Cell-cell interactions during osteoclastogenesis

Bloemen, V.

2010

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
CHAPTER 1

General Introduction

Dans le port d'Amsterdam  
Y a des marins qui chantent  
Les rêves qui les hantent  
Au large d'Amsterdam

Amsterdam – Jacques Brel
Bone remodeling

Bone structure
Bone is a dynamic tissue that consists of various structures. Cancellous or woven bone is a porous network of trabeculae that provides the flexibility to the tissue. It often changes into a dense bone structure during development. This compact bone is called cortical bone and ensures the bone’s rigidity. Into the cortical bone small tubular canals, the Haversian systems, are present. These canals contain blood vessels and provide the inner tissue with nutrients \(^1\)-\(^3\).

Bone is in close contact with bone marrow, a tissue found in the centre of bones. A distinction can be made between red bone marrow and yellow bone marrow. The latter is almost exclusively made of fat tissue whereas the former is rich of blood cells \(^2\),\(^4\),\(^5\).

Adaptation of bone
The main function of bone is to provide support for the body and protect the organs. To do so, this tissue is able to adapt its mass and structure to the mechanical loading that is exerted on it \(^6\),\(^7\). When a high load is applied, bone mass will increase in order to be able to support the increased load whereas in situations of a reduced mechanical loading, e. g. in space, bone mass will be reduced. The mechanism behind this unique property is exerted at a cellular level and can be summarized as a close interplay between the different bone cells wherein the osteocyte plays a central role \(^8\),\(^9\).

Bone cells
Bone contains four types of bone cells: the osteocyte, the bone lining cell, the osteoclast and the osteoblast. Osteocytes are the cells that are most abundantly present, about 90% of all bone cells are osteocytes. These cells develop from osteoblasts that become entrapped in the bone matrix and are characterized by their unique shape. When enclosed by matrix, osteocytes develop long, cytoplasmic extensions, the so called processes. With these processes they are in contact with each other, this way forming an extensive osteocyte network.

The main function of osteocytes is to send signals to other bone cells in a response to changes in mechanical loading. The osteocyte is
surrounded by a small layer of interstitial fluid and when mechanical loading changes, small deformations in the fluid layer surrounding the osteocyte will result in intercellular signaling to alter the activity of osteoblasts and osteoclasts.\textsuperscript{8,10-12}

Osteoblasts are cuboidal cells that are responsible for the formation of bone. When mechanical loading is increased, osteoblasts will start to synthesize bone matrix, the osteoid, and deposit the newly formed bone at places where extra support is needed. Osteoclasts, on the other hand, are the cells that degrade bone matrix. These flattened cells are characterized by their multinuclearity and a ruffled border at the site where resorption occurs.\textsuperscript{13}

The bone lining cells are fibroblast/osteoblast-like cells that cover the bone surface. After bone resorption has taken place, these cells enter the Howship’s lacunae and clean the surface in order to make it possible for the osteoblasts to deposit new bone matrix. Furthermore, it has been shown that bone lining cells play an important, supporting role in the formation of osteoclasts. They are characterized as ICAM-1\textsuperscript{+} cells and osteoclast precursors adhere to these cells. After adhesion, bone lining cells are able to retract and create the space needed for the osteoclast precursors to migrate to the bone surface.\textsuperscript{14-18}

Even when not subjected to changes in mechanical loading, bone is continuously remodeled. The interplay between osteocytes which send signals to osteoclasts to resorb bone, the bone lining cells that in turn clean the surface after bone resorption and osteoblasts that enter the lacunae to deposit new matrix makes bone a “young” and dynamic tissue.

