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REVIEW ARTICLE

The inflammatory response to bone infection – a review based on animal models and human patients

FREJA LEA LÜTHJE,[1,2] LOUISE KRUSE JENSEN,[1] HENRIK ELVANG JENSEN[1] and KERSTIN SKOVGAARD[2]

[1]Department of Veterinary and Animal Science, University of Copenhagen, Frederiksberg C; [2]Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark

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Bone infections are difficult to diagnose and treat, especially when a prosthetic joint replacement or implant is involved. Bone loss is a major complication of osteomyelitis, but the mechanism behind has mainly been investigated in cell cultures and has not been confirmed in human settings. Inflammation is important in initiating an appropriate immune response to invading pathogens. However, many of the signaling molecules used by the immune system can also modulate bone remodeling and contribute to bone resorption during osteomyelitis. Our current knowledge of the inflammatory response relies heavily on animal models as research based on human samples is scarce. *Staphylococcus aureus* is one of the most common causes of bone infections and is the pathogen of choice in animal models. The regulation of inflammatory genes during prosthetic joint infections and implant-associated osteomyelitis has only been studied in rodent models. It is important to consider the validity of an animal model when results are extrapolated to humans, and both bone composition and the immune system of pigs has been shown to be more similar to humans, than to rodents. Here *in vivo* studies on the inflammatory response to prosthetic joint infections and implant-associated osteomyelitis are reviewed.

Key words: osteomyelitis; inflammation; prosthetic joint infection; cytokines; immune response.

Freja Lea Lüthje, Ridebanevej 3, 1870 Frederiksberg C, Denmark. e-mail: frejal@sund.ku.dk

INTRODUCTION

Each year joint replacements increase the quality of life for millions of people. In Scandinavia, more than 150 000 knee arthroplasties were carried out from 1999 to 2007 with a continuous increase every year (1). In the US, the number of hip and knee arthroplasties is expected to rise from around 600 000 procedures in 2003 to more than 4 million in 2030 (2). The increasing number of joint replacements has resulted in an increase in prosthetic joint infections (PJI) (3). Along with joint prosthetics, other types of bone implants are also frequently used for fixation of bone fractures and infections of such implants are called fracture-related infections or implant-associated osteomyelitis (IAO). Bone infections like PJI and IAO are severe long-term conditions and recurrence of infection is common (4, 5). When a chronic implant-related bone infection is established, it is unlikely to be resolved by antibiotic treatment alone and, therefore, requires surgical intervention (6). Furthermore, careful debridement of necrotic bone is necessary to avoid recurrent infections, and two-stage exchange is recommended in PJI for the same reason (7). This entails surgical removal of the infected implant and necrotic bone, followed by 4–6 weeks of antibiotics, before a new implant can be inserted. Thus, the current treatment is a prolonged and painful affair. In addition, it can also be difficult to diagnose PJI as it can be hard to distinguish from aseptic implant failure (8). Bone infections are very complex diseases where both local bone cells and the infecting pathogen can modify the immune response. Therefore, it is highly relevant to study the local osseous immune response in animal models and osteomyelitis patients in order to develop better treatments and diagnostic tools. This review

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aims to describe how bone remodeling can be affected by the immune response raised toward bone infection and to summarize the regulation of inflammatory genes found *in vivo*. Animal studies have been limited to those involving PJI and IAO models, while all studies of human bone infections have been included.

NORMAL BONE HOMEOSTASIS

Bone is a dynamic tissue under constant remodeling to adapt to changes in mechanical loading and to repair microcracks. The remodeling consists of bone resorption carried out by osteoclasts and bone formation by osteoblasts. Osteoclasts are the primary cells to degrade mineralized bone and are thus essential in handling damaged bone. They belong to the hematopoietic lineage like macrophages. Fully differentiated osteoclasts are giant polykaryons. Osteoblasts belong to the mesenchymal lineage, and their primary function is to form bone, that is, osteoid (9). Terminally differentiated osteoblasts surrounded by mineralized osteoid become osteocytes, which are the most numerous cell type in bone tissue and are believed to play a pivotal role in sensing bone damage and initiating remodeling (10).

