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Infection, Inflammation, and Bone Regeneration: a Paradoxical Relationship

ABSTRACT

Various strategies have been developed to promote bone regeneration in the craniofacial region. Most of these interventions utilize implantable materials or devices. Infections resulting from colonization of these implants may result in local tissue destruction in a manner analogous to periodontitis. This destruction is mediated *via* the expression of various inflammatory mediators and tissue-destructive enzymes. Given the well-documented association among microbial biofilms, inflammatory mediators, and tissue destruction, it seems reasonable to assume that inflammation may interfere with bone healing and regeneration. Paradoxically, recent evidence also suggests that the presence of certain pro-inflammatory mediators is actually required for bone healing. Bone injury (*e.g.*, subsequent to a fracture or surgical intervention) is followed by a choreographed cascade of events, some of which are dependent upon the presence of pro-inflammatory mediators. If inflammation resolves promptly, then proper bone healing may occur. However, if inflammation persists (which might occur in the presence of an infected implant or graft material), then the continued inflammatory response may result in suboptimal bone formation. Thus, the effect of a given mediator is dependent upon the temporal context in which it is expressed. Better understanding of this temporal sequence may be used to optimize regenerative outcomes.

KEY WORDS: infection, inflammation, regeneration, cytokines, bone.

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INTRODUCTION

Oral disease and trauma often result in tissue destruction. While it is desirable to regenerate lost tissue, the oral cavity is a challenging environment that is colonized by an impressive array of micro-organisms, many of which can colonize the implants often used in regenerative procedures. These implants include bone substitutes and grafts, metallic implants of various types, and guided tissue regeneration (GTR) barriers (Lynch, 2010). Infected implants pose significant problems for the patient and clinician (Darouiche, 2003).

These infections can be acute or indolent and chronic. Indolent infections may often result in local tissue destruction, as seen in periodontitis. It seems likely, then, that inflammation may result in suboptimal regenerative outcomes. This review examines the evidence regarding this assumption.

DOES INFECTION INTERFERE WITH BONE REGENERATION?

Under normal circumstances, inflammation is self-limited (Kumar *et al.*, 2005a). In the presence of a substrate (*e.g.*, an implant, graft, or tooth), microbial colonization may result in a biofilm that provides a sanctuary for resident flora and may prove hard to eliminate (Costerton *et al.*, 1999, 2005). Biofilms are involved in many human infections, including periodontitis and infections of medical implants (Kinane and Attström, 2005; Kornman, 2008). In this review, the term "implant" shall be used in the broadest sense, and will include any device or material implanted during a surgical procedure.

In periodontitis, bacteria produce a variety of products that elicit a host response consisting of the expression of various signaling molecules and mediators, and the recruitment of inflammatory cells (Nair *et al.*, 1996; Graves and Cochran, 2003). This process may culminate in tissue destruction and interfere with tissue regeneration and repair (see Fig.).

Elimination of the offending agent allows for resolution of the inflammatory response. If the microbial challenge cannot be eliminated, the inflammatory response will persist and become chronic, leading to tissue destruction, as in periodontitis (Offenbacher *et al.*, 2008). Given the association among infection, inflammation, and tissue destruction, it is not surprising that inflammation may interfere with the process of bone healing and regeneration (Newman, 1993; Garrett, 1996; Kumar *et al.*, 2005b). The evidence supporting this assumption is reviewed below.

