Interaction of *Staphylococcus aureus* with osteoblasts (Review)

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Abstract. Orthopedic infection is refractory to cure. *Staphylococcus aureus* (*S. aureus*) is the main causative pathogen responsible for orthopedic infection. *S. aureus* is capable of not only colonizing bone matrix, but also invading osteoblasts, which may play a significant role in the persistence and recurrence of osteomyelitis. Internalization requires the involvement of cytoskeletal elements, including actin microfilaments, microtubules and clathrin-coated pits. Microfilaments are most significant in the invasion process. *S. aureus* is capable of remaining alive in osteoblasts for a long period of time. Decreased sensitivity to antibiotics capable of penetrating host cells increases the difficulties of eradicating *S. aureus*. Osteoblasts, invaded by *S. aureus*, play a significant role in the initiation and maintenance of inflammatory immune responses. These osteoblasts recruit leukocytes and phagocytes to the site of inflammation via the expression of cytokines. Apoptosis is observed in osteoblasts invaded by *S. aureus*. Recruitment of osteoclasts and other immunocytes plays a crucial role in the resorption and destruction of bone.

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1. Introduction

Orthopedic infection is a refractory disease that is associated with progression and recurrence (1). With increasing numbers of foreign bodies implanted in orthopedic surgery, such as prosthetic joints, implant-related infection poses a threat to patients (2). Deep infection following arthroplasty is severe and thorough debridement and removal of the foreign body should be carried out (3).

Staphylococcus aureus (*S. aureus*) is the main causative pathogen responsible for orthopedic infection (4). *S. aureus* is capable of colonizing the matrix of the bone. This infection is difficult to eradicate since bacterial biofilm formation and bacteria embedded in biofilms become resistant to antibiotics, leading to the persistence of osteomyelitis (5). However, *S. aureus* was shown to invade osteoblasts, which may play a significant role in the persistence and recurrence of osteomyelitis (6-8).

The main function of osteoblasts is to synthesize bone matrix and regulate the activity of osteoclasts (9). However, the ability of *S. aureus* to be internalized by host cells and the expression of cytokines reveal the significant role of osteoblasts in the immune response.

2. Invasion of osteoblasts by *S. aureus*

Although not considered to be a typical intracellular pathogen, *S. aureus* was found to be capable of invading osteoblasts and other host cells *in vitro* (2,4,10-15). Internalization of bacteria by osteoblasts also occurs *in vivo* (16). In one experiment, *S. aureus* were injected subcutaneously under the skin of the scalp and the allantoic sac of 17-day-old chick embryos. Following 48 h, calvariae and tibiae were collected for transmission electron microscopy (TEM). *S. aureus* cells were found in approximately 14% of the calvarial osteoblasts following subcutaneous injection, and in 11% of calvarial and tibial osteoblasts following intra-allantoic injection (16). Internalization of *S. aureus* by osteoblasts have also been reported in a patient with recurrent, long-term osteomyelitis (17).

This phenomenon was considered to be another mechanism for the persistence and recurrence of chronic osteomyelitis. Bacteria and osteoblasts play a significant role in the invasion process. The attachment of *S. aureus* to osteoblasts is the first step of internalization and this attachment involves surface molecules of *S. aureus*. Fowler (18) proposed a model of attachment in which osteoblasts form a fibronectin bridge between surface-associated fibronectin-binding proteins of bacteria and host cell β1 integrins; this bridge then leads to the invasion of *S. aureus*. *Staphylococcus epidermidis* (*S. epidermidis*) was also shown to be capable of invading bone cells. However, unlike *S. aureus*, *S. epidermidis* was unable to gain

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entrance into bone cells through a fibronectin bridge between the integrin and a bacterial adhesin (19).

Different *S. aureus* strains have variable capacity in internalizing bacteria. This capacity is found to be correlated with σ B expressed by *S. aureus* (20). σ B, the only σ factor identified in *S. aureus*, is capable of prolonging the production of cell surface proteins, including fibronectin. σ B also plays a significant role in the regulation of virulence genes in *S. aureus* (21).

Internalization requires the involvement of cytoskeletal elements, including actin microfilaments, microtubules and clathrin-coated pits. Microfilaments are most significant in the invasion process (13). The process may be inhibited by monodansylcadaverine and cytochalasin D and, to some extent, by ouabain, monensin, colchicine and nocodazole (12). Notably, the invasion process does not require live bacteria (live and dead *S. aureus* are equally effective for invasion); however, live osteoblasts are required (22). The role of the osteoblast is positive in the process of invasion, and a 'zipper-type' mechanism has been proposed (23).