Osteoblasts also have an important role in regulating osteoclast activity. Osteoclast precursors require binding of receptor activator NF kappa B ligand (RANKL) to the receptor activator NF kappa B (RANK) to initiate the formation of multinuclear cells by cell fusions (11). RANKL is produced by osteoblasts, which can also synthesize the RANKL decoy receptor osteoprotegrin (OPG) that inhibits RANK-RANKL interactions (Fig. 1). Thus by expressing RANKL or OPG, osteoblasts can increase or decrease osteoclast activity, respectively, and the ratio between these molecules is therefore used as an indicator of bone erosion (12).

Osteoclast activity is also regulated by many other molecules (for an extensive review see Loi et al. (13)), and several inflammatory genes can affect osteoclastogenesis. To complicate matters further, RANK/RANKL is also involved in regulating the immune system (14).

Apart from regulating bone metabolism, osteoclasts and osteoblasts also interact with the immune system. In fact, the close interplay between the immune system and the skeletal system has led to a new interdisciplinary research field called osteoimmunology (15). Osteoclasts have been shown to produce interleukin (IL)-10, IL-6, transforming growth factor beta and tumor necrosis factor (TNF) in response to lipopolysaccharide and to function as antigen-presenting cells *in vitro* (16). In addition, osteoblast cultures have been reported to produce IL-6, IL-12, CXCL8, and CCL2 in response to *Staphylococcus aureus* and thus take part in immune activation (17–19). Osteoblasts have also been shown to activate T lymphocytes by expressing MHC class II molecules, which are normally only expressed by professional antigen-presenting cells (20). In addition, the antimicrobial peptides human beta-defensin-1, -2, and -3 can be produced by osteoblasts and are expressed in human bone (21–23). However, Chang et al. argues that it is actually resident tissue macrophages, called osteomacs, that respond to bacterial stimuli in osteoblast cultures (24).

OSTEOLYSIS

Bone loss is a major complication of osteomyelitis. Experiments in immune-deficient mice with periodontitis indicate that it is mainly the immune system and not the bacteria that is responsible for osteolysis in bacterial bone infections (25, 26). Thus, the bone loss can be considered collateral damage caused by excessive inflammation. *In vitro* studies suggest that bone infection leads to both increased osteoclast activity and decreased osteoblast activity. *Staphylococcus aureus* and *S. aureus* biofilm-conditioned media reduces osteoblast viability and inhibits bone formation. At the same time, the secretion of RANKL is upregulated, leading to increased bone resorption (27, 28) (Fig. 1). *Staphylococcus aureus* infected osteoblasts also upregulate cell surface receptors that can induce apoptosis (29) and downregulate the expression of OPG, which can lead to increased osteoclast activity. *S. aureus* also affect osteoclasts directly by promoting cellular fusion (30). Therefore, osteolysis in osteomyelitis has typically been ascribed to an increase in RANKL-induced osteoclastogenesis (28). However, the increase in RANKL expression and osteoclast activity has not been confirmed in human settings.

The only *in vivo* study of local RANKL expression during PJI revealed no difference in RANKL expression between patients with PJI and aseptic loosening of implants. A moderate upregulation of RANKL at the osteolytic cite compared with a distant site was also reported (31). In addition, there was no deference in the serum level of RANKL in patients with PJI and aseptic loosening (32).

The assumption that osteoclasts are solely responsible for the bone loss during osteomyelitis probably also builds on the false notion that osteoclast are the only cell type to degrade bone (33). However, monocytes, fibroblasts and neutrophils

