Introduction
Autotaxin (ATX) is a secreted protein produced by various tissues in the body including the liver, adipose tissue and bone. Autotaxin is an enzyme with a lysophosphatidylcholine phospholipase D activity responsible for cleavage of lysophosphatidylcholine (LPC) in lysophosphatidic acid (LPA). LPA is a bioactive phospholipid, which acts as a growth factor, affecting cell proliferation, migration and differentiation. It has been shown that biological effects of LPA could be the direct consequence of cell proliferation, migration and differentiation.

Recently, we have shown that LPA controls two steps of osteoclastogenesis: the fusion of monocyte progenitors and bone resorption capacity of osteoclasts. The aim of this study is to test if ATX produced by osteoclasts could play a direct role on osteoclastogenesis and in bone mass control.

Methods
We generated Ctsk-Cre+ ATXfl/fl mice and use these animals as a source of osteoclasts progenitors and ATX-deficient mature OC. Because LPA, LPC and ATX are massively present in sera, to explore either LPA or ATX putative effect in culture, the use of delipidated serum was mandatory.

Bone marrow from ATXfl/fl Ctsk-Cre+ or WT Mice

Monocytes purification

Osteoclasts differentiation (M-CSF + RANK-L)

Bone-mimicking matrix (hydroxyapatite)

Bone resorption capacity analyse

Results

Figure 1: ATXm RNA expression is decreased in ATXfl/fl Ctsk-Cre+ mice compared to WT mature OC. Bone marrow-derived monocytes of WT or ATXfl/fl Ctsk-Cre+ mice were maintained in αMEM medium during 3 and 5 days with M-CSF and RANK-L for differentiation into osteoclasts. At day 0, 3 and 5, mRNA was extracted from cells. Expression of Enpp2/ATX mRNA was measured by real-time quantitative RT-PCR. Quantifications were normalized to corresponding L32 RNA values. Results represent the mean with SD [*p<0.05, **p<0.01, ***p<0.001].

Figure 2: LPA and ATX (free of derived-LPA) are required for osteoclast differentiation

Bone marrow derived monocytes of WT mice were maintained in αMEM medium during 5 days with M-CSF and RANK-L for differentiation into mature osteoclasts. LPA (1 or 10μM), LPC (1µM), ATX (3 nM), KI16425 (10µM) and PF8380 (5 nM) were added to the cell media containing delipidated serum at day 2. At day 5, cells were TRAP-stained and osteoclasts were counted (TRAP positive cells with 3 nuclei). Results represent the mean with SD of osteoclasts number per well (*p<0.05, **p<0.01, ***p<0.001).

Figure 3: ATX is required for osteoclast bone resorption activity

Bone marrow derived monocytes of WT or ATXfl/fl Ctsk-Cre+ mice were maintained in αMEM medium during 4 days with M-CSF and RANK-L for differentiation into mature osteoclasts. At day 4, mature osteoclasts were detached from plastic and seeded on bone-mimicking 96-wells plates. At day 5, LPA (25µM), ATX (3 nM), KI16425 (10µM) and PF8380 (5 nM) were added to the cell media containing delipidated serum and resorption was quantified 48 hours later. Results represent the mean with SD resorbed area per well (*p<0.05, **p<0.01, ***p<0.001).

Conclusions
All together, and more specifically the results obtained in presence of LPC alone indicate that:

(i) ATX is secreted by mature osteoclasts
(ii) Endogenous OC-ATX is functionally involved in osteoclast activity but not in OC differentiation in vitro.

Current studies are conducted on ATXfl/fl Ctsk-Cre+ mice to fully characterize the role of ATX in the bone mass control in physiological and pathological conditions due to ageing (osteoporosis) and inflammation (rheumatoid arthritis).

References:
1-Nishimura S. et al Diabetes. 2014 Dec;63(12):4154-64

Pathophysiological implication of Autotaxin on osteoclast function
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