Impact of Bacterial Infections on Osteogenesis: Evidence From In Vivo Studies

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ABSTRACT: The clinical impact of bacterial infections on bone regeneration has been incompletely quantified and documented. As a result, controversy exists about the optimal treatment strategy to maximize healing of a contaminated defect. Animal models are extremely useful in this respect, as they can elucidate how a bacterial burden influences quantitative healing of various types of defects relative to non-infected controls. Moreover, they may demonstrate how antibacterial treatment and/or bone grafting techniques facilitate the osteogenic response in the harsh environment of a bacterial infection. Finally, it a well-known contradiction that osteomyelitis is characterized by uncontrolled bone remodeling and bone loss, but at the same time, it can be associated with excessive new bone apposition. Animal studies can provide a better understanding of how osteolytic and osteogenic responses are related to each other during infection. This review discusses the in vivo impact of bacterial infection on osteogenesis by addressing the following questions (i) How does osteomyelitis affect the radiographic bone appearance? (ii) What is the influence of bacterial infection on histological bone healing? (iii) How do bacterial infections affect quantitative bone healing? (iv) What is the effect of antibacterial treatment on the healing outcome during infection? (v) What is the efficacy of osteoinductive proteins in infected bones? (vi) What is the balance between the osteoclastic and osteoblastic response during bacterial infections? (vii) What is the mechanism of the observed pro-osteogenic response as observed in osteomyelitis? © 2019 The Authors. *Journal of Orthopaedic Research*® published by Wiley Periodicals, Inc. on behalf of Orthopaedic Research Society. J Orthop Res 37:2067–2076, 2019

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Osteomyelitis is a frequent complication of trauma or surgery, often in conjunction with prosthetic implantation, but may also occur secondary to vascular insufficiency or hematogenous infections.[1] Microorganisms that are either competent in binding to the surface of the host tissue/implant (i.e., coagulase-negative staphylococci), or specialized in evading the host defense system and destructing host cells/extracellular matrix (i.e., *Staphylococcus aureus*), are the most common causative pathogens in osteomyelitis.[1,2] After the formation of a persisting biofilm, osteomyelitis can manifest itself as a complicated clinical scenario, necessitating repeated surgical interventions to clear the infection.[3]

It is well-accepted that the exaggerated inflammatory response in osteomyelitis leads to drastic bone changes, which are the result of a dysregulation of the number of bone-forming osteoblasts and bone-resorbing osteoclasts.[4] The cytokine receptor activator of NF-κB ligand (RANKL) is a critical regulator of bone remodeling and regeneration by controlling osteoclast formation and activity.[5] An enhanced expression of RANKL is a hallmark of osteomyelitis, and occurs in direct response to bacterial antigens and their secreted products, or in an indirect response to exaggerated tissue inflammation.[5–7] Osteoblasts actively participate in the osteolytic process by internalizing bacteria[8,9] and aggravating the inflammatory response through secretion of pro-inflammatory[10–12] or osteoclast-modulating[6,13] factors. In addition to the enhanced osteoclast-mediated bone resorption, several processes may cause a lack of sufficient bone matrix-depositing cells at the bacterial burden.[14] Osteomyelitis is known to cause an uncontrolled cell death by compression of vascular channels[1] and the local release of nitric oxide.[15] Moreover, osteoblasts undergo programmed cell death and necrotic cell death after uptake of bacteria,[16,17] or after exposure to high levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferonγ (IFN-γ).[18,19] These many bone changes result into life-long diminished resistance of the tissue to bacterial adherence and infection, explaining the relatively high chance of reinfection even years after the first episode.[20]

In quantitative terms, the actual clinical impact of bacterial infections on bone regeneration remains largely elusive. Clinicians recognize that osteomyelitis impairs the regenerative response after injury,[1,4] but the underlying mechanism, the course of the disease, and the healing outcome cannot be measured in a standardized way in a clinical situation. Moreover, as bone infections are usually treated as soon as clinical manifestations occur, it is difficult to assess the long-term effect of infection on bone healing without any intervention. Animal models can provide valuable insight into the correlation between the bacterial burden, bone loss, and callus formation. Furthermore, infection