Infection and Guided Tissue Regeneration

GTR is a periodontal regenerative technique which promotes selective repopulation of a periodontal defect by those cells most likely to result in tissue regeneration (Nyman *et al.*, 1982; Needleman *et al.*, 2006). This is accomplished through the use of barrier materials (*e.g.*, membranes) that are used to

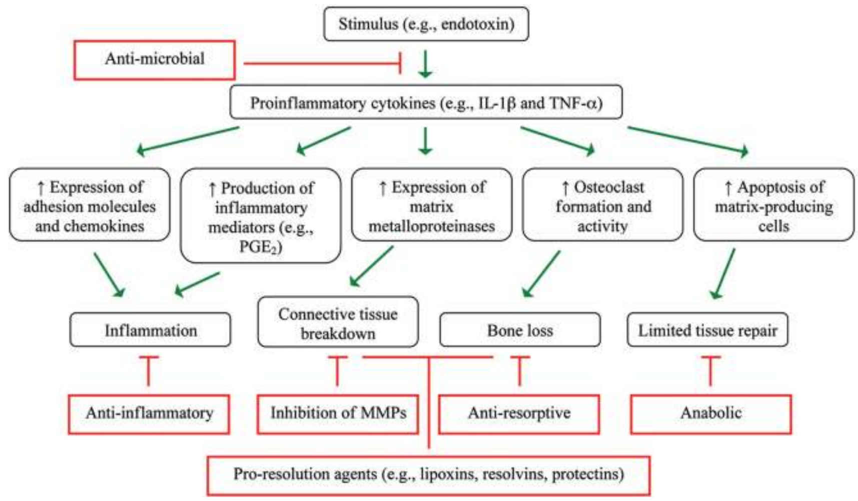

Figure.

exclude gingival epithelium from the root surface and provide physical space for the ingrowth of the desired tissues.

These barrier membranes can serve as substrates for biofilm formation. Premature membrane exposure is common in GTR procedures and results in microbial colonization of the membrane (Garrett, 1996). It is worthwhile to review the effects of such membrane exposure upon regenerative outcomes for better elucidation of the effects of indolent infections on bone healing. Non-resorbable membranes are rapidly colonized with periodontal pathogens following surgical placement (Sbordone *et al*., 2000). Many investigators have reported that such exposure is associated with poor regenerative outcomes, which may be clinically significant (Nowzari and Slots, 1994; Nowzari *et al*., 1995; Sander and Karring, 1995; Trombelli *et al*., 1995; Smith MacDonald *et al*., 1998; Yoshinari *et al*., 1998).

Membrane colonization may be a greater problem when multiple deep pockets are present during healing. Nowzari *et al*. reported that a group of patients who had all pockets surgically reduced to 5 mm or less had better outcomes than those with persistent deep pockets (Nowzari *et al*., 1996). The authors suggest that the persistent pockets served as bacterial reservoirs, thereby facilitating microbial colonization of the membranes.

Anti-infective Interventions and Regenerative Outcomes

The extent to which antimicrobial interventions improve regenerative outcomes provides additional evidence of the effect of infection upon tissue regeneration.

Antibiotics have been applied to barrier materials, which has often resulted in increased attachment gain (Pepelassi *et al*., 1991; DiBattista *et al*., 1995; Zarkesh *et al*., 1999; Zucchelli *et al*., 1999; Yoshinari *et al*., 2001). Systemic antibiotics have also been shown to improve regenerative outcomes (*i.e*., GTR) (Nowzari *et al*., 1995). Some antimicrobial agents affect connective tissue metabolism through mechanisms unrelated to their effects on bacteria, however (*e.g*., the effects of tetracyclines on some matrix metalloproteinases) (Ryan and Golub, 2000; Sorsa *et al*., 2006). As a result, data involving the use of some antimicrobial agents (*e.g*., tetracycline and its congeners) must be interpreted with caution.

In orthopedic surgery, antibiotics have been used to improve surgical outcomes. Various vehicles have been used to deliver antibiotics to surgical sites, and have exhibited favorable release kinetics (Mousset *et al*., 1995; Benoit *et al*., 1997). This group also reported that the release of vancomycin could be delayed by the coating of the calcium sulfate with a polymer. Moojen *et al*. found that tobramycin loading of a biomimetic HA coating on a titanium rod resulted in reduced infection and increased implant stability in a rabbit tibia model in which test sites were infected with *Staphylococcus aureus* prior to implantation (Moojen *et al*., 2009). Covalent attachment and controlled release of vancomycin from titanium rods have also been shown to reduce peri-prosthetic infection and related osteolysis (Antoci *et al*., 2007a,b,c, 2008; Edupuganti *et al*., 2007; Adams *et al*., 2009).

