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Review Article

# The osteoclast, a target cell for microorganisms

Brigitte Raynaud-Messina, Christel Verollet[1], Isabelle Maridonneau-Parini*,[1]

*Institut de Pharmacologie et Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France*
*International Associated Laboratory (LIA) CNRS "IM-TB/HIV" (1167), Toulouse, France*
*International Associated Laboratory (LIA) CNRS "IM-TB/HIV" (1167), Buenos Aires, Argentina*

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ABSTRACT

Bone is a highly adaptive tissue with regenerative properties that is subject to numerous diseases. Infection is one of the causes of altered bone homeostasis. Bone infection happens subsequently to bone surgery or to systemic spreading of microorganisms. In addition to osteoblasts, osteoclasts (OCs) also constitute cell targets for pathogens. OCs are multinucleated cells that have the exclusive ability to resorb bone mineral tissue. However, the OC is much more than a bone eater. Beyond its role in the control of bone turnover, the OC is an immune cell that produces and senses inflammatory cytokines, ingests microorganisms and presents antigens. Today, increasing evidence shows that several pathogens use OC as a host cell to grow, generating debilitating bone defects. In this review, we exhaustively inventory the bacteria and viruses that infect OC and report the present knowledge in this topic. We point out that most of the microorganisms enhance the bone resorption activity of OC. We notice that pathogen interactions with the OC require further investigation, in particular to validate the OC as a host cell *in vivo* and to identify the cellular mechanisms involved in altered bone resorption. Thus, we conclude that the OC is a new cell target for pathogens; this new research area paves the way for new therapeutic strategies in the infections causing bone defects.

## 1. The OC biology

Bones provide a mechanical support for locomotion. Bone fragility is a major health concern, as bone fractures have devastating outcomes in terms of decreased autonomy, healthcare costs and mortality. In addition, the high frequency of bone infection happening upon bone surgery or systemic spreading of pathogens is one of the main causes of altered bone homeostasis. Bone tissue comprises a stiff extracellular matrix mostly composed of type I collagen embedded in minerals, as well as bone cells, blood and nerve networks. It is subjected to continuous remodelling for growth, healing and regulation of phosphate and calcium metabolism, processes that are achieved by the concerted action of osteoblasts/osteocytes and osteoclasts (OC). Osteoblasts are involved in bone formation, they arise from mesenchymal stem cells and are located at the bone surface. Osteocytes are the terminally differentiated form of osteoblasts that are located in the bone matrix [1]. They are the most abundant cells in bone, survive for decades and regulate osteoblastic bone formation and osteoclastic bone resorption through the secretion of biochemical signals. An imbalance in bone resorption *versus* bone formation leads to skeletal diseases including osteoporosis. As infection is one of the major cause of bone defects, this review will focus on the effects of pathogens infecting OC.

Under physiological conditions, OC are the exclusive bone resorbing cells of the organism (Fig. 1A). They are multinucleated giant cells that differentiate from myeloid precursors mainly under the control of Macrophage Colony-Stimulating Factor (M-CSF) and Receptor Activator of Nuclear Factor-κB ligand (RANK-L) with a long lifespan [2,3]. It has been revealed very recently that during development, fetal OC rise from embryonic erythro-myeloid progenitor [2]. After birth, hematopoietic stem cells present in the bone marrow give raise to multipotent progenitors and subsequently to common myeloid progenitors. The latter produce OC, monocytes, macrophages and dendritic cells. In response to M-CSF and RANK-L, common myeloid progenitors differentiate into OC precursors that migrate to bone [4–7]. Thus, after birth, OC originate from precursors derived from blood monocytes that fuse with embryonic OCs and also from precursors that fuse together [2,8]. The cell fusion process involves proteins including DC-STAMP, hyaluronate receptor CD44 and proteases, as well as the remodelling of membrane phospholipids [9–11].

RANKL is a transmembrane protein expressed at the cell surface, which forms homotrimers [12]. It can be cleaved by MT1-MMP (also called matrix metalloproteinase 14, MMP14), releasing a soluble

* Corresponding author.
*E-mail address:* maridono@ipbs.fr (I. Maridonneau-Parini).
[1] These authors contributed equally to this work.

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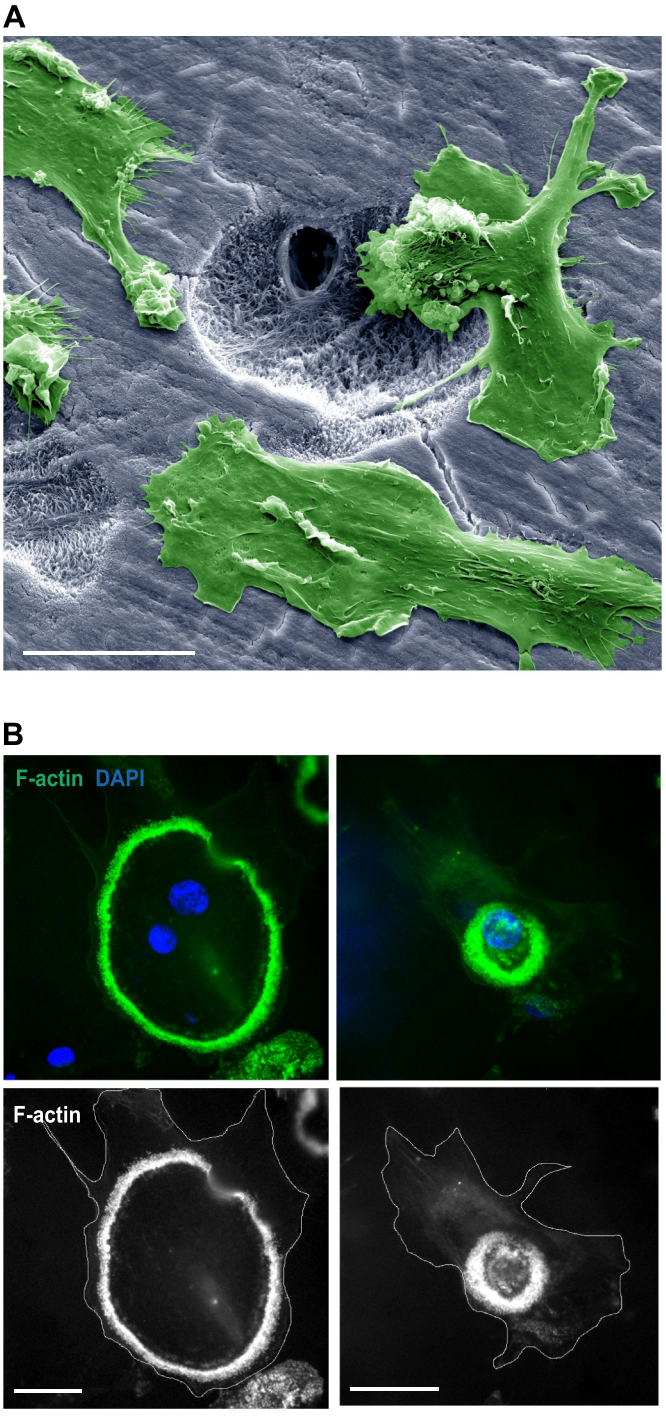


**Fig. 1.** Human OC mediate bone matrix degradation and form a particular cell structure: the sealing zone.
(A) Scanning electron microscopy image showing a cavity formed by degrading human OC (colored in green) and bone matrix (colored in grey) modification. Scale bar, 20 µm. (B) Immunofluorescence images showing sealing zones in mature human OC. Cells are delimitated in white. Scale bar, 10 µm. Briefly, human OC were differentiated from CD14+ human monocytes during 10 days in the presence of M-CSF and RANK-L, as described in [122]. Mature OC were detached with Accutase and seeded on bovine cortical bone slices for 48 h. In A, the cells were then fixed in 2.5% glutaraldehyde/3.7% paraformaldehyde (PFA)/0.1 M sodium cacodylate (pH 7.4), post-fixed in 1% osmium tetraoxide (in 0.2 M cacodylate buffer), dehydrated in a series of increasing ethanol concentration and then critical point-dried using carbon dioxide in a Leica EM CPD300. After coating with gold, cells were examined with a FEI Quanta FEG250 scanning electron microscope. In B, after 3.7% PFA fixation, cells were permeabilized and stained for F-actin (phalloidin Alexa-488, in green) and DAPI (in blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cytokine monomer which binds to RANK with a lower affinity than the trimeric RANKL conformation [9]. RANKL/RANK signaling activates Nuclear Factor Of Activated T Cells 1 (NFATc1), the master regulator of osteoclastogenesis, to induce osteoclastogenic gene expression [13]. This pathway is controlled by osteoprotegerin (OPG), which acts as a decoy receptor for RANKL. The ratio between OPG and RANKL determines the level of activation of RANK and, thereby, the degree of osteoclastogenesis [14]. Interestingly, both RANKL and OPG are regulated by several calcium-regulating hormones, such as sex hormones, the parathyroid hormone and vitamin D3. RANKL is secreted by bone marrow stromal cells and osteoblasts/osteocytes. In young mice, osteoblasts are the main source of RANKL with chondrocytes, whereas in adults, osteoblasts do not significantly contribute to the production of RANKL, which is mainly expressed by osteocytes [9]. While the bone environment is the major source of RANKL under physiological conditions, other sources such as B cells and T cells contribute to RANKL production under inflammatory conditions [14,15]. In animal models of arthritis and inflammatory osteolysis, the expression of RANKL by $CD4^+$ T cells mediates bone loss and joint destruction [16,17]. The exclusivity of RANKL as the osteoclastogenesis cytokine has been discussed, since other factors such as tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) have been described to participate in OC differentiation [18,19]. The increase in proinflammatory cytokines including TNF-α, IL-1β, interleukin (IL)-6, IL-17 provides a supportive environment for osteoclastogenesis but, importantly, they cannot substitute for RANKL [20]. These cytokines are also involved in the transdifferentiation of dendritic cells into OC [9,15,21], a process restricted to inflammatory conditions, including infections [21–25]. Of note, in physiological conditions, immature dendritic cells are rarely located in bone tissue and dendritic cell-deficient mice do not exhibit any bone defect [21,26].

