Osteoimmunological Aspects of Periodontal Diseases

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Abbreviations

AgP	Aggressive periodontitis
C3	Complement component 3
CP	Chronic periodontitis
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible NO-synthases
LPS	Lipopolysaccharide
M-CSF	Macrophage colony-stimulating factor
MMP	Matrix metalloproteinases
NO	Nitric oxide
OPG	Osteoprotegerin
RANK	Receptor activator of NF-kB

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RANKL   Receptor activator of nuclear factor "kappa-light-chain-enhancer" of the
         activated B cell ligand
TGF   Transforming growth factor
Th   T helper cells
TIMP   Tissue inhibitors of metalloproteinase
TLR   Toll-like receptors
TNF   Tumor necrosis factor
Treg   Regulatory T cells

12.1 Osteoimmunological Aspects of Periodontal Diseases

Osteoimmunology describes the cross-talk of cells of the musculoskeletal and the immune system during the pathogenesis of various diseases; among the most prevalent ones is *periodontitis*, a chronic infectious inflammatory disease of the tooth-supporting structures, i.e., the periodontium consisting of the gingiva, alveolar bone, periodontal ligament, and root cementum. Periodontal disease is initiated by oral pathogens that accumulate at the gingival margin of the teeth and thereby trigger a response of the innate and adaptive immunity. In contrast to other osteoimmunological disorders (e.g., osteoporosis, rheumatoid arthritis), the immune system plays a two-sided role in the pathogenesis of periodontitis: it controls the infection and protects the organism from bacterial invasion but also propagates the destruction of the soft and hard tissues surrounding the tooth. Periodontitis, if left untreated, finally results in tooth loss.

The present chapter discusses, in a concise manner, the interplay of pathogens, mediators, and enzymes, and host cells involved in the pathogenesis of periodontitis. Further, a short overview on some of the potential "osteoimmunological targets" for the treatment of periodontitis is included.

12.1.1 Anatomy of the Periodontium

The term "*periodontium*" is derived from the Greek terms "peri-" (around) and "-odous" (tooth); it consists of four main components, which differ in their histological structure but act functionally as a single unit:

- Gingiva
- Periodontal ligament
- Alveolar bone
- Root cementum

The gingiva is via the junctional epithelium attached to the tooth surface and thereby forms a barrier against external factors (e.g., bacterial invasion). The other three components of the periodontium are forming the "attachment apparatus"; the

Fig, 12.1 Thin ground section of the periodontium of two adjacent teeth, stained with toluidine blue, in health (**a**) and disease (**b**). *E* enamel, *CEJ* cementoenamel junction, *P* plaque (due to overhanging crown margins), *G* gingival fibers, *D* dentin, *L* periodontal ligament, *B* alveolar bone, *C* root cementum. (**c**) Thin ground section of the lateral teeth of the upper jaw (By courtesy of late Prof. Dr. mult. K. Donath and Prof. DDr. Ulm)
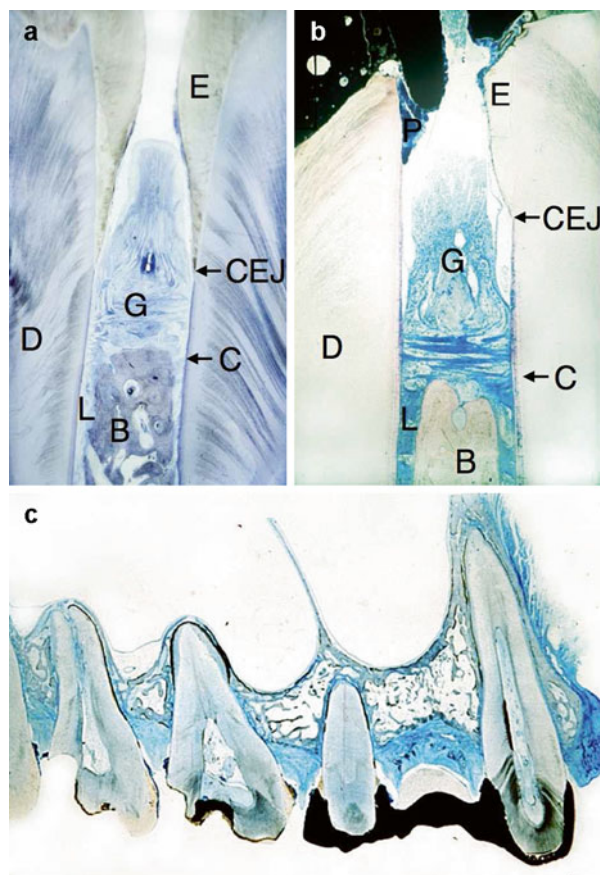
periodontal ligament consists of a rich vasculofibrous connective tissue, with collagen fibers incorporated in the root cementum and in the alveolar bone, and provides tooth support and tooth function (Fig. 12.1a–c).

