Dual Impact of Live *Staphylococcus aureus* on the Osteoclast Lineage, Leading to Increased Bone Resorption

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**Background.** Bone and joint infection, mainly caused by *Staphylococcus aureus*, is associated with significant morbidity and mortality, characterized by severe inflammation and progressive bone destruction. Studies mostly focused on the interaction between *S. aureus* and osteoblasts, the bone matrix–forming cells, while interactions between *S. aureus* and osteoclasts, the only cells known to be able to degrade bone, have been poorly explored.

**Methods.** We developed an in vitro infection model of primary murine osteoclasts to study the direct impact of live *S. aureus* on osteoclastogenesis and osteoclast resorption activity.

**Results.** Staphylococcal infection of bone marrow–derived osteoclast precursors induced their differentiation into activated macrophages that actively secreted proinflammatory cytokines. These cytokines enhanced the bone resorption capacity of uninfected mature osteoclasts and promoted osteoclastogenesis of the uninfected precursors at the site of infection. Moreover, infection of mature osteoclasts by live *S. aureus* directly enhanced their ability to resorb bone by promoting cellular fusion.

**Conclusions.** Our results highlighted two complementary mechanisms involved in bone loss during bone and joint infection, suggesting that osteoclasts could be a pivotal target for limiting bone destruction.

**Keywords.** osteoclastogenesis; bone and joint infections; *Staphylococcus aureus*; resorption.

Bone is a mineralized tissue that is constantly remodeled under the simultaneous, coordinated action of 3 cell types: the bone matrix–resorbing osteoclasts, the bone matrix–forming osteoblasts, and osteocytes embedded in the mineralized bone matrix [1]. This physiological process is tightly regulated and crucial to maintaining a constant bone mass in adults. This balance, however, can be impaired to favor resorption in pathological conditions, such as osteoporosis or bone and joint infection (BJI). *Staphylococcus aureus* is the leading cause of BJIs, such as osteomyelitis or prosthetic joint infections [2–4]. Clinical observations pinpoint that such staphylococcal infection leads to the local recruitment of immune cells and is marked by progressive bone loss [5].

Numerous studies have investigated the direct impact of *S. aureus* on osteoblasts [6]. It is now clear that this microorganism is able to adhere to, become internalized by, and intracellularly survive in and/or induce cell death of osteoblasts, depending on its virulence factor equipment [7, 8]. The role of microbial surface components that recognize adhesive matrix molecules has been explored. For instance, the bacterial fibronectin-binding proteins (FnBPs) are necessary and sufficient for the internalization of *S. aureus* in osteoblasts through their interaction with integrin αvβ3 [9, 10]. Claro et al have also recently demonstrated that *S. aureus*
protein A can bind directly to preosteoblastic cells via tumor necrosis factor receptor 1 (TNFR-1), resulting in osteoblast apoptosis, which prevents new bone formation [11–13]. Moreover, the interaction between S. aureus and osteoblasts promotes the secretion of osteoclastic cytokines such as receptor activator of NFκ-B ligand (RANK-L), which enhances osteoclastogenesis. Similarly, Sanchez et al. have shown that soluble factors that are present in the S. aureus biofilm formed on prosthetic materials are able to increase RANK-L expression and affect bone formation and bone resorption related to the inhibition of osteoblastic differentiation [14]. Furthermore, using a murine model of S. aureus osteomyelitis, Cassat et al. recently showed that infected femurs lost approximately 10%–20% of their total cortical volume near the infectious focus, and they allocated the responsibility of this bone loss exclusively to the impact of the bacteria on osteoblastic cells [15].