The reciprocal impact of OC on osteoblasts and osteocytes to maintain bone homeostasis has been the object of recent reviews that detail the current knowledge on the OC-osteoblast coupling *via* the so-called basic multicellular units [27–29]. Briefly, the bone remodelling process inside basic multicellular units involves the coordinated action of the bone lining cells, an alternative source of osteoblasts that form a monolayer covering quiescent bone surface, together with osteocytes, OC and osteoblasts [30]. Factors such as TGF-β and insulin-like growth factor 1 (IGF1), which operate as chemotactic, proliferating and differentiating agents for osteoblasts, are stored in the bone matrix and liberated upon bone resorption by OC. In addition, clastokines such as the complement factor C3a or Collagen Triple repeat containing 1 (CTHCR1) are secreted by OC to stimulate osteoblast progenitor recruitment and differentiation, as well as bone formation [27]. Reciprocally, osteoblasts produce both inhibitory and stimulatory factors that stimulate OC differentiation [28].

The bone resorption machinery of the mature OC (highly positive for tartrate resistant acid phosphatase (TRAP)) is a sophisticated system that releases, in a confined space, inorganic and organic bone degrading factors, including $H^+$, $Cl^-$, cathepsins and metalloproteases. This space, called the resorption lacunae, consists of an actin ring, the sealing zone, surrounding the ruffled border membrane, the OC resorptive organelle. It maintains the concentration of resorbing factors and prevents them from diffusing into the surroundings with potential damaging effects [3,31,32]. On the other way, molecules over *M*r 10,000 in the incubation medium do not diffuse under OC indicating that the plasma membrane tightly adhere to the substratum and form a diffusion barrier [33,34]. The ruffled border above the resorption lacunae has the morphology of a scrubbing brush. It hosts both the exocytosis of proteases and V-ATPase and the endocytosis of degraded bone materials [35–40].

The sealing zone consists of podosomes (Fig. 1B). Podosomes are actin-rich cell structures of 1 µm diameter, perpendicular to the matrix, that form constitutively in a few cell types, including macrophages, immature dendritic cells and OC [41–45]. They consist in a core of F-

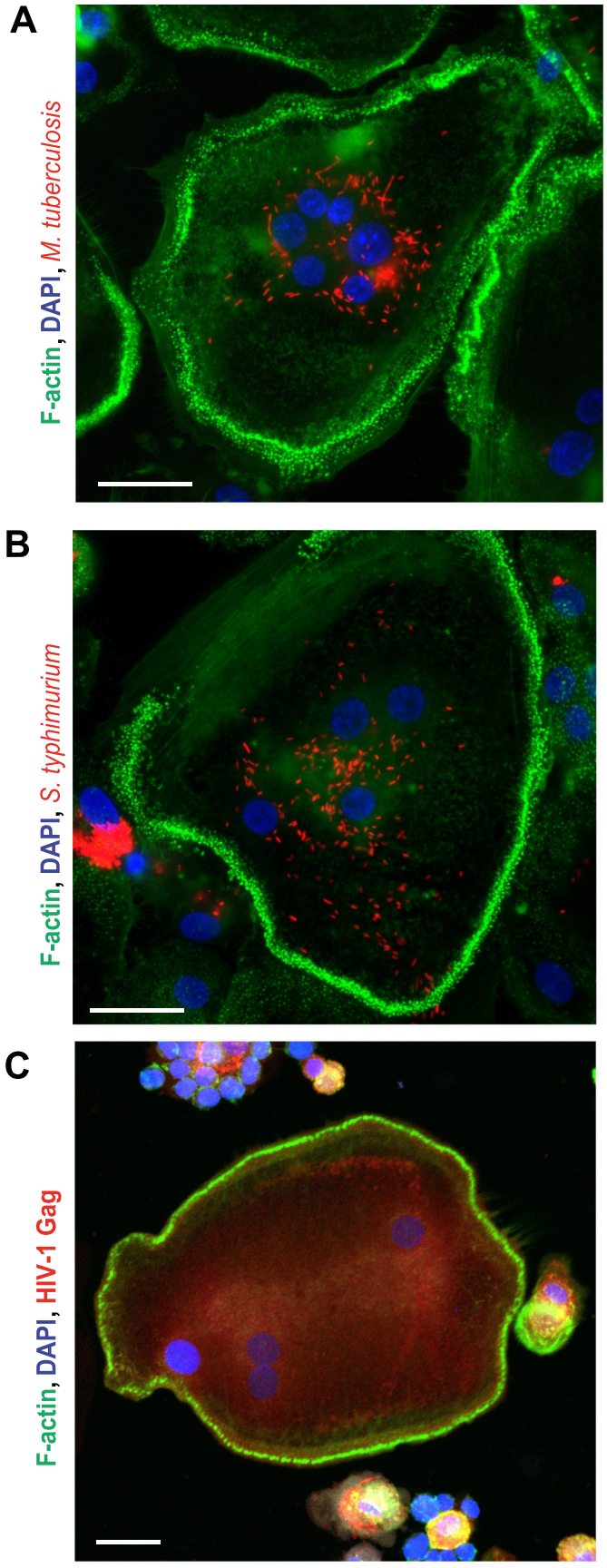


**Fig. 2.** Several pathogens can be internalized by human OC.
Immunofluorescence images showing mature human OC (prepared as in Fig. 1, except that they were seeded on glass) infected with (A) *Mycobacterium tuberculosis* (mCherry-H37Rv, Multiplicity Of Infection (MOI) 6, fixed 24 h post-infection), (B) mCherry-*Salmonella thyphimurium* (MOI 15, fixed 24 h post-infection) and (C) HIV-1 (ADA strain, MOI 0.5, fixed 8 day post-infection). *Mycobacterium tuberculosis* and HIV-1 infections were performed in multi-pathogen BSL3 facility at IPBS. Upon 3.7% PFA fixation, cells were permeabilized and stained for F-actin (phalloidin Alexa-488, in green) and DAPI (in blue). In C, cells were stained for HIV-1 Gag/p24 (in red), as in [122]. Scale bar, 15 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

actin surrounded by an adhesion ring that links integrins to actin. Podosomes exhibit adhesion, protrusion and mechanosensing activities [41–45]. They are mostly scattered in dendritic cells and macrophages, and occasionally organized as clusters, rings and rosettes [45–47]. In mature OC, when cells adhere to glass or plastic coated or not with extracellular matrix proteins, podosomes form large circular structures at the periphery of the OC, called belts (see Fig. 2). When OC are seeded on bone or bone-like surfaces (hydroxyapatite, dentine), tightly packed podosomes connected to each other by a very dense actin network (cloud) form sealing zones (Fig. 1B) [32,48–52]. The OC sealing zone attaches to the bone matrix through integrin αvβ3 and CD44. Localization of the two adhesion proteins has been analysed in OC belts showing that αvβ3 is located outside F-actin while CD44 co-localizes with it [39,51,53,54]. Podosomes are dynamic cell structures with a lifespan of 10–12 min in macrophages and about 2 min in OC [55,56]. The sealing zones are also dynamic structures that assemble to mediate bone resorption and disassemble for OC motility [50]. This corresponds to the “classical model” in which OC start migrating after bone resorption, and lose the sealing zone during migration. However, recent studies suggest that some OC can also resorb bone during migration [32,57]. A recent elegant study that monitors *in vivo* the spatiotemporal changes in bone resorption activity of OC, using intravital microscopy of pH variation, showed that resorbing OC are mostly static and suggests that they control motility to determine bone resorptive activity before the formation of acidic compartments [58]. The tyrosine kinase Src is a master regulator of the OC dynamic adhesion and, consequently, impacts their bone resorbing activity. It is localized at the cell membrane where it binds to and activates actin regulatory proteins such as FAK, Pyk2, p130Cas involved in the formation of podosomes and so, of the sealing zone. Src-deficient mice do not form the sealing zone and are osteopetrotic [54,59–64].