12.1.2 Periodontitis: Disease Initiation

The oral cavity provides a habitat for about *750 different microbial species* and is one of the most complicated microbial ecosystems in the human body (Jenkinson and Lamont 2005). It provides huge amounts of nutrients through the saliva, gingival crevicular fluid, and food remnants, particularly those containing sugar. The first steps of dental plaque and *biofilm formation* occur within minutes after tooth brushing. A pellicle, consisting mainly of salivary proteins, attaches first at surface pits and fissures of the tooth and at the smooth tooth surfaces of the interdental area and the gingival margin, which are not affected much by the constant movement of the cheek and tongue. Thereafter, early bacterial colonizers such as *Streptococci* species,

Actinomyces species, *Capnocytophaga* species, and *Veillonella* species are able to attach to salivary pellicle receptors (Foster and Kolenbrander 2004; Listgarten 1994; Ritz 1967; Teles et al. 2013). The primary bacterial attachment may still be reversible, but if not interrupted, the bacteria start to build up a biofilm by multiplying, forming multilayers, and secreting extracellular matrix. This extracellular matrix helps the bacteria to stick together, form the biofilm architecture, and provide each other nutrients. Further, the early colonizers provide binding sites for the next colonizers (e.g., *Fusobacterium nucleatum*). *F. nucleatum*, which is an obligate anaerobic bacterium, plays a highly relevant role in biofilm maturation. *F. nucleatum* co-aggregates with multiple other bacteria, including early as well as late colonizers, and aerobic as well as anaerobic species and is present in both periodontally healthy sites and in diseased sites, however, in larger amounts in diseased sites. The co-aggregation with *F. nucleatum* allows late colonizers, which are mainly gram-negative facultative and obligate anaerobic bacteria (e.g., *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*), to survive in an aerated environment due to oxygen gradients in the biofilm structure (Bradshaw et al. 1998; Holt and Ebersole 2005).

Biofilm formation at the gingival margin causes in the beginning gingivitis, which is a reversible condition affecting only the soft tissues (Löe et al. 1965). In susceptible patients, however, increasing biofilm accumulation provokes progression to periodontitis, which involves periodontal attachment and bone loss, mostly irreversible. Recently the *"polymicrobial synergy and dysbiosis"* model was proposed for periodontal disease pathogenesis (Hajishengallis et al. 2012; Hajishengallis and Lamont 2012). Herein, single pathogens, so-called keystone pathogens, are able to initiate a shift of the entire microbial colonization from homeostasis to dysbiosis, i.e., the imbalance of a microbial flora in an ecosystem associated with the presence of a disease, thereby causing a strong immune response. Thus far, *P. gingivalis* is the best-documented keystone pathogen. Its presence, although not necessarily in high amounts, elevates the pathogenicity of the entire oral microbial community, e.g., by an increased expression of various virulence factors (Hajishengallis 2014; Hajishengallis and Lamont 2012). An experimental periodontitis model with mice demonstrated that presence of *P. gingivalis* in less than 0.01 % of the total bacterial amount was still enough for periodontal disease initiation (Hajishengallis et al. 2011). However, after this initiating step, the major portion of periodontal tissue degradation is not caused by the biofilm; in fact, it is the host immune response that propagates the loss of periodontal structures.