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(e.g., bacterial species or load) and clinical parameters (e.g., type of bone defect and the use of antibacterial treatment) can be reproduced in a controlled setting allowing comparison with an appropriate control group. Animal experiments also offer the advantage that callus formation can be more easily monitored[21–23] and quantified with high-resolution imaging techniques,[23,24] and that the impact on biomechanics can be assessed.[24–26]

From a basic point of view, the current review summarizes how bacterial infection affects the radiographic and histological appearance of bone tissue in the context of fracture healing, and whether this is related to changes in mechanical stability and callus formation. Whereas bone fracture healing normally is a well-orchestrated event, it is unclear if, and to what extent, the sequential stages of fracture healing (i.e., inflammation, repair, and remodeling) are disturbed following bacterial contamination. Moreover, it is discussed how bone loss and new bone formation coincide as part of the local tissue response to a bacterial infection, and how these seemingly opposite processes are related to each other. Together, this can elucidate which cellular processes are most affected in osteomyelitis. From a more translational point of view, this review addresses how antibacterial treatment and/or bone grafting techniques facilitate the osteogenic response in the harsh environment of a bacterial infection. In this respect, there are several uncertainties that complicate the decision-making in osteomyelitis treatment. For example, although early removal of an implant increases the chance of bacterial eradication,[27] defect healing is compromised by defect instability following implant removal.[28–30] It remains to be answered whether non-critical size bone defects can fully consolidate despite infection, provided that they are adequately stabilized. Furthermore, it is not fully clear how antibiotic treatment, either or not in combination with bone grafting, contributes to the recurrence of healing during or after infection, and whether or not this is related to full bacterial eradication.

Using available in vivo data, we aim to answer the following questions (i) How does osteomyelitis affect the radiographic bone appearance? (ii) What is the influence of bacterial infection on histological bone healing? (iii) How do bacterial infections affect quantitative bone healing? (iv) What is the effect of antibacterial treatment on the healing outcome during infection? (v) What is the efficacy of osteoinductive proteins in infected bones? (vi) What is the balance between the osteoclastic and osteoblastic response during bacterial infections? (vii) What is the mechanism of the observed pro-osteogenic response as observed in osteomyelitis?

LITERATURE SEARCH STRATEGY

A literature search was performed in the PubMed database. The following search terms were used: bone OR callus OR osteogenesis AND animal OR in vivo OR preclinical OR pre-clinical AND infection OR bacterial OR contamination OR osteomyelitis. Non-English articles or articles published before 2000 were excluded. The resulting ±150 articles were screened for relevance, that is, whether the effect of bacterial infection on bone healing, osteolysis, or new bone formation was reported in a quantitative manner. The main findings from the remaining 40 references were summarized and critically reviewed. The key reports are shown in Supplementary Table S1 and Table S2. Additional clinical or in vitro articles were included to interpret the findings.

