Host-Pathogen Interactions in Progressive Chronic Periodontitis

ABSTRACT
Periodontitis is an infection characterized by the occurrence of supporting tissue destruction with an episodic nature. Disease progression is often determined by the loss of attachment level or alveolar bone, and sequential probing of periodontal attachment remains the most commonly utilized method to diagnose progressive destruction of the periodontium. The tolerance method has been the most extensive clinical method used in recent years to determine site-specific attachment level changes. There is abundant evidence that major tissue destruction in periodontal lesions results from the recruitment of immune cells. Considerable effort has been made to study the host cell and mediator profiles involved in the pathogenesis of chronic periodontitis, but the definition of active sites, where current periodontal breakdown occurs, and consecutive characterization of the mediators involved are still among the main concerns. In the present review, we summarize periodontopathic bacteria and host factors, including infiltrating cell populations, cytokines, and host matrix metalloproteinases, associated with undergoing episodic attachment loss that could partly explain the mechanisms involved in destruction of the supporting tissues of the tooth.

KEY WORDS: chronic periodontitis, immune response, cytokines, progressive lesions.

INTRODUCTION
Human periodontal diseases (i.e., gingivitis, periodontitis) result from the interaction of heterogeneous etiologic factors, including the formation of a complex biofilm in the subgingival microenvironment, social and behavioral modulations, and genetic or epigenetic traits of the host, each of which is influenced and/or modulated by the host's immune and inflammatory response. Although chronic bacterial and endotoxin exposure is a prerequisite for gingival inflammation and periodontal tissue destruction, its presence alone is not enough to explain disease initiation and progression (Offenbacher, 1996).

Evidence indicates that periodontal disease has dynamic states of exacerbation and remission, which can be described in terms of patterns of disease progression and regression (Goodson et al., 1984). Loss of attachment recorded by sequential probing assessments is thought to reflect the cumulative effect of such repeated episodes, and the clinical tolerance method represents the most commonly used clinical approach to identify progressive periodontal disease (Haffajee et al., 1983).

The major destruction of soft and hard tissues associated with periodontitis progression results from the activation of the host's immune-inflammatory response to the bacterial challenge, and thereby the destructive character of the disease is determined mainly by the nature of these inflammatory responses. During periodontal activity episodes, leukocyte activation involves the active expression of inflammatory mediators, including cytokines, capable of stimulating alveolar bone resorption and soft periodontal tissue destruction via host-derived matrix metalloproteinases (MMPs) (Offenbacher, 1996). However, the pathological mechanisms underlying progression of periodontal lesions remain unclear and require further investigation. Therefore, this review will focus on the nature of the host-pathogen interactions in periodontitis, with emphasis on the host immune and inflammatory response and its potential role over disease progression.

PERIODONTAL PATHOGENS
Few available studies have attempted to associate microbial species with periods of disease activity, but they contribute to highlight the specific
periodontopathogens that might be involved in attachment loss episodes. Among them, it has been shown that the prevalence of Porphyromonas gingivalis is associated with progressive periodontitis patients (N = 56). P. gingivalis was found in 96% of the affected individuals, 75% of them in active sites and 59.7% in inactive sites (Lopez, 2000). Recently, different P. gingivalis K serotypes have been associated with the induction of a distinct type of immune response, suggesting a role for the capsule over activation of dendritic cells (Vernal et al., 2009). Thus, this virulence factor may be an important pathogenic determinant in the initiation, progression, and/or the severity of periodontitis.

Analysis of the levels of Actinobacillus actinomycetemcomitans, Tannerella forsythia, and P. gingivalis in periodontal progressive sites by culture techniques demonstrated that active sites had significantly higher prevalence of P. gingivalis among total anaerobic micro-organisms, when compared with inactive sites (17.9% and 1.9%, respectively) (Silva et al., 2008). Similarly, P. gingivalis, T. forsythia, and Treponema denticola were more likely to be detected in subgingival plaque samples by real time-PCR in sites that experienced periodontal disease progression than in the matching controls (Byrne et al., 2009). The authors concluded that the levels of P. gingivalis and T. denticola in subgingival plaque are potentially useful for identifying sites at significant risk of periodontitis progression. Accordingly, comparison of the rate of attachment loss in periodontally healthy individuals under a preventive regimen and individuals with periodontitis and enrolled in a maintenance program showed that the latter group had significantly higher levels of P. gingivalis, A. actinomycetemcomitans, and T. forsythia and underwent disease progression even after a three-year period of a supportive periodontal care program (Teles et al., 2008). A specific T. forsythia prtH genotype, which encodes a virulence factor corresponding to a cysteine protease, was found to be significantly higher in individuals with periodontitis who experienced attachment loss compared with control individuals, suggesting that levels of this prtH genotype at baseline may be predictive of future attachment loss (Hamlet et al., 2008).