Calcium channels are also crucial in the invasion of osteoblasts, which are involved in the rearrangement of the cytoskeleton (24). The invasion of osteoblasts by *S. aureus* resulted in an increase in the phosphorylation of the extracellular signal-regulated protein kinases (ERK 1 and 2) (25). Activation of ERK 1 and 2 may result in the phosphorylation of numerous different substrates, including the transcription factors ATF-2, Elk-1 and c-Jun (26). ERK 1 and ERK 2 phosphorylation may also activate phospholipase A2, resulting in the production of leukotrienes (27). Leukotrienes may then open calcium channels on the host cell membrane (28).

3. The fate and viability of *S. aureus* following invasion

The fate of *S. aureus* following internalization by osteoblasts may correlate with the clinical manifestation of osteomyelitis. If *S. aureus* is capable of remaining alive in the intracellular environment, a thorough cure of osteomyelitis is difficult due to the difficulty of eradicating the bacteria in the osteoblast. Rifampin, chloramphenicol and clindamycin are the most active intracellular antibiotics. The majority of other antibiotics currently used are inactive intracellularly (e.g., lincomycin) or are incapable of penetrating cells (e.g., penicillins, cephalosporins and aminoglycosides) (29). Therefore, bacteria internalized by the osteoblast may be sequestered from the majority of antibiotics as well as the immune system.

Certain antibiotics are capable of eukaryotic cell penetration and have intracellular functions. However, similar to the bacteria embedded in the biofilms, the internalized bacteria may change their characteristics and decrease their sensitivity to antibiotics following the invasion of host cells and become situated in the intracellular environment. Investigators have used clindamycin and rifherampin to treat the *S. aureus* internalized in the osteoblast (30). The two antibiotics are capable of penetrating eukaryotic cells. Although the antibiotics may decrease or kill *S. aureus* internalized in the osteoblast at immediately following the invasion, the bacteria become less sensitive to the two antibiotics 12 h following the invasion (30). The observed structural changes were considered to be responsible for the change in sensitivity; however, metabolic change may also play a significant role. The change in sensitivity of *S. aureus* to antibiotics in the intracellular environment increases the difficulties of eradicating the pathogen.

Since *S. aureus* is capable of remaining alive in the osteoblast for a long period of time (4,22), the bacteria released from the dead osteoblast may induce the recurrence of osteomyelitis (6). A previous study also showed the lack of decreased viability of *S. aureus* in invading a second osteoblast *in vitro* (31).

4. The role of osteoblasts in the immune response

As is commonly known, the primary roles of osteoblasts are to synthesize the components of the bone matrix and to regulate osteoclasts, which are bone resorption cells (32). However, there is increasing understanding of the function of osteoblasts in the initiation and maintenance of the inflammatory immune response. Osteocytes may recruit leukocytes and phagocytes to the site of inflammation via the expression of cytokines.

Cultured mouse and human osteoblasts infected with *S. aureus* were found to express high levels of interleukin (IL-6 and IL-12p75) (33). IL-12 is capable of stimulating T lymphocytes and natural killer (NK) cells to secrete significant amounts of interferon (IFN), activating macrophages and T lymphocytes to augment a Th1 response (33). Although IL-12 is known for its ability to protect against intracellular pathogens, it may contribute to the process of organ-specific autoimmune diseases (34). Monocyte chemoattractant protein-1 (MCP 1), produced by osteoblasts, also has the ability to recruit macrophages and certain T lymphocytes to areas of inflammation (35,36). Osteoblasts may respond to bacterial infection by upregulating the expression of the chemokine CXCL10 (IP-10). IP-10 may then recruit T lymphocytes to the sites of bone infections (37). Dexamethasone, PGE(2) and T(h)2 cytokines are potential down-regulatory mediators of the chemokine (38).

Expression of NLRP3 in osteoblasts invaded by *S. aureus* was found in a study by McCall *et al* (39). The active NLRP3 inflammasome drives the innate immune response towards invading pathogens and cell damage, and regulates an adaptive immune response (40). The expression of NOD, a novel intracellular pattern recognition receptor, and Rip2 kinase, a critical downstream effector molecule for NOD signaling, was observed in osteoblasts invaded by *S. aureus* (41). NOD may regulate pro-inflammatory pathways in response to bacteria by inducing signalling pathways including nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs) (42). However, certain authors believe it is the attachment, not invasion or secreted soluble factor(s) that activates NF-κB in human osteoblasts (43,44).

