ImmunoTools *special* Award 2015

Florence Kauffmann, PhD-student

Supervisor: Prof. Dr. Stefan Magez

VIB Structural Biology Group/VUB, Laboratory of Cellular and Molecular Immunology, Pleinlaan 2, 1050 Brussels, Belgium

Towards understanding the immunopathology upon *Leishmania* infections.

Leishmaniasis is a neglected tropical disease, transmitted by the bite of *Plebotomus spp.* sand flies. The disease has several forms, including cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). *L. major* causes debilitating wounds months after infection, leaving ugly scars on the skin of the patient, whereas *L. donovani* is fatal if left untreated. Currently employed drugs against leishmaniasis are associated with high cost, severe toxic side effects and increasing parasite drug resistance. To date no vaccine has been commercialized for use in humans. It is of uttermost importance to understand the immunity generated against these pathogens, as they might be used for cross-protection in future vaccination studies.

1. "Characterization of a new *L. donovani* strain in the mouse and hamster model".

Most of the murine infections have been carried out with the *L. donovani* reference strains L82/LV9. Infection in the liver is controlled, whereas the spleen displays uncontrolled amastigote growth, massive micro-architecture remodelling, leading to severe immunosuppression. Few *L. donovani* strains are currently used for *in vivo* studies of visceral leishmaniasis, due to the difficulty of adapting these strains to the *in vivo* hamster/mouse model. The ITM has provided us with a clinical field *L. donovani* strain (BPK282) from a Nepalese patient. This strain has recently been adapted to hamsters. The aim of this study is to describe the infection characteristics of this BPK282 strain *in vivo* and compare it with the reference L82 strain by looking at parasitemia levels, hepatosplenomegaly, cellular composition at the level of B cell, T cell and myeloid cell subsets and the histological integrity in the spleen and the liver during the course of infection. To do so, we are routinely using anti-mouse antibodies for the detection of different B cell subsets (using anti-B220, AA4.1, CD1d, CD21, CD23 and CD138), T cell subsets (using anti-TCRb, CD3, CD5, CD4, CD8), NK cells (anti-NK1.1 and NKp46) and myeloid cell types (using anti-CD11b, Ly6C, Ly6G, MHCII, F4/80) for flow cytometry.

Tumor Necrosis Factor (TNF) is an important factor for granuloma formation and hence parasites control in the liver and a mediator in the destruction of the splenic

micro-architecture during *L. donovani* infections. Moreover, $TNF^{-/-}$ mice infected with *L. donovani* have been shown to have higher parasitemia levels in spleen and liver, less granulomas in the liver and to preserve the splenic micro-architecture upon *L. donovani* infection. Moreover, $TNF^{-/-}$ have been reported to succumb between 6-10 weeks after *L. donovani* infection. In order to elucidate whether TNF is differentially involved in the immune response upon infection with either of these two *L. donovani* strains, we have infected TNF-deficient mice with either L82 or BPK282. Hence, we continuously use anti-TNF antibodies for ELISA assays.

We also have set up a flow cytometric assay to study CD4 T cells, CD8 T cells and B cells in the hamster spleens. Therefore, we use cross-reactive antibodies against CD4, CD8 and MHCII to be used for flow cytometry.

2. "Refining the role of Th1/Th2-associated molecules in the immunopathology during *L. major* infections".

CL exhibits a typical T helper 1 (Th1) / T helper 2 (Th2) dichotomy. C57BL/6 resistant mice develop self-healing lesions mediated by a Th1 response that leads to the production of IL-12 and IFN-γ, which lead in turn to the production of nitric oxide (NO) by parasite-containing macrophages. As a consequence, the parasite is eliminated. In contrast, in the susceptible BALB/c mouse strain the T-cell response is dominated by Th2-cells and the production of IL-4 and IL-13. This Th1/Th2 paradigm makes *L. major* an interesting tool for studying the impact of T-helper cell differentiation on the *L. major*-associated healing/non-healing phenotype. In this project, we aim to elucidate the impact of impairing either the Th1- or Th2-differentiation process or both simultaneously by using several knock-out mice, on the outcome of the disease caused by *L. major* intradermal footpad infection, using IL-12p40 (a Th1-associated cytokine), STAT6 (a Th2-associated transcription factor) and IL-12p40/STAT6 double KO mice on a susceptible BALB/c background that we infect with the *L. major* IR75 strain. Parameters such as footpad swelling, parasitemia, cytokine production and immune cell composition are currently being investigated.

In order to conduct our investigation, we use several anti-mouse antibodies for flow cytometric analysis, immunhistofluorescence and ELISA (anti-Ly6G, F4/80, CD11b, MHCII, CD45, Ly6C, IFNy, IL-4, IL-13, IL-12).