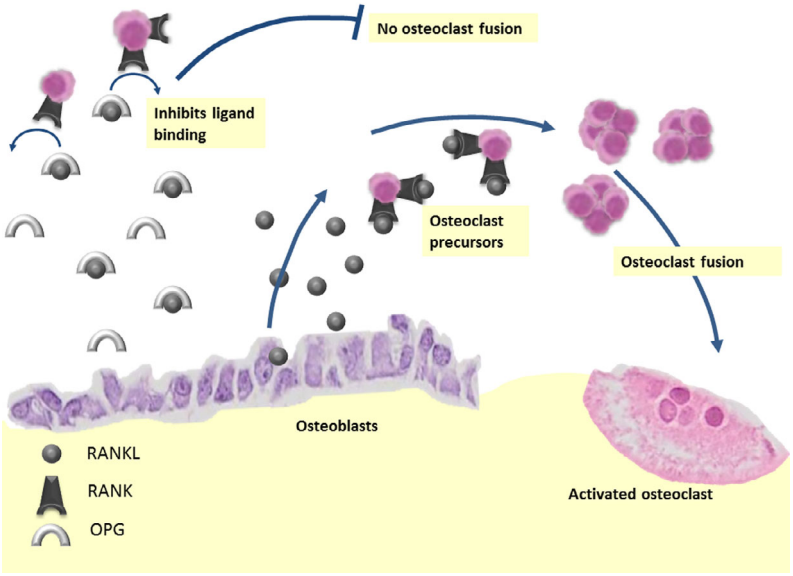

Fig. 1. Differentiation of mature osteoclast requires stimulation of RANK by RANKL. Osteoblasts produce both RANKL and the decoy receptor OPG, which inhibits the stimulation of RANK. Thus, osteoblasts have an important role in regulating the rate of osteoclastogenesis.

have all been shown able to degrade bone matrix (34–37).

STAPHYLOCOCCUS AUREUS

Staphylococcus aureus, one of the most common etiologies of bone infections (38–40), is the preferred pathogen in animal models of osteomyelitis (Table 1). The persistence of *S. aureus* infections is primarily attributed to the formation of biofilms. Biofilms consist of microorganisms organized in a matrix of extracellular polymeric substances. The matrix contains polysaccharides, proteins, lipids, and nucleic acids excreted by bacteria (41) but can also include compounds from the host (42). Both diagnosis and treatment of biofilm infections can be difficult and requires special clinical attention (43). Biofilm formation is a survival strategy that protects the bacteria from UV radiation, pH stress, chemical exposure, dehydration, and antibiotic treatment (41). The tolerance to antibiotics has been reported to increase 100- to 1000-fold in biofilms compared with the same planktonic bacterial strain (44). Biofilms also protect bacteria from phagocytosis by macrophages and neutrophils (45, 46).

It is also well established, that *S. aureus* can be taken up by osteoblasts *in vitro* and there are a few reports of *in vivo* internalization in human osteocytes (47, 48). It has been speculated that internalization is a virulence factor protecting the bacteria from the immune system and antibiotics (49, 50). However, the prevalence of *S. aureus* uptake by osteoblasts and osteocytes *in vivo* is unknown and, therefore, it is difficult to assess the importance in the recalcitrance of bone infections.

In addition, *S. aureus* have several strategies to evade both the innate and adaptive immune response (51, 52): For example, it can prevent complement activation (53), induce apoptosis in B cells by staphylococcal protein A (54) and in macrophages by degrading neutrophil extracellular traps (55).

INFLAMMATION

Inflammation is a fundamental defense response to injury and infection and is essential for pathogen defense and tissue healing. However, it is important that the inflammatory response is tightly regulated as immune dysregulation can cause tissue damage. As the host in many cases is unable to clear the bone infection, the infection turns chronic and subsequently the inflammatory processes leads to osteolysis (56). Clinically, there is no clearly defined line between acute and chronic osteomyelitis (57). Acute osteomyelitis describes recent bone infections causing systemic inflammation (58). In contrast, chronic osteomyelitis has a limited systemic response and is associated with avascular necrotic bone (7).