OC functions beyond bone resorption have been reviewed [27]. As bone resorbing cells, OC are involved in the formation and maintenance of the hematopoietic stem cell niche [27]. They are also involved in the control of calcemia and phosphatemia [65]. OC release many factors involved in angiogenesis (Vascular Endothelial Growth Factor, Fibroblast Growth Factor), they exert paracrine stimulation of endothelial cells and play a central role in the formation of bone transcortical vessels [65,66]. OC, monocytes/macrophages and dendritic cells originate from the common myeloid precursor, and as such, the OC is considered as an immune cell belonging to the innate immunity. They have the property to be immunosuppressive and to act as antigen-presenting cells [67,68], as they express class I and class II MHC and co-stimulatory molecules that activate T cell responses [27,65].

OC are viewed as resident macrophages of bones, like Kupffer cells in the liver or microglia in the brain. In addition to OC, a population of resident macrophages, osteal macrophages, has been described. They represent 15–20% of the cells in bone, are involved in regulating bone mineralization mediated by osteoblasts and are thought to be the major bone sentinels for pathogens. They can be distinguished from OC in mice by F4/80 expression and the lack of RANK expression [69–71]. Although it is not clear why OC that are involved in bone degradation should share common properties with macrophages, innate immune

cells, we propose that the following properties are advantageous for OC functions: the ability to fuse and form giant cells increases the bone surface that is degraded, the capacity to release lysosomal enzymes *via* a regulated secretory pathway optimizes their delivery in the resorption lacunae [72,73], and specific podosome organisation is instrumental for bone resorption [39]. Podosomes generate protruding forces that should be an advantage for OC to perform tight sealing onto the bone surface [41,42]. Thus, the monocyte/macrophage properties of OC are likely an opportunity for bone degradation.

OC have been poorly studied as cells of innate immunity. An interesting review dealing with the question why bone needs a cell belonging to the immune system to degrade the bone matrix is available [65]. In the context of the present review, we would like to highlight that OC perform phagocytosis, their phagosomes mature into phagolysosomes and they produce reactive oxygen species. In particular, the level of Nox2 (the NADPH oxidase specialized in bactericidal activity) expressed in murine OCs is almost double of that in phagocytes [74]. Thus, OC are likely involved in immune surveillance. However, as observed in macrophages, several pathogens use OC as a host cell to grow. In this review, we exhaustively report the microorganisms that have been described so far to infect OC (Table 1), and we emphasize that most of them enhance their bone resorption activity, creating bone diseases.

## 2. Bacterial infection of OC

A variety of bacteria (*Salmonella thyphimurium, Cutibacterium acnes, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Mycobacterium tuberculosis, Staphylococcus epidermis*, and most commonly *Staphylococcus aureus)* is involved in bone pathologies such as osteomyelitis, the medical term for bone infection by bacteria, with major consequences on the life of patients. Of note, periodontitis, a tooth infection induced by several bacteria including *Porphyramonas gingivalis*, is one of the most prevalent bone diseases which can affect osteoclastogenesis [75,76]. Several reports have studied how bacteria cause bone defects: most of them describe the infection of osteoblasts by bacteria that can cause their apoptosis, autophagy and necrosis. Osteoblast infection also leads to an imbalance in the production of RANKL and OPG that impacts osteoclastogenesis [77]. Regarding OC, most studies describe an effect of bacterial products, including LPS, on OC differentiation/function [78,79]. Only three reports describing the infection of OC by live *Staphylococcus aureus (S. aureus), Mycobacterium tuberculosis (M. tuberculosis)* and *Cutibacterium acnes (C. acnes*) are available to date.

Osteomyelitis can be acute or chronic. In children, acute bacterial bone infections most commonly occur in the long bones of the arms and legs and most likely spread to other bones through blood circulation. In adults, the hips, spine, and feet are preferentially infected, and chronic osteomyelitis often follow an open injury near a bone, a skin infection or an infection elsewhere in the body, and occurs most of the time upon surgery. These bone infections can break out readily or develop a long time after the initial contamination, depending on the type of bacteria and the immune status of the patient. If they are not properly treated, bone infections can lead to permanent bone damage. Treatment of osteomyelitis is challenging due to a variety of factors, including the poor bioavailability of antibiotics in bone tissue, antibiotic resistance of bacterial pathogens, and the formation of bacterial biofilms that are particularly insensitive to antibiotics. Due to these limitations, osteomyelitis is devastating for many patients, with painful long-term consequences and limited treatment options [78,79].

### 2.1. C. acnes

The pathogenesis of *C. acnes* has long been restricted to skin; however, some specific strains of *C. acnes* are increasingly recognized as pathogens in foreign-body infections, including arthroplasty, prosthetic hip or spinal instrumentation-associated infections [80]. Eleven strains of *C. acnes* are internalized by osteoblasts. Among them, two strains (CC18 and CC28) tested in OC were found to be efficiently internalized and to survive intracellularly for at least 96 h [80]. The mineral resorption ability of infected OC was found to decrease, contrasting with the two bacteria described below, which increase osteoclastogenesis.

### 2.2. S. aureus

The most common bacterium causing chronic osteomyelitis is *S. aureus* [79]. Clinical observations indicate that this infection induces the local recruitment of immune cells and causes progressive bone loss. The effects of *S. aureus* on osteoblasts have been largely described (for a review, see [81]), involving over-production of chemokines, cytokines and growth factors. *S. aureus* is internalized by osteoblasts, survives intracellularly and decreases the activity and viability of osteoblasts, through the induction of apoptosis-dependent and independent mechanisms. *In vivo*, it has been observed that infected femurs lose about 20% of their cortical bone volume, and proposed that it could be due to osteoblast infection causing malfunction [82]. *S. aureus* extracts or cell wall virulent factors such as protein A [83–85] show divergent effects on bone degradation. The impact of *S. aureus* on OC has been poorly studied. It has been shown *in vitro* that both precursors and mature OC derived from mouse bone marrow internalize *S. aureus,* with a higher internalization rate in mature OC than in OC precursors [86]. The bacteria were rapidly eliminated in both cell types in 72 h. How *S. aureus* is ingested by OC is not known. In contrast to osteoblasts, which internalize the bacteria *via* the interaction of bacterial fibronectin-binding proteins (FnBP) and αVβ1 integrin, FnBP is not necessary for OC infection by *S. aureus* [86]. Regarding the effects of *S. aureus* on bone degradation, two mechanisms were identified. First, infection of OC precursors modified the secreted proinflammatory cytokine profile with a marked increase in monocyte chemoattractant protein 1 (MCP-1), Macrophage Inflammatory Proteins (MIP)-1α, MIP-1β, RANTES/CCL5, which promote the osteoclastogenesis of uninfected precursors. Second, the infection of mature OC enhanced their capacity to fuse and, importantly, when infected OC were seeded on bone slices, their capacity to degrade the bone matrix was increased over 3-fold compared

**Table 1**
Effects of pathogens when internalized by OC.

| Pathogens | Effects on OCs upon entry | Refs |
|---|---|---|
| Bacteria | | |
| *Cutibacterium acnes* | Survives intracellularly at least 96 h decreases mineral resorption activity | [80] |
| *Staphylococcus aureus* | Eliminated in 72 h, modifies the cytokine/chemokine profile enhances osteoclast fusion | [86] |
| *Mycobacterium tuberculosis* (Pott's disease) | Grows rapidly and escapes from phagosome, modifies the cytokine/chemokine profile | [90] |
| Viruses | | |
| Dengue virus (Dengue) | Increases the release of inflammatory cytokines, increases bone resorption activity | [94–99] |
| Measles virus (Paget's disease) | Increases the release of inflammatory cytokine IL-6, enhances bone degradation | [103,106–108] |
| HIV-1 | Increases osteoclast migration, increases OC fusion, enhances OC adhesion and bone degradation, modifies sealing zone stability | [121,122] |

to non-infected OC [86]. This study is an important first step in the understanding of the role of OC infection in *S. aureus*-induced osteomyelitis. Further work is required to characterize the mechanisms of infection, bacterial killing and the enhanced osteolytic activity of OC.

