### THESIS TOPIC

**Subject N° (to be completed by the ED):**  
FUNDING: [ ] Requested  [x] Acquired  
Funding origin: Ecole Doctorale  

**Thesis title:**  
C – UN - A novel gut-derived microbiota-specific circulating human Treg subset: study of its role(s) in homeostasis and diseases  

**Unit / team:** UMR 1232 – Team 5 (Frédéric Altare)  
**Supervisor’s name:** Frédéric Altare & Emmanuelle Godefroy  
**Phone number:** 06 31 49 09 74 & 07 68 68 69 58  
**Email address:** frederic.altare@inserm.fr, emmanuelle.godefroy@inserm.fr  

**Keywords:** Immunology, Regulatory T cells, Microbiota  

**Socio-economic and scientific context (approximately 10 lines):**  
Microbiota affects immune homeostasis and susceptibility to diseases. It is therefore key to understand the underlying mechanisms. In this context, we have identified a subset of microbiota-induced FoxP3-negative regulatory T cells, named DP8α. We have also shown that their suppressive function depended on the CD39 ectonucleotidase activity. Moreover, our preliminary results showed that these Tregs induced naive B cells to secrete IgA *in vitro*, which support a contribution of DP8α Treg in host/microbiota mutualism. DP8α Tregs are abundant in the colon mucosa but also recirculate in the blood. Importantly, we have observed drastic alterations of this subset in several disorders, in terms of frequency and/or function, which support its role in preventing inflammatory bowel diseases and, kidney transplant rejection and Graft Versus Host Disease. These data strongly support that microbiota-induced DP8α Tregs play so far ignored roles in health and diseases. An in-depth characterization of these cells should provide diagnostic, prognostic and/or therapeutic applications for selected patient’s populations.  

**Working hypothesis and aims (approximately 8 lines):**  
DP8α Treg cells seem to play major roles in intestinal homeostasis, as shown in mice via *Clostridium-induced* FoxP3+ Tregs, and, more surprisingly, in allogeneic graft tolerance. It is thus of utmost importance to better characterize this subset and its regulatory functions. Main aims will be:  
1/ to identify the transcriptomic signature of colon- and blood-derived DP8α Tregs, as compared with Foxp3-positive Tregs  
2/ in-depth characterization of DP8α suppressive functions: CD39/CD73 pathway and other regulatory mechanisms such as cytotoxicity and suppressive cytokine production  
3/ identification of major physiological roles of DP8α cells: in gut IgA production and homeostasis as well as in transplantation tolerance  

**Main milestones of the thesis (approximately 12 lines):**  
- To reach the 1st aim, the 3’seq RNA profiling approach, developed in Nantes by the GenoBird core facility, will be applied to DP8α and Foxp3+ Tregs freshly FACS-sorted from the colonic lamina propria and the blood of 6 healthy donors.  
- To reach the second aim, we will delineate the role of adenosine production and/or eATP degradation through CD73 and/or CD39 activities using DP8α Treg clones already available (and to be produced). Clones will be used to study the contribution of cytotoxicity and IL-10 secretion in their suppressive function. The roles of these different pathways will be validated using CRISPR-Cas9 modified clones.  
- To reach the 3rd aim, we will determine the mechanism of IgA Class Switch Recombination (CSR). To this end, and following the identification of candidate molecules through transcriptome analysis, we will test the role of these molecules in an *in vitro* IgACSR assay using inhibitors and or CRISPR-Cas9 modified clones. The physiological contribution of DP8α Tregs in *in vivo* IgA production will be determined in collaboration with Pr Gorochov and Fieschi, who work on selective IgA-deficiency, looking for potential functional alteration of DP8α Tregs in these patients.  
Finally, the role of DP8α Tregs in transplantation tolerance will be addressed using an additional cohort of kidney transplanted patients by asking whether graft rejection can be predicted based on the following 3 variables: percent of DP8α Tregs among blood T cells, percent of DP8α Tregs expressing CD39 and percent of DP8α Tregs expressing CD73, as we have shown recently by logistic regressions analysis using PBMC from 30 tolerant and 20 rejection patients.  

**Scientific and technical skills required by the candidate (2 lines):**  
- Tissue culture, Cellular cloning, Biomarkers, Cell sorting, Multi-parameter cytometry, Immuno-staining, ELISA, Functional assays.  
- Immunology, Bibliography, Oral presentations, Scientific writing (publications, grants, patents)
3 publications from the team related to the topic (last 5 years):


**National and international collaborations:**

- Harry Sokol, UMR 1319 Micalis, Gastroenterology Department, St Antoine hospital, Paris
- Guy Gorochov, Pitié Salpêtrière, INSERM U1135, Paris
- Philippe Langella & Jean-Marc Chatel, ProbiHote team INRA, Micalis, Jouy en Josas
- Patrice Chevallier, Hematology Department, CHU of Nantes, Nantes
- Régis Josien & Jérôme Martin, U1064, CRTI, Nantes