Neutrophil-derived MRP-14 is up-regulated in infectious osteomyelitis and stimulates osteoclast generation

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ABSTRACT
Bone infections of patients with joint replacement by endoprostheses (so called “periprosthetic joint infection”) pose a severe problem in the field of orthopedic surgery. The diagnosis is often difficult, and treatment is, in most cases, complicated and prolonged. Patients often require an implant exchange surgery, as the persistent infection and the accompanying inflammation lead to tissue damage with bone degradation and consequently, to a loosening of the implant. To gain insight into the local inflammatory process, expression of the proinflammatory cytokine MRP-14, a major content of neutrophils, and its link to subsequent bone degradation was evaluated. We found MRP-14 prominently expressed in the affected tissue of patients with implant-associated infection, in close association with the chemokine CXCL8 and a dense infiltrate of neutrophils and macrophages. In addition, the number of MRP-14-positive cells correlated with the presence of bone-resorbing osteoclasts. MRP-14 plasma concentrations were significantly higher in patients with implant-associated infection compared with patients with sterile inflammation or healthy individuals, advocating MRP-14 as a novel diagnostic marker. A further biologic activity of MRP-14 was detected: rMRP-14 directly induced the differentiation of monocytes to osteoclast cells, thus linking the inflammatory response in implant infections with osteoclast generation, bone degradation, and implant loosening. J. Leukoc. Biol. 98: 575-582; 2015.

Introduction
MRP-14 (also known as S100A9 or calgranulin B), was described originally as a chain of a heterodimeric, abundant cytosolic protein of neutrophils or monocytes [1]. As a Ca\(^{2+}\) ion-binding protein, MRP-14 and its dimeric partner MRP-8 participate in numerous intracellular regulatory processes, but they are also released from cells, particularly in the context of infection and inflammation (reviewed in ref. [2]). Antimicrobial activities have been attributed to the complex of MRP-14/MRP-8 [3, 4], and a variety of biologic activities affecting the innate host response has been described. These include chemotaxis for neutrophils, increased cellular adherence through activation of the adhesion molecule CD11b [5, 6], growth factor functions [7], or enhanced phagocytosis [8]. MRP-14 is found in the peripheral blood of patients with inflammatory diseases, such as rheumatoid arthritis or inflammatory bowel disease (reviewed in ref. [9]). In line with the observation that it is released in response to cellular stimuli, MRP-14 is now regarded as an endogenous danger signal or damage-associated molecular pattern [10].

In the present study, we addressed expression, regulation, and functional aspects of MRP-14 in the context of implant-associated osteomyelitis, a bacterial infection of the bone, occurring in ~2% of patients receiving a total joint replacement and in up to 5% of patients requiring osteosynthesis after bone fractures. The “common cause” of implant-associated infection is the formation of bacterial biofilms on the endoprosthesis or the osteosynthesis material (plates, nails, screws) [11, 12], which display relative resistance toward many antibiotics, thus limiting the therapeutic options [13, 14]. Persistent infection elicits a progressive and destructive inflammatory reaction, which eventually results in bone degradation (reviewed in ref. [15]) and requires the removal of the implant. This procedure allows an access to the infected site and an analysis of the local tissue response. We found massive infiltration of activated neutrophils and to a lesser extent, of monocytes and T cells [16-18] and evidence for local synthesis of proinflammatory cytokines [19-21]. Moreover, we found that the proinflammatory environment promotes the generation of bone-resorbing osteoclasts and hence, the degradation of the bone [19, 20]. Although the local host defense mechanisms are activated, they apparently fail to eliminate the bacterial biofilm in some of the patients (reviewed in ref. [22]).

We now found local gene and protein expression of MRP-14 in osteolytic tissue of patients with implant-associated...
osteomyelitis, as well as in the peripheral blood of these patients. In vitro experiments revealed that MRP-14 is released from neutrophils in response to bacterial challenges. Moreover, we show that MRP-14 induces the differentiation of precursor cells to osteoclasts, thus linking the local host response to bone degradation.

**MATERIALS AND METHODS**

**Patients**

Patients who underwent revision surgery as a result of an implant infection and patients suffering from noninfectious (“aseptic”) loosening of a total joint replacement were included in the study. Diagnosis of loosening was based on patients’ complaints, clinical examination, and examination by conventional X-ray and/or computed tomography scan. Bacterial growth was assessed by conventional methods, and leukocyte count and CRP serum concentrations were determined by standard clinical laboratory methods. Informed consent was obtained from the patients, and the study was approved by the local ethic committee.

