Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Creation of gene-deficient retrogenic mice

Synopsis: Several events contribute to the process of T cell activation and differentiation. Many component of the activation system are known in detail and conserved between mouse and human. Protein interactions are critical in these events, undertaking translocation, degradation, post-translational modification and conformational changes. Expectantly, we discovered a novel player affecting T cell activation. This protein is highly expressed in immune cells, particularly lymphocytes, but its function is largely unexplored.

To understand its role in lymphocytes we crossed gene floxed mice with mice expressing Cre recombinase in lymphoid cells (therefore, these mice lack its expression only in T and B cells). In these animals, conditional deletion results in a marked reduction of T cells in the periphery, while B cells numbers largely unaffected. The decrease is evident both in the CD4 and CD8 lineage, is more marked with age and affects the naïve T cell compartment predominantly. Remarkably, its-deficiency completely abolishes TCR- and ionophore- induced calcium flux in T cells, but not in B cells. *Therefore, we hypothesise that this protein is a novel and critical factor in T cell activation and homeostasis.*

In order to test this hypothesis, this project will create TCR transgenic cells that are deficient for our protein of interest (or proficient). This is achieved by transducing bone marrow cells from protein-deficient mice with the sequence coding for the TCR of interest (see 10.1038/nprot.2006.61). These animals will be an invaluable tool to address the role of the novel protein in TCR activation, as we will have complete control over the TCR cognate peptide.

The first goal of the project will be to produce the retrovirus coding for the TCR sequence. Techniques: cell culture, bacteria transformation, DNA purification, DNA transfection.

Once the virus is produced, the student will optimize the transduction of murine bone marrow cells with such retrovirus. These cells will then be purified and transferred into a recipient mouse in order to create a chimera mouse in which all T lymphocytes will express one particular TCR (as opposed to polyclonal wild type mice). Tasks: bone marrow harvesting, processing, and purification, cell culture, flow cytometry and cell sorting.

Finally, the protein-of-interest-deficient retrogenic mice will be studied at steady-state as well as in the context of infection, memory formation, etcetera. Splenocytes will also be used for in vitro experiments.
Tasks: collect, process and purify lymphocytes from mouse spleens; culture lymphocytes *in vitro* for several days; flow cytometry, cell count, qRT-PCR.

The original paper describing the protocol we are trying to reproduce: 10.1038/nprot.2006.61

Requirements:
-previous experience with *in vitro* cell culture is essential

Instituto de Medicina Molecular João Lobo Antunes

-previous experience with DNA work is highly favored
-basic flow cytometry skills are a plus but not necessary
-The project involves some animal handling
-independent and inquisitive
-organized, accurate and good planner
-flexible with working hours
-able to communicate well in English

Supervisor: *Silvia Ariotti, Marc Veldhoen Lab, silvia.ariotti@medicina.ulisboa.pt*
Co-Supervisor: *Marc Veldhoen, Marc Veldhoen Lab, marc.veldhoen@medicina.ulisboa.pt*
Webpage of the group