OSTEOIMMUNOLOGY

Skeletal homeostasis depends on a dynamic balance between the activities of the bone-forming osteoblasts and bone-resorbing osteoclasts. This balance is tightly controlled by various regulatory mechanisms, such as the endocrine system and the immune response (Sato and Takayanagi, 2006). An imbalance in favor of osteoclasts leads to pathological bone resorption, which can be associated with attachment loss (Lopez, 2000). Recently, different CD4+ T-cells have been characterized: the Th1 and Th regulatory (Treg) cells. The Th17 subset displays pro-inflammatory and pro-resorptive activities through the secretion of IL-6, IL-21, IL-22, IL-23, IL-26 (Vernal and Garcia-Sanz, 2008), and particularly IL-17 and RANKL, both of which are involved in the differentiation and activation of osteoclasts and bone resorption (Vernal et al., 2004, 2006). In contrast, the Treg cells subset displays suppressor functions producing IL-10, transforming growth factor (TGF)-β1, and cytokotoxic T-lymphocyte antigen-4 (CTLA-4) (Vernal and Garcia-Sanz, 2008).

During progressive periodontitis, increased numbers of CD4+ lymphocytes are detected in active periodontal lesions compared with inactive lesions (Silva et al., 2008). However, analysis of T-lymphocyte populations by flow cytometry in progressive periodontitis has revealed similar CD4+/CD8+ ratios between active and inactive periodontal lesions (Silva et al., 2008). In this context, it could be speculated that particular CD4+ T-cell phenotypes are associated with active periodontal attachment loss. In fact, an increased expression of T-bet, the ‘master-switch’ gene controlling Th1 differentiation, has been detected in active periodontal lesions, and this over-expression correlates positively with the increments in IL-1β and IFN-γ expression (Dutzan et al., 2009). More recently, a Th17-type of response has also been associated with periodontitis-induced tissue destruction (Vernal et al., 2005).

Our group has recently analyzed the role of Th17 and T reg phenotypes during progressive periodontitis (Dutzan et al., 2009). The over-expression of transcription factor orphan nuclear receptor C2 (RORC2), the master-switch gene controlling the Th17 differentiation, and transcription factor forkhead box P3 (Foxp3), the master-switch gene controlling the Treg differentiation, was associated with active periodontal lesions during progressive periodontitis. In fact, IL-17 and RANKL were over-regulated and IL-10 and TGF-β1 were down-regulated in active periodontal lesions compared with inactive lesions.

Analysis of the associations between different genes yielded significant positive correlations between RORC2 and RANKL and between RORC2 and IL-17; in contrast, Foxp3, IL-10,
that Foxp3+ T-cells that do not bear regulatory functions may view of the down-regulation of IL-10 and TGF-β1, we speculate TGF-β1, and CTLA-4 did not show a positive correlation. In TNF = tumor necrosis factor.

through synthesizing RANKL, thereby inducing an increment in osteoclast differentiation/osteoblastic cells. In addition, Th17 cells may also contribute to alveolar bone loss directly as MMP-8, MMP-9, MMP-2, and MMP-13. MMPs act in cascades that are switched on during periodontal inflammation, amplifying and perpetuating soft and hard periodontal tissue breakdown by MMP-mediated proteolytic activation and by the removal of collagen I. In the context of an unresolved infection, an adaptive immune response is established, and Th17 cells may be activated, contributing to indirect bone destruction by secreting IL-6 and IL-17, which, in turn, increase the inflammatory response and induce RANKL expression by osteoblastic cells. In addition, Th17 cells may also contribute to alveolar bone loss directly through synthesizing RANKL, thereby inducing an increment in osteoclast differentiation/maturation and alveolar bone resorption. IL = interleukin; MMP = matrix metalloproteinase; TNF = tumor necrosis factor.