In summary, ample evidence exists to suggest an association between infection and suboptimal regenerative outcomes. Presumably, this effect is mediated *via* the tissue-destructive aspects of the inflammatory response.

MECHANISTIC CONSIDERATIONS

An understanding of the mechanisms by which inflammation causes tissue destruction is helpful in understanding the interface between inflammation and regeneration. The effects of inflammation upon bone healing are the result of the actions of various mediators and are reviewed below.

Arachidonic Acid Metabolites

Arachidonic acid (AA) metabolites have been associated with periodontal attachment loss, especially prostaglandin E_2, or PGE_2 (Offenbacher *et al*., 1984, 1990, 1993; Preshaw and Heasman, 2002; Kirkwood *et al*., 2006; Nisengard *et al*., 2006). Given the association of PGE_2 with periodontal tissue destruction and bone loss, it seems logical to suggest that the presence of PGE_2 would be inimical to bone formation. The evidence is compelling on the molecular/mechanistic level, since PGE_2 is a potent mediator of bone resorption. This is due, in part, to a positive effect on osteoclastogenesis by promotion of the expression of RANKL and the inhibition of osteoprotegerin (OPG) (Raisz, 1999; Horowitz *et al*., 2005). Non-steroidal anti-inflammatory drugs (NSAIDs), which interfere with PGE_2 synthesis, may slow the rate of periodontal destruction (Williams *et al*., 1985, 1989; Jeffcoat *et al*., 1988; Weber *et al*., 1994; Paquette *et al*., 1997; Salvi and Lang, 2005).

Thus, it seems that inhibitors of prostaglandin synthesis (*viz*., NSAIDs) would be likely to promote bone regeneration. As a result of this premise, new local delivery forms of NSAIDs have been developed to enhance periodontal and bone regeneration (Harten *et al*., 2005; Reynolds *et al*., 2007).

However, the literature supporting this assertion is ambiguous and contradictory. Although PGE_2 has been shown to stimulate bone resorption, prostaglandins have also been shown to inhibit osteoclast function (Fuller and Chambers, 1989; Chambers *et al*., 1999). Administration of cyclo-oxygenase (COX) inhibitors (*i.e*., NSAIDs) impairs fracture healing in a dose-dependent manner (Harder and An, 2003; Gerstenfeld *et al*., 2007; Vuolteenaho *et al*., 2008). This effect has been demonstrated for a variety of NSAIDs, including indomethacin (Allen *et al*., 1980; Keller *et al*., 1987; Dimar *et al*., 1996), ibuprofen (Lindholm and Tornkvist, 1981; Tornkvist *et al*., 1984; Obeid *et al*., 1992), ketorolac (Glassman *et al*., 1998; Martin *et al*., 1999), and celecoxib (Bergenstock *et al*., 2005; Leonelli *et al*., 2006; Simon and O'Connor, 2007). It may be worth noting that eicosanoids other than PGE_2 may affect bone metabolism, including prostacyclin (PGI_2) (Nakalekha *et al*., 2010) and leukotrienes (Cottrell and O'Connor, 2009; Wixted *et al*., 2009). However, relatively little literature exists on this topic.

Cytokines

Cytokines involved in the inflammatory response include (but are not limited to) IL-1, IL-6, IL-11, IL-18, and TNF-α (Havemose-Poulsen and Holmstrup, 1997; Horowitz *et al*., 2005; Graves, 2008). These cytokines are released in a "temporally and spatially controlled manner" (Gerstenfeld *et al*., 2003; Mountziaris and Mikos, 2008). Inflammatory cells are then recruited to the site, and angiogenesis occurs (Gerstenfeld *et al*., 2003; Rosenberg, 2005). Dependent upon the site, osteoprogenitor cells may also undergo differentiation and proliferation (Dimitriou *et al*., 2005).