**Collection of tissue and blood samples**

From 6 patients with infected knee prostheses and 6 patients with aseptic loosening of the prostheses, tissue samples were taken from the synovia and tibia and after removal of the implant, from the intramedullary space. For comparison, tissue from macroscopically unaffected muscle was taken. The tibia and after removal of the implant, from the synovia and tissue samples were taken before surgery from patients with implant-associated osteomyelitis (n = 38), from patients with aseptic loosening (n = 28), and from healthy volunteers (n = 10) in heparinized tubes. An aliquot was used for gene expression analysis and another to collect plasma for ELISA

**Immunohistochemistry**

Human tissue biopsies from patients with infectious (n = 4) and aseptic (n = 5) prosthetic loosening were collected and embedded in paraffin. Then, standard H&E staining, as well as immunohistochemistry, was performed. The following primary antibodies were used: anti-CD68 (M0876, 1:50, retrieval condition: pH 6.0; Dako, Glostrup, Denmark), anti-CD14 (IS062, 1:20, retrieval condition: pH 6.0; Dako), antiactin K (sc-83353, 1:50, retrieval condition: pH 9.0; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-MRP-14 (LS-C10781, 1:100, retrieval condition: pH 9.0, LifeSpan Biosciences, Seattle, WA, USA). As secondary antibody, the Histofine Simple Stain universal polymer was used (Nichirei, Tokyo, Japan), followed by the color reaction with liquid permanent red (Zytomed, Berlin, Germany) and counterstain with hematoxylin. The number of positive cells was quantified by counting 5 representative HPFs.

**Quantitative real-time PCR**

mRNA was isolated from cells with the MagNA Pure LC device by use of the mRNA standard protocol for cells. Tissue samples were disrupted with Thermo Hybusaid Ribolyser devices (Thermo Scientific, Heidelberg, Germany), and mRNA was isolated with the MagNA Pure LC device by use of the mRNA standard protocol for cells. mRNA was reversely transcribed by use of avian myeloblastosis-RT and oligo(dt) as primer (First Strand cDNA synthesis kit; Roche Diagnostics, Minneapolis, MN, USA), according to the manufacturer’s protocol. Primer sets optimized for the LightCycler (Roche Applied Science, Mannheim, Germany) were purchased from Search-LC GmbH (Heidelberg, Germany). The PCR was performed with the LightCycler FastStart DNA SYBR Green I kit (Roche Applied Science), according to the protocol provided. The copy number was calculated from a standard curve, obtained by plotting known input concentrations of 4 different plasmids at log dilutions to the PCR cycle number, at which the detected fluorescence intensity reaches a fixed value. To correct for differences in the mRNA content, the transcript numbers were normalized, according to expression of the housekeeping gene PPIB. Values were given as transcripts/1000 transcripts of PPIB.

**Bacteria**

Staphylococcus aureus (Seattle 1945, ATCC 25922; American Type Culture Collection, Wesel, Germany) and Staphylococcus epidermidis (RP62a, ATCC 35984; American Type Culture Collection) were grown overnight on blood agar plates at 37°C (BP5039A; Thermo Scientific, Wesel, Germany). For opsonization, bacteria were incubated with normal human serum (10% v/v).

To generate biofilms, 6 × 106 bacteria were suspended in 1 ml tryptic soy broth (Becton Dickinson, Heidelberg, Germany) and placed into 24-well culture plates for 48 h. Formation of biofilms was controlled by staining with MIRA-2-TON (Hager & Werken, Duisburg, Germany).

**Isolation of monocytes and PMN**

Blood was layered on Polymorphprep (Axis Shield, Oslo, Norway), and the fractions containing PMN or mononuclear cells, respectively, were harvested. For further purification, anti-CD15 microbeads were used to select positively PMN, anti-CD14 beads for monocytes (Miltenyi Biotec, Bergisch Gladbach, Germany). The procedure yielded ~99.0% pure cells, as assessed by flow cytometry.

