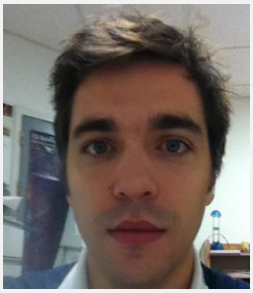
ImmunoTools *special* Award 2015



Alexis Chenouard, PhD-student

Supervisor: Dr Sophie Brouard

UMR 1064 Center of research in transplantation and immunology, 30 bd Jean Monnet 44093 Nantes, France

Blood T follicular helper cells in operational tolerance

In humans, long-term acceptance of mismatched kidney allograft after immunosuppressive drug withdrawal, defined as operational tolerance, can occasionally be observed. However, the mechanism behind this state is yet unknown. Operationally tolerant patients display a “B cell footprint” with a higher number of blood B cells and transcriptional B cell signature compared to transplant patients with a good graft function under immunosuppression (stable patients). Recently, we have shown that B cells from drug-free tolerant patients did not fully terminally differentiate into plasma cells, which might be deleterious in transplantation by donor-specific antibodies secretion.

Blood T follicular helper (Tfh) cells play a crucial role in regulating adaptive immune response against foreign antigens. Recently described in autoimmunity and infectious diseases, this subset of $CD4^+$ T cells induce B cell differentiation, principally through secretion of IL-21. Our hypothesis is that blood Tfh cells may play a role in the defect of B cell differentiation observed in operational tolerance. In this application, our objectives are to describe the blood Tfh cells (phenotype and cytokine secretion) and to investigate their role particularly on B cell differentiation in blood from tolerant patients compared to stable transplant patients under immunosuppression.

1) Patients and healthy volunteers

We compare 2 groups of patients: 1) Tolerant patients (TOL) with a stable kidney graft function (blood creatinemia < 150 µmol/L and proteinuria < 1g/24h) in the absence of immunosuppressive treatment for at least one year; 2) Kidney transplant recipients with

stable graft function under standard immunosuppression (STA) (proteinuria < 1g/24h and a stable creatinemia, variations < 25% for at least 3 years).

2) Characterization of blood Tfh cells

Firstly, we perform an immunophenotyping of blood Tfh from tolerant and stable patients with these anti-human monoclonal antibodies: **anti-CD4**, anti-CXCR5, anti-CXCR3, anti-CCR6, **anti-CD45RA**, anti-CCR7 and anti-PD1. In order to more precisely define these blood Tfh cells, we assess the intracellular expression of cytokines typically associated with Th2, Th1 and Th17 phenotype (i.e. IL-4, IFN-γ and IL-17) as well as IL-21, following PMA/ionomycin stimulation. Blood Tfh ($CD4^+$ $CXCR5^+$) are sorted using flow cytometry. Supernatants are then collected and analysed for the presence of **IL-4, IL-6, IL-21 and IFN-g by ELISA tests.**

3) Role of blood Tfh on B cell differentiation

To investigate the role of blood Tfh on B cell differentiation, we use a co-culture model. PBMCs from transplant patients are stained with **anti-CD4**, anti-CXCR5, **anti-CD45RA**, **anti-CD19** and **anti-CD27**. Blood Tfh ($CD4^+$ $CXCR5^+$) and naive B cells ($CD19^+$ $CD27^-$) are then sorted using flow cytometer and co-cultured (5.10^4 cells/well, ratio 1:1) with endotoxin-reduced staphylococcal enterotoxin B (SEB). Plasmablast (**$CD20^{lo}$ $CD38^+$**) differentiation is analysed at day 7 by flow cytometry. In the same manner, supernatants are collected to determine immunoglobulin concentrations.

Taken together, our investigation aim to characterize blood Tfh in tolerant patients and increase our knowledge on operational tolerance in kidney transplantation.