**Osteoclasts: specialized bone resorbing cells**

**Morphology and function**

Osteoclasts are large cells that can contain a high number of nuclei. These multinucleated cells have an apical and basolateral pole that differ both morphologically and functionally. The basolateral side faces the local cellular environment and has receptors for distinct hormones and growth factors. The apical pole is characterized by a clear zone that attaches to the bone matrix to isolate an extracellular microenvironment where bone has to be resorbed.\textsuperscript{13,19-21} In the center of the clear zone resorption takes place adjacent to the so-called ruffled border. This site is characterized by a high
expression of V-H\textsuperscript{+}-ATPase, a protein that is required for the transportation of H\textsuperscript{+} to the extracellular environment. The secretion of H\textsuperscript{+}-ions leads to an acidification of the ruffled border and a pH of 4-5 can be reached. Acidification causes solubilization of mineral crystallites from the bone matrix\textsuperscript{22-24}. To ensure neutrality of ions in this compartment, the osteoclast is equipped with a chloride pump (ClC7) which transports Cl\textsuperscript{-} ions to the H\textsuperscript{+}-rich clear zone\textsuperscript{25,26}.

The H\textsuperscript{+} ions are formed by conversion of carbon and water in bicarbonate and H\textsuperscript{+}. This process is mediated by the enzyme carbonic anhydrase II, an isoform of carbonic anhydrase that is almost exclusively found in the osteoclast. The intracellular pH is kept neutral by specialized anion exchangers such as AE2\textsuperscript{27,28}.

After calcium has been dissolved from the bone matrix, bone degrading enzymes will further degrade the non-mineralized substances by cleaving matrix molecules such as collagen type -1. Matrix metalloproteinases (MMPs) and cathepsins are two important groups of bone degrading enzymes with each a different optimal working pH\textsuperscript{29-31}. Cathepsins (such as cathepsin K) are active in an acidic milieu (pH 4-5) whereas MMPs (such as MMP-9, MMP-13) degrade collagen type I in a neutral environment (pH 7). The latter enzymes are thought to play a role in bone resorption at specific sites. It has been suggested that they are involved in calvarial bone resorption but not in the resorption of bone in long bones\textsuperscript{32}.

In order to facilitate the high energy demands required in this process, mitochondria are abundantly present in osteoclasts\textsuperscript{33-36}. A recent study has demonstrated the involvement of the peroxisome proliferator activator receptor- gamma co-activator PGC-1\textbeta in the formation of mitochondria during osteoclastogenesis\textsuperscript{37}. However, little is known about the expression of other mitochondria-related genes and how biogenesis of these organelles is regulated in these cells has not been elucidated yet.

**Osteoclast formation**

**Sequence of events**

Osteoclast precursors are hematopoietic in origin and derive from the monocyte/macrophage lineage\textsuperscript{38,39}. Mononuclear cells are able to differentiate into cells that express tartrate resistant acid phosphatase
(TRACP), a marker enzyme for osteoclastogenesis. These mononuclear cells are present both in peripheral blood and in the marrow spaces. They can differentiate into functional osteoclasts when cultured in the presence of macrophage colony stimulating factor (M-CSF). Also human granulocyte-macrophage progenitor cells can differentiate into osteoclast-like, bone resorbing multinucleated cells when cultured with GM-CSF for a week and with \(1,25(\text{OH})_2\text{VitD}_3\) for an additional two weeks. Since recently peripheral blood mononuclear cells (PBMCs) are a commonly used source for human osteoclast precursors. Stimulated with M-CSF and RANKL or co-cultured with osteoblast-like cells, these mononuclear cells form TRACP-positive, multinucleated cells that resorb bone.

Osteoclast precursors are selected by the osteoblast-like bone lining cells and after selection the osteoclast precursors will adhere to these supporting bone lining cells. Adhesion between these two cell types will lead to a cascade of signals to stimulate the osteoclast precursor to further differentiate towards the osteoclast lineage. Following adhesion and partial differentiation, osteoclast precursors prepare to migrate to the bone surface. Simultaneously, bone lining cells retract to create a space in order to select the site were resorption has to occur and to facilitate the migration of osteoclast precursors. The latter cells migrate to the bone surface and finally fuse with fellow precursors. How fusion is regulated and how multinucleated cells interact with osteoclast precursors and other osteoclasts is not known yet.