Surprisingly, the direct impact of S. aureus on osteoclasts, which are the only cells known to be able to degrade bone, has not been extensively explored. Mature osteoclasts are large, multinucleated cells that are generated from the fusion of precursors belonging to the monocyte/macrophage lineage. These cells form an actin-rich sealing zone that delimits the resorption lacuna and into which H⁺ protons and multiple proteases are secreted. The fast-growing field of osteoimmunology [16, 17] has demonstrated an unsuspected link between the immune system and osteoclasts. For instance, osteoclasts share conserved signaling pathways with monocytes/macrophages; they are antigen-presenting cells [18]. We and others have shown that dendritic cells are able to transdifferentiate and fuse to give rise to mature resorbing osteoclasts [19, 20]. These cellular properties suggest that osteoclasts might play a major role in staphylococcal BJI in the local bone resorption, as well as in the control of inflammation. Nevertheless, the very few studies that have explored the direct interaction between S. aureus and osteoclasts have only tested staphylococcal extracts (so-called surface-associated material) or specific, purified staphylococcal components, such as lipoteichoic acid [21]. The results from these studies are divergent and depend upon the protocols used [22, 23]. For instance, Kim et al. demonstrated that bacterial lipopeptides enhanced osteoclast differentiation, whereas Yang et al. showed that staphylococcal lipoteichoic acid inhibited osteoclastogenesis [24]. These conflicting data suggest that the impact of staphylococci on osteoclastogenesis cannot be evaluated only through one bacterial protein or inactivated staphylococci. Indeed, damages induced by S. aureus on eukaryotic cells are caused by a combination of virulence factors. This led us to investigate the direct and global impact of live S. aureus on osteoclastogenesis and resorption activity, using an in vitro infection model of precursors and mature osteoclasts. Our results describe 2 complementary mechanisms suggesting that live S. aureus indirectly and directly enhances bone resorption mediated by osteoclasts.

**MATERIALS AND METHODS**

**Bacterial Strains**
The methicillin- and gentamicin-susceptible S. aureus reference strain 8325-4 and its mutant DU5883 deleted for fiabA/B genes, were generous gifts from Tim Foster [25]. S. aureus strain 6850 (53 657; ATCC, Manassas, VA) was also used. For specific experiments, heat-killed bacteria were prepared by exposing bacteria to a temperature of 95°C for 20 minutes.

**Infection of Osteoclasts by S. aureus**
Mice were maintained in our animal facility and cared for in accordance with French laws. All animal work was approved by the Direction Départementale des Services Vétérinaires (French national agreement B691230303).

Murine primary bone marrow precursors were differentiated into osteoclasts using macrophage colony-stimulating factor (M-CSF) and RANK-L as described elsewhere [26]. Osteoclast precursors and mature osteoclasts were infected (for 2 hours at 37°C) at a multiplicity of infection (MOI) of 10:1 to allow the adhesion and internalization of bacteria. Then, as previously described, we used the gentamicin protection assay for selection of intracellular S. aureus [27].

**Flow Cytometric Analysis of Mononuclear Tartrate-Resistant Acid Phosphatase (TRAP)–Negative Cells**
The characterization of TRAP-negative mononuclear cells obtained after infection of precursors was based on (1) membrane marker identification and (2) cytokine release profiling. Briefly, the cells were labeled with a combination of antibodies to CD11b (N418, ebiosciences, Paris, France), LY6C (AL21, BD Biosciences, San Jose, CA), LY6G (1AB, BD Biosciences), and F4/80 (BM8, ebiosciences) immediately after bone marrow extraction, 4 or 10 days after infection in presence of M-CSF with or without RANK-L. Controls were also realized with uninfected cells. Flow cytometry acquisition was performed using a LSRII flow cytometer (BD Biosciences), and analysis was performed using FlowJo Software (Tree Star, Stanford, CA). In addition, secretion of cytokines/chemokines was measured in cell-culture supernatants, using a Luminex analyzer (Luminex, Austin, TX) and Mouse Cytokine 23-plex Panel Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer’s recommendations.

**Cell Staining, Microscopy, and Quantification**
To investigate the impact of S. aureus infection on osteoclast fusion, the cell surface area and number of nuclei per mature osteoclast were analyzed using immunofluorescence labeling (Supplementary Materials).

