ImmunoTools *special* Award 2016

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Towards the understanding of the interaction between Innate Lymphoid Cells (ILCs) and the endothelium

Innate Lymphoid Cells (ILCs) were recently identified as a distinct family of lymphocytes playing an important role in immunity. In contrast to adaptive lymphocytes, ILCs are devoid of antigen receptors and are stimulated by the cytokine environment. They are divided into 3 subgroups, i.e. ILC1, ILC2 and ILC3, which mirror the functional specialization of CD4 T cells. ILCs were shown to cross talk with other immune cells and to influence tumor growth in mouse models. ILCs seem to exert both pro- or anti-tumorigenic effects, depending on the ILC subset and the tumor type.

Interestingly, one of the mechanisms responsible for their anti-tumorigenic roles involves their capacity of modulating blood vessels' permeability and adhesion molecule expression on endothelial cells in the tumor microenvironment, facilitating the infiltration and eraditcation of the tumor by adaptive immune cells. Yet, ILCs characterization and their role in anti-tumor immunity in humans remain to be investigated.

The aim of my project will be to investigate the molecular interactions between human innate lymphoid cells and human blood and lymphatic endothelial cells. To gather this main aim, I will answer to two different questions: (i) are ILCs able to influence the permeability of the vessels?, and (ii) are ILCs able to modulate the capacity of endothelial cells to form new vessels?

ILC lines (either total ILCs or ILC subsets) will be generated after direct *ex-vivo* sorting of peripheral blood ILCs by a protocol established in the host laboratory. For the identification and the subsequent sorting I will use a large panel of antibodies (FITC conjugated anti-human CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD33, CD203c, FcεRI; PE conjugated anti-human CRTH2 (CD294); APC conjugated cKit (CD117)).

ILCs will be co-cultured with endothelial cells (blood and lymphatic cell lines (HUVEC/LEC/BEC)) either in resting or after pre-activation with specific recombinant cytokines that can specifically activate the different ILC populations. In particular, ILC1 will be stimulated by combinations of rh IL-12 and rh IL-18, ILC2 by combinations of rh IL-25, rh IL-33, rh TSLP and rh PGD2, ILC3 by combination of rh IL-1β and rh IL-23. After 4, 24, 48 and 72 hours of co-cultures I will assess the expression of endothelial markers by qPCR and flow cytometry. As positive control I will use endothelial cells stimulated by rhTNF-α. The following markers will be considered for

the analysis: VE-cadherin, CD105, ICAM-1, VCAM, MadCAM, JAMs, ESAM, IL-22R, Podoplanin. VEGFR3, and STAT3 activation. If results with HUVEC will be negative, microvascular EC (e.g. skin-derived) will also be used.

In parallel, the ability of ILCs to influence the *in vitro* capacity of endothelial cells to form vessel structures in matrigel will be assessed and monitored by microscopy observation. Also in this setting I will use endothelial cell pre-cultured in presence of either ILC1, ILC2 or ILC3 cell lines. ILCs will be pre-activated or not as described above with different combinations of specific human recombinant cytokines. As positive control, endothelial cells will be cultured in presence of either rh VEGF-121 or rh VEGF-165.

In a second step, in order to elucidate if ILCs act in a contact-dependent or soluble factor-dependent manner, experiments will be performed either by using transwells and/or by adding ILC-conditioned medium to the endothelial cells. The supernatants will be collected for the analysis of secreted factors. The putative modulators will be blocked in subsequent assays by using neutralizing antibodies (e.g. anti-TNFα, anti-IFNγ,…).

ImmunoTools anti-human antibodies for flow cytometry that fit with my project: FITC - conjugated anti-human CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD33 will allow (partially) the detection and the sorting of ILCs. ImmunoTools recombinant human cytokines that fit with my project: rh IL-1beta /IL-1F2, rh IL-12, IL-23A, rh TNFα, rh TSLP, rh VEGF-121, rh VEGF-A/ VEGF-165 will allow (largely) the stimulation of the different ILC subsets and of the endothelial cells to have a positive control for the assays that I have planned to perform. ImmunoTools human ELISA-set (for one 96 plate) that fit with my project: human MCP-2 (CCL-8), human MIP-4 (PARC) will allow to test the presence of modulators of the lymphocytes/monocytes migration that I have not yet tested. Their modulation would be an intriguing possibility, since in my preliminary experiment I saw a modulation of MCP-1 by ILCs.

ImmunoTools *special* AWARD for **Giulia Vanoni**

includes 21 reagents

FITC - conjugated anti-human CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD33

recombinant human cytokines: rh IL-1beta /IL-1F2, rh IL-12, IL-23A, rh TNFα, rh TSLP, rh VEGF-121, rh VEGF-A/VEGF-165

human ELISA-set: human MCP-2 (CCL-8), human MIP-4 (PARC) for 96 wells, (each 3 reagents)

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