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Chemokines and bone: Lessons from *in vitro* and *in vivo* studies

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Avhandlingen kommer att försvaras på svenska.

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Abstract
Bone homeostasis is maintained by the balanced activity of bone-forming osteoblasts and bone-resorbing osteoclasts. Inflammation in the vicinity of bone disturbs the balanced bone remodeling process, which often results in bone loss. The chemokine C-C motif chemokine ligand 11 (CCL11) is associated with several conditions that inflict bone loss and it increases the recruitment and activity of osteoclasts. Osteoclasts express high levels of the CCL11 receptor C-C motif chemokine receptor 3 (CCR3). Although chemokines and chemokine receptors are demonstrated to be involved in both physiological and pathological bone turnover, their roles in skeletal growth, maturation, and bone remodeling are only partially understood.

The overarching aim of this thesis was to investigate if CCR3 regulates osteoclast and osteoblast differentiation and function *in vitro*, and if it affects bone modeling and remodeling *in vivo*. Furthermore, this project aimed to elucidate the molecular mechanisms by which CCL11 interacts with and affects osteoclasts.

In murine cell culture experiments, we identified that CCR3-deficient osteoclasts became larger and had more nuclei compared to CCR3-proficient osteoclasts. This was accompanied by an increased bone resorption activity, although none of the investigated osteoclast-associated genes were affected by the absence of CCR3. On the other hand, CCR3-deficient osteoblasts demonstrated an increased expression of osteoanabolic genes and the crucial osteoclast differentiation factor regulator of nuclear factor kappa B ligand (RANKL).

Using micro-computed tomography, we demonstrated that CCR3-deficient adolescent and adult male mice had thinner cortical bones and lower cortical bone volumes compared to CCR3-proficient mice. Interestingly, no skeletal phenotype was detected in female mice. Among juvenile CCR3-deficient mice, neither males nor females showed a skeletal phenotype, which indicates that the observed phenotype in CCR3-deficient adolescent and adult mice was not acquired due to an impaired early bone modeling process. In addition, histomorphometric analyses showed an increased cortical mineral apposition rate in both adolescent and adult male mice, and an increased cortical bone formation in adult male mice, whereas osteoclast numbers and size were not affected *in vivo*.

Advanced microscopy analyses were used to assess the membrane binding and internalization of fluorescent CCL11 in pre-osteoclasts and osteoclasts. We detected that CCL11 was rapidly internalized in pre-osteoclasts, whereas the initial CCL11 interaction in mature osteoclasts mainly involved surface binding to actin-rich protrusions on the cell membrane. Live-cell imaging demonstrated an overall increased cell motility and speed of pre-osteoclasts exposed to CCL11. Using an immunobased array screening of pre-osteoclasts stimulated with CCL11, we also detected alternations in the signaling network of cytoskeletal proteins coupled to cell migration and adhesion.

In conclusion, we discovered that the absence of CCR3 regulates both osteoclast and osteoblast differentiation and function *in vitro*. This was reflected *in vivo*, since CCR3-deficiency caused a cortical femoral bone phenotype in adolescent and adult male, but not female, mice. Furthermore, we demonstrated that CCL11 increases osteoclast motility and identified that CCL11 regulates cytoskeletal protein signaling in osteoclasts.

Keywords
Bone, osteoclast, osteoblast, CCL11, CCR3, gene knockout, chemokine signaling

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