-6, mIL-33, mIL-22, mIL-15, mTNFα, mIL-19 and mIL-10 to simulate a pro- or anti-inflammatory microenvironment, and since we are planning to transpose the whole study also on human, we would need also the human cytokines hIL-6, hIL-22, hIL-15, hIL-19, hTNFα and hIL-10. Then, in order to evaluate the levels of human mast cells mediators released in the co-cultures, we would need ELISA kit for h-IL-6 and h-TNFα.

Finally, our next goal will be to add to our co-culture model also B cells derived from mouse spleen. For this reason, we will need m-NK-cell antibody PE, and m-CD4 PE to isolate B cells from the spleen FACS-sorting, and m-CD19 APC and m-CD80 PE to analyze the purity and activation status of B cell population after the co-culture.

ImmunoTools *special* AWARD for **Dr. Chiara Dal Secco** includes 25 reagents

Mouse recombinant cytokines:	mIL-6, mIL-33, mIL-22, mIL-15, mTNFα, mIL-19, mIL-10
anti-mouse Antibodies:	m-NK-cell PE, m-CD4 PE, m-CD19 APC, m-CD80 PE
Human cytokines:	hIL-6, hIL-22, hIL-15, hIL-19, hTNFα, hIL-10
Human ELISA set:	h-IL-6 and h-TNFα

DETAILS more AWARDS

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