Table 1. The cytokine response in animal models of *Staphylococcus aureus*-induced implant-associated osteomyelitis or prosthetic joint infections

Model	*S. aureus* strain	Implant	Observation	References
BALB/c mice	UAMS-1	None	IL-6↑ in bone and the expression co-localize with osteoblasts	(112)
BALB/c mice	UAMS-1	None	CCL2 (called MCP-1)↑ in bone and the expression co-localize with osteoblasts	(113)
BALB/c mice	ATCC 29213	Titanium locking plate	IL-6↑in lavage fluid from soft tissue but IL-6 → in blood	(86)
C57BL/6 and BALB/c mice	MRSA-M2	Insect pin	Th2/Treg response protects against chronic *S. aureus* infections. IL-4↑ and IL-10↑ in infected BALB/c mice compared to C57BL/6 mice	(100)
C57BL/6 mice	MRSA-M2, UAMS-1	Insect pin	IL-2↑, IL-12 p70↑, TNF↑, IL-6↑ and IL-17↑ in bone	(71)
C57bl/6 mice	JAR06.01.31	Titanium locking plate	IL-17↑ in bone. TNF↑, IL-1β↑ and IL-4↓ in bone and soft tissue.	(73)
C57BL/6 mice	USA300 LAC::*lux*	Nickel-titanium wire	Myeloid-derived suppressor cells (MDSCs) suppress the immune response to *S. aureus* biofilm infections	(114)
C57BL/6 mice	USA300 LAC::*lux*	Nickel-titanium wire	IL-10 production by MDSCs contributes to the persistence of *S. aureus* biofilm infection	(99)
C57BL/6NCr mice	USA300 LAC::*lux*	Nickel-titanium wire	IL-12p40↑, IL-1β↑, TNF↑, CSF3 (called G-CSF)↑, CXCL2↑, and CCL5↑ in tissue around knee joint. Increased infiltration of MDSCs and reduced infiltration of monocytes, macrophages and T cells.	(78)
CD1 mice	ATCC 49230	Silk suture	Osteoclasts do not absorb damaged bone in infected animals but are active at the margins of the infected site, resulting in sequestra formation	(97)
ICR mice	E-31461	Silk suture	IL-1β↑, IL-4↑, IL-6↑, and TNF↑ in bone	(72)
Wistar rats	9213	Stainless steel needle	Antibiotic treatment affects the cytokine expression (IL-1α, IL-6, and IL-10).	(115)
Wistar rats	9213	Stainless steel needle	IL-2↑ and IL-10↑ in blood of young rats (three months) and IL-6 ↑ in blood of old rats (22 months). IL-10↑in tibia of young rats and IL-1β↑ in old rats	(111)
Mandorin rats	1098	Dental gypsum	TNF↑ in bone	(116)

→: no significant change, ↑: increased level, ↓: decreased level.

The host immune response to PJI and IAO is affected by multiple contributing factors. These include the bone damage from implant insertion, the implant *per se* and the pathogens. The implant insertion procedure causes osteonecrosis due to high temperatures (59) while the implant as an foreign body also elicits a molecular immune response (60). Following implantation, plasma proteins immediately coat the implant, the compliment and the coagulation systems are activated, immune cells are recruited and cytokines are released. All these action are part of the foreign body reaction (61).

Even though immune cells, especially neutrophils and macrophages (61, 62), migrate to the implant, the presence of a foreign body lowers the number of pathogens necessary to cause an infection (63, 64). This can be explained by reduced phagocyte function at the implant site. As the neutrophils are unable to phagocytose the implant they release pro-inflammatory cytokines and neutrophil extracellular traps, which can cause neutrophil apoptosis (62). Macrophages, upon adhesion to the implant, release reactive oxygen intermediates, degradative enzymes and acids in an attempt to degrade the foreign body. The macrophages are left unsuccessful and exhausted unable to produce more biocidal agents (61). In addition, the surface chemistry of the implant, especially hydrophilic and anionic surfaces, can induce apoptosis in macrophages (65). Moreover, oxygen availability is important for the neutrophilic microbicidal function (46) and the oxygen supply is limited during bone infections (66). The vascular channels in bone are compressed by inflammatory processes, such as pus formation, leading to restricted blood perfusion (67). At the same time, both neutrophils and the infecting microorganism can consume large amounts of oxygen resulting in hypoxia around biofilm infections (68).