**Bone Resorption Assays**
Murine bone marrow precursors were differentiated into osteoclasts for 5 days on a mineralized matrix (OsteoCorning, Corning, MA) with M-CSF and RANK-L. Then, mature osteoclasts...
were infected by *S. aureus* as described above. Seventy-two hours after infection, the cells were lysed by osmotic shock, and OsteoCorning Assay plates were stained with phosphate-buffered saline/5% silver nitrate (Sigma-Aldrich) to quantify resorption, using a Leica DMI6000 microscope (Nanterre, France) and Fiji software [26].

**Statistical Analysis**

Comparisons of the medians of the quantitative data were performed using nonparametric Mann–Whitney tests (XLStat software v7.5.2; Addinsoft, Paris, France). The significance threshold was set at 0.05.

**RESULTS**

**S. aureus Is Internalized Into Precursors and Mature Osteoclasts Independently of the Presence of FnBP**

Bone marrow–derived osteoclast precursors and mature osteoclasts were infected with 2 strains of live *S. aureus*, 8325-4 and DU5883 (Δfnb), to evaluate whether the infection mechanisms were similar to those involving osteoblasts, in which endocytosis of the bacteria occurred after the FnBP–α1β1 integrin interaction. Then, intracellular bacteria were counted 3, 24, 48, or 72 hours after infection. The results were expressed as percentages of inoculum. Three hours after infection, the mean internalization rates (±SD) of the 8325-4 and DU5883 strains in the precursor cells were 0.53% ± 0.16% and 0.78% ± 0.43%, respectively, and decreased gradually over time (Figure 1A). In comparison, the rate of internalization was 18-fold higher in mature osteoclasts than in precursor cells (mean ± SD, 13.33% ± 9.12% and 16.54% ± 8.57% for 8325-4 and DU5883, respectively) (Figure 1B). However, the bacteria were cleared as efficiently as in the precursors in <72 hours. These results were confirmed by microscopic evaluation showing that *S. aureus* organisms were adherent on and internalized into mature osteoclasts (Figure 1C and 1D, respectively). Taken together, these data showed that live *S. aureus* could be efficiently internalized into osteoclast precursors or mature osteoclasts, but not at the same

![Figure 1. Live *Staphylococcus aureus* binds to and is internalized by bone marrow–derived cells and mature osteoclasts. Murine bone marrow precursors (A) or mature osteoclasts (B) were infected with *S. aureus* reference strain 8325-4 for 2 hours at a multiplicity of infection of 10:1. Cell invasion was quantified using a gentamicin protection assay at 3, 24, 48 and 72 hours after infection of the osteoclast precursors (A) or mature osteoclasts (B). The mean number (±SD) of colony-forming units per well was expressed as a percentage of the inoculum and was derived from 3 independent experiments in duplicate. After infection, microscopic evaluation showed that *S. aureus* was able to adhere to (C) and be internalized by (D) mature osteoclasts. For the adhesion assay, extracellular bacteria (closed arrow), 2 hours post-infection, were labeled using the membrane-impermeable fluorochrome vancomycin-Bodipy FL (green; C). For the invasion assay, performed 2 hours after infection, extracellular bacteria were disrupted by lysozaphin treatment (for 1 hour at 37°C), the cells were permeabilized, and intracellular bacteria (open arrow) were labeled with VBFL (D). The actin cytoskeleton of the cells was counterstained with phalloidin (red), and nuclei were stained with Hoechst (blue). Bars represent 10 μm.](image-url)
rate. In contrast to osteoblast, the \textit{S. aureus}–uptake mechanism in osteoclasts is independent of FnBP.