### 2.3. M.tuberculosis

*M. tuberculosis,* the infectious agent causing tuberculosis (TB), is also involved in extrapulmonary infections including spinal tuberculosis or Pott's disease [78,79]. Pott's disease is located in spinal vertebrae. As such, it is a granulomatous disease in response to *M. tuberculosis* infection characterized by chronic inflammation and massive bone resorption [87,88]. In many parts of the developing world, TB is the most common cause of vertebral body infection, with the majority of cases seen in patients under the age of 20. Patients usually suffer of back pain, deformation and sensation loss in the affected bones or joints and lower limb weakness/paraplegia. Constitutional symptoms such as fever and weight loss are not as pronounced as with osteomyelitis caused by other bacteria. Spinal TB is believed to be initiated by an abnormal activation of OC in spinal bones, which leads to bone loss [89]. This was correlated with the histological observation of an enormous amount of multinucleated OC-like cells around deteriorated bones. OC activation in Pott's disease is assumed to result from the abnormal activation and recruitment of *M. tuberculosis*-infected macrophages and their precursors to the infection site, although no experiment has been done to sustain this hypothesis and these macrophages have not been characterized. In addition, another hypothesis is that OC are directly infected by *M. tuberculosis*, as demonstrated *in vitro* by [90] and by us (Fig. 2A). Hoshino and co-workers found that a virulent strain of *M. tuberculosis* (H37Rv) is internalized by OC precursors and by mature OC. The bacteria grow rapidly in OC and then they escape from phagosomes. Infected OC are highly fused multinucleated cells that overexpress the osteolytic enzyme cathepsin K and MMP9. *M. tuberculosis* infection of OC precursors results in a strong increase in the production of pro-inflammatory cytokines (TNF-α, IL-6 and IL1-β), whereas the infection of mature OC incapacitates their production of pro-inflammatory cytokines and leads to a different activation pattern illustrated by high expression of chemokines (*e.g.* CCL5, CCL17 and CCL20). The authors propose on one hand that osteoclastogenesis of non-infected OC precursors coming from the blood is favored and, on the other hand, that resident OC are reprogrammed by *M. tuberculosis* toward overactivated OC [90]. Thus, bone destruction could not only results from changes in the cytokine/chemokine profile secreted by immune cells in spinal tuberculosis, but also from the infection of OC, which participates in microenvironment modifications [90]. However, it has not been tested whether intrinsic bone resorption activity of *M. tuberculosis*-infected OC is altered. As noted above for *S. aureus*, additional work to provide information about the phagocytic receptors and the OC phenotype upon infection will help understand their role in spinal tuberculosis. In addition, it would be interesting to investigate whether *M. tuberculosis* is able to affect phagolysosome maturation as described in macrophages since lysosome trafficking in OC markedly differs from macrophages [72].

## 3. Viral infection of OC

Numerous viruses have been reported to target bone tissue [77]. Mosquito-borne viral pathologies affecting bone represent a major public health problem due to the rapid reproduction rate of mosquitoes and their high efficiency to act as a vector to viruses, such as Ross River virus, the chikungunya virus and the dengue virus (DV) [78]. Viruses that use other modes of transmission, such as the measles virus (MV) or the Human immunodeficiency virus (HIV), also generate bone defects. Abundant literature is available on the role of osteoblasts in these infections [77,81]. Like other stromal cells of the bone, OC could also be susceptible to viral infections because they share common receptors with macrophages, especially the ones used by viruses to bind to the cell surface. However, little is known about OC contribution to immunopathology and bone diseases.

### 3.1. Dengue virus (DG)

Dengue is one of the most important arthropod-borne viral diseases in the world, with about 390 million cases reported per year and an annual mortality rate of 25,000 [91]. It is mostly caused by one of the four serotypes of DV, single-stranded enveloped RNA Flaviviruses. While primary infection with any of the four serotypes results in mild fever and provides immunity to the infecting virus, a second DV infection is associated with an increased risk of developing hemorrhages, organ damage, and in some cases death [92,93]. Moreover, DV infection is often associated with severe bone and joint pain [78]. Myeloid cells such as monocytes, dendritic cells and macrophages are the primary targets of DV infection. Mannose-binding receptor (MR) and the C-type lectin receptor DC-SIGN are involved in DV binding and internalization, while the C-type lectin domain family 5 member A (CLEC5A) cooperates with these receptors to increase DV binding and acts as signaling receptor to stimulate proinflammatory cytokine release [94–98]. Recently, it was reported that serotype 2 DV infects and replicates in OC *in vitro* [99]. This infection stimulates osteolytic activity and the release of inflammatory cytokines such as TNF-α and IL-6. All these DV-induced effects appear to be dependent on CLEC5A [99]. Moreover, in STAT-1-deficient mice infected with a mouse-adapted DV strain [94], infection causes increased osteolytic activity and transient inflammatory reaction in bone. Again, these dysregulations are impaired in CLEC5A-deficient mice [99]. This study has thus proposed that DV, through OC infection, upregulates bone resorption activity *via* CLEC5A and participates in the clinical bone symptoms seen in DV-infected individuals.

### 3.2. Measles virus (MV)

Paget's disease (PD) of bone is the second most common metabolic disorder of bone after osteoporosis. This skeletal disease affects 2–5% of Caucasians over 55 years old. It is characterized by localized areas of strong bone resorption accompanied by exuberant bone formation, resulting in bone pain, deformity and weakness [100]. The cellular phenotype observed in PD resides in OC, which are increased in size, in number, in responsiveness to osteoclastogenic signals (namely vitamin D3, RANKL) and in bone resorption activity. These characteristics form the “pagetic phenotype”. The etiology of the disease is complex and involves both genetic and environmental factors [100]. In particular, in genetically susceptible individuals, an infection with paramyxoviruses such as MV has been proposed to trigger PD. Although this hypothesis is still discussed, numerous studies using various technical approaches report the presence of MV nucleocapsid transcripts or proteins in OC (or in their precursors) in a majority of PD patients [101–105]. Moreover, OC transduced with a retroviral vector expressing the MV nucleocapsid gene present the pagetic phenotype, namely hypersensibility to osteoclastogenic signals and increased bone resorption capacity [106]. Transgenic mice with the MV nucleocapsid gene targeted to OC develop localized bone lesions and OC phenotypes characteristic of PD [107]. These phenotypes are dependent on the production of the pro-inflammatory cytokine IL-6 by pagetic OC. Further, mice co-expressing the MV nucleocapsid protein and a mutation in p62 (to date, the only gene that has been clearly involved in the pathology) develop more dramatic PD-like bone lesions than do mice expressing only the MV nucleocapsid protein [103]. In these mice, knockdown of MV nucleocapsid protein expression is sufficient to abolish the pagetic phenotype [103]. Altogether, these data indicate that the infection of OC by MV may contribute to the development of PD, at least in a subset of susceptible individuals. Regarding the molecular mechanisms involved in MV-induced effects, a model proposes that the expression of the MV

nucleocapsid protein in OC precursors induces the activation of the IKK family members TANK-binding kinase 1 (TBK1), which leads in turn to the activation of NF-κB, an essential transcription factor for osteoclastogenic differentiation and IL-6 expression [108]. The activation of TBK1 also increases the expression of the transcription initiation factor TFIID subunit 12 (TAF12), which acts as a vitamin D receptor coactivator contributing to the hyper-susceptibility of the cells to vitamin D3 [100,109]. Recently, an upregulation of coupling factors between OC and osteoblasts (ephrinB2 and EphB4) was described in OC expressing the MV nucleocapsid protein and a high level of IL-6. This mechanism can explain both the excessive bone degradation and formation that are observed in PD patients [110]. Thus, OC infection by MV appears as a main factor in PD, acting not only directly on OC activity but also exerting bystander effects on OC precursors or other bone stromal cells such as osteoblasts.

