General Abstract

Do you find things are moving
Just a little too fast

Hazy Jane I – Nick Drake
General abstract

Bone is a dynamic tissue that constantly remolds to adapt its structure to external mechanical loading. Bone remodeling is mediated by bone formation and bone resorption, two major processes that are tightly balanced. The cells responsible for bone resorption are the so-called osteoclasts. The formation of these bone resorbing osteoclasts in vivo is a multistep process orchestrated by cells of the osteoblast lineage (bone lining cells) that provide the proper signals to osteoclast precursors. In an early stage of osteoclast formation, adhesion between bone lining cells and osteoclast precursors occurs and is established via an interaction involving intercellular adhesion molecule-1 (ICAM-1) on bone lining cells and leukocyte function-associated antigen-1 (LFA-1) on osteoclast precursors. M-CSF and RANKL, cytokines expressed by the osteoblast-like cells, then further stimulate the osteoclast precursors towards the osteoclast lineage. In this process migration is an essential step for both the precursors and the osteoclast itself. Yet, the dynamics of cell migration throughout osteoclast formation is largely unknown.

Differentiated osteoclasts are characterized as mitochondria-rich cells, a feature required to meet the energy demands during their bone resorbing activity. Although the huge number of these organelles is a recognized characteristic of osteoclasts, surprisingly little is known about their biogenesis during osteoclast differentiation. In this thesis we investigated the role of cell-cell interactions in several aspects of osteoclast formation, osteoclast migratory behavior and the genesis of mitochondria in these cells.

In chapter 2 we analyzed the expression of ICAM-1 on both human osteoblast-like cells and osteoclast precursors at different time points during osteoclastogenesis. We found that in the co-culture the protein expression on osteoclast precursors strongly increases whereas the osteoblast-like cells become ICAM-1 negative. Moreover, ICAM-1 on osteoclast precursors clusters and this clustered ICAM-1 is found to be associated with F-actin. The clusters are distributed at the baso-lateral membrane and remain present for several days. We suggest that ICAM-1 of bone lining cells is mainly involved in the initial adhesion of osteoclast precursors to bone lining cells. Clustered ICAM-1 and its association with F-actin on osteoclast precursors on the other hand suggest an involvement of this molecule in cell movement at a later stage. These findings led us to hypothesize that cell-cell interactions influence the expression of genes during osteoclastogenesis.

In chapter 3 we investigated the effect of cell-cell interactions on the mRNA expression of adhesion molecules and molecules involved in osteoclast differentiation. We further analyzed the formation of multinucleated, tartrate resistant acid phosphatase (TRACP) positive cells. Interestingly, gene expression of ICAM-1 and of osteoclastogenesis-related genes was highly up-regulated in the co-culture and reflected a synergistic increase due to direct cell-cell interaction. Subsequently, we analyzed the number of osteoclast-like cells that were formed in the co-culture and found that these numbers are significantly augmented compared to mono-cultures. These data indicate that cell-cell adhesion between osteoclast precursors and osteoblast-like cells significantly modulates the cellular response leading to an increase in expression of osteoclast differentiation genes and the ultimate formation of osteoclasts.

Based on these findings, we wondered whether a stimulation of the supporting osteoblast-like cells could indirectly lead to an increase in osteoclast formation. In chapter 4 we show that a short pre-incubation of these cells with IL-1β increased the adhesion of PBMCs.
mRNA expression of ICAM-1, macrophage colony stimulating factor (M-CSF) and IL-1β itself was highly increased. Pre-incubation with IL-1β further favors retraction of the osteoblast-like cells and concomitantly increased the formation of TRACP+ multinucleated cells. These findings show that a short pre-stimulation of only 6 hours of the supporting fibroblasts is sufficient to result in a significantly increased osteoclastogenesis at a much later time point, 21 days.

Cell-cell interactions during osteoclastogenesis also play a pivotal role in the migration of osteoclast precursors to the bone surface. It has been shown that the retraction of osteoblast-like cells is a prerequisite for the osteoclast precursors to migrate. In chapter 5 we investigated migratory behavior of osteoclast precursors at different time points. At an early time interval during differentiation, the cells migrated further away from their initial position compared to the later stage. At this later time point the cells were still highly motile, but did not travel long distances. We next monitored isolated osteoclasts and showed for the first time that osteoclasts can undergo fission and generate a number of functional multinucleated compartments. Besides, also compartments that contained apoptotic nuclei were shed from the osteoclast. Together, these findings suggest important migratory activities during osteoclastogenesis: First, the osteoclast precursor explores the environment in search for fusion partners whereas in a later stage the osteoclast precursor moves in a much more localized area, possibly preparing for an interaction with neighboring cells. The osteoclast splitting up into several multinucleated cells could suggest that this is needed to simultaneously control bone resorption at different sites. In addition, fission provides a means to shed apoptotic nuclei.

In chapter 6 we investigated the biogenesis and activity of mitochondria during osteoclastogenesis. More specifically, we determined the role of M-CSF and receptor activator of nuclear factor kappa B ligand (RANKL), important cytokines during osteoclastogenesis, in these processes. Based on mRNA expression profiles, we could distinguish macrophage and osteoclast differentiation in the M-CSF and M-CSF/RANKL cultures, respectively, and found a differential up-regulation of mitochondria-related genes during these processes. Differences between mitochondria in those two cell types also existed at the morphological level. The mitochondria of the osteoclasts were larger and more mitochondria were formed per cytoplasmic surface area in this culture compared to the other cultures. On the other hand, it was M-CSF that strongly stimulated the mitochondrial activity per cell and increased the percentage of cells with a high mitochondrial activity.

In conclusion, the data presented in this thesis show the importance of cell-cell interactions between osteoclast precursors and bone lining cells during osteoclast formation. They increase the gene expression of osteoclastogenesis-related genes and they regulate the migration dynamics of the different cell types. In addition, they also augment the ultimate formation of multinucleated osteoclasts. The important role of the supporting osteoblast-like cells in osteoclastogenesis has been further underlined by the results on the role of M-CSF in the biogenesis of mitochondria during this process and, finally, the intriguing finding of osteoclasts that undergo fission opens new paths for investigation.