12.1.3 Periodontitis: Prevalence and Risk Factors

Periodontitis represents besides caries the major reason for tooth loss and is vastly prevalent in the population. In Germany, the *prevalence of periodontitis* according to the case definitions developed by the Centers for Disease Control and Prevention and the American Academy of Periodontology (Eke et al. 2012; Page and Eke 2007) was 71 and 87 % in age cohorts of 35–44 and 65–74 years, respectively; 17 and 42 %,

respectively, suffered from severe forms of periodontitis, i.e., advanced loss of the periodontal structures and bone (Holtfreter et al. 2010). *Risk factors* and *risk indicators* for periodontal disease may be both nonmodifiable and modifiable; examples of nonmodifiable factors include age, male gender, ethnicity, and genetic factors, while modifiable factors include smoking, alcohol consumption, low educational level, poorly controlled diabetes, obesity and metabolic syndrome, osteoporosis, and stress and its coping mechanisms (Genco and Borgnakke 2013). Page and Kornman (1997) illustrated the interplay between the bacterial attack, the immune response, and the risk factors in the pathogenesis of periodontal disease as shown in Fig. 12.2.

12.1.4 Periodontal Disease Entities

The current classification system of periodontal diseases was established in 1999 at the International Workshop for the Classification of Periodontal Diseases and Conditions (Armitage 1999):

1. Gingival diseases
2. *Chronic periodontitis*
3. *Aggressive periodontitis*
4. Periodontitis as a manifestation of systemic diseases
5. Necrotizing periodontal diseases
6. Abscesses of the periodontium
7. Periodontitis associated with endodontic lesions
8. Developmental or acquired deformities and conditions

   This chapter deals with osteoimmunological aspects associated with alveolar bone loss occurring in patients with aggressive or chronic periodontitis. *Aggressive*
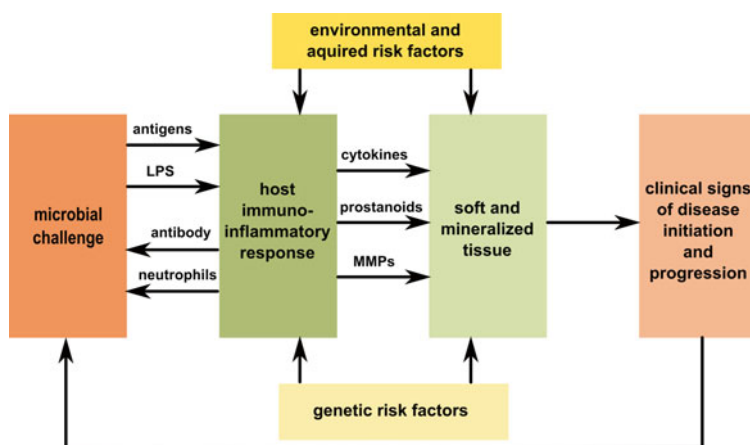

Fig. 12.2 Pathogenesis model of periodontal disease. (Modified from Page and Kornman 1997)
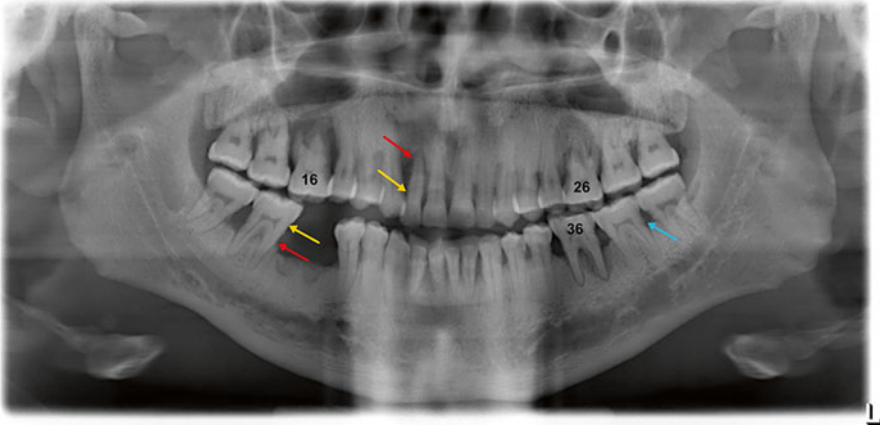
Fig. 12.3 Panoramic radiograph of a 24-year-old man with severe generalized aggressive periodontitis. *Yellow arrows* indicate the regular height of the alveolar bone in a healthy patient, while the *red arrows* indicate the bone level of this patient. The *light blue arrow* is indicating calculus, which is a rare finding in aggressive periodontitis. Severe bone loss is present around the first molar teeth (tooth number 16, 26, and 36; tooth 46 is already missing)