The signals, such as TNF-α, IL-1, and IL-6, are critical for the inflammatory response that triggers osteogenesis. TNF-α, IL-1, and IL-6 are important in this process. IL-1 and TNF-α exhibit a biphasic response, with high levels expressed immediately following injury that become undetectable within 72 hours. At approximately 3 to 4 weeks post-injury, both IL-1 and TNF-α exhibit peaks which may correspond to early phases of the remodeling process (Kon *et al*., 2001).

Gerstenfeld *et al*. demonstrated that bone healing was delayed in TNF-α-receptor-deficient mice (Gerstenfeld *et al*., 2001). TNF-α regulates both osteoblasts and osteoclasts by means of the TNFR1 and TNFR2 cell-surface receptors, the latter of which is expressed only following injury and may be responsible for promoting bone formation (Kon *et al*., 2001; Balga *et al*., 2006). TNF-α-receptor-deficient mice exhibit decreased osteoclastogenesis in response to bacterial challenge, thus implicating TNF in this process (Graves *et al*., 2001). IL-1 and TNF-α play similar roles in these processes *via* different signaling pathways (Nanes and Pacifici, 2005). IL-1 (both α and β forms) binds to IL-1R/Toll-like receptors, which activate interleukin-receptor-associated kinase-1 (IRAK-1); IRAK-1 may activate NF-κB (Janssens and Beyaert, 2003).

NF-κB is well-established as essential for osteoclastogenesis (Boyce *et al*., 1999). Boyce *et al*. showed that mice deficient in functional NF-κB developed a condition akin to osteopetrosis due to the absence of osteoclasts. Recently, NF-κB has also been shown to affect bone formation through an effect on osteoblastic function (Chang *et al*., 2009). More specifically, Chang *et al*., reported that inhibition of the endogenous inhibitor of kappaB kinase (IKK)-NF-κB in a murine model significantly increased bone mass and bone mineral density. These authors suggest that NF-κB may be an attractive therapeutic target in the treatment of osteoporosis and various inflammatory bone disorders (*e.g*., periodontitis and arthritis). Such therapy might be particularly efficacious, since inhibition of NF-κB will not only suppress osteoclast-mediated bone resorption, but will also promote osteoblast function and bone formation.

TNF-α and IL-1 have also been shown to inhibit collagen synthesis (Harrison *et al*., 1998; Horowitz *et al*., 2005). IL-1 has been shown to repress promoter activity and collagen synthesis in a dose-related manner (Harrison *et al*., 1998). Interestingly, this effect was mitigated by the administration of indomethacin. TNF-α inhibits collagen synthesis *in vitro*, in addition to its previously mentioned effects on bone resorption (Bertolini *et al*., 1986).

IL-6 regulates osteoblast and osteoclast differentiation, influences the expression of vascular endothelial growth factor, and promotes callus mineralization and maturation (Horowitz *et al*., 2005; Yang *et al*., 2007). Mice deficient in IL-6 exhibit less bone loss on challenge from *P.g*. than do wild-type mice (Baker *et al*., 1999). IL-6 has been shown to overcome inhibition of GM-CSF inhibition of osteoclast differentiation *in vitro*, as does TNF-α (Gorny *et al*., 2004). However, mice deficient in gp-130 activator protein showed increased numbers of osteoclasts (Kawasaki *et al*., 1997). Because IL-6 shares this protein in the receptor complex (Manolagas *et al*., 1995), other members of this family may be responsible for the effects observed by Kawasaki *et al*. Blanchard *et al*. have noted that the actions of IL-6 on bone are like a "double-edged sword" in that this cytokine can promote either bone formation or resorption, depending on the context (Blanchard *et al*., 2009).