Figure. Role of MMPs, Th17 cells, and RANKL in periodontal tissue destruction induced during progressive periodontitis. As a consequence of infection, an inflammatory response is established in periodontal tissues, characterized by the synthesis of inflammatory cytokines, such as IL-1β, IL-12, and TNF-α, and, consequently, of matrix metalloproteinases (MMPs), such as MMP-8, MMP-9, MMP-2, and MMP-13. MMPs act in cascades that are switched on during periodontal inflammation, amplifying and perpetuating soft and hard periodontal tissue breakdown by MMP-mediated proteolytic activation and by the removal of collagen I. In the context of an unresolved infection, an adaptive immune response is established, and Th17 cells may be activated, contributing to indirect bone destruction by secreting IL-6 and IL-17, which, in turn, increase the inflammatory response and induce RANKL expression by osteoblastic cells. In addition, Th17 cells may also contribute to alveolar bone loss directly through synthesizing RANKL, thereby inducing an increment in osteoclast differentiation/maturation and alveolar bone resorption. IL = interleukin; MMP = matrix metalloproteinase; TNF = tumor necrosis factor.

TGF-β1, and CTLA-4 did not show a positive correlation. In view of the down-regulation of IL-10 and TGF-β1, we speculate that Foxp3+ T-cells that do not bear regulatory functions may have a role in periodontal progressive destruction (Dutzan et al., 2009).

Although Treg and Th17 cells play different roles during the pathogenesis of infections, it has been demonstrated that a reciprocal developmental pathway exists for their generation. Naive T-cells exposed to TGF-β up-regulate Foxp3 and become induced Treg cells; however, when cultured with TGF-β and IL-6, naïve T-cells generate Th17 cells with pathologic activities (Yamazaki et al., 2007). Thus, when the immune response is not activated, TGF-β favors the generation of induced Treg cells, which suppress inflammation; however, when an infection is established—for instance, during either gingivitis or an early phase of periodontitis—IL-6 is synthesized during the innate immune response, inhibiting the generation of Treg cells and inducing the differentiation of pro-inflammatory and pro-resorptive Th17 cells in the presence of TGF-β (Bettelli et al., 2007).

Numerous cytokines have demonstrated an ability to stimulate bone resorption, including IL-1α, IL-1β, IL-6, IL-11, IL-15, and IL-17, whereas others, such as IL-4, IL-10, IL-13, IL-18, GM-CSF, and TGF-β1, are inhibitors (Walsh et al., 2006). In line with IL-17, IL-1β and IFN-γ were significantly over-expressed in active periodontal lesions compared with inactives (Dutzan et al., 2009), whereas increased GCF interleukin-1 levels were associated with active sites (Gamonal et al., 2000). In addition to cytokines, levels of chemokine monocyte chemotactrant protein (MCP)-3 (Dezerega et al., 2010) and prostaglandin E2 were found to be significantly higher in GCF of active sites compared with inactives (Offenbacher et al., 1993). In this context, functional characterization of 3 novel members of the TNF-ligand and receptor superfamily, RANKL, its receptor RANK, and the soluble decoy receptor of RANKL, OPG, has significantly contributed to the establishment of osteoimmunology, and their participation as key modulators of physiological and pathological bone resorption. RANKL exerts its biological effects through inducing osteoclast differentiation, maturation, and activation, and inhibiting their apoptosis (Lacey et al., 1998).

The identification of RANKL as the cytokine TRANCE (TNF-related activation-induced cytokine), previously identified in T-cells, has allowed us to envisage the possibility that CD4⁺ T-cells may have the capacity to induce osteoclast differentiation and activation by acting directly on osteoclast precursors and mature osteoclasts, through synthesis of RANKL during bone-resorptive diseases (Vernal et al., 2004). Furthermore, many well-known osteotropic factors, including TNF-α, IL-1β, IL-6, and IL-17, exert their osteoclastogenic activity by inducing RANKL expression on osteoblasts and CD4⁺ T-cells (Boyle et al., 2003). Th17 cells promote osteoclastogenesis primarily through the production of IL-17 and RANKL (Sato et al., 2006). Furthermore, IL-17 facilitates local inflammation by recruiting and activating immune cells, leading to an abundance of inflammatory cytokines, such as IL-1β and TNF-α, and by enhancing RANKL expression (Weaver et al., 2006) (Fig.). In fact, an increased synthesis of RANKL and over-expression of RORC2, RANKL, and IL-17 have been detected in active lesions during progressive periodontitis (Vernal et al., 2004; Dutzan et al., 2009).