**Osteoblast-osteoclast precursor interactions**

Osteoclast precursors derive from monocytes that are selected and recruited by bone lining cells at time points when bone resorption and thus osteoclast formation is required. To do so, bone lining cells release mediators, also called chemokines, which attract specific subpopulations of monocytes.

Examples are CCL5 (or RANTES) and CCL2 (or MCP-1), the latter being hardly expressed in healthy tissue but being up-regulated on stromal cells under inflammatory conditions.

After selection, adhesion of osteoclast precursors to the bone lining cells takes place. The ability of cells to recognize and adhere to one another has been widely described and several groups of adhesion molecules have been identified. In the perspective of osteoclast formation and adhesion between osteoclast precursors and bone lining cells, integrin- and cadherin-
based adhesions have been identified\textsuperscript{17,18,53-56}. The heterotypic integrin interactions include two major members which have been found to play important roles during osteoclastogenesis. Vascular adhesion molecule-1 (VCAM-1) was first found on endothelial cells and can bind to its receptor very late antigen-4 (VLA-4) on several cell types including leukocytes, T-and B-cells, eosinophils and monocytes. Next, VCAM-1 expression was also reported for bone lining cells and inhibition of VCAM-1 expression on these cells inhibited osteoclast formation in a co-culture of stromal cells and osteoclast precursors\textsuperscript{45}. However, the most described adhesion molecule involved in osteoclast precursor-bone lining cell interactions is intercellular adhesion molecule -1 (ICAM-1). ICAM-1 is a 90 kDa transmembrane glycoprotein that belongs to the immunoglobulin (Ig) superfamily. It consists of five extracellular IgG-like domains and a short cytoplasmic tail that associates with cytoskeletal proteins.

The critical role of this molecule in the formation of osteoclasts has been shown by a study of Tanaka et al.; they distinguished ICAM-1\textsuperscript{+} and ICAM-1\textsuperscript{-} osteoblasts and showed that osteoclasts were only formed in co-cultures of osteoclast precursors and ICAM-1\textsuperscript{+} osteoblasts\textsuperscript{18}. The main ligands of ICAM-1 are leukocyte function-associated antigen-1 (LFA-1) and Mac-1, β2 integrins, and are expressed by osteoclast precursor cells\textsuperscript{53-55}. Shedding of membrane-bound ICAM-1 leads to the release of soluble ICAM-1, sICAM-1, in the serum. Elevated levels of sICAM-1 have been found in patients suffering from e.g. multiple sclerosis and rheumatoid arthritis which could contribute to the increased bone resorption found in these patients\textsuperscript{57}.

Endothelial cells release sICAM-1 upon stimulation \textit{in vitro} and sICAM-1 is able to block lymphocyte attachment to endothelial cells which is normally regulated by membrane-bound ICAM-1. Knockout studies with mice genetically deficient for ICAM-1 resulted in attenuated myocardial necrosis and reductions in myocardial neutrophil infiltration. They showed impaired inflammatory and immune responses due to the lack of ICAM-1\textsuperscript{58}. Whether depletion of ICAM-1 also leads to a specific bone phenotype in these mice is not known.

Besides heterotypic interactions also homotypic calcium-dependent cadherin binding has been described during osteoclast differentiation. Cadherin-6 mediates the interaction between murine osteoclast precursors and stromal cells. Both cell types express cadherin-6 isoforms and inhibition of the cadherin-mediated adhesion results in an
impairment of osteoclastogenesis. Besides cadherin-6, no involvement of cadherins has been reported with regard to the adhesion of osteoclast precursors to osteoblast-like bone lining cells.

Adhesion between those two cell types induces a cascade of signals and it is plausible that this is associated with the release of the two critically important cytokines expressed by the bone lining cells; macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-β (RANKL).