Nitric Oxide

Nitric oxide (NO) is another inflammatory mediator that has been shown to have a paradoxical relationship with bone. For example, although excessive production of NO may be associated with bone loss in some inflammatory conditions, NO also mediates some of the beneficial effects of estrogen on bone *via* the NO/cyclic guanosine monophosphate (cGMP) pathway (Wimalawansa, 2008, 2010). Knockout mice deficient in endothelial nitric oxide synthase (eNOS) exhibit osteoporosis as a result of a defect in bone formation (van't Hof and Ralston, 2001). Similarly, mice that are deficient in inducible NOS (iNOS) exhibit altered bone healing (Saura *et al*., 2010). Increased bone mineral density has been reported in mice deficient in all three isoforms of NOS (Sabanai *et al*., 2008). The transcription factor Cbfa-1 and the mitogen-activated protein kinase (MAPK) pathway are crucial for osteoblastic cell differentiation, and NO plays a significant role in this process (Zaragoza *et al*., 2006). NO inhibition delays remodeling of an autogenous bone graft (Diwan *et al*., 2010) and also modulates bone loss subsequent to apical periodontitis infection (Fukada *et al*., 2008).

NO is implicated in inflammation-related bone loss. Mice lacking iNOS exhibited no maxillary bone loss on challenge with *Porphyromonas gingivalis*, while the wild-type mice did (Gyurko *et al*., 2005). *In vitro*, iNOS-deficient cells developed 51% fewer osteoclast-like cells than did the wild-type. The authors concluded that iNOS promotes bone resorption during bone development as well as after bacterial infection, and that it is involved in osteoclast differentiation. Other workers have shown that iNOS is also involved in the pathogenesis of inflammation-mediated osteoporosis (Armour *et al*., 2001). Thus, NO also has a paradoxical association with bone metabolism.

Growth Factors and Morphogens

Chen *et al*. investigated the effects of recombinant human osteogenic protein-1 (rhOP-1) and bone morphogenetic protein 2 (rhBMP-2) on osteogenesis in a chronically infected site (Chen *et al*., 2006, 2007). Both proteins maintained their osteoinductivity in the presence of infection, although this property was enhanced by systemic antibiotic therapy, thus suggesting that infection interfered with bone formation. In the infected sites, no substantial callus formation was observed in the absence of either rhOp-1 or rhBMP-2. Infected femoral defects exhibit reduced expression of collagen I and II and osteocalcin mRNAs, as well as BMP receptor II (Brick *et al*., 2009). Aseptic inflammation negatively affects the osteoinductivity of BMP-2, which can be mitigated by the utilization of a composite graft composed of BMP-2 and collagen (Ji *et al*., 2010).

Pro-inflammatory Disease States and Bone Healing

Several non-infectious diseases are associated with derangements of bone metabolism. Diabetes has been shown to increase the risk of fracture and is also associated with impaired fracture healing (Schwartz, 2003). Several of the effects of diabetes are due to the presence of advanced glycation end-products (AGEs). AGEs result from the non-enzymatic reaction between glucose-derived precursors and intra- and extracellular proteins. AGEs are capable of binding to a specific receptor (RAGE). RAGE is expressed on various inflammatory cells, and the AGE-RAGE interaction results in the release of pro-inflammatory cytokines, some of which are involved in bone resorption. The potential significance of the AGE-RAGE interaction in the pathogenesis of periodontal bone destruction is underscored by the finding that blockade of RAGE decreased bone loss in *P.g.*-infected diabetic mice (Lalla *et al*., 2000). Additionally, AGEs in bone have been shown to increase osteoclast-mediated bone resorption (Miyata *et al*., 1997), inhibit markers of osteoblast activity (Katayama *et al*., 1996), and stimulate IL-6 production in bone cells (Takagi *et al*., 1997).