periodontitis (AgP) is characterized by rapid attachment loss, which is most often inconsistent with the amount of microbial deposits. AgP patients are systemically healthy, i.e., no specific immune compromising condition, but familial aggregation is characteristic. AgP appears clinically as localized and generalized AgP, currently considered to represent two different disease entities. Localized AgP is primarily restricted to first molars and incisors and has a circumpubertal onset, and patients show a high serum antibody response against the infecting agents. In contrast, generalized AgP affects individuals around 30 years of age, but patients may be older; further, generalized AgP patients present a poor serum antibody response against the infecting agents and a pronounced episodic destruction of periodontal attachment (Armitage 1999; Armitage and Cullinan 2010; Tonetti and Mombelli 1999) (Fig. 12.3).

Chronic periodontitis (CP) is the most common form of periodontitis in adults and it is characterized by consistency between the amount of periodontal tissue destruction and the presence of local factors, i.e., large amounts of biofilm. Further, a variable microbial pattern and a slow to moderate rate of progression with possible burst periods of rapid attachment loss are observed. Localized and generalized CP are considered as the same disease, but with different manifestation in terms of percentage of teeth affected, i.e., up to or more than 30 % affected teeth, respectively (Armitage 1999; Armitage and Cullinan 2010; Flemmig 1999) (Fig. 12.4a, b).

In general, the biochemical and histological characteristics, as well as the pathological processes and involved mediators and cells in the lesions, are rather similar between AgP and CP. However, certain, partly genetic, but yet not fully clarified, differences are causing the existing variations in the onset, magnitude, extent, and progression rate of tissue destruction (Kulkarni and Kinane 2014).

Fig. 12.4 Panoramic radiograph (**a**) and clinical picture (**b**) of a 52-year-old woman with severe generalized chronic periodontitis. The *yellow arrow* indicates the regular height of the alveolar bone in a healthy patient, while the *red arrows* indicate the bone level of this patient. The *orange arrows* are indicating the lost bone between the roots of the lower molars, i.e., furcation involvement, and the light *blue arrow* is indicating calculus
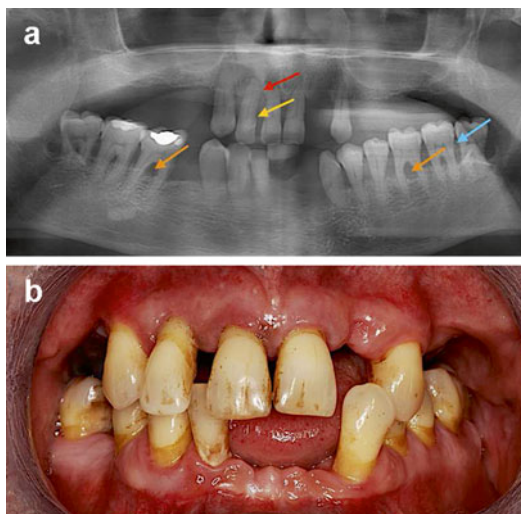
12.1.5 Periodontal Disease Progression