M-CSF is a glycoprotein growth factor that induces the proliferation and differentiation of cells of the macrophage and monocyte lineages. It binds to its receptor c-fms, expressed by osteoclast precursors. The critical role of this differentiation factor in osteoclast biology has been elucidated by studies with osteopetrotic op/op mice. The bone defect was found to be associated with a mutation in the M-CSF gene which made the protein inactive. Due to the lack of activity of M-CSF in these mice, osteoclast formation was impaired and a reduced bone resorption led to osteopetrosis. In co-cultures of osteoblastic cells from the op/op mice and normal spleen cells, the inhibited osteoclast formation could be restored by the addition of recombinant M-CSF. Administration of this recombinant cytokine to op/op mice also rescued them from osteopetrosis, indicating that M-CSF plays a critical role in osteoclast formation in vitro as well as in vivo.

The second crucial cytokine needed for osteoclast formation is RANKL, a membrane bound cytokine and member of the tumor necrosis factor (TNF) - family. RANKL binds to its receptor RANK on osteoclast precursors and the RANKL/RANK pathways in osteoclast biology were also confirmed by mouse genetic studies. Depletion of RANK or RANKL resulted in osteopetrotic mice due to reduced osteoclast formation and subsequent osteoclast activity.

Besides the membrane bound form, RANKL also exists as a soluble factor. sRANKL can be formed by shedding of mRANKL by so called sheddases such as matrix metalloproteinase-14 (MMP-14).

A decade ago, several studies reported the discovery of a soluble member of the tumor necrosis factor receptor superfamily which was able to bind to RANKL and suppress osteoclast formation both in vitro and in vivo. This decoy receptor was named osteoprotegerin (OPG). The binding of OPG to RANKL blocked its activity by competitively suppressing the RANKL/RANK interaction. The ratio OPG/RANKL is therefore considered
crucial in the process of osteoclast formation. When an excess OPG is produced, osteoclastogenesis will be inhibited whereas a low OPG/RANKL ratio favors the formation of osteoclasts \(^{62-64}\).

Also after fusion of osteoclast precursors into a multinucleated cell, the interactions between osteoblast-like cells and osteoclasts seem to be important. The osteoblastic expression of M-CSF and RANKL has found to have an anti-apoptotic effect on osteoclasts \(^{65}\). However, little is known about the interactions between multinucleated osteoclasts and the supporting bone lining cells.

**Osteoclast precursor-osteoclast precursor interactions**

Another important type of cell-cell interaction during osteoclast formation is the interaction among osteoclast precursors. Osteoclast precursors originate from a heterogeneous group of monocytic cells. It has been suggested that osteoclast precursors arise from a CD14\(^+\) CD16\(^-\) subpopulation, but an exclusive cell marker has not been elucidated yet \(^{39}\). Moreover, it is not known what the role is of the other monocytic cells during the differentiation of osteoclast precursors.

A critical process that also involves cell-cell communication and that has been only limitedly studied so far is the trafficking of osteoclast precursors to and from the bone surface. A recent study shows the involvement of spingosine-1-phosphate (S1P), a molecule that had previously been found to control cell migration in other tissues \(^{66}\). However, the migration dynamics during osteoclastogenesis are still not known yet, as well as the type of cell-cell interactions during migration.