Several pro-inflammatory and anti-inflammatory cytokines have been examined during bone

Table 2. The human immune response to osteomyelitis

Patient group	Control	Samples	Observation	References
Children with acute hematogenous OS caused by MRSA	Healthy age- and gender-match children	Blood	Genes related to neutrophil activity, coagulation, and inflammation↑ while genes linked to activity of T cells, NK-cells, and B cells↓.	(117)
Acute or chronic OS	Blood bank donors	Blood	IL-6↑, IL-1α↑, and TNF → in serum.	(82)
Chronic staphylococcal OS	Healthy volunteers	Blood	IL-6↓ in peripheral blood mononuclear cell supernatant and NO↓ in serum.	(118)
Chronic OS	Orthopedic patients with non-infectious or inflammatory disorders	Blood and pus from infected bone	IL-6↑, CXCL8 (called IL-8)↑, and TNF↑ in pus compared to plasma, but no systemic differences to controls	(119)
Patients with chronically infected mandibles	Patients undergoing plastic surgery	Bone	Human beta-defensin-1, -2, and 3↑ in bone	(21)
Acute and chronic posttraumatic OS	Patients with fresh fractures without infection	Blood and tissue from infected bone	CXCL8↑, IL-6↑, TNF↑, IL-1β↑, and LTB4↑ in acute OS samples compared to chronic bone samples. All measured inflammatory mediators↑ in the plasma of patients with acute OS compared to chronic and control group	(58)
IAO	Healthy donors (only blood)	lavage from infected implant and blood	Lavage contained 1–2 × 10^7 leukocytes, predominantly (75–90%) activated polymorphonuclear neutrophils	(120)
IAO	Healthy donors (only blood)	Lavage from infected implant and blood	CD8 + T cells ↑ in blood	(121)
PJI	Patients with aseptic loosening	Blood and tissue samples from synovia, tibia, intramedullary space and unaffected muscle	S100A9 (called MRP-14)↑ and CXCL8↑ in synovial and tibial tissue. S100A9↑ in blood	(92)
PJI	Patients with aseptic loosening	soft tissue from osteolytic, adjacent, and distal site, and a muscle sample.	CXCL8↑, IL-1β↑, and CXCL2↑ at the osteolytic site	(31)
PJI	Patients with aseptic loosening	Blood, tissue from site of osteolysis and unaffected muscle	CXCL2↑ and CCL3 → at osteolytic site	(122)
PJI	Aseptic loosening	Bone	CSF3↑, IL-1β↑, IL-6↑, CXCL8↑, and CD40L↑ in bone	(78)
PJI in acetabular bone	Sample from uninfected iliac wing of the same patients	Bone	CCL5↑, CXCL9↑, CXCL10↑, and CXCL11↑ in bone and viable *S. aureus* are present inside osteocytes	(47)
OS caused by *S. aureus*	Healthy volunteers	Blood	miR-24↓ in blood	(123)
Bacterial OS	Healthy relatives or patients with non-inflammatory disorders	Blood	IL-6→, TNF→, total leukocyte count↑, erythrocyte sedimentation rate↑, C-reactive protein↑, and plasma albumin↓ in blood	(124)
Bacterial OS	Healthy age-match individuals	Serum	IgA→, IgM→, and IgG → in serum	(125)

Table 2. *(continued)*

Patient group	Control	Samples	Observation	References
Unspecified OS	Healthy tissue from hip replacements	Bone	Human β-defensin-2 ↑ in bone	(22)
Unspecified OS	Healthy tissue from hip replacements	Bone	Human β-defensin-3 → in bone	(23)

→, no significant change; ↑, increased level; ↓, decreased level; IAO, implant-associated osteomyelitis; MRSA, Methicillin-resistant *Staphylococcus aureus*; OS, osteomyelitis; PJI, prosthetic joint infection.