In summary, the first defense mechanism against the biofilm is represented by the *innate immune system*; while at later stages, the *adaptive immune system* becomes more relevant. After the initial step of biofilm accumulation at the tooth surface, biofilm components are perceived as "danger signals" by cells of the *innate immune system*; i.e., leukocytes and resident cells (e.g., gingival epithelial cells, periodontal ligament cells) recognize microbial lipopolysaccharide (LPS), DNA, or peptidoglycans via toll-like receptors (TLR). TLR are indispensable for the host to recognize the bacterial attack and their activation induces an intracellular signal pathway, expression of transcription factors, and subsequently release of pro-inflammatory cytokines and chemokines; altogether, an immune response focused on the infecting agents. TLR-2 and TLR-4 are primarily responsible for sensing periodontal pathogens (e.g., *A. actinomycetemcomitans* or *P. gingivalis*) (Kikkert et al. 2007). Peptidoglycans and atypical LPS from *P. gingivalis* activate TLR-2, LPS from gram-negative bacteria in general activate TLR-4, while *A. actinomycetemcomitans* activates both receptors (Akira and Takeda 2004; Ford et al. 2010). As a response to biofilm accumulation, a massive influx of neutrophils occurs in the tissues and, through the epithelium, in the periodontal sulcus. Neutrophils, except from phagocytosing bacteria, express a variety of pro-inflammatory cytokines, resulting in additional recruitment of various immune cells, including macrophages and T cells, thus propagating inflammation. Among the T cells, mainly CD4+ T cells (T helper cells; Th) are involved in periodontal disease pathogenesis; more specifically the cells of the innate immune system activate Th1 and Th17 cells, which results in a strong pro-inflammatory immune response. Later on Th2 cells promote the establishment of a lesion with predominantly B and plasma cells. Additionally, regulatory T cells (Treg) are activated and exert an anti-inflammatory role.
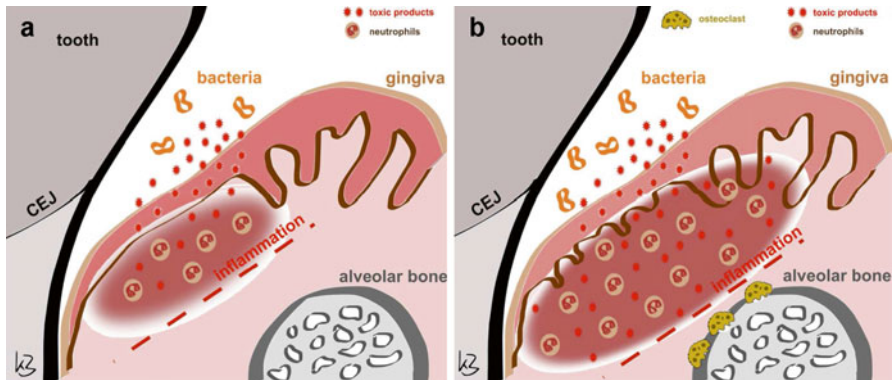
Fig. 12.5 (**a**) The inflammatory reaction is restricted to the gingiva; (**b**) the inflammatory process reaches the proximity of the alveolar bone and induces osteoclastogenesis and alveolar bone loss. (Modified by Graves et al. 2011; Schröder and Lindhe, 1981). *CEJ* cemento enamel junction

As already mentioned, the immune response initially causes a local inflammatory reaction, resulting in tissue destruction restricted to the gingiva, i.e., gingivitis. Yet, if the bacterial invasion cannot be controlled in susceptible hosts, the inflammatory reaction proceeds from the gingival margin apically towards the alveolar bone; i.e., the inflammatory response causes detachment of the junctional epithelium and its conversion into an ulcerated pocket epithelium, loss of the connective tissue attachment to the root, and loss of alveolar bone, i.e., periodontitis. An inflammatory process in close proximity to bone regularly supports differentiation and activation of osteoclast precursors, which results in uncoupling of the physiological balanced bone formation and bone resorption, in favor of the latter (Fig. 12.5a, b).

If this, often non-self-resolving, inflammatory process is left untreated, alveolar bone is continuously lost until tooth exfoliation. Hence, the immune response initiated by the bacterial attack is primary responsible for the extent, rate, and severity of host tissue degradation (Bartold et al. 2010; Brook 2003; Ford et al. 2010; Stabholz et al. 2010).