After migration, the osteoclast precursors prepare to fuse. To do so, they interact with each other and several molecules that are thought to be involved in cell fusion have been described. The first molecule identified was signal regulatory protein-\(\alpha\) (SIRP\(\alpha\)), a transmembrane protein that belongs to the immunoglobulin superfamily. SIRP\(\alpha\) interacts with CD47 and SIRP\(\alpha\)/CD47 interaction seems to play a role in cell-cell recognition prior to the actual cell fusion process \(^{67,68}\). However, recent work from our group demonstrated that the SIRP\(\alpha\) deficient mouse contains an equal number of multinucleated osteoclasts \textit{in vivo} as the wild-type mouse. Also \textit{in vivo}, osteoclastogenesis was comparable as in wild-type mice. SIRP\(\alpha\) knock-out osteoclasts formed \textit{in vitro} were more active as demonstrated with increased bone resorption and an increased number of actin rings per osteoclast \(^{69}\).
Dendritic cell-specific transmembrane protein (DC-STAMP) on the other hand, has been identified as a key regulator of cell fusion between osteoclast precursors. DC-STAMP−/− mononuclear cells are largely impaired in their ability to fuse with each other. No multinucleated osteoclasts are formed in vivo in DC-STAMP−/− knockout mice. In addition, formation of multinucleated giant cells was severely hampered in an experimental model. Although this suggests an important role for DC-STAMP in cell fusion, the mechanisms by which DC-STAMP regulates osteoclast fusion still remain unclear. Other molecules that are suggested to be involved in the fusion of osteoclast precursors include the vo subunit of v-ATPase (ATP6vo2), CD44, CD98, MCP-1 and E-cadherin.

Recently, new light has been shed on the whole process of osteoclast fusion. Time-lapse analyses showed that large, already multinucleated cells were able to ‘catch’ mononuclear osteoclast precursors to subsequently being followed by fusion between those two cells. It thus seems that not only osteoclast precursors fuse, but that cell-cell interaction and fusion also play an important role between multinucleated osteoclasts and their precursors.

Osteoclast formation and inflammation

Diseases

Periodontitis is a chronically inflammatory disease that affects the tissues surrounding the teeth and is hallmarked by alveolar bone loss. The pathogenesis of this disease starts with a bacterial infection that leads to inflammation of the gums. This in turn activates the innate immune system and proinflammatory factors are released. A failure in reducing the inflammation will lead to an expansion of the inflamed site and will drive the destruction of connective tissues and alveolar bone.

Proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-11, IL-17 and tumor necrosis factor – α (TNF-α) have a stimulatory effect on the bone resorbing activity of osteoclasts. Moreover, these factors can induce RANKL expression by osteoblast-like cells which results in an increased formation of osteoclasts. Likewise, gram-negative bacteria and their products (such as lipopolysaccharide (LPS)) can also induce osteoclastogenesis. Inflammation-induced osteoclast formation can either
directly affect the number of osteoclast precursors, or indirectly, by affecting the bone lining cells.

Periodontal ligament fibroblasts are known to act as bone lining cells and support osteoclastogenesis. Recent studies have reported a down-regulation of the OPG/RANKL ratio by P. gingivalis in these fibroblasts as well, denoting their stimulatory effect on osteoclastogenesis in periodontitis.

Another chronic inflammatory disorder with effects on bone remodeling is rheumatoid arthritis (RA). This systemic disease primarily targets the joints and leads to pathological cartilage and bone loss. The synovial inflammatory tissues induce the local formation of bone-resorbing osteoclasts and the synovial membrane of RA contains many monocytes which can differentiate towards the osteoclast lineage. Similar to the pathogenesis in periodontitis, leads the invasion of proinflammatory factors in RA to an up-regulation of RANKL and an overall lowering of the OPG/RANKL ratio in the synovial joint.

A third very common disease wherein osteoclast formation is affected is osteoporosis. Osteoporosis is, in contrast with periodontitis and rheumatoid arthritis, principally a bone disease. This bone disorder is characterized by a reduced bone mineral density (BMD) which increases the risk of fractures. The reduction in BMD is caused by an imbalance in bone formation and bone resorption, with a decrease in formation and an increase in resorption, respectively.