Rheumatoid arthritis (RA) is a chronic inflammatory disorder which may affect many organ systems, but its orthopedic manifestations chiefly occur because of its effect on the synovial linings of various joints. RA causes destruction of articular cartilage and bone (an effect that is likely due to an imbalance between pro- and anti-inflammatory cytokines) (McInnes and Schett, 2007). NF-κB is also believed to play a critical role. T-helper cells, especially the Th17 subset, are believed to be critical in the pathogenesis of RA (Koenders *et al*., 2006). Th17 cells produce IL-17, which is a potent inducer of other cytokines (*e.g.*, IL-1 and TNF-α). IL-17 activates NF-κB, which induces the expression of numerous cytokines and chemokines (Brown *et al*., 2008). Th17 cells and their hallmark cytokine, IL-17, are likely to prove of seminal importance in understanding the pathogenesis of many inflammatory disorders (reviewed by Weaver *et al*., 2007; Brown *et al*., 2008; Gaffen, 2008; Garrett-Sinha *et al*., 2008). It has recently been suggested that this newly described subset of cells and their associated cytokine, IL-17, may also be of interest in describing the pathogenesis of periodontal diseases (Gaffen and Hajishengallis, 2008).

In conclusion, various inflammatory mediators have an ambiguous and somewhat paradoxical relationship with bone formation and healing (see Table).

RESOLUTION OF THE PARADOX

Inflammation and bone resorption are normal antecedents to bone healing. The requirement for bone resorption during bone healing can be inferred from the observation that delayed healing is observed in a setting of impaired osteoclast function or number. Such conditions include osteopetrosis and bisphosphonate-related osteonecrosis of the jaws (BRONJ) (Landa *et al*., 2007; Del Fattore *et al*., 2008; Filleul *et al*., 2010). Thus, pro-inflammatory cytokines may be necessary for bone repair and regeneration. In particular, IL-1, IL-6, TNF-α, and various eicosanoids (especially PGE_2) seem to be required for optimal bone formation. Given the apparent requirement for the presence of pro-inflammatory mediators, why is infection-associated inflammation associated with bone loss and impaired regeneration?

The answer to this paradox can likely be found in the carefully orchestrated sequence of events that occurs during bone healing. A temporal "window" exists immediately subsequent to the tissue insult (*e.g.*, regenerative surgical intervention or a fracture). At this stage, several pro-inflammatory mediators initiate the repair cascade. If these requisite mediators are absent, then bone formation may be impaired (Gerstenfeld *et al*., 2001). Inhibition of cyclo-oxygenase, leading to decreased PGE_2, may explain the impaired bone healing often noted when NSAIDs are given in the early healing phase following various types of orthopedic surgical interventions (Harder and An, 2003; Vuolteenaho *et al*., 2008). The effect is reversible, however, and normal strength is eventually attained when the use of COX inhibitors is discontinued (Gerstenfeld *et al*., 2007).

In a non-infected surgical site, the initial inflammatory reaction quickly resolves, after which the reparative phase predominates. The resolution of inflammation is not a passive process, but rather it is dependent upon specific chemical mediators, including lipoxins, resolvins, and protectins (Serhan, 2007, 2009). These "pro-resolution" molecules, like the pro-inflammatory eicosanoids, are derivatives of AA. During inflammation, activation of 15-lipoxygenase leads to "class switching" of AA metabolism and subsequent synthesis of these pro-resolution agents. However, in an infected site, the inflammatory response may persist and become chronic. This occurs in periodontal diseases. In the case of regenerative sites, the

Table.