5. Apoptosis of osteoblasts following the internalization of *S. aureus*

Osteoblasts invaded by few bacteria were found to be able to remain alive and differentiate into osteocytes (16). However, apoptosis or programmed cell death was found in osteoblasts invaded by *S. aureus*, but the process may also be induced by pathogens. Tucker *et al* (45) used light microscopy to examine morphological changes in the osteoblasts following the internalization of *S. aureus*. Cell rounding was observed, and dark centers, due to condensation of chromatin, were noted. Apoptotic nuclei were also present.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is crucial in the process of cell apoptosis. Alexander *et al* (8) examined the ability of *S. aureus* to induce the production of TRAIL by osteoblasts. Results demonstrated that *S. aureus* was capable of inducing TRAIL expression by osteoblasts. A dose-dependent response was observed 30 min following exposure to bacteria. Attachment of *S. aureus* to osteoblasts is necessary for optimal TRAIL induction (46). Messenger RNA (mRNA) molecules encoding TRAIL receptors were also expressed by osteoblasts. The expression of NLRP3 in osteoblasts invaded by *S. aureus* also provides a potential mechanism of apoptotic cell death of the host cells (39). Osteoblast apoptosis results in decreased matrix deposition and destruction of the bone (8).

6. Destruction of the bone due to inflammation and osteoclasts

Normal bone remodeling requires the coordinated regulation of the genesis and activity of osteoblast and osteoclast lineages (47). Therefore, apoptosis of osteoblasts is not the only cause of bone destruction. Recruitment of osteoclasts and other immunocytes play a significant role in the resorption and destruction of bone. In *S. aureus* infection, bone resorption is caused by proteins rather than lipoteichoic acid or muramyl dipeptide. The surface-associated protein fraction may stimulate fibroblasts or monocytes to release osteolytic cytokines and chemokines (48), including MCP-1, colony-stimulating factors (CSFs) and interleukins. Dexamethasone, PGE(2) and T(h)2 cytokines have potential down-regulatory mediation of these chemokines (38).

Although MCP-1 is capable of recruiting macrophages and certain T lymphocytes to areas of inflammation (35,36), T lymphocytes and macrophages may also be responsible for bone loss and contribute to the development of the progressive inflammatory damage (35). IL-6, produced by osteoblasts invaded by *S. aureus*, may directly or indirectly modulate the activity of osteoclasts, resulting in the induction of osteoclast differentiation or osteoclast-mediated bone demineralization (49). IL-6 expressed in the osteoblasts may be suppressed by certain agents including epigallocatechin gallate, a constituent of tea, which plays a role in the suppression of inflammation and decrease of bone resorption (50). IL-12 also contributes to the process of organ-specific autoimmune diseases (34). CSFs have a profound effect on osteoclastogenesis, high levels of granulocyte-macrophage-CSF (GM-CSF) and G-CSF secretion by osteoblasts challenged by *S. aureus*, and may induce osteoclastogenesis, resulting in bone resorption (51).

7. Conclusion

The interaction of *S. aureus* with osteoblasts is initiated from the attachment of the bacteria to osteoblasts, followed by the internalization of *S. aureus* by osteoblasts. Signal transduction and cytokine expression play a vital role in the process of inflammatory damage and destruction of the bone.

S. aureus, internalized in the osteoblasts, may be sequestered from the majority of antibiotics and the immune system. However, the sensitivity change of the bacteria to the antibiotics capable of penetrating eukaryotic cells results in the failure of antibiotic therapy. Therefore, metabolic characteristics of *S. aureus* in the intracellular environment should be further studied to develop novel antibiotics to kill the bacteria in the osteoblasts. In addition, the internalization process should also be considered; if we are able to prohibit the invasion process and locate the bacteria outside the host cells, then eradication of these bacterias becomes simpler.

Osteoblasts challenged by *S. aureus* play a significant role in the initiation and maintenance of the immune response. The process is complicated and involves numerous cytokines and pathways that conduct the signal. Although recruitment of leukocytes and phagocytes may be helpful in killing the pathogens, inflammatory damage may accumulate. Apoptosis of osteoblasts induced by *S. aureus* and the increased activity of osteoclasts are responsible for bone resorption (39). Down-regulation of cytokines may inhibit the destruction of bone; however, down-regulation of cytokines also inhibits the immune response, which may be helpful in the process of eradicating bacteria (32). Therefore, it appears more promising and easier to inhibit the attachment and internalization of *S. aureus* by osteoblasts in treating osteomyelitis. Further investigation into the interaction of *S. aureus* with osteoblasts should therefore be performed in order to decrease the rate of orthopedic infection.

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