**Generation of osteoclasts**

Mononuclear cells were seeded into 24-well dishes (Nunc, Wiesbaden, Germany) at a concentration of 5 × 106 cells/ml in RPMI containing 10% FCS, 1% glutamine, and 1% penicillin/streptomycin (medium and supplements were obtained from Gibco Life Technologies, Darmstadt, Germany). After 24 h, nonadherent cells were removed, and the medium was replaced by RPMI plus supplements plus rhM-CSF (25 ng/ml; R&D Systems, Minneapolis, MN, USA). For stimulation, either rhRANKL (50 ng/ml; PeproTech, Rocky Hill, NJ, USA) or rhMRP-14 (5 μg/ml; Hölzel Diagnostika, Cologne, Germany) was added. To confirm that rhMRP-14 was endotoxin free, we performed a limulus-amebocyte assay (Pierce LAL Chromogenic Endotoxin Quantitation Kit; Thermo Scientific, Rockford, IL, USA) that showed no LPS contamination. After 12–16 h, cells were fixed and stained with phalloidin-FITC (Sigma, Taufkirchen, Germany) and DAPI (Invitrogen, Carlsbad, CA, USA). Cells with an actin ring and more than 2 nuclei were counted as osteoclasts. In addition, cells were stained with anticalcethesin K (1:50, overnight; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a secondary anti-mouse Cy3 (1:1; DianoVA, Hamburg, Germany). Functionality of osteoclasts was tested by differentiating monocytes on ivory slices for 18 days, followed by toluidine blue staining and quantification of the resorption pits (ivory was obtained from the Bundesamt für Umweltschutz, Bonn, Germany, and was cut in 100 × 100 mm slices, ~500 μ thick).

**Western blot NFATc1**

Mononuclear cells were stimulated as described above for 48 h. Cells were lysed by use of radioluminoprecipitation assay buffer (Santa Cruz Bio-technology, Santa Cruz, CA, USA), separated by SDS-polyacrylamide gel (9%), and blotted to a nitrocellulose membrane (Whatman, Dassel, Germany). NFATc1 was detected by anti-NFATc1 (Santa Cruz Biotechnology) by use of peroxidase-labeled anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) as secondary antibody. Antibody binding was visualized by Amersham ECL plus Western Blotting Detection System (GE Healthcare, Munich, Germany). As loading control rabbit anti-β-actin (Abcam, Cambridge, MA, USA) was used, followed by peroxidase-labeled anti-rabbit IgG (Jackson ImmunoResearch Laboratories).

**Stimulation of PMN with S. aureus or S. epidermidis**

PMN (1 × 106/1 ml in HBBS) were incubated with bacteria (S. aureus or S. epidermidis) in a ratio of 20, 100, or 500 bacteria/cell. After incubation for 2 h...
at 4°C or 37°C, cells were washed twice with PBS, and 400 μg vancomycin was added to 1 ml culture medium for 30 min at 37°C. The medium was replaced by medium containing 20 μg vancomycin, and culture was continued for 24 h or 48 h at 37°C. Supernatants were collected and stored at −20°C for ELISA (all experiments were carried out in duplicates and repeated at least 3 times with cells of different individuals). For stimulation on biofilms, 1 ml PMN (1 × 10^5 or 5 × 10^5) was added to an established biofilm, and after 24 or 48 h, supernatants were harvested for ELISA.

Figure 1. Gene expression of MRP-14, CXCL8, CD14, and cathepsin K in biopsies of patients. In tissue of 6 patients with implant-associated osteomyelitis, gene expression of (A) MRP-14 (B) CXCL8, (C) CD14, and (D) cathepsin K was determined. For MRP-14, CXCL-8, and CD14, expression in the affected sites (synovia, tibia) differed from expression in muscle (largest P value, 0.035). (D) Expression of cathepsin K was seen mainly in the tibia sample. Intramedullary. (E) Gene expression in the affected sites (synovia, tibia) of patients with implant-associated infection of MRP-14 and CXCL8 was higher compared with patients with aseptic loosening (n = 6); expression of CD14 was moderately enhanced, and cathepsin K expression did not differ (n.d.).
ELISA

MRP-14 in cell culture supernatants and in serum samples was determined by use of a commercially available ELISA kit and the protocol provided by the manufacturer (Immundiagnostik, Bensheim, Germany).

Statistical analysis

Differences between groups were calculated by use of Mann-Whitney test. Significance level was $P < 0.05$. Data were presented as box blots (Origin 9.0 software). Correlations were calculated by use of Spearman’s rank test.