12.1.6 Mediators and Enzymes Involved in the Pathogenesis of Periodontal Disease

12.1.6.1 RANKL/RANK/OPG System

The receptor activator of nuclear factor "kappa-light-chain-enhancer" of the activated B cell ligand (RANKL)/receptor activator of NF-kB (RANK)/osteoprotegerin (OPG) system (*RANKL/RANK/OPG system)* is regulating the coupling of bone formation and resorption. RANKL, a member of the tumor necrosis factor (TNF) family, induces osteoclastogenesis by binding to its receptor RANK on osteoclast precursors. This step is promoted by macrophage colony-stimulating factor

(M-CSF), which is stimulating RANK expression on osteoclast precursors. The activation of RANK by RANKL is essential for osteoclast formation, differentiation, and activity. The soluble counterpart OPG, expressed by osteoblasts, acts as a decoy receptor binding to RANKL and thereby can inhibit the binding of RANKL to RANK (Boyle et al. 2003).

During periodontal disease, elevated levels of RANKL and/or decreased levels of OPG, resulting in a RANKL/OPG ratio that favors bone resorption, are observed (Bartold et al. 2010; Cochran 2008; Crotti et al. 2003). For example, in a preclinical trial on experimental periodontitis in mice, the expression level of RANKL correlated well with the level of various pro-inflammatory cytokines, i.e., interleukin (IL)-1, TNF-α, and interferon (IFN)-γ, during active bone loss (Garlet et al. 2006). Elevated levels of RANKL are also found in active periodontal lesions in patients, i.e., sites showing progressive alveolar bone loss; in contrast, no remarkable change from the physiological RANKL/OPG ratio was found in samples of gingivitis sites, i.e., sites without alveolar bone loss (Menezes et al. 2008). Osteoblasts, dendritic cells, B and T cells, and resident cells (e.g., periodontal ligament cells, gingival fibroblasts) are considered as important sources for the elevated levels of RANKL in periodontal lesions upon pro-inflammatory stimuli and/or TLR activation (Bar-Shavit 2008; Belibasakis et al. 2007; Kawai et al. 2006).

12.1.6.2 Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are a family of calcium- and zinc-dependent proteases, which are expressed by immune cells (e.g., neutrophils, macrophages). In healthy periodontal tissues, MMPs control the physiological turnover of extracellular matrix and are regulated by their inhibitors, i.e., *tissue inhibitors of metalloproteinases (TIMPs)*, which are expressed by resident and immune cells (e.g., macrophages, fibroblasts, endothelial cells). During inflammation, presence of pro-inflammatory cytokines deregulates the MMP/TIMP balance, resulting in higher levels of MMPs and/or lower levels of TIMPs. This imbalance leads to increased degradation of periodontal soft tissues (Giannobile 2008; Verstappen and Von den Hoff 2006). Specifically, MMP-8 – being a collagenase – is strongly associated with periodontal disease; progressively increasing levels of MMP-8 are found in sites with increasing severity of disease, i.e., from healthy to gingivitis and to periodontitis. Thus MMP-8 is considered as a strong biomarker for detecting alveolar bone loss (Ebersole et al. 2013; Gursoy et al. 2013; Leppilahti et al. 2014).

12.1.6.3 Pro-inflammatory Interleukins

IL-1 is a strict pro-inflammatory key cytokine expressed by resident (e.g., fibroblasts, epithelial cells) and immune cells (e.g., neutrophils, macrophages). IL-1 stimulates the expression of other pro-inflammatory and chemotactic factors (e.g., IL-6, TNF-α, prostaglandins) and promotes osteoclastogenesis by upregulating RANKL expression. Further, IL-1 enhances matrix degradation by inducing MMPs and impairs the regenerative potential of the tissues by inducing apoptosis of matrix producing cells (Cochran 2008; Garlet et al. 2006; Graves 2008; Graves and Cochran 2003; Wei et al. 2005). Several studies have shown increased levels in gingival

crevicular fluid, saliva, and periodontal tissue samples of periodontitis patients (Duarte et al. 2007; Ebersole et al. 2013; Gamonal et al. 2000; Gursoy et al. 2009; Mogi et al. 1999).