Abnormalities in endocrine, metabolic and mechanical factors (such as insufficient vitamin D or calcium intake, changes in the level of parathyroid hormone, long term administration of glucocorticoids and menopausal changes in sex hormones) are attributing to the development of the disease. Recent observations showed a striking coincidence between the presence of osteoporosis and the occurrence of systemic inflammation. It has been recognized that T-lymphocyte subsets are altered in osteoporotic menopausal women, indicating a role for these cells in this process. T cell deficient nu/nu mice were found to be protected from ovariectomy-induced osteoporosis and can thus be considered as important modulators of bone remodeling. These findings raised the question whether osteoporosis could also be identified as an inflammatory process. Several epidemiological studies showed an increased risk of developing osteoporosis in inflammatory diseases such as HIV infections, rheumatoid
arthritis, and inflammatory bowel diseases. Moreover, bone loss induced by estrogen deficiency was strongly inhibited in knock-out mice deficient for TNF-α which could indicate a prominent role for this inflammatory factor in the disease 74,86.

TNF-α, as well as IL-1, not only stimulates osteoclast formation but also promotes the bone resorbing activity of mature osteoclasts. RANKL expression is increased by the osteoblasts and OPG production is lowered in the absence of estrogen, leading to an increase in the number of osteoclasts formed 87.

**Inflammatory factors and bone**

Tumor necrosis factor-α (TNF-α) is a member of a large family of inflammatory cytokines and TNF-producing T-cells are thought to be the genuine source of TNF-α 84. However, TNF-α is expressed by a variety of cells including monocytes/macrophages. The synthesis of the cytokine is triggered by lipopolysaccharide (LPS), immune complexes, IL-1 and as an autoimmune regulator also by TNF-α itself. TNF-α is synthesized *de novo* after cell activation and this is regulated at a transcriptional or post-transcriptional level. A pool of TNF-α mRNA that is not expressed as protein can be present in the cell, which will be translated after the cell gets activated; thus facilitating a rapid cellular response 74.

TNF-α binds to a TNF-α receptor and it functions mainly as a regulatory cytokine or as a mediator involved in inflammatory reactions 88. In an immune response this cytokine triggers the production of other cytokines such as IL-1, TGF-α and TGF-β. TNF-α also stimulates phagocytosis and favors adhesion to endothelial cells 89,90. The expression of the adhesion molecule ICAM-1 by the endothelial cells increases under influence of TNF-α which promotes the adherence of PMNs to the endothelium 91,92.

TNF-α plays also a crucial role in osteoclast formation and activity. As already mentioned earlier, this cytokine promotes the expression of RANKL by osteoblast-like cells and is also capable of stimulating the expression of M-CSF by these cells. There are conflicting findings about whether its effect on osteoclastogenesis is dependent on RANKL/RANK signaling or not 93-97. Though, a recent study by Kim et al. provides evidence that bone marrow precursors from RANKL-, RANK or TRAF6-null mice can differentiate into functional osteoclasts *in vitro* after stimulation with TNF-α in the presence of cofactors such as TGF-β. This suggests that alternative
pathways, independent of the RANKL-RANK axis, can lead to osteoclast formation\(^98\).

Whereas TNF-\(\alpha\) plays a favoring role in osteoclastogenesis, it impairs the activity of bone-forming osteoblasts and induces pathological bone loss in both ways. This cytokine blocks the differentiation of new osteoblasts and regulates the expression of several genes. The production of type I collagen is strongly inhibited as well as the mRNA expression of osteocalcin, another skeletal matrix protein. TNF-\(\alpha\) also inhibited normal mineralization by affecting the regulation of alkaline phosphatase \(^{99-102}\). Taken together, this inflammatory cytokine plays a central role in the pathophysiology of bone loss.

Interleukin-1\(\beta\) (IL-1\(\beta\)) has often been compared with TNF-\(\alpha\) because of similar actions on bone metabolism. It has been demonstrated that pro-inflammatory cytokines such as IL-1\(\beta\) and TNF-\(\alpha\) can modulate the expression of ICAM-1 in several cell types \(^{18,103,104}\) including osteoblast-like cells. Whether this results in an augmented adhesion of osteoclast precursors or whether a short stimulation with these factors can also affect the later stages of osteoclast formation is not known.

