PROJECT 2

DoS: ALESSANDRA MORTELLARO

Title: Unraveling the role of Nod-like receptors and inflammasomes in maintenance, proliferation and lineage specification of multipotent hematopoietic stem and progenitor cells

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page: http://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/pathogenesis-and-therapy-of-primary-immunodeficiencies/index.html

Project description:

The innate immune response serves as the first line of defense against microbial infections by detecting pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) through pattern recognition receptors (PRRs). PRRs belonging to the nucleotide oligomerization domain (NOD)-like receptors (NLRs) are a specialized group of proteins that play a critical role in the recognition of intracellular PAMPs and DAMPs. Some NLR proteins form large complexes, called inflammasomes, which activate caspase-1 and induce the production of active IL-1β and IL-18. Activation of NLR proteins can also cause cell death. Among NLR inflammasome complexes, the NLRP3 inflammasome has been the most widely characterized. Gain-of-function mutations in genes encoding inflammasome proteins, including NLRP3, NLRC4, and Pyrin, cause various periodic hereditary syndromes or autoinflammatory diseases [cryopyrin-associated periodic syndrome (CAPS) for NLRP3, familial Mediterranean fever (FMF) and pyrin-associated autoinflammation with neutrophilic dermatosis for MEFV]. The selective blockade of IL-1β with anakinra (IL-1 receptor antagonist) demonstrated the efficacy of targeting IL-1β for treatment of these conditions. However, some symptoms did not resolve, suggesting that they may be caused by excessive IL-18 production or inflammatory cell death. Therefore, alternative therapeutic options for CAPS are needed. Bone marrow transplant and gene therapy may represent potential therapy that can offer long-term correction of inflammasome hyperactivation. However, whether NLRs and inflammasomes regulate the function of hematopoietic stem and progenitor cells (HSPCs) is not clear. Therefore, a better understanding of the underlining mechanisms regulated by NLRs and inflammasomes in HSPCs will significantly contribute to the identification of targeted therapeutic strategies for these autoinflammatory syndromes. In this context, the goals of this Ph.D. project are:

1) To investigate the physiological consequences of unrestrained NLR and inflammasome activation in HSPCs during steady-state and "emergency" hematopoiesis, and infectious stress.
2) To determine the feasibility and efficacy of a genome editing–mediated correction of NLRP3 cryopyrinopathies in mouse HSPCs and induced pluripotent stem cells derived from patients' fibroblasts.

To address these aims, the student will be supported by a team of renowned experts comprising research scientists with experience in basic and translational research in the field of NLR/inflammasome, auto-inflammation, hematopoiesis, and genome editing.

Skills to be acquired by the student:

- Molecular biology techniques: cDNA cloning in a plasmid, gene delivery, DNA and RNA sequencing, RNA isolation and real-time qPCR, gel electrophoresis.
- Cellular biology techniques: ELISA, Western blot, immunoprecipitation of proteins, immunofluorescence microscopy, flow cytometry and cell sorting.
- Cell culture techniques: isolation of immune cells from blood, mouse bone marrow and spleen, maintenance of immune and non-immune cell lines.

- Basic and advanced in vivo experimentation techniques: mouse handling, generation and maintenance of colonies, genotyping, administering injections, bone marrow transplant, isolation of immune organs.
- The student will be prepared to integrate scientific method and critical thinking, to present in English information clearly and effectively to small/medium audience. The student will be empowered with effective writing skills.

References

- Viganò E, Diamond CE, Spreafico R, Balachander A, Sobota RM, Mortellaro A. Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. *Nat Commun.* 2015 Oct 28;6:8761.
- Khameneh HJ, Mortellaro A. NLRC4 gets out of control. *Nat Genet.* 2014 Oct;46(10):1048-9.
- Zambetti LP, Laudisi F, Licandro G, Ricciardi-Castagnoli P, Mortellaro A. The rhapsody of NLRPs: master players of inflammation...and a lot more. *Immunol Res.* 2012 Sep;53(1-3):78-90.