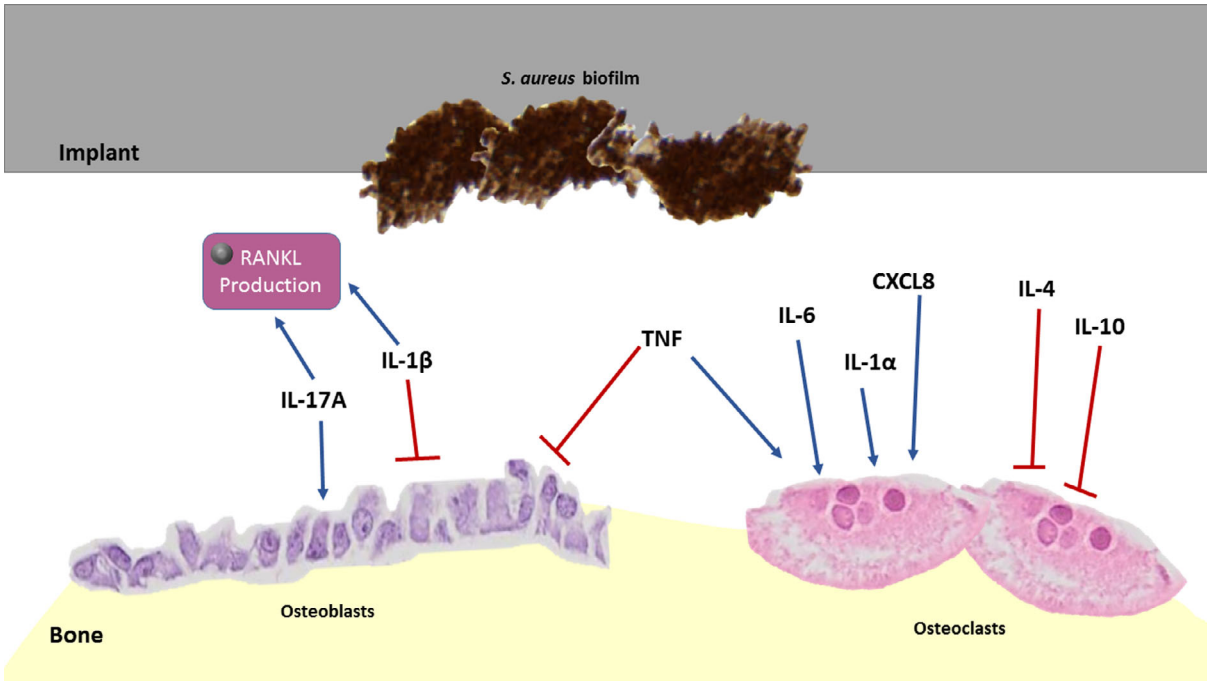

Fig. 2. Based on *in vivo* and clinical studies, the mentioned pro- and anti-inflammatory cytokines have been shown upregulated during bone infection. The effects of these cytokines on bone cells (osteoblasts and osteoclasts) are based on *in vitro* studies and arrows indicate a stimulatory effect while T-bars indicate inhibition.

infections in animal models and human patients (Tables 1 and 2). The results of these studies are reviewed in the following two sections. Apart from modulating the inflammatory response many of the cytokines have been shown to affect bone metabolism *in vitro* (69). The known *in vitro* effect on bone cells of the cytokines upregulated *in* vivo is summarized in Fig. 2.

PRO-INFLAMMATORY CYTOKINES IN OSTEOMYELITIS

TNF is a pro-inflammatory cytokine that together with IL-1 and IL-6 is essential for initiating an inflammatory response to trauma and infection (70). TNF is upregulated in the bone of patients with PJI compared to patients with aseptic loosening of implants (31) and in the bone of several murine IAO models (71–73). *In vitro* TNF stimulates osteoclastogenesis (74), inhibits osteoblast differentiation (75, 76), and induces IL-6 production in osteoblasts (77).

IL-1β is highly elevated in bones during IAO in mice (72, 73) and in humans with PJI (78). IL-1β-deficient mice with PJI showed increased bacterial burdens and fewer neutrophils in the infected joint compared with wild-type (WT) mice (79). Apart from attracting neutrophils and maintaining inflammation, IL-1β also affects bone remodeling. Like TNF, IL-1β inhibits osteoblast differentiation (76), while it stimulates the production of IL-6 and RANKL, leading to increased osteoclast activity (77, 80). In addition, IL-1α, which binds to the same receptors as IL-1β, can directly stimulate osteoclast development in cultures without

osteoblasts (81), and IL-1α is upregulated in the serum of osteomyelitis patients (82).

