Short-term Staff Exchange - TwinnToInfect

Activity plan Report

iMM Lisboa Lab	Silva-Santos lab
iMM Lisboa Staff	Julie Ribot (post-Doc) and Pedro Papotto (PhD student)
Partnering Lab	Gitta Stockinger lab, Francis Crick Institute, London
Expected duration of the activity	5 days
Expected starting date	25/07/2016

Objectives of the activity

The main focus of our project is to characterize the peripheral homeostasis of IL-17 producing γδ T cells. We are currently reaching a stage of our project where we needed a reliable model to confirm our working hypothesis at steady state conditions. For this purpose, we benefited from the IL-17-eYFP fate mapping reporter mice developed by Prof. Gitta Stockinger and available in her research lab from the partner institution.

Activity plan

Monday, 25th July:
Julie: Dissect meningis from 4-5 IL-17 reporters and analysis by FACS (Fortessa x20) for the production of IL-17 by γδ T cells.
Staining : (FITC chanel is left free for eYFP signal) LiveDead, CD45, CD3, TCRd, CD4, CD8, IL-17, IFN-g.
Pedro: Dissect LNs and spleens from the same 4-5 IL-17 fate reporters and sort (Aria) live CD3+γδ +YFP- T cells. Culture sorted cells with type 17-driving cocktails (IL-1b+IL-23; IL-1b+IL23+aCD3; IL-7)

Tue 26th July:
Same as the day before (for both Julie and Pedro), including FACS analysis and sorting

Thu 28th July:
Pedro: Experiment 1 analysis (FACS Fortessa x20)

Fri 29th July:
Pedro: Experiment 2 analysis (FACS Fortessa x20)

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Activity Final Report

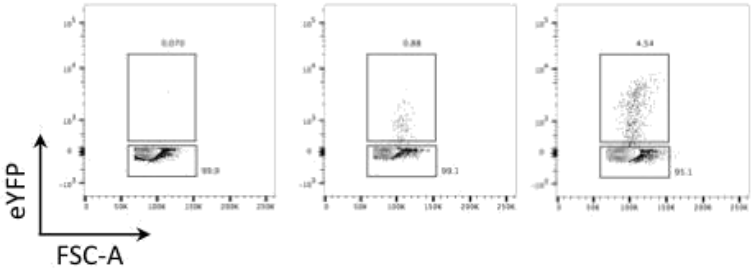
Were the initial objectives achieved?

The initially predicted objectives were achieved and no deviations to the initial activity plan have been needed. Moreover, the expected benefits for iMM Lisboa Lab as well as for the staff involved in the activity were fulfilled, as described below.

1- DE NOVO DIFFERENTIATION OF eYFP+ ©™ T CELLS FROM eYFP(-) PRECURSORS

Our working hypothesis is that there are two types of IL-17+ γδ T cells: “natural” which develop in the thymus from fetal liver progenitors (as shown in Haas et al. Immunity 2012); and “induced” bone marrow-derived peripheral IL-17 producers. This is supported by Pedro’s bone marrow chimeras which generate IL-17+ γδ T cells only upon EAE induction (but not in the steady-state).The data collected in Gitta Stockinger’s lab allowed us to validate this hypothesis *in vitro*; and identify factors required for their differentiation: the minimal condition being IL-1b + IL-23 and the optimal conditiona cocktail of innate cytokines + TCR stimulation.

Below are some of the key plots:



IL-7	+	+	+
IL-21	-	+	+
IL-6	-	+	+
TGF-β	-	+	+
IL-1β	-	+	+
IL-23	-	+	+
α-CD3	-	-	+

2- FATE MAPPING OF IL-17 PRODUCING γδ T CELLS IN THE MENINGIS

We have recently identified a population of IL-17 producing γδ T cells that infiltrate the brain meningis and impact on the learning/memory capacities of WT mice. We thus took advantage of the IL-17-eYFP fate mapping reporter mice available at the partner institution in order to document this in situ IL-17 production by meningeal γδ T cells.

Below are some of the key plots: