DIVISION OF RHEUMATOLOGY

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Arginine and the Metabolic Control of Osteoclast Generation

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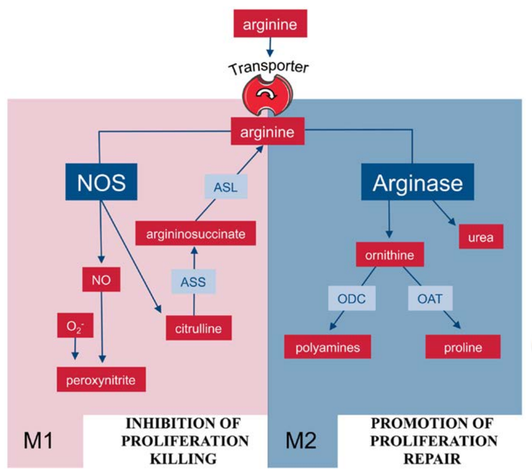
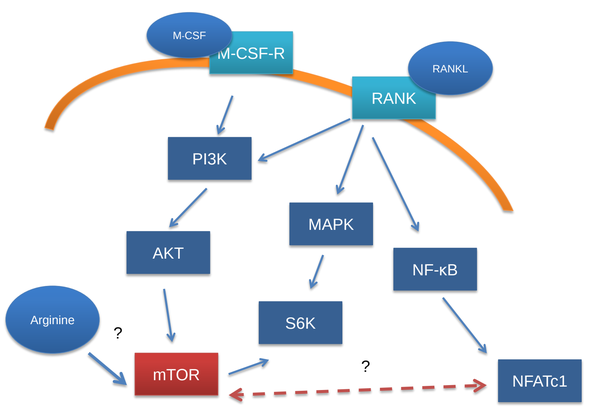
Background:

L-arginine is a semi-essential amino acid, known for its prominent role in cellular metabolism. Availability and catabolism of arginine have been implicated with immune cell biology, skewing inflammatory responses within myeloid cells in a pro- or anti-inflammatory manner. While the role of arginine within certain myeloid lineages such as macrophages is well appreciated, its role within osteoclasts is relatively unknown.

Methods:

We analyzed osteoclastogenesis of C57BL/6J or BALB/c wildtype cells in vitro in the presence and absence of recombinant arginase I (recARG1) and its inhibitor nor-NOHA. This approach was complemented via qPCR analysis of relevant marker genes. We further investigated the potential of the enzyme to induce cell death via flow cytometry analysis of 7-AAD and Annexin V.





Arginine's central role in the M1-M2 macrophage polarization paradigm and its proposed influence on osteoclast differentiation

The Importance of L-Arginine in Osteoclastogenesis

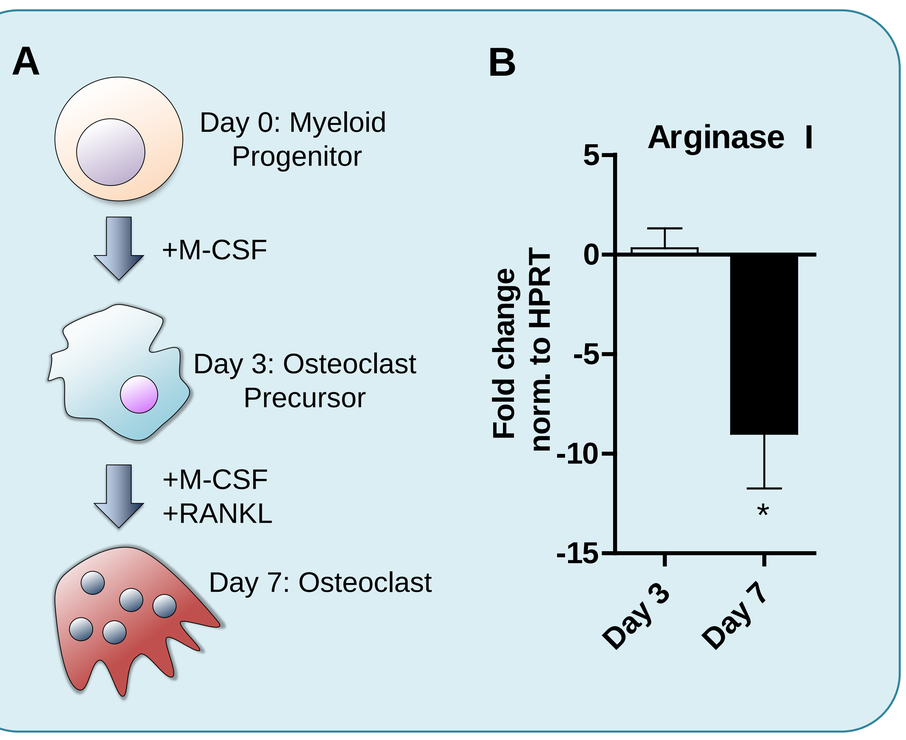


Figure 1: Expression levels of arginase I after induction of osteoclastogenesis. A) Schematic of an *in vitro* osteoclast assay. **B)** Messenger RNA levels of arginase I decrease after addition of RANKL on day 3 of osteoclast differentiation.

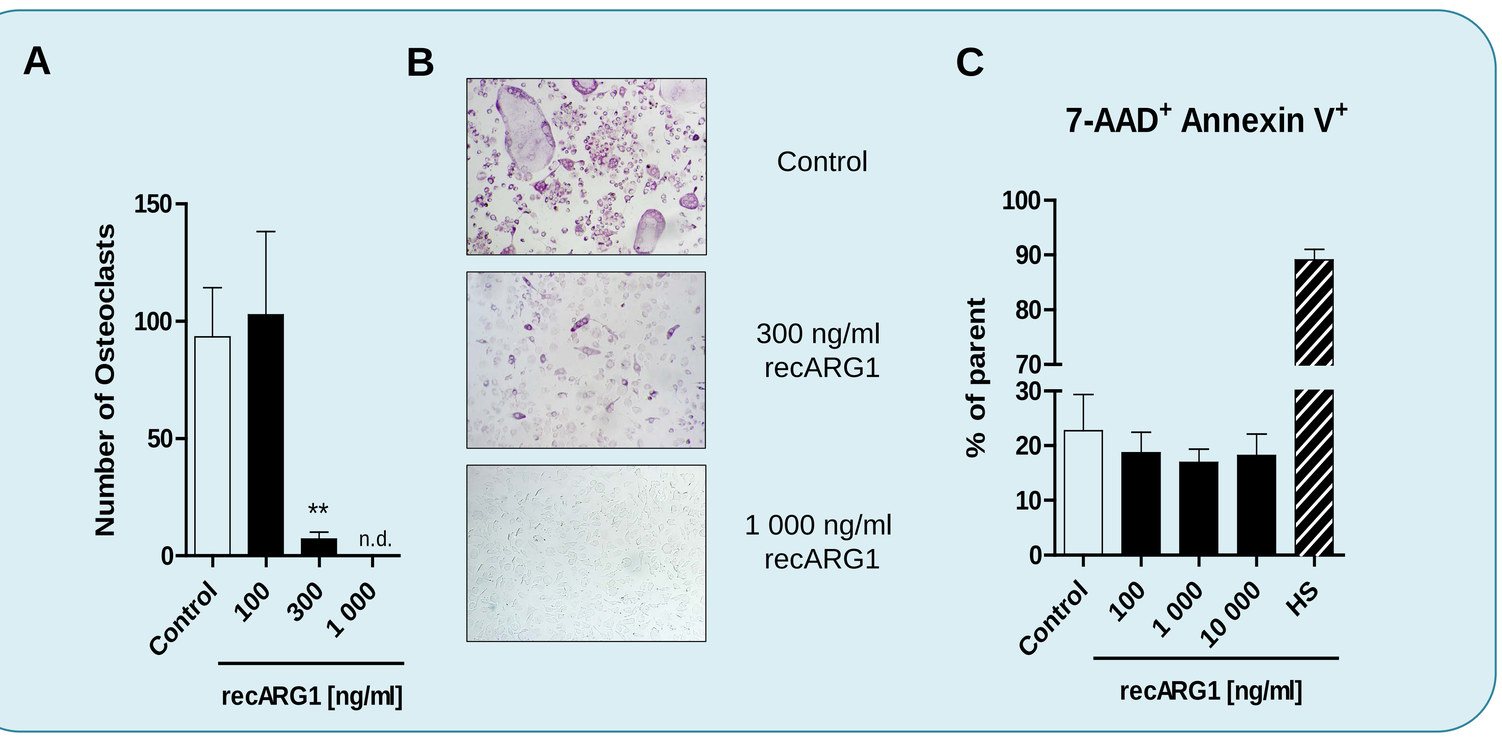


Figure 2: Osteoclast assay of differentiated wildtype bone marrow derived macrophages treated with recARG1. A) Counted osteoclasts containing at least 3 nuclei. **B)** Representative microscope pictures taken from the same experiment, stained for tartrate-resistant acid phosphatase (TRAP), a marker for osteoclasts. **C)** Flow cytometry analysis of 7-AAD and Annexin V double positive cells, harvested on day 4 after differentiation, one day after incubation with RANKL and recARG1 (HS: Heat shock).