Mediator	Pro-regenerative Effect	Pro-resorptive Effect	Mechanism of Action
IL-1	Initiates repair cascade (Kon *et al.*, 2001)	Induces synthesis of IL-6, GMCSF, and MCS	IL-1 (both α and β) binds to IL-1R/Toll-like receptor family, which activates interleukin receptor-associated kinase-1 (IRAK-1); IRAK-1 may activate NF-κB (Janssens and Beyaert, 2003)
	Promotes collagen synthesis and cross-linking (Kon *et al.*, 2001)	Inhibits collagen synthesis (Horowitz *et al.*, 2005)	Enhances synthesis of prostaglandins
	Stimulates angiogenesis (Kon *et al.*, 2001)		
IL-6	Promotes callus mineralization and maturation (Yang *et al.*, 2007)	Promotes bone resorption (Blanchard *et al.*, 2009)	Gp-130 activator protein
	Mice lacking gp-130 activator protein have increased osteoclasts; however, this receptor is shared by all members of IL-6 family, so this complicates interpretation of this finding (Kawasaki *et al.*, 1997)	Has been shown to cause increase in osteoclastogenesis (Gorny *et al.*, 2004). Regulates differentiation of progenitor cells into osteoclasts (Horowitz *et al.*, 2005)	
	Promotes bone healing (Blanchard *et al.*, 2009)	IL-6-deficient mice showed less bone loss secondary to *P.g.* challenge than did wild-type mice (Baker *et al.*, 1999)	
TNF-α	Initiates repair cascade (Kon *et al.*, 2001)	Bone formation inhibited *via* inhibition of osteoblast differentiation, suppression of osteoblast function, and induction of osteoblast resistance to 1,25-(OH)D$_3$ (Nanes and Pacifici, 2005)	TNF-α regulates both osteoblasts and osteoclasts *via* the TNFR1 and TNFR2 cell-surface receptors
	Bone healing is delayed in TNF-α receptor-deficient mice (Gerstenfeld *et al.*, 2001)	Enhanced osteoclastogenesis and increased protease production by osteoclasts (Graves *et al.*, 2001; Nanes and Pacifici, 2005)	Enhances synthesis of prostaglandins
PGE_2	May be needed for normal bone repair (inferred from effect of NSAIDs on fracture healing)	Potent stimulator of bone resorption and osteoclastogenesis (Raisz, 1999; Horowitz *et al.*, 2005)	Bone resorption may be mediated by c-AMP-dependent mechanism *via* EP4 receptor (Miyaura *et al.*, 2000)
	Inhibit osteoclast function (Chambers *et al.*, 1999)		
Nitric oxide	Knockout mice deficient in endothelial nitric oxide synthase (eNOS) exhibit osteoporosis as a result of a defect in bone formation (van't Hof and Ralston, 2001)	Increased bone mineral density has been reported in mice deficient in all three isoforms of NOS (Sabanai *et al.*, 2008)	Exact mechanisms unknown, but increased bone resorption in iNOS(-/-) mice is correlated with increased expression of receptor activator NF-κB (RANK), stromal-cell-derived factor-1 alpha (SDF-1 alpha/CXCL12), and reduced expression of osteoprotegerin (OPG) (Fukada *et al.*, 2008)
	Mice deficient in inducible NOS (iNOS) have altered osteogenesis and bone healing (Saura *et al.*, 2010)	iNOS-deficient mice did not have bone loss on challenge with *P.g.*, while wild-type mice did (Gyurko *et al.*, 2005)	
	NO inhibition delays remodeling of autogenous bone grafts (Diwan *et al.*, 2010)	NO involved with inflammation-associated osteoporosis (Armour *et al.*, 2001)	
	NO-deficient mice have greater osteolysis and inflammatory cell recruitment than do wild-type mice (Fukada *et al.*, 2008)		

persistent presence of indolent infection and chronic inflammation has a deleterious effect on regeneration.

These concepts provide the resolution to the seemingly paradoxical relationship among infection, inflammation, and bone regeneration. Inflammation is needed early in the regenerative process to initiate the repair cascade. If healing occurs normally, then the inflammatory response is resolved promptly and tissue regeneration can occur. If, however, the site becomes infected and the inflammatory response persists and becomes chronic, then an adverse effect on bone formation will likely be observed. The likelihood of chronic infection may be enhanced when materials or devices are implanted into the surgical site, since these provide a substrate for potential microbial colonization. The role of various inflammatory mediators is context-specific with regard to the temporal sequence of the injury-repair continuum.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Interventions which interfere with microbial colonization of the surgical site are likely to promote regeneration. Some of these are simple, such as proper aseptic technique during surgery. Some, such as the use of prophylactic antibiotic coverage to enhance bone healing by suppressing indolent infections, should be further investigated. Another concept lies in the idea of submergence of certain regenerative materials (*e.g.*, non-resorbable membranes), although this does not appear necessary in the case of certain titanium implants (Buser *et al*., 1997, 1999; Brocard *et al*., 2000).

