The Etiology of Generalized Osteoporosis in Rheumatoid Arthritis

General Summary
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Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects 0.5-1% of the world's population. Generalized osteoporosis is an extra-articular complication of RA that results in increased fracture risk and associated morbidity, mortality, and healthcare costs. The incidence of generalized osteoporosis among patients with rheumatoid arthritis is 15-20%. Generalized osteoporosis in RA is in part caused by immobility and corticosteroid therapy, but it has also been attributed to the effects of systemic inflammation, such as elevated levels of circulating cytokines, chemokines, and signaling molecules.

Serum of patients with active RA contains altered levels of cytokines, biological proteins, and signaling molecules such as soluble receptors, receptor antagonists, antibodies, and autoantibodies. Individual inflammatory cytokines are known to affect bone cell formation and function, thereby affecting bone homeostasis. However the effect of serum from patients with active RA (active RA-serum) containing a complex mixture of cytokines, chemokines, biological proteins, and signaling molecules, on bone cell formation, function, and communication is unknown. Therefore in this thesis we investigated the effect of active RA-serum on bone cell formation, function, and communication towards other cell types. In chapter 2, we describe the existing research methods that can be used to test the effects of active RA-serum on bone cell formation, function, and communication in vitro.

In chapter 3, we investigated the effect of active RA-serum on osteochondrogenic differentiation of two different precursor cell types, i.e. mouse chondrogenic ATDC5 cells and human periosteum-derived mesenchymal stem cells. We found that serum from patients with active RA inhibits differentiation of osteochondrogenic precursor cells, as shown by decreased cartilage matrix accumulation and changes in gene expression of bone and cartilage markers. Such an inhibition may explain, at least in part, the decrease in bone formation and delayed fracture healing in patients with RA, since inflammatory cytokines and signaling molecules present in the circulation of patients with RA affect differentiation of mesenchymal stem cells towards a bone or cartilage xenotype.

In chapter 4, we studied whether a complex mixture of circulating inflammatory mediators present in the serum of patients with active RA alters osteoblast function and communication towards osteoclast precursors compared with serum from the same patients in clinical remission. We demonstrated that active RA-serum inhibits osteoblast proliferation and differentiation. We also found that active RA-serum enhanced IL-6 and receptor activator of nuclear factor kappa-B ligand (RANKL) gene expression in osteoblasts, as well as osteoblast-mediated osteoclastogenesis. Blocking of IL-6 and RANKL in co-cultures of RA-serum-pretreated osteoblasts and osteoclast precursors reduced the number of
osteoclasts. Such an inhibition of osteoblast function and altered osteoblast-to-osteoclast communication by serum containing inflammatory mediators might explain the catabolic effect of systemic inflammation on bone mass.

Elevated levels of CXCL8 and CCL20 are found in RA-serum, but their effect on osteoblasts is unknown. Therefore, in chapter 5, we analyzed whether the chemokines CXCL8 and CCL20 potentially affect osteoblast proliferation and differentiation, and osteoblast-to-osteoclast communication. We found that CXCL8 and CCL20 did not inhibit osteoblast proliferation nor gene expression of the main matrix proteins. However, CXCL8 and CCL20 might have an effect on bone homeostasis via other mechanisms. We demonstrated that chemokines CXCL8 and CCL20 enhanced osteoblast-mediated osteoclastogenesis partly via IL-6 production by osteoblasts. Interestingly, these results correspond with data using RA-serum (chapter 4). Moreover conditioned medium from osteoblasts cultured with CCL20, but not CXCL8, enhanced osteoclast activity. Based on the results from chapter 4 and 5, we speculate that RA-serum-induced osteoblast-mediated osteoclastogenesis might be partly caused by CXCL8 and CCL20 present in active RA-serum. This qualifies these chemokines as interesting targets for future research on personalized medicine for RA patients who are not responding to the existing treatments.

Osteocytes are highly mechanosensitive; after mechanical stimulation they alter the production of a range of signaling molecules, that modulate recruitment, differentiation, and activity of osteoblasts and osteoclasts. Therefore the cytokines and signaling molecules produced by osteocytes play a crucial role in bone adaptation. In chapter 6, we investigated whether active RA-serum affects the intrinsic capacity of osteocytes to sense mechanical stimuli as well as osteocyte-to-osteoclast communication, and whether mechanical loading of osteocytes can alter the effect of RA-serum on osteocyte-to-osteoclast communication. We used primary human bone cells containing a mixture of osteoblasts and osteocytes like cells. Since osteocytes are more mechanoresponsive to mechanical stimuli than osteoblasts, we designated these cells “osteocytes” in chapter 6. We found that active RA-serum did not affect the intrinsic capacity of osteocytes to sense mechanical stimuli. We demonstrated that active RA-serum enhanced the RANKL/OPG gene expression ratio by osteocytes as well as osteocyte-mediated osteoclastogenesis, and that mechanical loading of osteocytes reversed these effects. These findings suggest that physical exercise, that can be performed by RA patients without pain, or other forms of bone loading, e.g. vibrating platforms, could have therapeutic potential for the prevention of osteoporosis in RA and other inflammatory diseases.

Activation of canonical Wnt signaling exclusively in osteocytes induces bone anabolism and triggers Notch signaling. Osteocytes have a function in phosphate homeostasis. For this purpose osteocytes produce a range of cytokines
and signaling molecules. In previous chapters, a mixture of osteoblast-like and osteocyte-like cells were used for experiments, but we would like to have a more pure culture of human osteocytes, preferably in their own environment. Unfortunately there is lack of a proper human osteocyte models to investigate their function in vitro. In chapter 7, we cultured human bone chips containing osteocytes embedded in their native matrix, and analyzed the effect of exogenous recombinant cytokines, chemokines, and active RA-serum on osteocyte signaling. We found that exogenous recombinant inflammatory cytokines enhanced gene expression of the cytokines IL-1β, IL-6, IL-8, TNFα, FGF23 which is involve in phosphate homeostasis, and Wnt antagonist SOST, while the chemokine CCL20 enhanced TNFα, IL-6, and IL-8 gene expression by osteocytes. Similarly, serum from patients with active RA enhanced gene expression of IL-1β, TNFα, FGF23, SOST, and Wnt antagonist DKK1 by osteocytes. These results suggest that osteocytes might play a key role in bone mass regulation during systemic inflammation, and therefore could represent an excellent target to prevent loss of bone mass in inflammatory diseases.

In this thesis we demonstrated that diluted (1:10) active RA-serum already affected osteoblast function, osteoblast-to-osteoclast communication, osteochondrogenic differentiation of precursor cells, osteocyte signaling, and osteocyte-to-osteoclast communication, indicating even more pronounced effects in vivo. Such effects may disturb bone homeostasis causing systemic bone loss and increasing fracture risk in vivo. Mechanical stimulation of osteocytes was able to attenuate the active RA-serum–mediated enhanced osteocyte-to-osteoclast communication, suggesting mechanical stimulation of osteocytes as a possible therapeutic option for bone loss in RA. The results described in this thesis contribute to opening a new research direction in osteoimmunology because failure of existing drugs to mitigate inflammation and bone loss are frequently experienced in the clinic in too many patients. This in turn might lead to the development of drugs for new targets and interventions such as antibodies against CXCL8 and CCL20, which might be more effective to mitigate inflammation-related bone loss when existing drugs are not effective. Such drugs can help to design more personalized treatments for generalized bone loss in systemic inflammation.