IL-6 has a multitude of both innate and adaptive immune functions and have both pro-inflammatory and anti-inflammatory properties (83). In an *ex vivo* study of fetal mouse calvaria, IL-6 was shown to increase bone resorption and osteoclast numbers (77). However, IL-6 have also been shown to inhibit osteoclastogenesis by skewing monocytic cells away from osteoclast differentiation and toward the macrophage linage (84). Two studies of IL-6-deficient mice with periapical infection showed opposing results, one found a greater bone loss in the IL-6-deficient mice than in WT mice (83), while the other failed to find any bone loss in the IL-6-deficient infected mice (85). Thus, the effect of IL-6 *in vivo* during bone infections is still unclear and awaits further studies. The expression of IL-6 is upregulated in several mouse models of IAO (71, 72, 86). Both human and murine osteoblasts increase the production of IL-6 in response to *S. aureus* (17, 18, 87). In patients with posttraumatic osteomyelitis, the expression of IL-6 was found to be higher both in serum and in the infected bones of acute cases compared with chronic cases (58). Asensi et al. (2004) also found a higher level of IL-6 in serum from osteomyelitis patients (etiology not specified) compared with healthy controls.

IL-17A is a pro-inflammatory cytokine involved in the immune response against bacterial infections (88), and it is highly upregulated in murine IAO models (71, 73). IL-17A induces the differentiation of osteoblasts and the production of RANKL both *in vitro* and *in vivo*, thus promoting both bone formation and degradation (89–91).

The chemokine CXCL8 (a.k.a. IL-8) is upregulated locally in PJI patients compared to patients with aseptic loosening of implants (31, 92). In addition, human osteoblast cell lines exposed to *S. aureus* upregulate the expression of CXCL8 (17, 93). CXCL8 is an important chemoattractant for neutrophils during infection and enhances osteoclastogenesis (94). Despite the central role of CXCL8 in the early immune defense of pig and human, this chemokine has not been identified in mice and is an example of differences in human and murine innate immune systems (95).

The chemokine CXCL2 is also upregulated in patients with PJI compared to patients with aseptic loosening (31). This upregulation has also been shown in a murine model of IAO (78). In addition, CCL5, colony stimulating factor 3 and IL-12 are all upregulated in murine IAO models (71, 78), and CCL5, CXCL9, CXCL10, and CXCL11 are upregulated in infected bone samples of patients with PJI compared with samples from uninfected bone (47).

ANTI-INFLAMMATORY CYTOKINES IN OSTEOMYELITIS

IL-4 inhibits osteoclast differentiation *in vitro* (96) and reduced the number of osteoclasts in an murine model (97). This anti-inflammatory cytokine was upregulated at day 14 in a murine model of IAO (72) and in the bone during aseptic bone healing (73).

IL-10, like IL-4, suppresses the development of osteoclasts *in vitro* (98). IL-10 was upregulated in a murine IAO model from day 5, and it was concluded that IL-10 enhanced the persistence of biofilm infection with *S. aureus* in C57BL/6 mice after 14 days (99). In contrast, a study comparing the immune response in C57BL/6 and BALB/c mice with IAO concluded that BALB/c mice benefited from a higher IL-4 and IL-10 expression after 49 days (100). Thus, the length of the experiment greatly affects the overall conclusion. In the comparison of Th2-biased BALB/c mice and Th1-biased C57BL/6 mice, Prabhakara *et al*. found no difference between the mouse strains at day 14, where the study by Heim *et al*. ended. However, at day 21, more than half of the BALB/c mice (with an upregulation of IL-4 and IL-10 compared with the C57BL/6 mice) had cleared the infection spontaneously. In addition, biofilm was only formed on the implants of C57BL/6 mice. As Heim *et al*. measured the bacterial burden via plate counts dormant cells which are common in biofilms was not considered. Since the study by Heim *et al*. only measured bacteria in a culturable state, the results are mainly applicable for acute infections with planktonic bacteria, while the study by Prabhakara *et al*. indicates that upregulation of IL-4 and IL-10 can prevent development of chronic biofilm infections in mice. Another possible explanation for the opposing conclusions is that they used different bacterial strains (Table 1) that could contain different virulence genes.

