Title of the PhD project

Impact of autotaxin (ATX) interaction with integrins in inflammatory rheumatisms.

Disciplines: Cell Biology, Inflammation, Preclinical models.
Laboratory : INSERM U1033 director Pr Roland Chapurlat Web: http://www.lyos.fr/
Doctoral school: Interdisciplinary Doctoral program in health-sciences (EDISS) - ED 205

Scientific background and rationale:
Rheumatoid arthritis (RA) is a common chronic inflammatory disease of the joints that progressively leads to cartilage and bone destruction. Despite the fact that inflammation may be controlled in patients with RA, those in a state of sustained clinical remission or low disease activity may continue to accrue erosions, which supports the need for new treatments that would be suitable for long-lasting inhibition of osteoclasts, the specialized bone resorbing cells, without altering their physiologic function in bone homeostasis. In addition, RA may be associated with extra-articular manifestations of which diffuse interstitial lung disease (DIL), similar to idiopathic pulmonary fibrosis (IPF), is responsible for a significant excess of mortality. Often unrecognized, there is no validated therapeutic management due to a lack of knowledge of its pathophysiology. The project is based on recent published works from the lab and preliminary in vitro and in vivo results using proprietary neutralizing monoclonal antibodies against Autotaxin (ATX).

Aim:
Autotaxin (ATX) is a soluble enzyme that through its lysophospholipase D (lysoPLD) activity is responsible for the synthesis of a bioactive lipid, lysophosphatidic acid (LPA). The ATX/LPA axis controls multiple pathophysiological processes including bone degradation in RA and lung fibrosis in IPF.
The project will have both a discovery phase and a preclinical therapeutic phase. In objective (1) the project will characterize through in silico and in vitro analyses the binding domains of ATX with integrins of the beta1 and beta3 families using Incucyte X5 technology. In objective (2) the project will validate the therapeutic potential of targeting these domains in pre-clinical animal models of RA using pegylated peptides and recombinant antibodies addressing both bone degradation and pulmonary fibrosis using live animal imaging and histological technologies.

Description of the project methodology:
Molecular biology & biochemistry: The project will consist in site directed mutagenesis experiments on Autotaxin, Beta1 and Beta3 integrin subunit cDNAs from a list of already identified key amino acids. Then, mutATX will be generated and purified by LPLC. The impact of ATX mutations will be characterized by protein crystallography (collaboration with EMBL Grenoble). mut-Beta1 and mutBeta3 cDNAs will be transfected in beta1 deficient COBb1-/- and beta3 CHO cells, respectively and synthetic pegylated peptides containing targeted amino acids or domains will be purchased.
Cell biology : All mutated proteins, pegylated peptides and already generated anti-ATX recombinant antibodies will be used on cell adhesion, proliferation, migration, gel contraction assays using Incyte X5 technology.
Preclinical animal models : Inflammatory mouse models: (1) Chemically induced inflammatory bone loss (LPS, CAIA) and (2) spontaneous bone erosion and lung fibrosis in transgenic mice. Animals will be challenged with blocking pegylated peptides and with anti-ATX recombinant antibodies. Analyses will be carried out on live animals by microcomputed tomography (µCT) and bone tissue histology.

Expected results: The project will determine the specific domains of ATX that interact with integrins. The project will define new therapeutic strategies that will be validated at a preclinical level.

Perspectives: In close collaboration with the rheumatologists of the INSERM U1033 and the department of the Hospices Civils de Lyon the perspective of the project will be the launch of clinical trials on RA patients to prevent inflammation, bone degradation and lung fibrosis.

Skills required: Experimental and basic knowledges on cell biology, molecular biology and protein analyses will be required.

Bibliography: Leblanc et al., Blood 2014, Oncotarget 2018, Flammier et al., Arthritis & Rheumatology 2019, Peyruchaud et al., Cancers 2019, Coury et al., Frontiers in Immunology 2019.

Key-words: Molecular and cellular biology, Inflammation, Osteoclasts, Bone resorption, Fibrosis, Integrins

Contact: **Dr. O. Peyruchaud** DR2 INSERM (HDR) Team LeaderApplication should include: CV, application letter, Names and addresses of two references. The application file should be sent before May 1st, 2022 to: (Olivier.peyruchaud@inserm.fr). The open competitive recruitment process is in two steps: 1. Internal laboratory procedure. 2. Interdisciplinary jury of EDISS