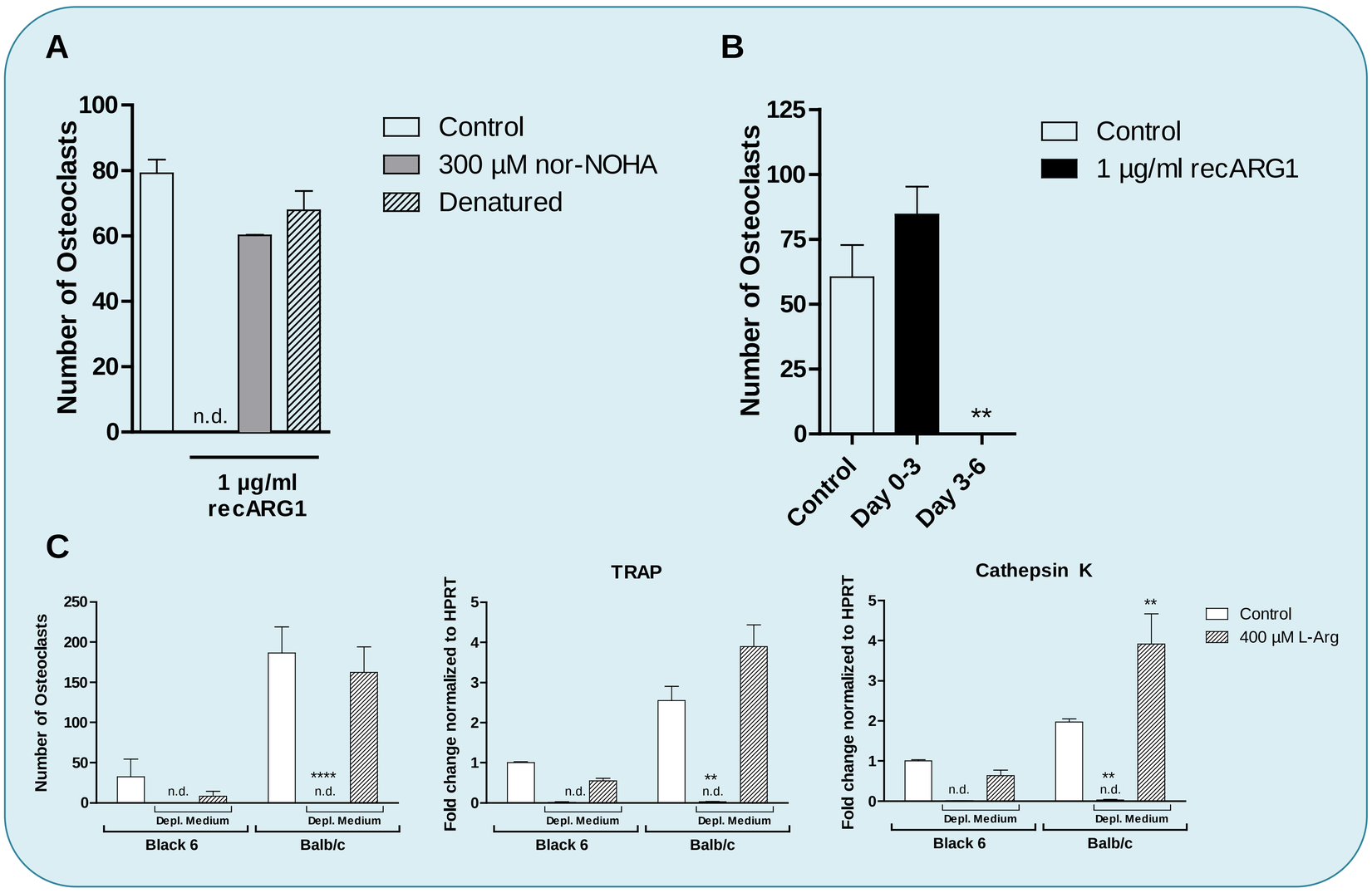


Figure 3: Effects of recARG1 on osteoclastogenesis are linked to RANKL signaling and its enzymatic activity. The effects can be rescued by L-arginine resupplementation. A) Inhibition of the enzymatic activity via denaturation or inhibition of the enzyme rescues osteoclastogenesis. **B)** Incubation with 1 µg/ml recARG1 for the first three days during MCSF incubation does not influence later osteoclast formation. **C)** Counted osteoclasts containing at least 3 nuclei and fold induction in developing osteoclasts incubated with depleted medium or resupplied medium after 7 days of differentiation shown for TRAP and cathepsin K.

Results:

We incubated day 3 osteoclast precursors with recARG1 and observed that addition of 1 000 ng/ml recARG1 abolished the formation of mature osteoclasts. Further, recARG1 did not affect the generation of osteoclasts when added only in the M-CSF-dependent first phase of the osteoclast differentiation assay. Strikingly, osteoclastogenesis could be completely restored upon combined treatment of recARG1 with the arginase I specific inhibitor N^{ω}-hydroxy-nor-l-arginine (nor-NOHA) or by denaturing the enzyme, confirming that the enzymatic activity of recARG1 is necessary for the inhibitory effect. We depleted osteoclast generation cell culture medium of L-arginine using recARG1, then blocked the enzyme using nor-NOHA and found osteoclastogenesis to be completely blocked using this "depleted" medium. Osteoclastogenesis could be restored by reconstituting the depleted medium with L-arginine.

Conclusion:

We propose that the essential amino acid L-arginine is critical for the development of osteoclasts from myeloid precursors and hypothesize that its abundance might influence development and severity of osteoporosis.