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Title of the research project :

Role(s) of DC subsets in maternal-fetal immune tolerance

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Research Unit

Name : Immunology Immunopathology Immunotherapy Lab

Code *(ex. UMR xxxx)* :

UMR_S 959

Doctorate School

Thesis supervisor's doctorate school (candidate's futur doctoral school) : ED 394 - Physiologie, physiopathologie et thérapeutique

PhD student currently supervised by the thesis supervisor (number, year of the first inscription) :

Abstract: Successful pregnancies depend on maternal-fetal tolerance. Understanding the immune mechanisms of infertility and recurrent miscarriage is mandatory to improve care. We previously established that maternal regulatory T cells (Tregs) are necessary to protect the fetus from maternal immune response [1]. We also discovered that self-specific thymic-derived Tregs accumulate in the uterus-draining lymph nodes (LNs) and uterus and protect embryos at implantation [2, 3]. The homeostasis of Tregs as well as natural killer cells (NKs), both major players in successful pregnancies, is dependent on their interactions with dendritic cells (DCs). Specifically, we have shown that the interaction with DC depends on MHCII for Tregs [4] and on IL15 for NKs [5]. Because DCs control the homeostasis of the two major lymphocyte subsets involved in the success of pregnancy, we predicted that DCs themselves play an important role during pregnancy.
Our preliminary results indeed reveal that the pregnancy, both in humans and in mice, modulates general DC homeostasis. Moreover, we find that in pathological pregnancies there is a correlation between Tregs/NKs and DCs numbers going down. Our hypothesis is that the modulation of the homeostasis of specific DC subsets affects the outcome of pregnancy and can be used to cure implantation failure and multiple miscarriages.
There are several subsets of DCs with distinct functions [6], but the role of each DC subset in fetal-maternal tolerance is unexplored. During this PhD. project, the candidate will examine the dynamics of DC subsets in the placenta and uterine LNs from pregnant and non-pregnant mice and humans, and will deplete them in genetically-modified mice. The goal is to define the biological mechanisms behind DC-mediated recruitment of Tregs and NKs in the uterus during pregnancy. Our team has successfully used this approach to map out DC/Treg/NK axis at the steady state [4] and in the context of cancer [5]. The candidate will use animal models and molecular tools for the precise depletion or boost of specific subsets of DCs. We successfully used these tools to study the role of DC in various physiological and pathological conditions, but they were never before applied to study infertility and miscarriage. The PhD. student will also have access to relevant biological samples (blood, vaginal rub, decidua) from non-pregnant and pregnant women, as well as from patients suffering from implantation failure or multiple miscarriages. Informed by the animal studies, he/she will examine patient samples for presence of equivalent DC subsets and determine their correlation with Tregs, NKs, and disease. We predict that the results of this PhD. project will help to improve prenatal diagnosis and serve as a foundation for designing innovative biotherapies for patients with infertility problems and multiple miscarriages.

Keywords: Maternal-fetal immune tolerance and disruption - dendritic cells - NK lymphocytes - regulatory T cells - implantation problems in assisted reproductive technology (ART) - multiple miscarriages

Rationale and Aims of the project: Successful pregnancies depend on the correct and timely establishment of the maternal-fetal tolerance. Understanding the immune mechanisms of infertility and recurrent miscarriages is mandatory to improve care. We previously established that maternal regulatory T cells (Tregs) are necessary to protect the fetus from maternal immune response [1]. We also found that human fetus contains functional fetal thymic-derived Tregs that arise with the first wave of T cell differentiation, as early as at 13 weeks of gestation [7], some of them specific to maternal alloantigens [8]. Further characterization of maternal Tregs in mouse models led us to conclude that self-specific thymic-derived Tregs accumulate in the uterus-draining LNs and uterus and protect embryos at implantation [2, 3], while others implicated peripherally induced Tregs specific to paternal alloantigens [9]. The homeostasis of Tregs as well as natural killer cells (NKs), both major players in successful pregnancies, is dependent on their interactions with dendritic cells (DCs). Specifically we have shown that Tregs interact with DCs in a MHCII-dependent fashion [4], and NKs interact with DCs in a IL15-dependent manner [5]. These studies implicated DCs in the immune mechanisms leading to successful pregnancy.

