PROJECT 1

DoS: Guido Poli

Title: **Role of M1/M2 Polarization of Human Macrophages in Pathogenic RNA Virus Infection**

Curriculum: BAIO

Link to OSR/UniSR personal page:
https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/human-immuno-virology.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Macrophages are plastic cells capable of activate differential programs in response to environmental signals such as those induced by pro-inflammatory and anti-inflammatory/tissue remodeling cytokines, to give rise to "M1" and "M2" cells, respectively. Both polarization profiles have been already implicated in the defense, but also pathogenesis of certain infections and cancer. We have also earlier investigated the role of primary human monocyte-derived macrophages (MDM) polarization in HIV-1 infection by short-term (18h) stimulation with pro-inflammatory cytokines (IFN-gamma plus TNF-alpha) or IL-4 in order to generate M1 and M2-MDM that have been then infected with HIV-1. Both M1 and M2 polarization resulted in variable levels of restriction of virus replication, with M1 polarization being significantly more profound and associated, among other anti-viral features with the upregulation of apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)-3A, a candidate restriction factor (RF) previously associated with the limited capacity of monocytes to support productive HIV-1 infection [1]. We have recently published that a second round of M1 polarization several days after the infection lead to almost undetectable levels of virus production, while stimulation of these cells by allogeneic T cell blasts rescued viral replication [2]. In addition, we have preliminary results indicating that Zika virus (belonging to Flaviviridae) productively infect human MDM and it is restricted by M1, but not M2 polarization and that the human coronaviruses responsible for SARS can also replicate in these cells (unpublished observations). Based on these findings, the project will pursue both specific and general goals. In particular:

Aim 1 (HIV). Dissecting out the potential role of APOBEC-3A, and of related RF such as APOBEC-3G, -3F and -3H as well as of SAMHD1 and TRIM22 [3], in the restricted phenotype of $M1^2$–MDM infected with HIV-1 siRNA knock-down as first approach. Investigating whether cytokines or current latency-reversing agents could mimic allogeneic T cell blast in terms of rescuing virus production from $M1^2$–MDM.

Aim2 (Zika Virus, human coronavirus). Define whether M1/M2 polarization can influence the replicative and cytopathic profile of these viruses with human MDM and define potential molecular correlates of viral restriction.

Aim 3. Define similarities and differences in M1/M2 polarization of human MDM in terms of modulation of multiple virus replication and cytopathicity.

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

- Working safely in a biosafety level 3 (BSL-3) environment;
- Safe manipulation of infectious viral strains (HIV-1, Zika Virus, SARS-CoV-1 and SARS-CoV-2);
- Purification of monocytes from peripheral blood leukocytes, their differentiation into MDM and polarization according to M1/M2 protocols;
- Multiparametric FACS analysis of cell surface and intracellular markers of macrophage differentiation and viral protein expression;

- Molecular biology techniques for quantification of HIV nucleic acids (RNA, DNA) and of host cell RF and transcription factors;
- Protein characterization by Western blotting and immunoprecipitation.

References (max. 15)

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3. E. Saba, P. Panina-Bordignon, I. Pagani, M. Origoni, M. Candiani, C. Doglioni, G. Taccagni, S. Ghezzi, J. Alcami, E. Vicenzi & G. Poli. 5-Hydroxytyrosol inhibits HIV-1 replication in primary cells of the lower and upper female reproductive tract. *Antiviral Research 2017, 142:16-20, 2017.*

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