Our findings have demonstrated that the total amount of RANKL detected in gingival crevicular fluid of individuals undergoing periodontitis progression was higher in active periodontal lesions than in inactive lesions, proposing this pro-resorptive factor as a marker of active alveolar bone resorption associated with periodontal infection (Vernal et al., 2004;
Dutzan et al., 2009). This finding was corroborated when cytokine mRNA analysis was performed with quantitative real-time PCR. In active periodontal lesions, RANKL was significantly over-expressed compared with inactive periodontal lesions (Dutzan et al., 2009).

In response to periodontopathogens that have been strongly associated with periodontitis progression—for instance, *P. gingivalis* and *A. actinomycetemcomitans* (Silva et al., 2008)—RANKL expression has been reported to increase in CD4+ T-lymphocytes infiltrating periodontally affected tissues (Yamamoto et al., 2006). In this context, bacterial LPS induces production of RANKL and several pro-inflammatory cytokines in T-cells through a TLR-mediated signaling pathway, where Cot/Tp12, a member of the MAPK family of serine threonine kinases, plays a central role. In fact, it has been established that the extent of alveolar bone loss and osteoclastogenesis observed in an animal model of ligature-induced periodontitis was decreased in Cot/Tp12-deficient mice, and this decrement was associated with a reduction of RANKL expression in periodontal tissues, suggesting that Cot/Tp12 is essential for RANKL-mediated alveolar bone loss and osteoclastogenesis during periodontitis (Ohnishi et al., 2010).

The role of Th17 cells in host defense against periodontopathogens is just emerging, particularly in terms of their destructive potential in progressive periodontitis. Th17 lymphocytes constitute an early defense against infections and represent a bridge between innate and adaptive immunity. Moreover, Th17 cells seem to antagonize Treg cell development, thereby amplifying the inflammatory response, and thus playing a crucial role in the progression of inflammatory and autoimmune disorders (Bettelli et al., 2006). Th17 cells represent the osteoclastogenic Th phenotype involved in progressive periodontitis, mainly by inducing osteoclastogenesis and bone resorption through synthesizing IL-17 and RANKL in active periodontal lesions; however, several aspects of Th17 activation, function, and regulation remain unclear and are currently being investigated.

**MATRIX METALLOPROTEINASES**

Extracellular proteolysis and tissue remodeling are integral features of periodontal homeostasis, involving a tight regulation between protease activity and inhibition. Matrix metalloproteinases (MMPs) can synergistically degrade almost all extracellular matrix and basement membrane components and regulate several cellular processes, including inflammatory and immune responses, representing the most prominent and widely studied protease family associated with periodontal diseases (Folgueras et al., 2004). MMPs are genetically distinct but structurally related zinc-dependent metalloendoproteinases. The 23 MMPs expressed in humans can be classified into different subgroups based on their primary structures and substrate specificities, including collagenases (MMP-1, -8, -13), gelatinases (MMP-9, -2), membrane-type MMPs (MT-MMPs, MMP-14, -15, -16, -17, -24, -25), and other MMPs (Folgueras et al., 2004). Overall, the pathophysiological significance of increased MMP expression in periodontitis will rely on their regulation by activating enzymes and endogenous inhibitors (Sorsa et al., 2006; Hernandez Rios et al., 2009).

Type I collagen is the main extracellular matrix component of soft and hard periodontal tissues, and thus, collagen degradation is regarded as one of the key factors in uncontrolled destructive lesions (Golub et al., 1997). Although numerous cross-sectional studies have indicated that excessive collagenase levels parallel the severity of periodontal lesions and inflammation (Sorsa et al., 2006), there are far fewer data from longitudinal studies associating MMPs with ongoing destruction of periodontal supporting tissue.

The major collagenolytic MMPs associated with severity of periodontal disease are MMP-8 and MMP-13 (Sorsa et al., 2004, 2006; Leppilahit et al., 2011). Despite the genetic background underlying periodontal diseases, most MMP gene polymorphism studies undertaken in different ethnic populations have been conducted without concluding associations with susceptibility to periodontitis. An exception could be the -1607 2G MMP-1 polymorphic allele that has been related to susceptibility to develop severe chronic periodontitis and -519 AG and GG MMP-1 genotypes in the outcome of periodontal therapy in the Turkish population (Pirhan et al., 2008; Ustun et al., 2008).