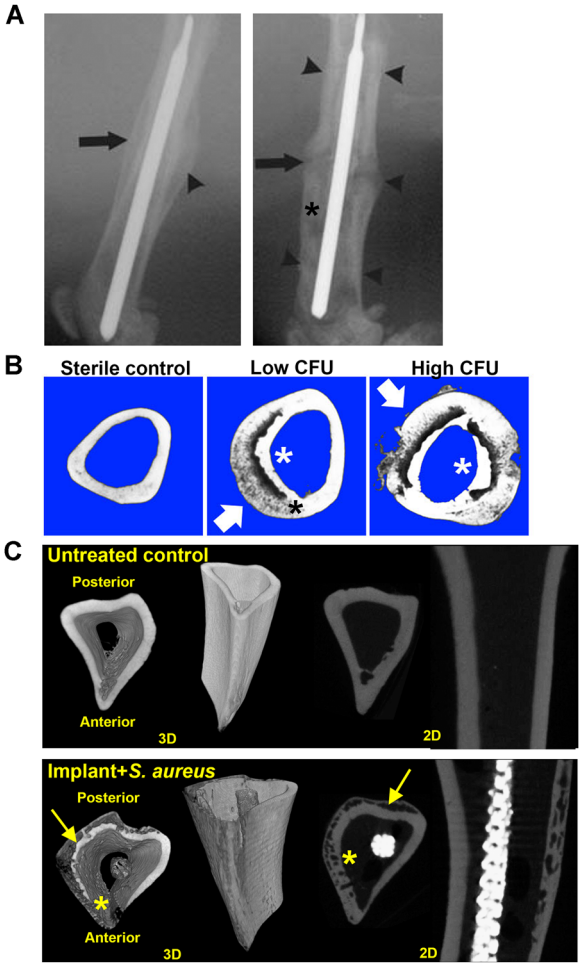

Figure 1. Commonly observed radiographic signs of osteomyelitis. (A) A closed fracture was created in the rat femur and stabilized with an intramedullary nail, either with (right image) or without (left image) *Staphylococcus aureus* contamination. After 3 weeks, the radiographs show complete healing in the absence of infection. In the presence of infection, the defects were unable to heal. Osteolysis (asterisk) is seen in proximity of the fracture gap (arrow), while pronounced periosteal bone formation (arrowheads) can be seen distally and proximally to the defect. Reprinted from Robinson et al.[44] (B) *S. aureus* infection in a rabbit tibia model of periprosthetic infection leads to periosteal bone formation (arrows) and osteolysis (asterisk) as observed by micro-CT. The amount of periosteal bone formation and cortical resorption is associated with the number of colony-forming units (CFU) after 4 weeks. Reprinted from Croes et al.[49] (C) Micro-CT image showing an untreated contralateral rat tibia or *S. aureus*-contaminated rat tibia receiving intramedullary implant. Osteolysis (asterisk) and new bone formation (arrows) were indicative of osteomyelitis after 4 weeks. Reprinted from Croes et al.[48] [Color figure can be viewed at wileyonlinelibrary.com]

HOW DOES OSTEOMYELITIS AFFECT THE RADIOGRAPHIC BONE APPEARANCE?

Bone resorption, cortical thickening, and periosteal new bone apposition are the key signs of osteomyelitis by plain radiography or computed tomography (CT) imaging.[31–35] The osteomyelitis-related bone changes are consistent among small or large animal models, and are similar to those seen in patients[36,37] (Fig. 1). This leads to the suggestion that also the underlying pathologic abnormalities (i.e., bacterial spread through the cortex, induction of an inflammatory milieu, abscess formation)[37] are comparable from mice to men. Periosteal new bone formation generally has an earlier onset than cortical osteolysis.[31,33,38] As an explanation, reactive bone formation occurs in a direct response to the local inflammatory milieu,[39] while in comparison, cortical osteolysis is also caused by bacterial spread into the cortical Haversian and Volkmann canals.[36,37] Several studies have utilized radiographic scoring systems to quantify the severity of osteomyelitis. These grading systems can clearly distinguish between infected animals and non-infected controls.[31,40–44] Nevertheless, there is no evident relationship between the radiographic score and the actual bacterial count, making radiography by itself an unreliable tool to assess the effectiveness of antibacterial strategies.[45–48] There are several explanations as to why the radiographic appearance of bone is not directly related to the bacterial burden. First, even if the bacterial burden is completely removed, the bone would require several weeks to remodel to its original architecture.[49] Second, bacterial effects on bone remodeling are very dependent on the specific strain being utilized, for example, by having a different capacity to secrete toxins modulating osteogenesis or osteoclastogenesis,[4,40] or by engaging different immune response through extracellular or intracellular pathogen-associated molecular patterns (PAMPs).[50–53] Third, the presence of a foreign body or delivered compounds alone can already lead to osteomyelitis-like bone changes and exaggerate the radiographic scores, as has been observed with silver nanoparticle-based implant coatings.[48]

WHAT IS THE INFLUENCE OF BACTERIAL INFECTION ON HISTOLOGICAL BONE HEALING?

In this section, we discuss how the complex immunological and tissue response to bacterial infection impacts the process of histological bone healing. In the normal situation, fracture healing follows a characteristic time-course, with the three overlapping phases of inflammation, repair, and remodeling.[54] The phases of bone repair are similar among different species, although it should be realized that small animals generally show faster fracture healing as compared to larger animals or humans.[54,55] Furthermore, the type of bone healing depends on the anatomical location of the fracture. As compared with diaphyseal bone healing, metaphyseal/epiphyseal fracture healing occurs in a direct manner, with limited or no periosteal callus formation.[56] The acute inflammatory response, essential for the recruitment of progenitor cells from distant sources, also seems less important for metaphyseal bone healing.[57] Since fracture (infection) models commonly involve defects created in the diaphyseal bone in rodents, the current discussion focuses on bone healing at this anatomical location.

In response to tissue injury and blood clot formation, an inflammatory response is initiated (duration: 0–5 days), which is critical for bone healing.[55,58,59] Neutrophils enter the injured site already within minutes.[60] Their main contribution is to phagocytose bacteria, remove cellular debris, and produce cytokines and chemokines to direct the infiltration of monocyte/macrophages.[60,61] Macrophages play a key role in the inflammatory response, as they produce key regulatory factors needed for fibroblast and mesenchymal stem cell (MSC) recruitment from their local niches, and growth factors inducing osteogenesis and angiogenesis.[58,62] The initial fracture hematoma is replaced by granulation tissue with proliferating fibroblasts and MSCs in the first-week post-fracture. The subsequent repair stage (duration: 1–4 weeks) involves a combination of both direct (intramembranous) and indirect (endochondral) forms of bone healing, with the relative contribution of these processes being influenced by the mechanical stability of the defect, that is, with relative more intramembranous healing when there is less interfragmentary motion.[55] Chondrocytes contribute to the formation of a soft callus, that is, cartilage tissue accompanied by fibrotic tissue. This serves as the template for subsequent endochondral bone formation. At the same moment, direct bone formation originates from recruited and periosteal-derived progenitor cells that differentiate into osteoblasts to deposit woven bone. The stability provided by the initial fibrocartilage and woven bone scaffold allows the formation of hard callus, characterized by hypertrophy of chondrocytes, revascularization, and the replacement of the cartilage scaffold by woven bone. In the final stage (duration: 5–8 weeks), the woven bone and cartilage is removed by osteoclasts and replaced by the typical osteon and Haversian bone structure under influence of the mechanical stresses applied to the bone.[55,58,63]

Most of the in vivo studies have characterized the histological appearance of the bone after 4 or more weeks, which corresponds to the reparative (1–4 weeks) or remodeling (5–8 weeks) phases of healing. The studies collectively show that the well-orchestrated event of callus formation is disrupted in osteomyelitis. Instead of the expected fibrocartilage and woven bone, an inflammatory medullary reaction is observed composed of fibrous tissue and abscess formation with only occasionally fibrocartilaginous tissue deposition. The cortical response is characterized by osteolysis, necrotic bone sequestra, and periosteal new bone formation. In contrast to non-infected controls, active inflammation is always observed in infection animals, irrespective of the time point of evaluation.[21,22,25,33,34,44,64–68] Whereas neutrophils and

monocytes normally only play a role in the acute phase of healing, they remain predominant cell populations in infected defects even after weeks to months.[43,68,69]

The total absence of hyaline cartilage in the defects of severely-infected animals compared with low-grade[44,65] or non-infected[43,44] animals, leads to the suggestion that bacterial contamination already disrupts the early stages of callus formation. Histology reports suggest that the sustained presence of neutrophils may be a limiting factor for normal callus formation. Neutrophils are normally only short-lived (hours-days), and are quickly replaced by macrophages in the fracture hematoma.[22,70,71] In osteomyelitis, they remain present in high numbers both locally and systemically, outnumbering monocytes and lymphocytes.[25,38,43,44,65,68,72] Recent reports have shed light on possible mechanisms by which neutrophils could inhibit callus formation. First, a sustained presence of neutrophils will dysregulate the osteogenic differentiation and matrix production of bone progenitor cells.[61,73] Second, sustained neutrophil activity can limit the recruitment of monocytes/macrophages to the defect site, thus delaying the angiogenic and osteogenic responses.[60,72]

