Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: The role of a new protein in the activation of T and B lymphocytes

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, we want to compare T (and B) cell activation of wild type and protein-deficient lymphocytes. According to our hypothesis, we would predict that deficient T cells do not respond to activation stimuli (such as aCD3 antibody) as efficiently as wild type cells; in fact, we have collected preliminary *in vitro* data that confirm this. Surprisingly though, we did not observe significant differences between knock out and wild type animals in the *in vivo* experimental setting.

The first goal of the project will be to confirm and consolidate the in vitro and in vivo data. Tasks: collect, process and purify lymphocytes from mouse spleens; culture lymphocytes *in vitro* for several days; analysis T-cell activation by flow cytometry, cell count, qRT-PCR.

In parallel, the student will optimize the transduction of murine lymphocytes with lentiviral constructs coding for the protein in order to confirm that deletion of it is the cause of the observed phenotype (and therefore reintroduction of the protein rescues the lymphocytes). Tasks: transfection of cell lines for lentiviral production, transduction of lymphocytes.

The in vitro and will be validated in vivo and to understand intrinsic defect and pathways in which this protein is involved, for example the protein-deficient T cells cannot produce a cytokine upon stimulation; this would cause death in vitro, while in vivo other cells could provide IL-2 and rescue lymphocytes. Alternatively, strong non-productive TCR engagement could cause T cell apoptosis unless supported by a plethora of co-stimulating molecules (available in vivo but not in purified T-cell cultures).

In this second phase the student is invited to propose biologically relevant hypotheses for the potential *in vitro/ in vivo* action, as well as design experiments to test such hypotheses.

Requirements:

-strong interest in Immunology
-previous experience with *in vitro* cell culture is a must
-basic flow cytometry skills favored but not essential
-the extent of the involvement in the *in vivo* work will be discussed will involve some animal handling
-independent and inquisitive
-organized 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