Our preliminary results and recent literature [10] show a modulation of DCs homeostasis during pregnancy in humans and in mice, and a correlation between Tregs/NKs and DCs numbers going down in pathological pregnancies. Our hypothesis is that the modulation of the homeostasis of specific DC subsets can affect the outcome of a pregnancy and can be used to cure implantation failure and multiple miscarriages.

There are several subsets of DCs with distinct functions [6], but the role of each DC subset in fetal-maternal tolerance is uncharacterized. The PhD. candidate will examine DC subsets in the placenta and uterine LNs from pregnant and non-pregnant mice and humans. To define the biological mechanism behind recruitment of Tregs and NKs in pregnancy, he/she will use genetically-modified mice to deplete individual DC subsets, an approach that we have successfully applied in the context of cancer [5]. The candidate will have access to an arsenal of animal models and molecular tools for the precise depletion/increase of specific subsets of DCs, which we have used to study anti-tumor responses, but which has never before been applied to

study infertility and miscarriage. He/she also have access to relevant biological samples (blood, vaginal rub, decidua) from non-pregnant and pregnant women, as well as from patients suffering from implantation failure or multiple miscarriages. Informed by our animal studies, he/she will examine patient samples for presence of equivalent DC subsets and determine their correlation with Tregs, NKs, and disease. We predict that these translational studies will serve as a foundation for designing innovative biotherapies for patients with infertility problems and multiple miscarriages.

Specific Aims of the PhD. student:

- Identify parameters of immunopathological deregulation of DC subsets, Tregs and NK in spontaneous abortion-prone mice as well as in patients with implantation failures or multiple miscarriage pathologies, by flow-cytometry-based evaluation of DC (and other APC), Treg and NK frequencies and activation status as well as transcriptome in biological samples (blood, vaginal rub, decidua).
- Determine critical parameters of immunological homeostasis in pregnant mice and non-pregnant controls using animals deficient or proficient for various subsets of DCs (all cDCs, cDC1, cDC2, pDCs and MoDCs), or overexpressing them (through Flt3-L (FL) or GM-CSF treatment) by flow-cytometry-based evaluation of APCs, Treg and NK frequencies and activation status at the fetal-maternal interface and in the conceptus-draining lymph nodes. Their *in-situ* interactions will be evaluated by bi-photonic microscopy.
- Establish and validate immunological transcriptional signatures of healthy and pathological fetal-maternal interaction by computational analysis of RNA sequencing data from human and mouse biopsies.

Expected Results: Realization of this PhD. project will determine which DC subset(s) are responsible for the recruitment of NK and Treg leading to successful pregnancies, or are instead associated with pathological conditions. The PhD. student will characterize the DC subsets and the biological mechanisms that alter pregnancy course and are responsible for implantation failure, multiple miscarriage or even IUGR in mice and humans, and then identify new cellular targets for therapies, as well as new tools for obstetric diagnoses, such as biomarkers for PMA patients and patients experiencing infertility or spontaneous miscarriage.

Relevance of the project: Approximately 15% of pregnancies end in miscarriage, and Immune dysregulation seems to be clearly linked to infertility, an important public health problem. The absence of an effective treatment for these conditions makes our proposal even more relevant to the research priorities of the Biomedicine Agency. Today, DCs research in maternal tolerance is virtually non-existent. This represents an important gap in our knowledge, as DCs are critical in the regulation of the homeostasis of Treg and NK cells, both confirmed major players in successful pregnancies. To close this gap, we have assembled an arsenal of genetic and molecular tools, unique in Europe; formed a team with skills and expertise in both immunological and clinical aspects of the project. and generated solid preliminary data to accelerate the success of this project, and to assure the excellent translational potential, as several therapeutic candidate molecules (already approved for use in humans) are available to modify DC homeostasis in patients.