**Live \textit{S. aureus} Infection Inhibits Osteoclastogenesis**

We then evaluated whether bacterial infection of the precursors could impact osteoclastogenesis. Freshly isolated murine bone marrow cells were cultured in control conditions or in the presence of live 8325-4 or DU5883 \textit{S. aureus} for 2 hours. The cells were then grown with M-CSF and RANK-L to induce osteoclast differentiation. Four days later, multinucleated, TRAP-positive osteoclasts were formed in the control, uninfected conditions (Figure 2A). In contrast, after internalization of live 8325-4 or DU5883 \textit{S. aureus} into the precursors, only a mean (±SD) of 4.42% ± 3.64% and 3.25% ± 2.88% of the cells, respectively, were multinucleated and TRAP positive, compared with uninfected cells (control, 100% [\(P < .0001\); Figure 2B and 2C). Again, this dramatic osteoclastogenesis inhibition was independent of FnBP expression. Because strain 8325-4 is an rsbU mutant that expresses high levels of hemolysins and proteases, we also tested whether osteoclastogenesis inhibition was also observed with \textit{S. aureus} harboring wild-type rsbU. Use of live 6850 \textit{S. aureus} again revealed that only a mean (±SD) of 3.35% ± 1.91% of the cells were multinucleated and TRAP positive. Of note, cell viability, as measured by an MTT-based cytotoxicity assay (Supplementary Materials), was not significantly changed in any of the subgroups (data not shown), which demonstrated that the \textit{S. aureus} inhibition of osteoclastogenesis was not associated with a cytotoxic effect of the infection.

Because culturing of bone marrow–derived osteoclast precursors with nonbiological particles has been reported to inhibit
osteoclastogenesis [28, 29], we tested whether, in our model, the live S. aureus impact was due to an effect of particulates or was due to the activity of the live bacteria. For that purpose, using the same protocol used with live S. aureus, osteoclast precursors were grown for 2 hours in the presence of either latex beads within the size range of staphylococci (0.5 and 2 μm; Life Technologies, Carlsbad, CA) or heat-inactivated S. aureus or supernatant of S. aureus 8325-4. The results in Figure 2C show that supernatant or heat-killed staphylococci only partially inhibited osteoclastogenesis (mean ± SD, 81% ± 24.22 and 61% ± 23.33 of TRAP-positive cells respectively) compared with uninfected cells (100%). Conversely, in similar conditions, latex beads did not significantly affect osteoclastogenesis (Figure 2D). Altogether, these data demonstrated that infection of

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**Figure 3.** Mononuclear, tartrate-resistant acid phosphatase (TRAP)–negative cells obtained after Staphylococcus aureus infection of precursor cells are inflammatory macrophages. A. Bone marrow–derived osteoclast precursors of C57/B1/6J mice were either uninfected or infected for 2 hours with the S. aureus 8325-4 strain. The precursors were then grown in presence of macrophage colony-stimulating factor (M-CSF) alone to differentiate into macrophages or in the presence of M-CSF and receptor activator of NFκB ligand (RANK-L) to induce osteoclast differentiation. The cells were fixed after bone marrow extraction (day 0) or after 4 or 10 days of differentiation to analyze the expression levels of membrane markers (F4/80, CD11b, Ly6C, and Ly6G) by flow cytometry. B. To identify the cytokine-release profiles, osteoclast precursors were infected with live or heat-killed S. aureus 8325-4 (multiplicity of infection, 10:1) 24 hours after extraction and cultivated in the presence of M-CSF and RANK-L. The supernatants were collected 4 days later, and the cytokine levels in the cell culture supernatants were quantified using the Luminex assay. The data represent 1 experiment that was performed in triplicate and is representative of the 2 other experiments. Cytokine release in cells infected with live S. aureus 8325-4 was significantly higher than in cells infected with heat-killed S. aureus 8325-4; cytokine release in S. aureus–infected cells (live or heat killed) was also higher than in noninfected cells (P < .05 for all pairwise differences with respect to each tested cytokine). Abbreviations: G-CSF, granulocyte colony-stimulating factor; IL-12p40, interleukin 12p40; MCP-1, monocyte chemotactic protein 1; MIP-1α, macrophage inflammatory protein 1α; MIP-1β, macrophage inflammatory protein 1β; KC, keratinocyte chemoattractant; RANTES, regulated on activation, normal T cell expressed and secreted.
bone marrow precursors by live *S. aureus* dramatically inhibited osteoclastogenesis.