RESULTS AND DISCUSSION

Gene expression of MRP-14 in tissue of patients with implant-associated osteomyelitis and detection of MRP-14 in biopsies by immunohistochemistry

The innate immune system plays a key role in implant-associated osteomyelitis. PMN, monocytes, and to a lesser extent, T cells are attracted to the infected site, where a bacterial biofilm is attached to the implant [16]. In response, chemokines, such as CXCL8, IL-1β, or the macrophage inflammatory proteins CXCL2 and CCL3, are produced and released, which subsequently attract and activate more leukocytes, creating an inflammatory microenvironment, which promotes the generation of bone-resorbing osteoclasts [17, 19, 21].

In the present study, tissue samples from patients with infected knee endoprostheses were recovered during surgery. Gene-expression analysis revealed MRP-14 in synovial and tibial tissue (Fig. 1A). In these tissues, CXCL8 was also found, as was CD14, indicative of the presence of monocytes (Fig. 1B and C). In the adjacent, unaffected muscle, tissue expression of MRP-14, CXCL8, and CD14 was minimal. Cathepsin K, which is typically expressed by osteoclasts, was found primarily in tissue derived from the tibia (Fig. 1D). To assess whether MRP-14 expression was associated with infection, tissues from patients with aseptic loosening of knee prostheses were analyzed. A lower number of transcripts for MRP-14, CXCL8, and CD14 were found, whereas cathepsin K expression did not differ (Fig. 1E).

Furthermore, biopsies from the affected sites were examined histopathologically and by immunohistochemistry for the presence of cathepsin K as a marker for osteoclasts, CD68 as a marker for macrophages, CD15 as a marker for neutrophils, and for cells expressing MRP-14 (examples are shown in Fig. 2A; the respective normal tissue is shown in the insets). Compared with the noninfected tissue, the number of neutrophils (CD15+) and monocytes/macrophages (CD68+) was higher in tissue from patients with implant infection, and more MRP-14-expressing cells were found as well. MRP-14 was mainly associated with neutrophils (the correlation between CD15+ and MRP-14+ cells was statistically significant by Spearman’s rank; correlation coefficient 0.80). Cathepsin K-positive cells occurred in tissue of patients with implant-associated osteomyelitis and with aseptic loosening as well (data summarized in Fig. 2B).

Thus, we were able to show that MRP-14 is generated in tissues surrounding an infected implant. In line with the fact that MRP-14 is mainly produced by myeloid cells, gene expression

Figure 2. Histologic evaluation of biopsies. (A) Soft tissue from the direct vicinity of an infected prosthesis was examined (magnification, 200×; left; zoomed images, right). (a and b) Following H&E staining, multinucleated osteoclasts are seen, marked by arrows. (c and d) The osteoclasts express cathepsin K (arrows). (e and f) Shown is a dense infiltration of CD15+ neutrophils. The zoom (f) shows their typical polymorphous nucleus (arrows). (g and h) CD68+-positive cells infiltrate tissue surrounding the infected implant. (i and j) MRP-14 is mainly expressed in neutrophils and to a lesser extent, in infiltrated macrophages. (B) In biopsies of patients with implant-associated osteomyelitis ($n = 4$; filled symbols; each symbol represents 1 patient) and patients with aseptic loosening ($n = 4$; open symbols), neutrophils (CD15+), monocytes/macrophages (CD68+), osteoclasts (cathepsin K'), and MRP-14-expressing cells were counted in 5 HPFs. The mean values are given (differences between groups were calculated by Mann-Whitney test).
correlated with that of CD14, which is typically expressed by monocytes and activated neutrophils [23]. MRP-14 expression also correlated, to some extent, with gene expression of CXCL8, which is also a product of myeloid cells. By immunohistology, infiltration of neutrophils (CD15+) and monocytes (CD68+) was verified, as was the association of MRP-14 with these cells.