ANIMAL MODELS

As seen from Table 1, all *in vivo* studies of cytokine expression in IAO and PJI have been carried out in mice or rats, so apart from studies using samples from human patients (Table 2), all knowledge about cytokine production and pathways involved in bone infections relies on rodent models. Therefore, it is important to consider how the rodent bone physiology and immune system differ to humans, when assessing the validity of rodent models. Aerssens et al. (1998) compared the composition and density of human, dog, pig, cow, sheep,

chicken, and rat cortical femoral bone and found rat bone to resemble human bone the least (101). The immune system of mice also differs from the human counterpart in several aspects, for example, the human genes IL-26, CXCL8, and CXCR1 have no homolog in mice and neutrophils are the dominating leukocyte in peripheral blood in humans while lymphocytes dominate in mice (102, 103). In addition, the transcriptional response to inflammatory conditions in mice does not always mimics the human response well. In fact, no correlation was found between the genes regulated under inflammatory conditions in humans and the orthologue genes in relevant murine models (104). Furthermore, inbreed strains are preferred in rodent studies and there can be major differences in the development of disease between different mouse strains (100). This means that extrapolation of results from rodents to humans should be done with care. Unfortunately, human bone samples of especially healthy control tissue are very difficult to obtain (Table 2). Therefore, more comparable animal models should be considered.

The use of porcine models of infectious diseases are becoming increasingly popular (105), and pigs have several advantages compared to mice as models for osteomyelitis. Their size makes it possible to operate with the same tools as in humans and adequate quantities of tissue can be sampled, so pooling of samples is unnecessary. In addition, the bone anatomy and physiology of pigs are similar to humans (106) and the porcine immune system also resembles humans more than that of rodents (95, 107). The limitations of porcine models includes fewer available molecular tools, considerably less scientific literature regarding the porcine genome and immune system compared with rodents, and a relatively high cost – often resulting in small sample sizes (108).

In a recent study of a porcine IAO model, we saw an upregulation in gene expression of many of the same inflammatory markers – including TNF, IL-1β, IL-6, and IL-17A (Lüthje et al., unpublished data) – as are seen in rodent models and patients with osteomyelitis (Tables 1 and 2). Furthermore, a significant upregulation of IL-26, which has no homolog in rodents but is present in humans (103), was found.

Regardless of the animal model, new results of the pro- and anti-inflammatory orchestration of bone infection must be confirmed in human samples whenever possible. Currently, the descriptions of the immune response during human osteomyelitis are at best incomplete, making it difficult to predict good biomarkers and treatment targets. Furthermore, most animal model studies are done in relatively young animals while it is mainly elderly people that require prosthetic joint replacements. It is known that both bone metabolism, inflammation and the immune system are affected by age (109, 110). In a rat IAO model, the cytokine expression was very different in young and old rats (111); consequently, age is a factor that further complicates the translation of results from the laboratory to the clinic.

CONCLUSION

The immune response toward an infection is a complex process, and this is especially true in bone tissue where the immune activation and signaling also affect bone remodeling. Thus, a delicate balance is necessary to regulate inflammation in bone infections. An inflammatory response is necessary to control the invading pathogens and dampening the inflammation can increase the bacterial burden (99). However, a too strong response can damage the host tissue, creating devitalized bone for *S. aureus* to develop biofilm on (56). Clearing of the infection is also complicated by the fact that classical immune cells are poorly equipped to degrade dead and infected bone tissue. Therefore, regulation of bone degradation is very important at the site of infection and many pro-inflammatory cytokines also promote osteolysis (69). However, the effect of cytokines on bone metabolism is mostly based on *in vitro* studies, which rarely provide the complete picture. Only a few studies of the human immune response to osteomyelitis have been conducted and rarely with samples from bone (Table 2). This is because it is difficult to take bone samples, especially healthy control tissue, without harming the patients. Therefore, it is important that the chosen animal models mimic the human immune response as much as possible. Furthermore, researchers should be aware of known differences in the immune response between the chosen animal species and humans.

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