**S. aureus** Infection of Osteoclast Precursors Promotes Their Differentiation Into Macrophages That Secret Proinflammatory Cytokines

Because osteoclasts are derived from the monocyte/macrophage pathway, we hypothesized that the infected, adherent, mononucleated, TRAP-negative cells obtained after infection of osteoclast precursors were macrophages. To test this hypothesis, we analyzed the cell surface markers (CD11b, Ly6C, Ly6G, and F4/80) of (1) uninfected bone marrow cells that were differentiated into macrophages with M-CSF alone, (2) *S. aureus*-infected bone marrow cells that were differentiated into macrophages with M-CSF alone, and (3) *S. aureus*-infected bone marrow cells that were cultured in the presence of RANK-L and M-CSF, which classically allows for differentiation of bone marrow cells into osteoclasts. TRAP-negative cells, which were present 4 and 10 days after infection and RANK-L/M-CSF induction, displayed a marker profile that included high-level expression of Ly6C, CD11b, and F4/80 similar to those of uninfected precursors or *S. aureus*-infected precursors that were differentiated in macrophages by using M-CSF alone (Figure 3A). These findings suggested that after *S. aureus* infection, bone marrow–derived osteoclast precursors were no longer able to differentiate into osteoclasts, even in the presence of pro-osteoclastic cytokines (M-CSF/RANK-L), but differentiated into macrophages (Figure 3A).

We then determined whether these *S. aureus*-infected macrophages were activated macrophages and exhibited a modified cytokine profile. Measurement of the cytokines/chemokines secretions by use of LumineX revealed that among the tested cytokines (interleukin (IL) 1α [IL-1α], IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL12-p70, IL-13, IL-17, eotaxin, granulocyte macrophage colony-stimulating factor [G-CSF], interferon γ, keratinocyte chemoattractant [KC], macrophage inflammatory protein 1 α [MIP-1α], MIP-1β, regulated on activation, normal T cell expressed and secreted, [RANTES] and tumor necrosis factor α), steady-state uninfected osteoclasts secreted mostly monocyte chemotactic protein 1 (MCP-1). Their profiles matched those that were obtained with osteoclasts whose precursors had been exposed to heat-killed *S. aureus* for 2 hours (Figure 3B). In contrast, macrophages obtained from precursors that had been cocultured with live *S. aureus* for 2 hours secreted quantitatively more proinflammatory cytokines, especially RANTES, MIP-1α, MIP-1β, G-CSF, KC, IL12p40, and MCP-1 (Figure 3B). Such a cytokine profile is classically found in activated macrophages.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Infected precursors release pro-osteoclastogenic factors. Bone marrow precursors, either infected or uninfected, were grown with receptor activator of NFκ-B ligand (RANK-L) and macrophage colony-stimulating factor (M-CSF). Supernatant was collected 4 days after infection and used as conditioned medium. New, bone marrow–derived osteoclast precursors were then grown in regular culture medium in the presence of M-CSF plus RANK-L, and conditioned medium was added at either a 25% or 50% concentration. After 4 days in the presence of conditioned medium, the number of tartrate-resistant acid phosphatase (TRAP)–positive, multinucleated osteoclasts was determined for each condition. The results are expressed as the percentage of TRAP-positive osteoclasts that were obtained in the presence of infected-conditioned medium versus the percentage of TRAP-positive osteoclasts obtained in the presence of uninfected-conditioned medium (100%). Horizontal bars denote means derived from 3 experiments realized in triplicate. Statistical analyses were performed using the Mann–Whitney test. *P < .05.
Results indicated that *S. aureus* infection of bone marrow precursors blocks osteoclastogenesis but promotes their differentiation into macrophages, which secrete a panel of proinflammatory cytokines.