### 3.3. Human immunodeficiency virus-1 (HIV-1)

The use of antiretroviral therapy has significantly improved life expectancy for HIV-1 infected individuals. Patient now live to an older age, which reveals the persistence of latent proviruses inside reservoir cells and long-term effects of the infection. Among these long-term complications, reduction of bone mineral density is frequent and often progresses to osteoporosis and a high prevalence of fractures. Although it has long been unappreciated, a 6-fold increased risk of low bone mineral density is observed in HIV-1-positive individuals compared to the general population [111–113], which means that over half of the HIV-infected population presents bone loss [114]. In HIV-1 patients, multiple factors including the life style and the antiviral therapy, especially during the first years, contribute to bone defects. One hypothesis is that following initiation of the therapy, T-cell repopulation and inherent production of RANKL represent major mechanisms involved in the observed bone loss [115]. In addition, there is evidence of bone deficit in non-treated patients, indicating that the virus by-itself alters bone homeostasis. The HIV-1-induced bone disorders are associated with an increase of blood biomarkers for bone resorption and minor changes in bone formation-specific markers, suggesting a major contribution of enhanced osteolytic activity [116,117]. Bone homeostasis is strongly dependent on the level of RANKL and of other osteoclastogenic cytokines, the levels of which are influenced by the immune system. Thus, it is not surprising that HIV-1, by perturbing the level of osteoclastogenic cytokine secretion by immune cells, contributes to bone loss. In particular, in HIV-transgenic rats and HIV-infected individuals, a marked elevation in RANKL production has been reported, concurrent with a marked depletion in OPG production by B cells, leading to an upregulation in the RANKL/OPG ratio that favors osteoclastogenesis [115,117–120]. Moreover, RANKL/OPG imbalance is associated with HIV-induced bone defects in patients harboring a sufficiently high number of CD4 T cells in spite of the infection [119]. In addition to these effects on immune cells, it has recently been shown that HIV-1 infects human OC *in vitro* with an infectivity comparable to that of macrophages [121,122] (Fig. 2C). OC infection has been confirmed *ex vivo* in human synovial explants and *in vivo* in a humanized murine model [122]. HIV-1 uses the receptor and co-receptor CD4 and CCR5 to enter OC, and then localizes in specific intracellular compartments, just like macrophages [123]. HIV-1 also infects OC precursors, which not only stimulates their migration in 3D environments and may favor their recruitment to bones, but also leads to the fusion of OC with a high number of nuclei. In infected OC, the structure and function of the sealing zone are modified and its basal subunit, the podosome, is enriched in F-actin; consequently, OC are more adherent and more active in resorbing the bone matrix. *In vitro*, all these effects are dependent on the viral protein Nef, which activates Src, a tyrosine kinase involved in podosome regulation and sealing zone assembly. Supporting these results, OC derived from Nef-transgenic mice display high osteolytic activity, and these mice exhibit an increased density of OC in bones associated with bone deficit [122]. Thus, HIV-infected OC contribute to bone defects by upregulating their osteolytic activity as a consequence of an enhanced activity of the bone resorption machinery. In addition, HIV-1 increases the motility of OC precursors, leading to a higher number of OC in bones.

## 4. Conclusion

Several viruses and bacteria have been described to cause bone defects [77,79]. Numerous actors can participate in skeletal diseases, including osteoblasts, inflammatory environments that stimulate osteoclastogenesis, and OC themselves. OC, which share the same progenitors as the phagocytic cells, macrophages and dendritic cells, should be considered as cells that ingest microorganisms, although infected OC are described in only a few reports, all mentioned here.

Until now, *S. aureus*, *M. tuberculosis*and *C. acnes* are the only example of bacterial infection of OC *in vitro*. It is likely that additional bacteria affecting bones are internalized by OC probably by phagocytosis as in macrophages. Actually, *Salmonella thyphimurium* is a good example of bacteria known to induce bone damages that is efficiently ingested by OC (as shown for the first time in Fig. 2B). Thus, further work is required to determine whether OC are targets for additional bacteria causing osteomyelitis and to verify that it is also the case *in vivo*. Similarly, the interaction of only a small number of viruses with OC has been explored. Viruses such as hepatitis C and chikungunya should be examined as they are associated to bone alteration. It is also important to better decipher internalization routes of these different pathogens. To this purpose, a precursor work from Vaananen's group reported that rat OC supported *in vitro* Sendai, vesicular stomatitis virus and influenza virus infections [36,124]. Glycoproteins of these viruses were used to identify specific membrane domains for pathogen docking or entry.

Among the microorganisms impacting bones, several of them have developed mechanisms to dysregulate the microbicidal activity of macrophages or to escape it. Whether they also developed survival strategies in OC should be examined as well as the mechanisms involved. For example, *M. tuberculosis* escape phagosomes in OC and grow in the cytosol like in macrophages [90], but the escape pathways elucidated in macrophages deserve additional studies in OC. Also, the docking and entry receptors of these pathogens in OC are not elucidated, except for HIV-1 and the Measles virus that appear to use common receptors with macrophages. In most cases, it is not yet clear how microorganisms impact the bone resorption machinery of OC. One example is HIV-1 which alters the cytoskeleton of OC, in particular podosomes, with consequences on the sealing zone [122].

Another question is why and how the microorganisms benefit from manipulating OC. It is not clear whether enhancement of bone degradation by infected OC is an advantage. A hypothesis is that OC present reservoir characteristics. The observations in mice that OC of embryonic origin acquire new nuclei, one at a time, every four to eight weeks from circulating blood monocytes by cell-cell fusion events indicate that embryonic OC are very long-lived cells [2,125]. Long lifespan is a major feature for a reservoir. In addition, if we consider HIV-1 for instance, it replicates moderately within OC and does not exhibit cytotoxic effects [118,122]. It also accumulates inside intracellular compartments in which it can retain its infectious potential for extended periods [122]. Finally, the unique anatomical characteristics of the bone tissue, where drug delivery is described as being limited, is clearly an advantage for microorganisms hiding in OC. Thus, OC should be considered as new reservoirs for HIV-1 and likely for other pathogens.

Hence, the perspective that OC is a cell target for pathogens opens a new research area and novel therapeutic approaches to infections that cause bone defects.