The histological appearance of the fractured bone has a large impact on its mechanical stability. Beyond the four-week time point, fibrous tissue formation and limited mineralized tissue formation is associated with biomechanically weaker bone based on torsional testing.[21,25,34,42] Studies even report that biomechanical analyses could not be performed on the healing calluses due to the unexpected lack of bone union in the presence of infection.[21,24] A reduced bone-implant fixation may result into further mechanical instability of the bones. Osseointegration studies have shown that bacterial contamination promotes high osteoclast activity and fibrous capsule development directly around the implant, reducing the strength of implant fixation already in the first-week.[26,32,35]

HOW DO BACTERIAL INFECTIONS AFFECT QUANTITATIVE BONE HEALING?

Plain radiography or micro-CT has been the main method to quantify healing outcomes in osteomyelitis models. As presented in Supplementary Table S1, the results of our literature search demonstrate that the impact of bacterial infections on osteogenesis has been mainly examined using closed fracture or non-critical size osteotomies in the long bones of rodents, in combination with a stabilizing metal implant or plate. The radiographic or micro-CT outcomes of the studies show that the defects generally bridge uneventfully under aseptic conditions. Strikingly, in spite of the relatively small defects that are often created, many studies report non-union at follow-up once a chronic infection is established.[25,34,43,44,64,68,74–76] This negative influence of bacterial infection on healing depends on the severity of infection,[23,43,44,66,77,78] and correlates with the initial bacterial inoculation dose.[43] In comparison, normal defect healing is often reported in cases where antibacterial treatment successfully eliminated all bacteria from the defect site.[65,76,77]

WHAT IS THE EFFECT OF ANTIBACTERIAL TREATMENT ON THE HEALING OUTCOME DURING INFECTION?

Next, it was asked how antibacterial treatment restores bone healing in osteomyelitis models. The data from animal models show that complete eradication of implant-associated infections is challenging using systemic antibiotics alone,[24,34,44,47] which is also not uncommon in the clinical situation.[79] Consequently, a partial reduction in bacterial burden only leads to a moderate improvement in callus formation in rats.[34,44] Although some studies have added a debridement and lavage step in their model, these interventions had little additive value in terms of the osteogenic response.[68,75,77] In comparison to systemic treatment, the coating of osteosynthesis plates with antibacterial agents has resulted in more successful bacterial killing and prevention of implant infections. In these specific models, similar healing can be seen as in non-infected controls.[65,76,77]

While aforementioned studies indicate that complete killing of bacteria is needed for optimal bone regeneration, there are also reports of contrasting effects of antibiotics in terms of infection or bone healing results. For example, Shiels et al.[80] showed that local application of vancomycin could not successfully reduce the bacterial count, but nevertheless, the clinicals signs of infection and radiographic bone healing were improved. In agreement, antibiotic treatment increases the effectiveness of bone-promoting growth factors without actual reduction in infection.[21,24,81] Finally, Lovati et al.[43] observed that a low-grade *S. aureus* infection did not reveal clinical signs of infection in rats, yet, the animals displayed impaired bone healing. These studies show that there can be incongruency in the effect of antibiotics on microbiological, clinical, and bone healing results during or latent or "silent" infection,[82] which can occur due to incomplete bacterial eradication or a persisting low-grade infection.

WHAT IS THE EFFICACY OF OSTEOINDUCTIVE PROTEINS IN INFECTED BONES?

The dual role of fracture fixation devices complicates the management of infected bone defects. On the one hand, the formation of an implant-associated biofilm contributes to the chronicity of osteomyelitis, and consequently, the removal of the fixation device facilitates bacterial clearance and gaining bone union.[3] On the other hand, the stability provided by the fracture fixation device aids in callus formation and healing outcome.[55] Hence, there is need of techniques that are capable of rescuing bone union under infectious conditions, but permit removal of the fixation device.[24] Different bone grafts can be applied to promote bone healing by directing osteoconduction and/or

osteoinduction; however, it is currently unclear what the effectiveness is of different bone grafts in the harsh environment of a bacterial infection. Even though autologous bone remains the gold standard bone graft, the current literature search did not yield any studies that evaluated the effectiveness of autologous bone in an osteomyelitis environment. The current section will, therefore, focus on the use of bone morphogenetic proteins (BMPs), and of which the BMP-2 (Infuse) and BMP-7 (OP-1) forms are clinically applied as bone graft extender/substitute.[83]