**Proinflammatory Cytokines Secreted by Macrophages Promote Osteoclastogenesis**

Because it is known that proinflammatory cytokines facilitate osteoclastogenesis, we determined whether the cytokine panel secreted by live *S. aureus*-infected macrophages could enhance osteoclastogenesis. Bone marrow precursors, either infected or uninfected, were grown with RANK-L and M-CSF. Supernatant was collected 4 days after infection and used as conditioned medium (at 25% or 50% concentration with complete culture medium) for osteoclast precursor culturing. After 4 days in the presence of conditioned medium, the number of mature osteoclasts recovered from the infected cells was 1.49- and 1.66-fold higher in the presence of 50% and 25% of supernatant, respectively, than in the presence of the same proportions of supernatant recovered from uninfected cells (*P* = .05 for both; Figure 4). These data demonstrated the pro-osteoclastogenic properties of the proinflammatory cytokines secreted by TRAP-negative, live *S. aureus*-infected macrophages.

Together, these results showed that infection of bone marrow-derived osteoclast precursors by live *S. aureus* directly blocks osteoclastogenesis but has an indirect effect on osteoclastogenesis through the release of proinflammatory cytokines.

**Infection of Mature Osteoclasts by Live *S. aureus* Induces Fusion and Enhances Bone Resorption Capacities**

We then decided to evaluate the impact of staphylococcal infection on mature osteoclasts. Imaging analyses, after labeling of nuclei and actin, showed that in the infected wells, the mature osteoclasts contained twice more nuclei (mean ± SD, 15.75 ± 4.19 vs 8.48 ± 4.59/multinucleated osteoclast; *P* = .012) and had a cellular area 4-fold greater, compared with findings in uninfected wells (mean ±SD, 20 010 ± 5966 vs 6766 ± 6390 μm²/multinucleated osteoclast; *P* = .005; Figure 5). The number of multinucleated osteoclasts was 4.2-fold higher in the infected wells than in the uninfected wells (mean ±SD, 225 ± 128 vs 53 ± 23 multinucleated osteoclasts/well, respectively; *P* < .001). These data...
suggested that *S. aureus* infection increased fusion events and led us to determine whether these cellular modifications had any impact on bone resorption capacities.

To evaluate the effect of infection on bone resorption, *S. aureus*–infected mature osteoclasts were cultured on a hydroxyapatite matrix. The mean resorbed area (±SD) by cells infected with 8325-4 or DU5883 was 2.93 ± 1.78-fold and 3.35 ± 2.8-fold higher, respectively, than the resorbed area in uninfected wells (\(P < .01\) for both; Figure 6). These results highlighted that independent of the presence of FnBP, *S. aureus* infection increased the capacity of resorption of osteoclasts.

**DISCUSSION**

Using an in vitro model to study the interaction between live *S. aureus* and osteoclasts, we described 2 complementary mechanisms that could contribute to bone loss during BJI (Figure 7). First, infection of osteoclast precursors hijacked their differentiation toward activated macrophages, which actively secrete proinflammatory cytokines. These cytokines are able to subsequently enhance the bone resorption capacity of uninfected mature osteoclasts and may promote the migration of osteoclastogenesis of new, uninfected precursors to the site of infection. Second, infection of mature osteoclasts by *S. aureus* directly enhances their ability for bone resorption.

In the literature, all published studies used synthetic bacterial proteins, inactivated *S. aureus*, or Toll-like receptor ligands to investigate the impact of *S. aureus* on osteoclasts and obtained divergent results [21–24]. These conflicting results revealed that the interaction of *S. aureus* and host cells is more complex than the simple extracellular interactions of isolated staphylococcal proteins with the host cell membrane. Here, we used short-term infections of osteoclasts (precursors and mature) by live *S. aureus* to take into account the global impact of this bacterium, including the influence of staphylococcal surface-expressed components and secretome. The 2 strains, 8325-4 and its isogenic mutant DU5883 (\(\Delta fnb\)), were internalized at the same rate, suggesting that the infection mechanisms were different from that for osteoblasts, which involves the endocytosis of the bacteria after the FnBP-\(\alpha\)V\(\beta\)1 integrin interaction. We hypothesized that the internalization mechanisms could be phagocytosis due to the phagocytic properties of osteoclasts [30]. Future studies are needed to identify the mechanisms involved. This infection of bone marrow–derived osteoclast precursors has a direct inhibitory effect on osteoclastogenesis. Contrary to live *S. aureus*, our results showed that short exposure to latex beads did not affect osteoclastogenesis and that heat-killed *S. aureus* or supernatant of *S. aureus* coculture only partially inhibited osteoclastic differentiation. Collectively, these results demonstrate that live *S. aureus* plays a major role in the inhibition of osteoclastogenesis of bone marrow–derived osteoclast precursors. Nevertheless, other osteoclast progenitors are found, notably in hematopoietic tissues, as well as in the bloodstream [31]. Others experiments, such as those involving spleen cells, are needed to confirm that *S. aureus* can also inhibit osteoclastogenesis from other progenitors.