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## References

[1] M. Capulli, R. Paone, N. Rucci, Osteoblast and osteocyte: games without frontiers, Arch. Biochem. Biophys. 561 (2014) 3–12.
[2] C.E. Jacome-Galarza, G.I. Percin, J.T. Muller, E. Mass, T. Lazarov, J. Eitler, M. Rauner, V.K. Yadav, L. Crozet, M. Bohm, et al., Developmental origin, functional maintenance and genetic rescue of osteoclasts, Nature 568 (2019) 541–545.
[3] N. Rucci, A. Teti, The "love-hate" relationship between osteoclasts and bone matrix, Matrix Biol. 52–54 (2016) 176–190.
[4] M.T. Engsig, Q.J. Chen, T.H. Vu, A.C. Pedersen, B. Therkidsen, L.R. Lund, K. Henriksen, T. Lenhard, N.T. Foged, Z. Werb, et al., Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones, J. Cell Biol. 151 (2000) 879–889.
[5] P. Hou, T. Troen, M.C. Ovejero, T. Kirkegaard, T.L. Andersen, I. Byrjalsen, M. Ferreras, T. Sato, S.D. Shapiro, N.T. Foged, et al., Matrix metalloproteinase-12 (MMP-12) in osteoclasts: new lesson on the involvement of MMPs in bone resorption, Bone 34 (2004) 37–47.
[6] N. Ortega, D. Behonick, D. Stickens, Z. Werb, How proteases regulate bone morphogenesis, Ann. N. Y. Acad. Sci. 995 (2003) 109–116.
[7] C. Verollet, A. Gallois, R. Dacquin, C. Lastrucci, S.N. Pandruvada, N. Ortega, R. Poincloux, A. Behar, C. Cougoule, C. Lowell, et al., Hck contributes to bone homeostasis by controlling the recruitment of osteoclast precursors, FASEB J. 27 (2013) 3608–3618.
[8] M. Kotani, J. Kikuta, F. Klauschen, T. Chino, Y. Kobayashi, H. Yasuda, K. Tamai, A. Miyawaki, O. Kanagawa, M. Tomura, et al., Systemic circulation and bone recruitment of osteoclast precursors tracked by using fluorescent imaging techniques, J. Immunol. 190 (2013) 605–612.
[9] T. Ono, T. Nakashima, Recent advances in osteoclast biology, Histochem. Cell Biol. 149 (2018) 325–341.
[10] M. Pereira, E. Petretto, S. Gordon, J.H.D. Bassett, G.R. Williams, J. Behmoaras, Common signalling pathways in macrophage and osteoclast multinucleation, J. Cell Sci. 131 (2018).
[11] A. Vignery, Macrophage fusion: the making of osteoclasts and giant cells, J. Exp. Med. 202 (2005) 337–340.
[12] J. Lam, C.A. Nelson, F.P. Ross, S.L. Teitelbaum, D.H. Fremont, Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity, J. Clin. Invest. 108 (2001) 971–979.
[13] M. Asagiri, H. Takayanagi, The molecular understanding of osteoclast differentiation, Bone 40 (2007) 251–264.
[14] X. Feng, S.L. Teitelbaum, Osteoclasts: new insights, Bone Res 1 (2013) 11–26.
[15] G. Mbalaviele, D.V. Novack, G. Schett, S.L. Teitelbaum, Inflammatory osteolysis: a conspiracy against bone, J. Clin. Invest. 127 (2017) 2030–2039.
[16] Y.Y. Kong, U. Feige, I. Sarosi, B. Bolon, A. Tafuri, S. Morony, C. Capparelli, J. Li, R. Elliott, S. McCabe, et al., Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand, Nature 402 (1999) 304–309.
[17] Y.T. Teng, H. Nguyen, X. Gao, Y.Y. Kong, R.M. Gorczynski, B. Singh, R.P. Ellen, J.M. Penninger, Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection, J. Clin. Invest. 106 (2000) R59–R67.
[18] K.H. Park-Min, Mechanisms involved in normal and pathological osteoclastogenesis, Cell. Mol. Life Sci. 75 (2018) 2519–2528.
[19] B. Zhao, S.N. Grimes, S. Li, X. Hu, L.B. Ivashkiv, TNF-induced osteoclastogenesis and inflammatory bone resorption are inhibited by transcription factor RBP-J, J. Exp. Med. 209 (2012) 319–334.
[20] S. Tanaka, RANKL-independent osteoclastogenesis: a Long-standing controversy, J. Bone Miner. Res. 32 (2017) 431–433.
[21] O. Laperine, C. Blin-Wakkach, J. Guicheux, S. Beck-Cormier, P. Lesclous, Dendritic-cell-derived osteoclasts: a new game changer in bone-resorption-associated diseases, Drug Discov. Today 21 (2016) 1345–1354.
[22] M. Alnaeeli, J.M. Penninger, Y.T. Teng, Immune interactions with CD4+ T cells promote the development of functional osteoclasts from murine CD11c+ dendritic cells, J. Immunol. 177 (2006) 3314–3326.
[23] A. Gallois, J. Lachuer, G. Yvert, A. Wierinckx, F. Brunet, C. Rabourdin-Combe, C. Delprat, P. Jurdic, M. Mazzorana, Genome-wide expression analyses establish dendritic cells as a new osteoclast precursor able to generate bone-resorbing cells more efficiently than monocytes, J. Bone Miner. Res. 25 (2010) 661–672.
[24] A. Rivollier, M. Mazzorana, J. Tebib, M. Piperno, T. Aitsiselmi, C. Rabourdin-Combe, P. Jurdic, C. Servet-Delprat, Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment, Blood 104 (2004) 4029–4037.
[25] A. Wakkach, A. Mansour, R. Dacquin, E. Coste, P. Jurdic, G.F. Carle, C. Blin-Wakkach, Bone marrow microenvironment controls the in vivo differentiation of murine dendritic cells into osteoclasts, Blood 112 (2008) 5074–5083.
[26] H.J. McKenna, K.L. Stocking, R.E. Miller, K. Brasel, T. De Smedt, E. Maraskovsky, C.R. Maliszewski, D.H. Lynch, J. Smith, B. Pulendran, et al., Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells, Blood 95 (2000) 3489–3497.
[27] J.F. Charles, A.O. Aliprantis, Osteoclasts: more than 'bone eaters', Trends Mol. Med. 20 (2014) 449–459.
[28] N.A. Sims, T.J. Martin, Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit, Bonekey Rep 3 (2014) 481.
[29] A. Teti, Mechanisms of osteoclast-dependent bone formation, Bonekey Rep 2 (2013) 449.
[30] I. Matic, B.G. Matthews, X. Wang, N.A. Dyment, D.L. Worthley, D.W. Rowe, D. Grcevic, I. Kalajzic, Quiescent bone lining cells are a major source of osteoblasts during adulthood, Stem Cells 34 (2016) 2930–2942.
[31] N.S. Soysa, N. Alles, Osteoclast function and bone-resorbing activity: an overview, Biochem. Biophys. Res. Commun. 476 (2016) 115–120.
[32] J. Takito, S. Inoue, M. Nakamura, The sealing zone in osteoclasts: a self-organized structure on the bone, Int. J. Mol. Sci. 19 (2018).
[33] G. Stenbeck, M.A. Horton, A new specialized cell-matrix interaction in actively resorbing osteoclasts, J. Cell Sci. 113 (Pt 9) (2000) 1577–1587.
[34] H.K. Vaananen, M. Horton, The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure, J. Cell Sci. 108 (Pt 8) (1995) 2729–2732.
[35] J.M. Halleen, S.R. Raisanen, S.L. Alatalo, H.K. Vaananen, Potential function for the ROS-generating activity of TRACP, J. Bone Miner. Res. 18 (2003) 1908–1911.
[36] M.T. Mulari, M. Nars, T. Laitala-Leinonen, T. Kaisto, K. Metsikko, Y. Sun, H.K. Vaananen, Recombinant VSV G proteins reveal a novel raft-dependent endocytic pathway in resorbing osteoclasts, Exp. Cell Res. 314 (2008) 1641–1651.
[37] H. Palokangas, M. Mulari, H.K. Vaananen, Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts, J. Cell Sci. 110 (Pt 15) (1997) 1767–1780.
[38] G. Stenbeck, M.A. Horton, Endocytic trafficking in actively resorbing osteoclasts, J. Cell Sci. 117 (2004) 827–836.
[39] S.L. Teitelbaum, The osteoclast and its unique cytoskeleton, Ann. N. Y. Acad. Sci. 1240 (2011) 14–17.
[40] H. Zhao, Membrane trafficking in osteoblasts and osteoclasts: new avenues for understanding and treating skeletal diseases, Traffic 13 (2012) 1307–1314.
[41] A. Bouissou, A. Proag, N. Bourg, K. Pingris, C. Cabriel, S. Balor, T. Mangeat, C. Thibault, C. Vieu, G. Dupuis, et al., Podosome force generation machinery: a local balance between protrusion at the core and traction at the ring, ACS Nano 11 (2017) 4028–4040.
[42] A. Labernadie, A. Bouissou, P. Delobelle, S. Balor, R. Voituriez, A. Proag, I. Fourquaux, C. Thibault, C. Vieu, R. Poincloux, et al., Protrusion force microscopy reveals oscillatory force generation and mechanosensing activity of human macrophage podosomes, Nat. Commun. 5 (2014) 5343.
[43] A. Labernadie, C. Thibault, C. Vieu, I. Maridonneau-Parini, G.M. Charriere, Dynamics of podosome stiffness revealed by atomic force microscopy, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 21016–21021.
[44] A. Proag, A. Bouissou, T. Mangeat, R. Voituriez, P. Delobelle, C. Thibault, C. Vieu, I. Maridonneau-Parini, R. Poincloux, Working together: spatial synchrony in the force and actin dynamics of podosome first neighbors, ACS Nano 9 (2015) 3800–3813.
[45] C. Wiesner, V. Le-Cabec, K. El Azzouzi, I. Maridonneau-Parini, S. Linder, Podosomes in space: macrophage migration and matrix degradation in 2D and 3D settings, Cell Adhes. Migr. 8 (2014) 179–191.
[46] E. Van Goethem, R. Guiet, S. Balor, G.M. Charriere, R. Poincloux, A. Labrousse, I. Maridonneau-Parini, V. Le Cabec, Macrophage podosomes go 3D, Eur. J. Cell Biol. 90 (2011) 224–236.
[47] E. Van Goethem, R. Poincloux, F. Gauffre, I. Maridonneau-Parini, V. Le Cabec, Matrix architecture dictates three-dimensional migration modes of human macrophages: differential involvement of proteases and podosome-like structures, J. Immunol. 184 (2010) 1049–1061.
[48] P. Jurdic, F. Saltel, A. Chabadel, O. Destaing, Podosome and sealing zone: specificity of the osteoclast model, Eur. J. Cell Biol. 85 (2006) 195–202.
[49] F. Saltel, A. Chabadel, E. Bonnelye, P. Jurdic, Actin cytoskeletal organisation in osteoclasts: a model to decipher transmigration and matrix degradation, Eur. J. Cell Biol. 87 (2008) 459–468.
[50] F. Saltel, O. Destaing, F. Bard, D. Eichert, P. Jurdic, Apatite-mediated actin dynamics in resorbing osteoclasts, Mol. Biol. Cell 15 (2004) 5231–5241.
[51] D. Georgess, I. Machuca-Gayet, A. Blangy, P. Jurdic, Podosome organization drives osteoclast-mediated bone resorption, Cell Adhes. Migr. 8 (2014) 191–204.
[52] C. Luxenburg, L. Addadi, B. Geiger, The molecular dynamics of osteoclast adhesions, Eur. J. Cell Biol. 85 (2006) 203–211.
[53] A. Chabadel, I. Banon-Rodriguez, D. Cluet, B.B. Rudkin, B. Wehrle-Haller, E. Genot, P. Jurdic, I.M. Anton, F. Saltel, CD44 and beta3 integrin organize two functionally distinct actin-based domains in osteoclasts, Mol. Biol. Cell 18 (2007) 4899–4910.