BMPs are contraindicated in the case of an active infection due to insufficient clinical comparison with autografting or allografting.[84] It, therefore, remains unanswered if BMPs are suitable candidates to promote healing in case of an infection. Clinical data indicate that BMPs may be particularly effective in promoting osteogenesis when the local environment is not favorable for healing,[85] or when there is an increased risk of non-union.[86] Several clinical trials have even indicated that the treatment of open tibial fractures with BMP-2 lowers the incidence of implant-related infections.[86,87] In addition to the aforementioned clinical studies, animal studies have investigated how BMPs stimulate bone formation in the presence of a clinically relevant infection.

BMP-2/Infuse is FDA-approved for lumbar fusions, however, it is contraindicated in the case of an active infection.[84] Miller et al.[88] evaluated the efficacy of these proteins in the setting of an infected fusion model in rabbits. Remarkably, following contamination with only 500 colony-forming units (CFU) *S. aureus*, BMP-2 failed to induce spinal fusion in all 12 rabbits, while fusion occurred in all 13 non-infected control rabbits. Although clinical investigations reported successful fusion rates for BMP-2 in vertebral osteomyelitis, this discrepancy in outcome can be related to the additional debridement and antibiotic treatments performed in these patients.[89,90]

More numerous in vivo studies have investigated the impact of *S. aureus* infection on BMP-induced osteogenesis in the long bones, as overviewed in Supplementary Table S2. Critical size defects, by definition, will not heal without intervention and therefore represent the clinical scenario of a non-union where osteoinductive factors may be introduced.[91] Chen et al.[21,24] investigated the efficacy of BMP-7 or BMP-2 in critical-size femoral defects in rats. By performing a debridement, either with or without subsequent delivery of systemic antibiotics, the authors could compare the effectiveness of the BMPs in high-grade (i.e., without antibiotics) or low-grade (i.e., with antibiotics) infection. In their model, successful defect bridging was only realized for the highest doses (200 μg) of BMP-2 or BMP-7, and only in combination with systemic antibiotics. The BMPs induced minimal callus formation within the infected defects in the absence of antibiotics. In a comparable model, Helbig et al.[42] found that neither BMP-2 or BMP-7 was effective in restoring bone healing without additional antibiotics treatment. In other critical size defect models, it was confirmed that BMP-2 was most effective in combination with local antibiotics[81,92] or antibacterial nanosilver.[23] A comprised of osteoinduction by BMP-2 has even been reported for relatively small defects or closed fracture models.[42,93] Together, these studies suggest that a bacterial infection is detrimental for the outcome of BMP-induced bone formation in clinically relevant bone defects, but that a beneficial response to BMPs can be realized in conjunction with appropriate antibacterial treatment.

WHAT IS THE BALANCE BETWEEN THE OSTEOCLASTIC AND OSTEOBLASTIC RESPONSE DURING BACTERIAL INFECTIONS?

Paradoxically, new bone formation often occurs in parallel to the bone loss in osteomyelitis,[31–35] suggesting that the local response to bacteria also activates pro-osteogenic pathways. However, it is unknown which of the two processes (i.e., osteolysis vs. osteogenesis) predominates during infection and if there are common pathways involved in both processes. While fracture models have demonstrated a clear negative correlation between bacterial burden and callus formation within the fracture, in the same studies, reactive new bone formation is often also apparent. Numerous reports even indicate a positive correlation between the measured bacterial CFU and the amount of reactive new bone formation.[23,39,43,44,66,77,78] Several lines of evidence indicate that new bone formation in osteomyelitis is usually not observed in vicinity of the bacterial burden, but that it often predominates at more distant sites. In the case of plate osteosynthesis, woven bone deposition is more evident in bone regions distant to the implant-related infection, that is, opposite or further away from the plate.[23,65,77,94] In the case of intramedullary fixation, bone apposition is observed in the periosteal region along the length of the implant.[34,44,93] Studies that have incorporated micro-CT algorithms to quantify bone formation and bone destruction separately from each other, have confirmed that significantly enhanced bone volume is measured more peripheral to the infected site.[35,40]

WHAT IS THE MECHANISM OF THE OBSERVED PRO-OSTEOGENIC RESPONSE AS OBSERVED IN OSTEOMYELITIS?