GCF MMP-8 accounts for the bulk of collagenases, followed by MMP-13 (Golub et al., 1997). Clinical trials testing antimicrobial dose doxycycline (SDD) treatment reported an association between improvement of clinical parameters, reduction of GCF total collagenase activity, and a decrease in the bone resorption fragment ICTP in an experimental (SDD) group composed of post-menopausal women exhibiting periodontitis and systemic osteopenia during a two-year follow-up (Golub et al., 2008). It is worth noting that the finding of a strong positive correlation between GCF ICTP, presumably released during progression of alveolar bone resorption, and total collagenase activity supports a role for collagenases in bone loss and thereby in disease progression that can be reflected in GCF.

Elevation of active MMP-8 has been previously associated with the conversion of gingivitis to periodontitis and the progression of established periodontitis (Mantyla et al., 2006). MMP-8, like MMP-9 and myeloperoxidase (MPO), is mainly released from neutrophils in a latent form and is induced and activated during periodontal inflammation by host inflammatory mediators like TNF-α, IL-1β, reactive oxygen species (ROS), including MPO-produced hypochlorous acid (Saari et al., 1990), and microbial and host-derived proteases (Sorsa et al., 1992). Recently, our group developed a study associating GCF MMP-8, MMP-14, myeloperoxidase (MPO), and TIMP-1 levels in GCF from progressive periodontitis patients, determined clinically by the tolerance method, at baseline and after conventional periodontal therapy (Hernandez et al., 2011). In agreement with previous findings, neutrophil MMP-8 was the predominant isotype (Golub et al., 1997, 2008). A significant strong positive correlation among total MMP-8 levels, active MMP-8 forms, and MPO was found in all progressive sites at baseline, whereas periodontal treatment consisting of scaling and root planing (SRP) resulted in significant MPO and MMP-8 reductions, together with a loss of MPO/MMP-8 association, despite the reduced sample size, except for the active sites. These results
point to the existence of an MPO-mediated oxidative pathway enhancing proMMP-8 underlying periodontitis progression. Furthermore, reactive oxygen species (ROS) can oxidatively inactivate TIMP-1 (Sorsa et al., 2006). In agreement with these findings, repeatedly elevated GCF MMP-8 concentrations determined by longitudinal monitoring of periodontal response during the maintenance phase after SRP were reported to be associated with a lack of improvement of clinical parameters (Mantyla et al., 2006). Furthermore, MMP-8 levels tended to decrease in treated stable sites (n = 3) and increased in unstable ones (n = 3) (Sorsa et al., 2009).

Increases in MMP-8 and IL-1β during the first year of periodontal maintenance have been associated with increased odds of subsequent attachment loss, but no associations were found between MMP-8 levels and bone loss (Reinhardt et al., 2010). Overall, it is plausible that high MMP-8 levels could reflect mainly soft periodontal tissue destruction and inflammation. Unchanged MMP-8 levels after periodontal treatment might be interpreted as a poor host response, representing sites at risk of further loss of periodontal support. Additionally, MMP-8/MPO association could reflect the persistence of MMP-8 activation and, consequently, the need for further treatment, follow-up, and a suitable target for pharmacological therapy (Sorsa et al., 2009; Hernandez et al., 2011).

In spite of the association between high MMP-8 levels and periodontitis, its biological functions have not been completely clarified. MMP-8 expression is not restricted to PMNs and soft-tissue mesenchymal cells, but it is also expressed in osteoblasts, osteocytes, and endothelial cells during early phases of bone healing, suggesting a role for the enzyme in connective tissue formation and degradation of bone matrix proteins (Itagaki et al., 2008). Development of P. gingivalis-induced periodontitis in a MMP-8-deficient mouse model showed unexpectedly extensive alveolar bone resorption (Kuula et al., 2009) and reduced expression of LIX/CXCL5 in gingival epithelium of MMP-8 null mice compared with wild types (Hernandez et al., 2010). LIX/CXCL5 represents the most potent PMN chemottractant in mice and might direct neutrophil influx to periodontal tissues at the oral interface (Van Den Steen et al., 2003).