[54] P.T. Lakkakorpi, H.K. Väänänen, Kinetics of the osteoclast cytoskeleton during the resorption cycle in vitro, J. Bone Miner. Res. 6 (1991) 817–826.
[55] O. Destaing, F. Saltel, J.C. Geminard, P. Jurdic, F. Bard, Podosomes display actin turnover and dynamic self-organization in osteoclasts expressing actin-green fluorescent protein, Mol. Biol. Cell 14 (2003) 407–416.
[56] R. Guiet, C. Verollet, I. Lamsoul, C. Cougoule, R. Poincloux, A. Labrousse, D.A. Calderwood, M. Glogauer, P.G. Lutz, I. Maridonneau-Parini, Macrophage mesenchymal migration requires podosome stabilization by Filamin A, J. Biol. Chem. 287 (2012) 13051–13062.
[57] K. Soe, J.M. Delaisse, Time-lapse reveals that osteoclasts can move across the bone surface while resorbing, J. Cell Sci. 130 (2017) 2026–2035.
[58] H. Maeda, T. Kowada, J. Kikuta, M. Furuya, M. Shirazaki, S. Mizukami, M. Ishii, K. Kikuchi, Real-time intravital imaging of pH variation associated with osteoclast activity, Nat. Chem. Biol. 12 (2016) 579–585.
[59] B.F. Boyce, T. Yoneda, C. Lowe, P. Soriano, G.R. Mundy, Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice, J. Clin. Invest. 90 (1992) 1622–1627.
[60] O. Destaing, A. Sanjay, C. Itzstein, W.C. Horne, D. Toomre, P. De Camilli, R. Baron, The tyrosine kinase activity of c-Src regulates actin dynamics and organization of podosomes in osteoclasts, Mol. Biol. Cell 19 (2008) 394–404.
[61] W.C. Horne, A. Sanjay, A. Bruzzaniti, R. Baron, The role(s) of Src kinase and Cbl proteins in the regulation of osteoclast differentiation and function, Immunol. Rev. 208 (2005) 106–125.
[62] T. Izawa, W. Zou, J.C. Chappel, J.W. Ashley, X. Feng, S.L. Teitelbaum, c-Src links a RANK/alphavbeta3 integrin complex to the osteoclast cytoskeleton, Mol. Cell. Biol. 32 (2012) 2943–2953.
[63] P. Soriano, C. Montgomery, R. Geske, A. Bradley, Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice, Cell 64 (1991) 693–702.
[64] W. Zou, H. Kitaura, J. Reeve, F. Long, V.L. Tybulewicz, S.J. Shattil, M.H. Ginsberg, F.P. Ross, S.L. Teitelbaum, Syk, c-Src, the alphavbeta3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption, J. Cell Biol. 176 (2007) 877–888.
[65] A. Cappariello, A. Maurizi, V. Veeriah, A. Teti, The Great Beauty of the osteoclast, Arch. Biochem. Biophys. 558 (2014) 70–78.
[66] A.e.a Grüneboom, A network of trans-cortical capillaries as mainstay for blood circulation in long bones, Nature Metabolism 1 (2019) 236–250.
[67] H. Li, S. Hong, J. Qian, Y. Zheng, J. Yang, Q. Yi, Cross talk between the bone and immune systems: osteoclasts function as antigen-presenting cells and activate CD4+ and CD8+ T cells, Blood 116 (2010) 210–217.
[68] H. Li, Y. Lu, J. Qian, Y. Zheng, M. Zhang, E. Bi, J. He, Z. Liu, J. Xu, J.Y. Gao, et al., Human osteoclasts are inducible immunosuppressive cells in response to T cell-derived IFN-gamma and CD40 ligand in vitro, J. Bone Miner. Res. 29 (2014) 2666–2675.
[69] L. Batoon, S.M. Millard, L.J. Raggatt, A.R. Pettit, Osteomacs and bone regeneration, Curr Osteoporos Rep 15 (2017) 385–395.
[70] M.N. Michalski, L.K. McCauley, Macrophages and skeletal health, Pharmacol. Ther. 174 (2017) 43–54.
[71] B.P. Sinder, A.R. Pettit, L.K. McCauley, Macrophages: their emerging roles in bone, J. Bone Miner. Res. 30 (2015) 2140–2149.
[72] T. Fujiwara, S. Ye, T. Castro-Gomes, C.G. Winchell, N.W. Andrews, D.E. Voth, K.I. Varughese, S.G. Mackintosh, Y. Feng, N. Pavlos, et al., PLEKHM1/DEF8/RAB7 complex regulates lysosome positioning and bone homeostasis, JCI Insight 1 (2016) e86330.
[73] E. van Meel, M. Boonen, H. Zhao, V. Oorschot, F.P. Ross, S. Kornfeld, J. Klumperman, Disruption of the Man-6-P targeting pathway in mice impairs osteoclast secretory lysosome biogenesis, Traffic 12 (2011) 912–924.
[74] K. Schroder, NADPH oxidases in bone homeostasis and osteoporosis, Cell. Mol. Life Sci. 72 (2015) 25–38.
[75] A. Kassem, P. Henning, P. Lundberg, P.P. Souza, C. Lindholm, U.H. Lerner, Porphyromonas gingivalis stimulates bone resorption by enhancing RANKL (receptor activator of NF-kappaB ligand) through activation of toll-like receptor 2 in osteoblasts, J. Biol. Chem. 290 (2015) 20147–20158.
[76] N. Scheres, T.J. de Vries, J. Brunner, W. Crielaard, M.L. Laine, V. Everts, Diverse effects of Porphyromonas gingivalis on human osteoclast formation, Microb. Pathog. 51 (2011) 149–155.
[77] C. Saint-Pastou Terrier, P. Gasque, Bone responses in health and infectious diseases: a focus on osteoblasts, J. Inf. Secur. 75 (2017) 281–292.
[78] Y.C. Cui, Q. Wu, S.W. Teh, A. Peli, G. Bu, Y.S. Qiu, G. Benelli, S.S. Kumar, Bone breaking infections - a focus on bacterial and mosquito-borne viral infections, Microb. Pathog. 122 (2018) 130–136.
[79] S.P. Nair, S. Meghji, M. Wilson, K. Reddi, P. White, B. Henderson, Bacterially induced bone destruction: mechanisms and misconceptions, Infect. Immun. 64 (1996) 2371–2380.
[80] G.G. Aubin, M. Baud'huin, J.P. Lavigne, R. Brion, F. Gouin, D. Lepelletier, C. Jacqueline, D. Heymann, K. Asehnoune, S. Corvec, Interaction of Cutibacterium (formerly Propionibacterium) acnes with bone cells: a step toward understanding bone and joint infection development, Sci. Rep. 7 (2017) 42918.
[81] J. Josse, F. Velard, S.C. Gangloff, Staphylococcus aureus vs. osteoblast: relationship and consequences in osteomyelitis, Front. Cell. Infect. Microbiol. 5 (2015) 85.
[82] J.E. Cassat, N.D. Hammer, J.P. Campbell, M.A. Benson, D.S. Perrien, L.N. Mrak, M.S. Smeltzer, V.J. Torres, E.P. Skaar, A secreted bacterial protease tailors the Staphylococcus aureus virulence repertoire to modulate bone remodeling during osteomyelitis, Cell Host Microbe 13 (2013) 759–772.
[83] L.R. Ren, H. Wang, X.Q. He, M.G. Song, X.Q. Chen, Y.Q. Xu, Staphylococcus aureus protein a induces osteoclastogenesis via the NFkappaB signaling pathway, Mol. Med. Rep. 16 (2017) 6020–6028.
[84] L.R. Ren, Z.H. Wang, H. Wang, X.Q. He, M.G. Song, Y.Q. Xu, Staphylococcus aureus induces osteoclastogenesis via the NF-kappaB signaling pathway, Med. Sci. Monit. 23 (2017) 4579–4590.
[85] Y. Wang, X. Liu, C. Dou, Z. Cao, C. Liu, S. Dong, J. Fei, Staphylococcal protein A promotes osteoclastogenesis through MAPK signaling during bone infection, J. Cell. Physiol. 232 (2017) 2396–2406.
[86] S. Trouillet-Assant, M. Gallet, P. Nauroy, J.P. Rasigade, S. Flammier, P. Parroche, J. Marvel, T. Ferry, F. Vandenesch, P. Jurdic, et al., Dual impact of live Staphylococcus aureus on the osteoclast lineage, leading to increased bone resorption, J. Infect. Dis. 211 (2015) 571–581.
[87] O. Boachie-Adjei, R.G. Squillante, Tuberculosis of the spine, Orthop Clin North Am 27 (1996) 95–103.
[88] S. Meghji, P.A. White, S.P. Nair, K. Reddi, K. Heron, B. Henderson, A. Zaliani, G. Fossati, P. Mascagni, J.F. Hunt, et al., Mycobacterium tuberculosis chaperonin 10 stimulates bone resorption: a potential contributory factor in Pott's disease, J. Exp. Med. 186 (1997) 1241–1246.
[89] D.R. Haynes, Bone lysis and inflammation, Inflamm. Res. 53 (2004) 596–600.
[90] A. Hoshino, S. Hanada, H. Yamada, S. Mii, M. Takahashi, S. Mitarai, K. Yamamoto, Y. Manome, Mycobacterium tuberculosis escapes from the phagosomes of infected human osteoclasts reprograms osteoclast development via dysregulation of cytokines and chemokines, Pathog Dis 70 (2014) 28–39.
[91] M.G. Guzman, D.J. Gubler, A. Izquierdo, E. Martinez, S.B. Halstead, Dengue infection, Nat Rev Dis Primers 2 (2016) 16055.
[92] S. Abel, B. Liautaud, A. Cabie, Dengue, N. Engl. J. Med. 367 (2012) 180–181 (author reply 181).
[93] J.A. Castillo, J.S. Naranjo, M. Rojas, D. Castano, P.A. Velilla, Role of monocytes in the pathogenesis of dengue, Arch. Immunol. Ther. Exp. 67 (2019) 27–40.
[94] S.T. Chen, Y.L. Lin, M.T. Huang, M.F. Wu, S.C. Cheng, H.Y. Lei, C.K. Lee, T.W. Chiou, C.H. Wong, S.L. Hsieh, CLEC5A is critical for dengue-virus-induced lethal disease, Nature 453 (2008) 672–676.
[95] P. Liu, M. Ridilla, P. Patel, L. Betts, E. Gallichotte, L. Shahidi, N.L. Thompson, K. Jacobson, Beyond attachment: roles of DC-SIGN in dengue virus infection, Traffic 18 (2017) 218–231.
[96] J.L. Miller, B.J. de Wet, L. Martinez-Pomares, C.M. Radcliffe, R.A. Dwek, P.M. Rudd, S. Gordon, The mannose receptor mediates dengue virus infection of macrophages, PLoS Pathog. 4 (2008) e17.
[97] A.K. Palucka, Dengue virus and dendritic cells, Nat. Med. 6 (2000) 748–749.
[98] M.F. Wu, S.T. Chen, A.H. Yang, W.W. Lin, Y.L. Lin, N.J. Chen, I.S. Tsai, L. Li, S.L. Hsieh, CLEC5A is critical for dengue virus-induced inflammasome activation in human macrophages, Blood 121 (2013) 95–106.
[99] Y.L. Huang, S.T. Chen, R.S. Liu, Y.H. Chen, C.Y. Lin, C.H. Huang, P.Y. Shu, C.L. Liao, S.L. Hsieh, CLEC5A is critical for dengue virus-induced osteoclast activation and bone homeostasis, J Mol Med (Berl) 94 (2016) 1025–1037.
[100] D.L. Galson, G.D. Roodman, Pathobiology of Paget's disease of bone, J Bone Metab 21 (2014) 85–98.
[101] W.E. Friedrichs, S.V. Reddy, J.M. Bruder, T. Cundy, J. Cornish, F.R. Singer, G.D. Roodman, Sequence analysis of measles virus nucleocapsid transcripts in patients with Paget's disease, J. Bone Miner. Res. 17 (2002) 145–151.
[102] M.T. Gordon, A.P. Mee, P.T. Sharpe, Paramyxoviruses in Paget's disease, Semin. Arthritis Rheum. 23 (1994) 232–234.
[103] N. Kurihara, Y. Hiruma, K. Yamana, L. Michou, C. Rousseau, J. Morissette, D.L. Galson, J. Teramachi, H. Zhou, D.W. Dempster, et al., Contributions of the measles virus nucleocapsid gene and the SQSTM1/p62(P392L) mutation to Paget's disease, Cell Metab. 13 (2011) 23–34.
[104] A.P. Mee, J.A. Dixon, J.A. Hoyland, M. Davies, P.L. Selby, E.B. Mawer, Detection of canine distemper virus in 100% of Paget's disease samples by in situ-reverse transcriptase-polymerase chain reaction, Bone 23 (1998) 171–175.
[105] A. Rebel, K. Malkani, M. Basle, C. Bregeon, Is Paget's disease of bone a viral infection? Calcif. Tissue Res. 22 (Suppl) (1977) 283–286.
[106] N. Kurihara, S.V. Reddy, C. Menaa, D. Anderson, G.D. Roodman, Osteoclasts expressing the measles virus nucleocapsid gene display a pagetic phenotype, J. Clin. Invest. 105 (2000) 607–614.
[107] N. Kurihara, H. Zhou, S.V. Reddy, V. Garcia Palacios, M.A. Subler, D.W. Dempster, J.J. Windle, G.D. Roodman, Expression of measles virus nucleocapsid protein in osteoclasts induces Paget's disease-like bone lesions in mice, J. Bone Miner. Res. 21 (2006) 446–455.
[108] Q. Sun, B. Sammut, F.M. Wang, N. Kurihara, J.J. Windle, G.D. Roodman, D.L. Galson, TBK1 mediates critical effects of measles virus nucleocapsid protein (MVNP) on pagetic osteoclast formation, J. Bone Miner. Res. 29 (2014) 90–102.
[109] G. Mengus, Y.G. Gangloff, L. Carre, A.C. Lavigne, I. Davidson, The human transcription factor IID subunit human TATA-binding protein-associated factor 28 interacts in a ligand-reversible manner with the vitamin D(3) and thyroid hormone receptors, J. Biol. Chem. 275 (2000) 10064–10071.
[110] J. Teramachi, Y. Nagata, K. Mohammad, Y. Inagaki, Y. Ohata, T. Guise, L. Michou, J.P. Brown, J.J. Windle, N. Kurihara, et al., Measles virus nucleocapsid protein increases osteoblast differentiation in Paget's disease, J. Clin. Invest. 126 (2016) 1012–1022.
[111] J. Compston, Osteoporosis and fracture risk associated with HIV infection and treatment, Endocrinol. Metab. Clin. N. Am. 43 (2014) 769–780.
[112] A.G. Cotter, P.W. Mallon, The effects of untreated and treated HIV infection on bone disease, Curr. Opin. HIV AIDS 9 (2014) 17–26.
[113] J. Hoy, B. Young, Do people with HIV infection have a higher risk of fracture compared with those without HIV infection? Curr. Opin. HIV AIDS 11 (2016) 301–305.
[114] S.S.L. Goh, P.S.M. Lai, A.T.B. Tan, S. Ponnampalavanar, Reduced bone mineral density in human immunodeficiency virus-infected individuals: a meta-analysis of

