Stromal immunoregulation of healthy and pathological adipose tissue remodeling

The white adipose tissue (AT) is a highly dynamic organ that responds to nutrient and environmental stress to regulate host metabolism. A healthy AT tissue remodeling in response to excess caloric intake is essential to stock excess lipid and fatty acids and limit their systemic toxicity. In response to excess caloric intake, pathological AT remodeling leads to adipocyte hypertrophy, hypoxia, a shift toward pro-inflammatory type I immunity and ECM accumulation, resulting in adipose tissue fibrosis. Obesity-induced fibrosis limits AT expansion/plasticity and is a major driver of AT dysfunction and metabolic diseases such as insulin resistance and type 2 diabetes[1].

AT tissue remodeling requires the crosstalk of adipocytes with other resident cells, including immune cells, blood vessels, stromal cells and adipocyte progenitors. Immune cells and proinflammatory cytokines are major inducers of fibrosis, however type 2 immune cells (such as M2 macrophages) are essential for AT homeostasis and healthy tissue remodeling, notably by promoting angiogenesis and repair. Type 2 cytokines IL-4, IL-5 and IL-13, play also a critical role in AT homeostasis by activating adipocyte progenitors towards a thermogenic beige lineage[2, 3]. The "beiging" of the white AT antagonizes obesity and metabolic disorders. While several profibrotic signals have been identified, the biological process leading to pathological AT remodeling/fibrosis are only partially understood. The major producers of ECM are stromal cells that express PDGFRα, consistent with a role for PDGFRα in fibrosis[4, 5]. They also express other mesenchymal markers such as Pdpn, which identifies stromal cells with essential roles in lymphoid organs and inflammation[6]. PDGFRα is also expressed in adipocyte progenitors. Recent single-cell transcriptomics in the AT have identified a broader heterogeneity, including immunoregulatory and antiadipogenic stromal subsets[7, 8], suggesting that the crosstalk of stromal cells with surrounding cells is determinant in AT homeostasis.

Further in vivo investigations have been hindered by the paucity of specific markers and genetic tools available, as PDGFRα is broadly expressed. To bypass this limitation, team 1 has generated reporter and deleter mice models for specific PDGFRα$^+$ stromal subsets with essential roles in tissue homeostasis, immunoregulation and response to injury[9-12]. When dysregulated, these stromal responses are involved in the pathogenesis of chronic diseases and fibrosis, notably by affecting macrophage functions and angiogenesis[9, 12, 13]. Our preliminary results show that specific stromal populations promote pathological adipose tissue remodeling and dysregulated metabolism upon high-fat diet (HFD), through a mechanism that involves the microbiota and myeloid cells, and will be the focus of this project. Team 2 has developed unique mouse models (notably enriched or depleted in mononuclear cells [14-17]) and pre-clinical models to decipher the complex interactions between mononuclear phagocytes, with particular emphasis on macrophages and dendritic cells, intestinal microbiota and lipid metabolism in the pathogenesis of chronic metabolic disorders[18-22], as well as metabolic phenotyping in the context of obesity and insulin-resistance. Combining the expertise and tools of both labs, **this project aims at investigating, in vivo, the cellular and molecular mechanisms regulating stroma-myeloid cells interactions in healthy and pathological AT remodeling, with a focus on obesity-induced fibrosis and associated metabolic disorders.** To that aim, we will use unique mice models to manipulate stromal and myeloid subsets in vivo, combined with transcriptomics at the single cells level, high-resolution imaging and metabolism studies to investigate the underlying molecular and cellular mechanisms. *This project will consolidate and extend the collaboration between the two labs following the obtention of a common ANR* grant. In the long term, this project may set the basis for the development of new therapeutic strategies to control AT inflammation and obesity-associated metabolic diseases. The specific aims of the project are to

Aim 1. **Define stromal control of metabolism dysfunction during obesity**. We will use reporter and deleter mice to assess the contribution of specific stromal and myeloid subsets to AT remodeling and host metabolism in different WAT depots at ND (normal diet) and HFD (early and late). The whole fat depot will be analyzed by single-cell transcriptomic profiling in different experimental conditions and by confocal microscopy on whole-mount clarified adipose tissue. Changes in metabolic parameters will be followed by NMR-TD, fat weight, adipocyte size/number, fat accumulation in the liver, food/water intake, indirect calorimetry spontaneous locomotor activity, intracellular insulin signaling, adipokines expression, lipid metabolism and fibrosis.

Aim 2. **Decipher the direct mechanisms of VAT regulation by stromal cells.** We will assess the role of specific subsets of stromal cells in adipogenesis. Using lineage tracing, we will test their capacity to differentiate into adipocytes at steady-state and upon external stimuli such as HFD, beta3 stimulation (beiging), inflammatory cytokines, starvation. In a second time, we will test their impact on adipogenesis by paracrine crosstalk, using cocultures of stromal subsets and adipocyte progenitors. The role of candidate molecules will be tested using neutralizing antibodies, siRNA-mediated inhibition or conditional ablation in vivo (using Cre mice). Transcriptomic analysis of adipocytes will be performed using a ribosome-tagging approach (RiboTag), by crossing the RiboTag mice to specific Cre mice , and to AdipoQ-Cre mice for total adipocytes signature.

Aim 3. **Identify the indirect mechanisms of VAT regulation by stromal cells through inflammation.** We will evaluate by FACS the local and systemic inflammatory response in mice lacking specific subsets of stromal cells. Ligand receptor analysis will be performed on scRNASeq performed in similar conditions (aim1). To further investigate the stromal-immune crosstalk, we will set up in vitro cocultures of stromal subsets with specific immune cell subsets (based on results obtained in aims 1 and 2) and notably their capacity to impact macrophage function/phagocytosis (using in vitro and in vivo assays). Identified signaling pathways, cytokines and immune populations with roles in stromal crosstalk will be tested in vivo by depleting specific immune populations, and in mice models lacking immune subsets.

Project management: All mice lines, experimental procedures and expertise are already in place. Experiments will be performed in collaboration and co-supervised based on the required expertise (Team 1: stroma, fibrosis, lineage tracing, scRNAseq; and team 2: metabolism, lipid, myeloid cells).

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