Local synthesis of MRP-14 and of CXCL8 was not restricted to infection but also occurred in tissue of patients with aseptic loosening, albeit to a lesser extent. Aseptic loosening is considered a sterile inflammation, induced by wear particles derived from the implant. Phagocytosis of wear particles presumably activates monocytes and subsequently induces the release of cytokines promoting a local inflammation (reviewed in refs. [24, 25]). Our data support the notion of a local cytokine generation also in aseptic loosening. Compared with the situation during infection, the reaction appears to be less prominent, which might explain the protracted time course of aseptic loosening [21]. Nevertheless, also in aseptic loosening, the proinflammatory environment is sufficient to induce osteoclast generation eventually, as indicated by synthesis of cathepsin K. The latter is induced when precursor cells differentiate toward an osteoclastic phenotype and hence, serves as a marker for osteoclast generation. Osteoclasts, the only cells known to have the capacity to resorb bone, are quite abundant in osteomyelitic lesions (see Fig. 2A). Normal bone tissue, without infiltrated inflammatory cells, as well as without an unphysiological amount of osteoclasts, is shown in the insets.

**MRP-14 in the peripheral blood of patients with infection and patients with aseptic loosening**

In peripheral blood cells, the number of transcripts for MRP-14 differed significantly between patients with infection and patients with aseptic loosening (Fig. 3A). This finding was unexpected, as we presumed a local synthesis. A possible explanation is the so-called “reverse migration,” defined as re-entering of neutrophils from infected sites to the peripheral blood of patients with infection or patients with aseptic loosening (Fig. 3A).

**TABLE 1. Release of MRP-14 from PMN in response to a bacterial challenge**

<table>
<thead>
<tr>
<th>PMN incubated with</th>
<th>Bacteria:cell ratio</th>
<th>MRP-14 in the supernatant (ng/ml) corrected for spontaneous release, mean ± sd of n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em> (ops)</td>
<td>50:1</td>
<td>11.75 ± 4.92</td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td>0.59 ± 0.23</td>
</tr>
<tr>
<td><em>S. epidermidis</em> (not ops)</td>
<td>50:1</td>
<td>Not detectable</td>
</tr>
<tr>
<td><em>S. aureus</em> (ops)</td>
<td>50:1</td>
<td>25.28 ± 4.55</td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td>1.19 ± 0.89</td>
</tr>
<tr>
<td><em>S. aureus</em> (not ops)</td>
<td>50:1</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

Bacterial biofilm of *S. epidermidis*  pg/ml (mean ± sd of n = 5)  631.4 ± 380.0

*a* ops, Opsonized with normal human serum.
peripheral blood [26, 27]. On the other hand, systemic neutrophil activation preceding the infiltration of cells cannot be ruled out. Irrespective of the underlying mechanism, gene expression of MRP-14 nicely differentiated between cells of patients with infection and those with aseptic loosening.

Correspondingly, plasma concentrations of MRP-14 were also higher in patients with implant-associated osteomyelitis compared with patients with aseptic loosening or healthy individuals (Fig. 3B). Furthermore, plasma concentrations of MRP-14 did not correlate with the CRP serum concentration nor with the white blood cell count (data not shown).

In that MRP-14 differs from CXCL8 and from other cytokines, e.g., CXCL2 and CCL3, which although generated locally, is not found regularly and in abundance in the peripheral blood of patients with implant-associated osteomyelitis or of patients with aseptic loosening [21]. A likely explanation is the exceptionally high cellular concentration of MRP-14, which is considered to be the most abundant cytoplasmic protein of neutrophils [2]. Possibly, MRP-14 is also rather stable, as data from patients with chronic inflammatory disease, such as rheumatoid arthritis, imply, where MRP-14 is considered as a “biomarker,” even predicting relapses (reviewed in ref. [9]). Our data suggest that MRP-14 in plasma is a promising, novel diagnostic marker for the detection of implant-associated infection.

MRP-14 is released from PMN in response to contact with bacteria

PMN, isolated from the peripheral blood of healthy donors, were incubated with *S. aureus* or *S. epidermidis* for 1–3 h. Then, release of MRP-14 into the supernatant was measured. Both bacteria species induced release of MRP-14, depending on the bacteria:PMN ratio. Opsonization of bacteria with serum was required (data of experiments with cells of different individuals are summarized in Table 1), as neutrophils do not recognize bacteria as such but the opsonizing antibody (via FcRs) and complement C3 (via C3Rs) [28]. Of note, the bacteria:PMN ratio was critical for inducing the release reaction. Presumably, phagocytosis of a high number of bacteria rapidly induces apoptosis of PMN and thus, prevents the release of intracellular content [29]. In another set of experiments, PMN were placed on bacterial biofilms. Again, release of MRP-14 into the supernatant was observed, in this case, independently of opsonization (Table 1). Concerning biofilms, apparently other recognition structures are displayed, possibly constituents of the extracellular matrix [30].