**Anti-inflammatory agents: glucocorticoids**

About half a century ago, Hench and colleagues discovered the anti-inflammatory action of glucocorticoid hormones \(^{105}\). Although in the meantime serious side-effects of long term glucocorticoid administration have been found, synthetic glucocorticoids are still the gold standard for anti-inflammatory and immunosuppressive therapy. These corticoids bind to a glucocorticoid receptor (GR) and inhibit the expression of cytokines, chemokines, adhesion molecules and many other genes involved in inflammatory and immune responses \(^{106,107}\). The most regularly used glucocorticoid agent is dexamethasone. High-dose treatment with dexamethasone is closely associated with severe bone loss, the most serious side effect of glucocorticoid-therapy \(^{108-111}\). Dexamethasone-induced osteoporosis affects both bone formation and bone resorption. The complex mechanism by which glucocorticoids induce a disturbed bone remodeling is still not fully understood.
Aim

The aim of the studies in this thesis was to investigate the role of cell-cell interactions during the differentiation of osteoclasts. Different aspects of osteoclast formation were investigated and the following questions were addressed:

1. Does the expression of adhesion molecules change during osteoclast differentiation and what is the effect of cell-cell interaction here upon?

2. What is the effect of cell-cell interaction on the expression of other osteoclastogenesis-related genes and does this effect change the ultimate formation of osteoclasts?

3. What is the influence of inflammatory cytokines on cell-cell interactions during osteoclastogenesis?

4. How do the different cell types interact with each other in relation to their migratory dynamics?

5. Are the osteoblastic cytokines M-CSF and RANKL involved in the biogenesis of mitochondria during osteoclast differentiation?

Thesis outline

In this thesis I will describe our results on the effect of cell-cell interaction during osteoclastogenesis. In chapter 2 I will focus on its role in the expression of the adhesion molecule ICAM-1. Hereby I suggest a possible new function for this molecule on the osteoclast precursor. Next, the importance of direct cell-cell contact between osteoclast precursors and periodontal ligament fibroblasts is investigated in chapter 3. mRNA and protein expression of osteoclastogenesis-related genes as well as the formation of bone resorbing multinucleated cells is investigated in the absence and presence of bone lining cells. Since osteoclastogenesis is highly susceptible to actions of inflammatory cytokines such as TNF-α and IL-1β, the study described in chapter 4 reports the effects of a short IL-1β stimulation of periodontal ligament fibroblasts and the implications for the ultimate formation of osteoclasts. Whereas in chapters 2-4 the focus was on heterotypic cell-cell interactions between bone lining cells and osteoclast precursors, chapter 5 touches upon the interactions between osteoclast precursors and osteoclasts in order to shed light on the migratory dynamics of these cells throughout the process of osteoclastogenesis. In chapter 6 I
have addressed the interesting though little studied biogenesis of mitochondria during osteoclast differentiation. The effects of the osteoclastic regulators M-CSF and RANKL on mitochondrial formation, activity and morphology were examined at different time points. In the final chapter 7, I will discuss the role of cell-cell interaction during osteoclastogenesis from different points of view and I will propose a new model of how bone lining cells and osteoclast precursors interact with each other.
References


63  Belibasakis GN, Bostanci N, Hashim A et al. Regulation of RANKL and OPG gene expression in human gingival fibroblasts and


70 Miyamoto T. The dendritic cell-specific transmembrane protein DC-STAMP is essential for osteoclast fusion and osteoclast bone-resorbing activity. *Mod. Rheumatol.* 2006; **16**: 341-2.


General Introduction


94 Fuller K, Murphy C, Kirstein B, Fox SW, Chambers TJ. TNFalpha potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. *Endocrinology* 2002; **143**: 1108-18.


104 Kurokouchi K, Kambe F, Yasukawa K et al. TNF-alpha increases expression of IL-6 and ICAM-1 genes through activation of NF-


107 Cooper MS, Hewison M, Stewart PM. Glucocorticoid activity, inactivity and the osteoblast. *J. Endocrinol.* 1999; 163: 159-64.