IL-2 is a pro-inflammatory cytokine and mainly expressed by Th1 cells. IL-2 affects the differentiation, growth, and activation of T, B, and natural killer cells (Kuziel and Greene 1990). Studies have reported both unaltered and increased levels of IL-2 in chronic periodontitis patients compared to healthy controls (Teles et al. 2009; Tymkiw et al. 2011).

IL-6 is another pro-inflammatory key cytokine, which is expressed by various resident and immune cells (e.g., fibroblasts, neutrophils, macrophages). IL-6 stimulates the expression of other pro-inflammatory factors and promotes – in a similar way as IL-1 – soft and hard tissue degradation by upregulating RANKL and MMP expression and inducing apoptosis of matrix producing cells (Cochran 2008; Garlet et al. 2006; Graves 2008; Graves and Cochran 2003; Wei et al. 2005). However, while IL-1 is a strictly pro-inflammatory cytokine, IL-6 has also a regulatory function; IL-6 was shown to regulate IL-1 and TNF-α by inducing IL-1 receptor antagonist and TNF-α soluble receptor (Irwin and Myrillas 1998). Several studies have shown increased levels of IL-6 in fluid and tissue samples of periodontitis patients (Duarte et al. 2007; Ebersole et al. 2013; Gamonal et al. 2000; Gursoy et al. 2009; Mogi et al. 1999).

IL-8 is a chemokine and secreted by monocytes, macrophages, fibroblasts, keratinocytes, and endothelial cells in the presence of microorganisms and related toxins. Its main function is to attract and activate neutrophils (Bickel 1993). Studies have reported significantly increased levels of IL-8 in periodontally diseased sites (Ertugrul et al. 2013; Gamonal et al. 2000).

IL-12, mainly expressed by immune cells (e.g., dendritic cells, macrophages, neutrophils), is a key mediator of cell-mediated immunity, necessary for the initiation and maintenance of Th1 cell response, including high IFN-γ expression (Park and Scott 2001). The levels of IL-12 in gingival crevicular fluid samples may not always differ significantly between periodontally diseased and healthy patients, but it has been shown that a significant reduction of IL-12 levels occurred after initial treatment in periodontitis patients (Thunell et al. 2010).

IL-17 is a pro-inflammatory cytokine secreted by Th17 cells and by various other cell types (e.g., neutrophils, dendritic cells, periodontal ligament cells). IL-17 increases the levels of MMPs and RANKL and amplifies the pro-inflammatory loop of IL-1−IL-6 and TNF-α (Cardoso et al. 2008; Cheng et al. 2014; Ford et al. 2010; Kotake et al. 1999; Sato et al. 2006). Further, IL-17 plays a role in the defense against pathogens by increasing mobilization and activation of neutrophils (Yu et al. 2007) and by improving the responsiveness of TLR in human gingival epithelial cells (Beklen et al. 2009). Indeed, induced *P. gingivalis* infection resulted in increased alveolar bone loss in IL-17 receptor knockout mice due to an impaired immune response (Yu et al. 2007). *P. gingivalis* is considered to promote a Th17 cell response with the corresponding IL-17 expression, and indeed, increased levels of IL-17 have been described in periodontally diseased subjects (Cheng et al. 2014).

IL-18 is a pro-inflammatory cytokine, expressed by immune cells, osteoblasts, and fibroblasts, and upregulates other pro-inflammatory and chemotactic mediators (e.g., IL-1, TNF-α, IL-8). Significantly higher levels of IL-18 have been shown in periodontitis patients compared to healthy controls (Banu et al. 2015; Ozçaka et al. 2011).

