Chapter 7

Summary

Osteoclasts play a crucial role in inflammation-related bone diseases such as rheumatoid arthritis and periodontitis. During inflammation, immune cells present in the inflamed tissue secrete a variety of inflammatory cytokines which affect osteoclast formation and subsequent osteoclast-mediated bone resorption. Some prevalent cytokines are interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α). Recently, various studies showed that osteoclasts at different locations are phenotypically different. These different osteoclasts could originate from different subsets of precursors. In this thesis, we focus on mouse bone marrow osteoclast precursors. Three subsets of bone marrow osteoclast precursors can be categorized according to the expression of CD31 and Ly-6C: early blasts, myeloid blasts and monocytes. The aim of this thesis was to unravel: (1) Whether the different osteoclast precursor subsets respond differently to the inflammatory cytokines IL-1β and TNF-α. (2) How an inflammatory mouse model affects the osteoclast precursor pool and (3) whether the morphology of osteoclasts differs at different skeletal sites in vivo.

In Chapter 2, it was shown that IL-1β had different stimulatory effects on the three mouse bone marrow osteoclast precursor subsets. (i) IL-1β accelerated early blasts’ proliferation, but not that of myeloid blasts and monocytes. In line herewith, the expression of cyclin D1, a regulatory gene for proliferation, was increased exclusively in early blast cultures. (ii) Regarding cell fusion, it was found that IL-1β stimulated this process of all three subsets, being the most pronounced in myeloid blast cultures. In the presence of IL-1β, myeloid blasts responded the earliest by forming large osteoclasts, and the highest number of large osteoclasts were found in these cultures. mRNA expression indicated that expression of most of the osteoclast-related genes, such as TRAcP, cathepsin K, DC-STAMP, as well as the receptor of IL-1, IL-1RI, were significantly upregulated in myeloid blast cultures. Together these findings suggested that myeloid blasts were the precursors most susceptible to IL-1 in the process of osteoclast formation. (iii) Although IL-1β also stimulated monocytes’ osteoclastogenesis as well as bone resorption, monocytes responded to IL-1β the latest and formed the lowest number of osteoclasts. However, monocyte-derived osteoclasts had the longest life span. Taken together, IL-1β has a stimulatory effect on all of the mouse bone marrow osteoclast precursor subsets, however the effect was different depending on the type of subset.
Whether the three osteoclast precursor subsets respond differently to another inflammatory cytokine, TNF-α, was studied in Chapter 3. Different from IL-1β, TNF-α only showed a stimulatory effect on early blasts and myeloid blasts, but inhibited monocytes’ osteoclastogenesis when cultured on plastic. However, when monocytes were seeded on bone, or were primed with M-CSF and RANKL followed by the addition of TNF-α, TNF-α stimulated monocytes’ osteoclastogenesis. qPCR and FACS analysis indicated that exposure to TNF-α led to decreased mRNA expression of RANK, NFATc1 and TRAcP, only in monocyte cultures, and the ratio of bound-RANK/unbound-RANK was changed in monocyte cultures. Therefore we proposed the following: (i) Under normal conditions, RANKL binds to RANK and induces the RANKL/ RANK signaling pathway for osteoclastogenesis; (ii) When TNF-α is added at the beginning together with M-CSF and RANKL on plastic, RANK signaling is not triggered, leading to an inhibited osteoclastogenesis; (iii) Such inhibitory effect can be prevented when monocytes were first cultured with M-CSF and RANKL before addition of TNF-α. After the RANKL/RANK signaling pathway is stimulated by binding of RANKL to RANK, TNF-α stimulates osteoclastogenesis by monocytes.

In Chapter 4, the osteoclast precursor pool of IL-1RA knockout mice was shown to be affected differently at different skeletal sites. The IL-1RA knockout mouse, which has a sustained IL-1 signaling, was reported to spontaneously develop arthritis. This study compared the osteoclast precursor composition as well as the osteoclastogenesis of bone marrow cells from long bone, calvaria, vertebra and jaw. It was found that the percentage of myeloid blasts and monocytes was increased in long bone, and the percentage of monocytes was enhanced in jaw of IL-1RA knockout mice. The bone marrow cells of IL-1RA knockout mice from each bone site showed a stimulated osteoclastogenic capacity and mineral-dissolution activity. Thus, different skeletal sites were affected differently with respect to osteoclastogenesis: deletion of IL-1RA stimulated the osteoclast precursor pool in long bone and jaw, which resulted in an enhanced osteoclast formation as well as bone resorption. In calvaria, the sustained IL-1 appeared to activate the precursors to a more IL-1 sensitive phenotype, resulting in strongly stimulated osteoclastogenesis and bone resorption.

In Chapter 5, the morphology of osteoclasts as well as bone metabolism were studied and compared between adseverin knockout mice and wildtype mice in vivo. Adseverin is an actin-severing/capping protein which is shown to mediate osteoclast formation in vitro. This in vivo study, however, showed
that the density of bone was not affected in adseverin knockout mice. Yet, the size of the osteoclasts in the adseverin knockout mice was smaller, despite the fact that the number of osteoclasts, the structure of the ruffled border and sealing zone were comparable between knockout and wildtype mice. The ultrastructure of osteoclasts was also compared between alveolar bone and long bone, and between a bacteria ligature-induced inflammatory condition and non-inflammatory condition: no distinct differences were found. Regarding the structure of the osteoclast nuclei, we found in adseverin knockout mice that the nuclei were smaller and they contained a higher percentage of heterochromatin. The findings suggest that adseverin contributes to osteoclast structure \textit{in vivo}.

In this thesis, we provide some insight into the osteoclastogenic potential of distinct precursors and their response to some inflammatory cytokines. We conclude that different osteoclast precursors respond differently to inflammatory cytokines, and their responses are different at different skeletal sites. The data presented may help to explain the diversity in bone site-specific osteoclasts and bone degradation under both physiological and inflammatory conditions.