its prevalence and risk factors, Osteoporos. Int. 29 (2018) 595–613.
[115] I. Ofotokun, K. Titanji, T. Vikulina, S. Roser-Page, M. Yamaguchi, M. Zayzafoon, I.R. Williams, M.N. Weitzmann, Role of T-cell reconstitution in HIV-1 antiretroviral therapy-induced bone loss, Nat. Commun. 6 (2015) 8282.
[116] N. Aziz, A.W. Butch, J.J. Quint, R. Detels, Association of blood biomarkers of bone turnover in HIV-1 infected individuals receiving anti-retroviral therapy (ART), J AIDS Clin Res 5 (2014).
[117] K. Titanji, A. Vunnava, A.N. Sheth, C. Delille, J.L. Lennox, S.E. Sanford, A. Foster, A. Knezevic, K.A. Easley, M.N. Weitzmann, et al., Dysregulated B cell expression of RANKL and OPG correlates with loss of bone mineral density in HIV infection, PLoS Pathog. 10 (2014) e1004497.
[118] I. Ofotokun, Deciphering how HIV-1 weakens and cracks the bone, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 2551–2553.
[119] K. Titanji, A. Vunnava, A. Foster, A.N. Sheth, J.L. Lennox, A. Knezevic, N. Shenvi, K.A. Easley, I. Ofotokun, M.N. Weitzmann, T-cell receptor activator of nuclear factor-kappaB ligand/osteoprotegerin imbalance is associated with HIV-induced bone loss in patients with higher CD4+ T-cell counts, AIDS 32 (2018) 885–894.
[120] T. Vikulina, X. Fan, M. Yamaguchi, S. Roser-Page, M. Zayzafoon, D.M. Guidot, I. Ofotokun, M.N. Weitzmann, Alterations in the immuno-skeletal interface drive bone destruction in HIV-1 transgenic rats, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13848–13853.
[121] J. Gohda, et al., HIV-1 replicates in human osteoclasts and enhances their differentiation in vitro, Retrovirology 12 (2015) 12.
[122] B. Raynaud-Messina, L. Bracq, M. Dupont, S. Souriant, S.M. Usmani, A. Proag, K. Pingris, V. Soldan, C. Thibault, F. Capilla, et al., Bone degradation machinery of osteoclasts: an HIV-1 target that contributes to bone loss, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E2556–E2565.
[123] J. Tan, Q.J. Sattentau, The HIV-1-containing macrophage compartment: a perfect cellular niche? Trends Microbiol. 21 (2013) 405–412.
[124] J. Salo, K. Metsikko, H. Palokangas, P. Lehenkari, H.K. Vaananen, Bone-resorbing osteoclasts reveal a dynamic division of basal plasma membrane into two different domains, J. Cell Sci. 109 (Pt 2) (1996) 301–307.
[125] Z.F. Jaworski, B. Duck, G. Sekaly, Kinetics of osteoclasts and their nuclei in evolving secondary Haversian systems, J. Anat. 133 (1981) 397–405.