Considering that the reactive bone formation starts almost immediately after the onset of infection,[39] the bone deposition is unlikely the result of biomechanical adaptation to compensate for osteolysis. Alternatively, it is known that pro-inflammatory signals can directly target bone-lining osteoprogenitor and immune cells to propagate osteogenesis,[39,95,96] which can occur uncoupled from an increased osteoclast activity.[39,97,98] The finding that infection-induced bone formation is enhanced in mice with metabolic syndrome strengthens

the hypothesis that the inflammatory milieu is a key mediator of the osteogenic response.[41]

The same cytokines that are needed for efficient antibacterial immune responses may also drastically affect the activity of bone cells.[99] It can be reasoned that the inflammatory response in bacterial infection is a double-edge sword in terms of its opposite effects on osteogenesis. On the one hand, a mild inflammatory milieu distant from the infection site may stimulate osteogenesis,[50,100,101] resembling the normal bone healing response after injury.[95] To illustrate, the pro-inflammatory cytokines TNF-α and interleukin (IL)-17 are upregulated during bacterial infection,[22] and their transient expression is known to have pro-osteogenic effects on osteoprogenitor cells.[50,100,102,103] On the other hand, increased cell death, comprised vascularization, and uncontrolled osteoclast activity will be most profound in the vicinity of the bacterial burden.[1,7,14,15,17] Moreover, the increased influx of immune cells can lead to the production of soluble factors hampering osteogenesis.[104] Histology performed on infected bone tissue generally shows a high number of neutrophils, and also monocytes/macrophages to a lesser extent.[38,41,68] Under acute or mild inflammatory conditions, neutrophils contribute to bone fracture healing via yet unknown mechanisms.[60,105] The finding that neutrophils inhibit the mineralized extracellular matrix production by MSC might explain how their prolonged activity at the infected tissue impairs the early reparative phase of fracture healing.[73] In the same line of reasoning, macrophages are considered the most important immune regulators in bone regeneration,[62,72] but can inhibit osteogenesis via IL-1β secretion during exaggerated inflammation. In this context, the activation of various pattern recognition receptors (PRRs) in osteomyelitis may be an important shared feature of the different, sometimes paradoxical, cellular responses seen in osteomyelitis. In recent years, it has been shown that PRRs not only play a key role in the antibacterial immune response,[106] but that they also modulate the osteoblastic and osteoclastic responses.[49,99]

SUMMARY

Animal studies collectively show a strong negative association between bacterial infection and bone regeneration. In the long bones, callus formation is impaired and the defect is instead replaced by fibrous tissue formation characteristic of an atrophic nonunion, even in the case of non-critical size defects. The lack of fibrocartilage and woven bone formation leads to significantly reduced mechanical stability. Neutrophils are the major immune cell type in the infected tissue, and their prolonged presence is associated with an abnormal bone healing response. The histopathology results agree with radiography or micro-CT imaging, showing that osteomyelitis-related bone changes are consistent among different animal models and humans. It should be noted that the outcomes of radiographic or histological scoring systems are not related to the actual bacterial counts, limiting them as a tool to assess the effectiveness of antibacterial strategies.

It was found that even relatively small defects do no consolidate when infected, despite the use of various fixation or stabilization techniques. Instead, the following prerequisites were found to exist to achieve successful bone regeneration of contaminated defects: for small defects—that is, closed fractures and small osteotomies—full bridging requires complete elimination of infection by adequate antibacterial treatment. For large segmental defects, an optimal environment for osteogenesis requires a combinatorial approach with antibiotics and an osteoinductive graft.