Lab and Partners profile: i3 Lab general objectives are (i) to advance the frontiers of knowledge in Immunology and (ii) to develop novel immunotherapies, with a dual reductionist and systems biology approach. Within this translational systems immunology project, our focus is on immune tolerance in health and disease. The specific themes are (i) to explore the immune system in physiological and pathological contexts, and to model the complexity of the immune system, (ii) to develop and validate new immunotherapies in mice, (iii) to develop and validate new immunotherapies in humans, and to perform clinical studies in the fields of Biotherapies and Immunology.
The lab has a recognized expertise in Treg and DC fundamental biology and related immunotherapies. Exploiting our experience in immunotherapies, we developed innovative immunotherapies with the specific aim of inducing immune tolerance and applying the strategies to treat autoimmune disease (AIDs), allergies, and fetal maternal tolerance. Different approaches were developed based on (i) fundamental research on Tregs, DCs and Tfh cells, (ii) pre-clinical studies like Treg-mediated therapies and a clinical trial based on low dose IL-2 therapy for patients with multiple miscarriages, as well as several trials based on this treatment for AIDs, or antigen-specific tolerogenic vaccination. Translational and clinical studies are

part of large and well-funded structures and projects, including the the Transimmunom LabEx, the RHU iMAP and the ERC TRiPoD.

Within the unit, the team dedicated to this project include :

1. Dr. Guillaume Darrasse-Jèze, is an associate professor. He has solid experience in the field of DCs and Tregs, and has co-authored several pioneering works in the field of fetal-maternal tolerance [1, 2, 3, 7]. His interest is focused on molecular basis of DC and Treg differentiation in the development of the fetal-maternal tolerance and other physiopathologies, such as cancer and autoimmune disorders. His long-term goal is to develop biomedical treatments based on his findings. He also authored the first report that revealed existence of the feedback regulatory loop between DC and Tregs [4], a central observation for the proposed research. He also characterized DC homeostasis and identified Zbtb46, the first known transcription factor specific for 'classical' DCs: [11]. Recently, his team used models described in this proposal to uncover a paradoxical role of DCs in antitumor immune response [5], submitted to Immunity. His team also developed R algorithmic tools to analyze effect of tumor infiltration on patient survival, looking at the data from 19 distinct immune cell populations in 36 different cancer types [12], submitted to JI. He collaborated with David Klatzmann in the discovery or uterine resident Tregs [3], in review in Cell Reports. Dr. Darrasse-Jèze will supervise the work of the PhD candidate. Gwladys Fourcade, Studies Engineer, will provide technical assistance for the experiments process and analyses. David Klatzmann, head of the laboratory and expert on Tregs will provide his expertise and some mouse models for the project.

2 - Dr. Nathalie Lédée, head of the clinical unit of reproductive medicine at the hospital Les Bluets (Paris), CEO of MatriceLab start-up company (Paris-Santé-Cochin), inventor of a method for increasing implantation success in assisted fertilization. Her field of research focuses on the immune mechanisms occurring at the time of the embryo implantation. Her clinical practice aims to increase live birth rate through an optimization of the initial immune dialogue between the embryo and the endometrium. She's working on the personalization of assisted reproductive treatments in function of each endometrial immune profile. Dr Lédée will provide her clinical and scientific skills to the project as well as human biological samples and data, especially from patients with implantation pathology.

3 - Dr. Arsene Mekinian, MCU-PH at Sorbonne University, in DHU Inflammation-Immunopathology-Biotherapy Department, and in the Department of Internal Medicine at Hôpital Saint-Antoine. He received an M.D. in internal medicine from the medical University of Lille II, and a Ph.D. in immunology from University Paris XIII. Assistant professor in internal medicine, his field of research focuses on the immune mechanisms of systemic sclerosis. His clinical practice aims to increase the live birth rate in unexplained or immune related recurrent miscarriages and implantation failures through an optimization of regulatory tolerance. He will provide his clinical and scientific skills to the project as well as human biological samples from patients with recurrent spontaneous abortions.

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