MRP-14 is released from neutrophils or monocytes/macrophages by a not-yet well-defined tubulin-dependent mechanism in response to various stimuli, including inflammatory cytokines [9, 31] and as we found now, also in response to bacterial challenge. Accumulation of MRP-14 in experimentally induced staphylococci infection had been shown before in rat or rabbit models [32], and together, the data underline a participation of MRP-14 in bacterial infection. An antimicrobial activity for MRP-14, in association with MRP-8 (calprotectin; L1 myelomonocytic antigen), has been described many years ago [3, 4]. Furthermore, other biologic activities have been attributed to MRP-14, alone or in association with MRP-8, for example, effects on neutrophils, including induction of chemotaxis, adhesion to extracellular matrix proteins, degranulation, and enhancement of phagocytosis [8, 33]. Moreover, MRP-14 has been implied as an antiapoptotic molecule, fine-tuning the neutrophil turnover [34]. Thus, MRP-14 enhances innate host-defense mechanisms. Rather, MRP-14 mice appeared to be protected from endotoxemia [35], suggesting that excessive MRP-14

**Figure 4. Generation of osteoclasts in response to MRP-14.** (A) Typical features of osteoclasts are actin ring formation (visualized by binding of phalloidin-FITC; green), multiple nuclei (blue), and expression of cathepsin K (detected by an antibody; red). (B) Given is the number of osteoclasts/HPF (*n* = 5), generated from peripheral blood monocytes of 2 individuals, cultivated with culture medium alone, with M-CSF (25 ng/ml), M-CSF plus MRP-14 (5 μg/ml), M-CSF plus RANKL (50 ng/ml), or MRP-14 (5 μg/ml) alone for 14 days. (C) Western blotting of whole cells lysates, prepared 48 and 72 h poststimulation with M-CSF and either RANKL or MRP-14, revealed a persistent up-regulation of NFATc1. (D) After stimulation with MRP-14, resorption pits on ivory slices confirmed functionality of osteoclasts.
concentration might be harmful, an observation that had also been made for a sudden increase of other cytokines [36].

**MRP-14 induces the generation of osteoclasts**

Monocytes were cultivated with rMRP-14 and for comparison with RANKL, a well-established inducer of osteoclasts. After 14 days in culture, osteoclasts were found, identified by the ring-like arrangement of actin, multiple nuclei, and expression of cathepsin K (example in Fig. 4A). Compared with monocytes cultivated with M-CSF only, addition of MRP-14 or RANKL enhanced the number of osteoclasts (Fig. 4B). Moreover, in response to MRP-14, NFATc1, which is a crucial transcription factor for osteoclast differentiation, was persistently up-regulated (Fig. 4C). Functionality of osteoclasts after stimulation with MRP-14 was confirmed by occurrence of resorption pits on ivory slices (Fig. 4D).

Our results demonstrate a further, not-yet described biologic activity of MRP-14, the induction of the differentiation of monocytes to osteoclasts. Basically, monocytes have the capability to differentiate to macrophages, dendritic cells, or osteoclasts. The microenvironment, particularly cytokines, drives the differentiation by inducing the respective transcription factors. In the case of osteoclast generation, a persistent up-regulation of NFATc1 is required [37–39]. In response to MRP-14, persistently enhanced NFATc1 expression, as well as osteoclast generation, was seen.

In conclusion, bacterial biofilms induce the local host defense and the accompanying inflammatory response. If the immune cells are incapable of adequately clearing the bacteria, then the inflammatory response persists. Thus, the ineffectiveness of the local host defense mechanisms is responsible for bone destruction. Consequently, the controlling of the local inflammation, in particular, proinflammatory cytokines, such as MRP-14, might be a valuable therapeutic option to prevent early bone loss.

**AUTHORSHIP**

U.D., T.G., S.M., S.S., B.P., G.M.H., and M.M.G. have made substantial contributions to conception and design, acquisition of data, and analysis and interpretation of data and have given final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. U.D., G.M.H., and M.M.G. have been involved in drafting the manuscript and revising it critically for important intellectual content.

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**DISCLOSURES**

The authors declare no conflict of interest.

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