Overall, maintenance of MMP-8 at physiological levels could promote a protective inflammatory process and the onset of the reparative phase, whereas pathologically elevated MMP-8 levels could contribute to excessive and sustained proteolysis, leading to periodontal supporting tissue breakdown.

MMP-13 was first cloned from breast carcinoma (Freije et al., 1994) and is expressed by sulcular epithelial cells, macrophage-like cells, fibroblasts, plasma cells, and osteoblasts (Rydziel et al., 2000; Hernandez et al., 2006). Longitudinal studies in disease progression demonstrated that, despite the fact that active and inactive sites displayed similar MMP-13 levels, MMP-13 activity significantly increased in active sites (Hernandez et al., 2006, 2007; Hernandez Rios et al., 2009). Based on these data, increasing levels of MMP-13 might be associated with stable periodontitis, whereas the occurrence of disease activity might be related to an imbalance between MMP-13 and their inhibitors, resulting in increased enzyme activity.

To address the potential mechanisms of the downstream MMP-13 activation cascade, our group studied the association among ICTP levels, the activation rates of proMMP-9 and proMMP-2, and MMP-13 activity (Hernandez Rios et al., 2009). According to our previous results, MMP-13 activity and ICTP levels increased along with low TIMP-1 levels in active sites compared with inactive and healthy sites, supporting a putative association between MMP-13 activity and alveolar bone resorption. Additionally, MMP-13 significantly increased proMMP-9 activation rate in diseased gingival tissue, and the further addition of an MMP-13 specific synthetic inhibitor, CL-82198, prevented proMMP-9 activation (Hernandez Rios et al., 2009). MMP-13 might potentiate bone resorption by generating collagen fragments activating osteoclasts (Hill et al., 1995) and by enhancing proMMP-9 activation in vitro (Knauper et al., 1997), which is also involved in periodontal tissue destruction and disease progression (Belmar et al., 2008). Recent evidence suggests that MMP-13 might also influence RANKL/OPG axis (Nannuru et al., 2010). Active MMP-9, in turn, further digests denatured collagen derived from MMP-13 activity (Hill et al., 1995), is thought to act over pre-osteoclast recruitment to sites for osteoclast differentiation and bone resorption, and activates proMMP-13 and proMMP-2. This, thus novel proteolytic cascade could perpetuate periodontal soft- and hard-tissue destruction in vivo in a ‘feed forward’ manner (Fig.).

CONCLUDING REMARKS

Despite its limitations, the “burst” theory (Goodson et al., 1982) is one of the most accepted models, whereas the tolerance method (Haffajee et al., 1983) represents the most prevalent clinical approach to determine site-specific changes of attachment level over time. The susceptibility to development of destructive/progressive periodontitis relies on the influence of multiple behavioral, environmental, and genetic factors. Assessment of microbiological profiles explains only a part of the disease phenotype. Development and progression of periodontal lesions involve an imbalance of pro-inflammatory molecules released by specific cell populations. Macrophages represent an important source of pro-inflammatory cytokines, including IL-1β and TNF-α, that, under dysregulation, contribute to host tissue destruction. Active periodontal lesions have also been characterized by a prominent infiltration of activated CD4+ T-cells over Treg, which express osteolytic-associated cytokines such as IL-17 promoting RANKL-mediated alveolar bone destruction. These cytokines are also capable of stimulating periodontal breakdown and collagenase-mediated destruction.

Oxidative and proteolytic activation cascades involving MMP-8 and MMP-13 at pathologically high levels lead to direct breakdown of periodontal supporting tissue, enhanced proteolysis, and perpetuation of inflammatory response, resulting in attachment loss. Involved inflammatory and proteolytic pathways characterizing active lesions may provide a biological signature of the underlying disease phenotype that might be useful for the development of adjunctive chair-side point-of-care diagnostics to predict future attachment loss and improve treatment decisions, and also for future drug development.
ACKNOWLEDGMENTS

This study was supported by project grants 1090046 and 1090461 from Scientific and Technologic Investigation Resource (FONDECYT), Santiago, Chile, and by grants from The Academy of Finland (TS) and the Research Foundation of Helsinki University Central Hospital (TS). The authors are grateful to Nicole Baden for her valuable collaboration in this study. The authors declare that they have no conflict of interests.

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