Osteoinductive BMPs support promote bone healing in rodent infection models, but relatively high amounts of BMPs are needed to overcome the detrimental effects of bacteria on bone healing.[92,107–109] This inevitably leads to increasing concerns about unwanted effects at supraphysiologic doses, such as the possibility for complications and ectopic bone formation.[83] Considering the species-specific requirements in the minimal BMP-2 dose,[110] more elaborate large animal studies are needed to answer whether BMPs are appropriate bone grafts in case of a clinically relevant infection. Even though it is the gold standard bone graft, it is impossible to draw any conclusions regarding the use of autologous bone transplantation in the context of osteomyelitis due to a gap in the current literature.

Paradoxically, animal studies show that antibiotics generally improve bone healing compared with untreated controls even if a "silent" *S. aureus* infection persists. Antibiotics may, therefore, inhibit some detrimental effects of infection without necessarily reducing the bacterial burden. Indeed, subinhibitory concentrations of antibiotics modulate the expression of global virulence loci in *S. aureus* (i.e., *SarA*, *Sae*, and *Agr*).[111] The resulting changes in the profile of secreted virulence factors may lead to a reduced impact on tissue degradation or cell cytotoxicity.[40,112,113] Alternatively, it is possible that antibiotics initially reduce the bacterial burden, allowing bone healing to be initiated before the infection reactivates.

While bacterial contamination impairs callus formation within the bone defect, excessive new bone formation is often seen more distant to the infection.[21,24,114] The dual effect of infection—that is, on the one hand, impaired bone healing and on the other hand new bone formation—is likely related to a different inflammatory response in the vicinity or more distant to the bacterial burden. The high density of osteoprogenitor cells found in the periosteum, and their responsiveness to inflammatory cues, can explain why reactive bone formation occurs predominantly in this tissue.

Only under mild inflammatory conditions may pro-inflammatory cytokines or bacteria-derived antigens promote osteoblast differentiation in resident bone-lining or recruited osteoprogenitor cells. In line with this hypothesis, it has been shown that a transient

inflammatory reaction to a low-dose of bacterial stimuli promotes periosteal or ectopic bone formation, but that the sustained inflammation caused by a high-dose of bacterial stimuli leads to predominantly osteolysis or impaired ectopic bone formation.[39,49,50,115]

Of final note, the current review shows that most of the in vivo bone infection studies have used rodent models. On the one hand, different forms of osteosynthesis can be applied in rodents,[91] with many research tools available for rodents to study the cellular and molecular aspects of bone healing. Whereas the stages of callus formation in rodents are comparable with humans, the speed of healing is species-dependent.[116] To illustrate, we found reports of normal regeneration of infected defects in selective rabbit models,[45,46] which could be due to the relatively high bone turnover in these particular species.[116,117]

The conclusions of the current review are to be interpreted in the context of the simplified models that have been used. Very few of the animal models have included all the multiple elements of traumatic bone infections in patients, that is, soft tissue damage, open fracture, or a delay in treatment. Moreover, since the current applied animal models leave the infected implant in place, they cannot support or challenge the general clinical consensus that bone healing is optimal when an infected fracture fixation system is retained,[28–30] even though the basic rule in prosthetic joint infections is to remove the implant to facilitate bacterial eradication.[1] As another limitation of the current bone infection models, they almost exclusively use *S. aureus* as the causative microorganism of infection, whereas in clinical practice, *S. aureus* is responsible for 30% of the fracture-related infections.[118] As bacterial species express unique molecules associated with osteoclastogenesis or osteoblastogenesis, the effect of bacterial infection on bone remodeling, regeneration, and new bone formation is likely very species-dependent.[51–53,119] Finally, inter-individual variations in the immune system are also not well-reflected in animal studies due to genetic similarities and housing conditions, even though immune status determines both resistance to bacterial challenge and bone healing capacity.[120,121]

AUTHORS' CONTRIBUTION

H.C.V. conceived the original idea. H.C.V. and M.C. made the research design. M.C. performed the literature research and wrote the manuscript in consultation with B.C.H. and H.C.V. All authors have read and approved the final submitted manuscript.

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