One simple expedient may be the aggressive treatment of oral foci of infection prior to undertaking any therapy that involves the implantation of any device or material (Nowzari *et al*., 1996). This is similar to the periodontal concept of "full-mouth disinfection," in which the entire mouth is treated in a short period of time, to prevent "re-infection" of treated pockets by flora from untreated pockets (Bollen *et al*., 1998; Koshy *et al*., 2004; Apatzidou, 2006; Quirynen *et al*., 2006).

Certain non-antimicrobial molecular agents may mitigate some of the effects of infection. As noted previously, infection can inhibit expression of collagen I and II and osteocalcin mRNAs, as well as BMP receptor II expression (Brick *et al*., 2009). However, all four genes were up-regulated in infected defects in the presence of rhBMP-2. Delivery of substantial doses of rhBMP-2 and rhOP-1 has also been shown to mitigate the effect of infection, although this effect was more pronounced when an antibiotic was used (Chen *et al*., 2006). Although not used in a bone site, it has been shown that catheter-related staphylococcal infections could be reduced by coating the substrate with basic fibroblast growth factor (Hirose *et al*., 2007).

Resolution of the inflammatory process appears to be an active process, modulated by various mediators such as lipoxins, resolvins, and protectins, which serve as "stop signals" (Serhan, 2009). Administration of such agents may permit the therapeutic modulation of the inflammatory process. *In vitro* studies have shown beneficial effects using lipoxins and resolvins to treat peritonitis and infection with *T. gondii* and *A. costaricensis* (Bandeira-Melo *et al*., 2000; Aliberti *et al*., 2002a,b; Spite *et al*., 2009). Of particular relevance to dental applications, resolvin E1, a "proresolution" molecule derived from omega-3 fatty acids, has been shown to provide protection from periodontitis (Hasturk *et al*., 2006).

One problematic area concerns post-operative control of pain and inflammation. NSAIDs are widely prescribed by many surgeons following regenerative interventions in the head and neck. For example, the use of NSAIDs has been part of the post-operative protocol in the University of Kentucky Periodontology Clinic for over a decade, and those outcomes that have been tracked indicate a high level of success (*i.e.*, endosseous implants; unpublished observations). This observation is consistent with a recently reported double-blind randomized clinical trial showing that systemic ibuprofen did not have a significant effect on the marginal bone around dental implants in the early healing period (Alissa *et al*., 2009).

Reconciliation of these positive outcomes with the negative association reported between NSAID use and bone healing in the orthopedic literature is difficult, although the limited duration of such therapy following most dento-alveolar interventions may mitigate the negative effect of NSAIDs on bone healing. Several considerations exist worth noting. First, much of the literature on the deleterious effects of NSAIDs is derived from long-bone fracture models. Second, the healing process may differ somewhat when one is considering the healing of surgical wounds in the membranous bones of the craniofacial region. It is obvious that additional work is needed in this area. Pending more definitive information, however, clinicians may wish to limit the duration and dosage of NSAIDs following surgical implantation in the oral cavity.

SUMMARY

The study of the relationship between inflammatory mediators and bone has been aptly termed "osteoimmunology" (Graves, 2008). The relationship between the host response and bone biology is complex. Inflammation, repair, and regeneration are carefully choreographed processes which occur in a specific temporal and physical context. These processes are modified on an *ad hoc* basis, as dictated by circumstances such as infection. A better understanding of these associations will allow for the identification of new therapeutic targets and the development of novel interventions to promote bone regeneration.

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