Additionally, we have shown that infection of osteoclast precursors with staphylococci redirected their differentiation from...
mature osteoclasts to macrophages. Moreover, these results are in accordance with previous studies indicating the existence of plasticity between the immune system and osteoclasts. We have also showed that these newly differentiated macrophages secrete high levels of proinflammatory chemokines that act as pro-osteoclastogenic factors. These cytokines, especially MIP-1α and MCP-1, are known to enhance the capacity of uninfected mature osteoclasts for bone resorption [32–37]. Thus, our results combined with those of previous studies, suggest that S. aureus infection of osteoclast precursors has an indirect effect on bone resorption via the hijacking of differentiation and the secretion of proinflammatory cytokines.

We also showed that direct S. aureus infection of mature osteoclasts increases the fusion of osteoclasts and bone resorption capacity. It is well documented that in pathological situations of bone loss, such as inflammatory arthritis or Paget disease, large, highly nucleated osteoclasts predominate in areas of excessive resorption. This phenomenon has led several teams to investigate the relationship between osteoclast size and resorptive activity [38–41]. They demonstrated that large osteoclasts resorbed significantly more efficiently than smaller cells, which suggested that large osteoclasts that were recovered after S. aureus infection were likely responsible for the enhanced bone resorption observed in vitro.

The direct and indirect effects of live S. aureus on osteoclasts add to the well-documented mechanisms responsible for bone loss after staphylococcal invasion of osteoblasts. Indeed, several studies have demonstrated that S. aureus not only decreases the viability of osteoblasts, but also inhibits osteogenic differentiation and reduces the capacity of osteoblasts for bone...
mineralization. Interestingly, it has also been shown that infection of osteoblasts with inactivated *S. aureus* leads to an increase of pro-osteoclastic factors, such as RANK-L and prostaglandin E2 [11, 42]. This "ménage à trois" - between osteoblast, osteoclast, and *S. aureus" - during in vivo bone loss is not taken into account in our in vitro model, which could be considered a limitation of our data. In fact, no in vivo model is able to specifically determine the exclusive contribution of osteoblasts or osteoclasts to bone destruction. This is why we have chosen an in vitro model to explore the specific interaction of *S. aureus* on osteoclasts. Our original data revealed that *S. aureus* is capable of a direct specific enhancement of bone destruction mediated by osteoclasts independently of the osteoblast contribution. Nevertheless, other models deserve to be developed to determine the role of each bone cell type during bone remodeling in BJI. Different potential strategies could be proposed to clarify this question, such as a 3-dimensional tissue culture model that uses primary osteoblasts and osteoclasts [43], a laser capture microdissection of ex vivo tissue [44], and an in vivo BJI model that involves intravital microscopy [45].

In conclusion, our data help to refine the model of *S. aureus* bone loss, in which direct interaction between bacteria and osteoclasts must be taken into account. *S. aureus* infection induces (1) an indirect increase in the number of osteoclasts at the site of infection, owing to proinflammatory and pro-osteoclastogenic factors that are secreted by immune cells, and (2) the direct up-regulation of the bone resorbing capacity of infected mature osteoclasts. This study suggests that osteoclasts could be a main target to limit bone destruction during BJI.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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