IL-23 is expressed by monocytes, macrophages, and dendritic cells and promotes Th17 cell differentiation. Progressively increased levels of IL-23 in the gingival crevicular fluid from healthy to gingivitis and to periodontitis sites have been reported (Gonzales 2015; Himani et al. 2014).

IL-33 is primarily expressed intracellularly, e.g., in monocytes and endothelial and epithelial cells. It is assumed that IL-33 release after cell necrosis represents an alarming signal for cell damage and, hence, causes a pro-inflammatory immune response and cytokine production (Moussion et al. 2008; Nile et al. 2010). In human gingival tissue biopsies, the levels of IL-33 were increased compared to healthy tissue samples, and in a preclinical trial on experimental periodontitis, IL-33 expression was increased in the presence of bacterial infection and mediated bone loss via the RANKL system (Malcolm et al. 2015).

12.1.6.4 Anti-inflammatory Interleukins

IL-4 is expressed by Th2 cells and at the same time also promotes Th2 cell differentiation; further, IL-4 promotes B cell activation, differentiation, and antibody production (Appay et al. 2008; Cronstein 2007). IL-4 exerts its anti-inflammatory and antiresorptive action by decreasing IFN-γ, MMP, and RANKL expression and by elevating IL-10, TIMPs, and OPG levels (Appay et al. 2008; Garlet et al. 2006; Ihn et al. 2002; Jarnicki and Fallon 2003; Saidenberg-Kermanac'h et al. 2004). Indeed, lower IL-4 levels have been recorded in gingival crevicular fluid of periodontally diseased patients compared to those in healthy controls (Pradeep et al. 2008).

IL-10 is an anti-inflammatory cytokine, which is expressed by various cell types (e.g., T and B cells, macrophages, dendritic cells). IL-10 reduces the activity of pro-inflammatory cytokines (e.g., IL-1−IL-17, IFN-γ) (Jovanovic et al. 1998; Naundorf et al. 2009) and upregulates TIMPs and OPG, thereby reducing soft and mineralized periodontal tissue destruction (Garlet et al. 2004, 2006; Zhang and Teng 2006). IL-10 knockout mice presented a remarkably higher susceptibility against *P. gingivalis* (Sasaki et al. 2004) and a reduced expression of osteoblast and osteocyte markers (Claudino et al. 2010), which indicates that, in addition to its anti-inflammatory and antiresorptive properties, IL-10 appears to also have a direct positive effect on bone formation. In the clinic, patients with a polymorphism reducing IL-10 mRNA transcription showed also reduced TIMP-3 and OPG transcription (Claudino et al. 2008), and therefore IL-10 polymorphism has been suggested as risk factor for chronic periodontitis (Zhong et al. 2012).

IL-13, expressed mainly by Th2 cells, exerts its anti-inflammatory action by downregulating inflammatory cytokine production in monocytes and supports B cell activation and antibody production (Abbas et al. 1996; de Waal Malefyt et al. 1993). During periodontal disease, IL-13 appears to correlate with IL-4 levels but shows in general higher levels (Johnson and Serio 2007).

12.1.6.5 TNF-α

TNF-α is expressed by various resident and immune cells and is, next to IL-1 and IL-6, a pro-inflammatory key cytokine in periodontitis pathogenesis. IL-1, IL-6, and TNF-α seem to somehow complement each other during inflammatory alveolar bone loss, since blocking of all three cytokines is shown to result in a higher inhibition of alveolar bone loss compared to single cytokine blocking (Graves 2008; Zwerina et al. 2004). TNF-α has the potential to induce osteoclastogenesis even in the absence of RANKL and also to diminish bone formation by inhibiting differentiation of osteoblast precursors and proliferation of mature osteoblasts (Bartold et al. 2010; de Vries et al. 2016; Graves et al. 2011; Graves and Cochran 2003; Lacey et al. 2009; Tomomatsu et al. 2009). Progressively increasing gingival crevicular fluid levels of TNF-α from periodontally healthy to gingivitis and to periodontitis patients have been reported (Ertugrul et al. 2013).

12.1.6.6 IFN-γ

IFN-γ is expressed by Th1 cells and natural killer cells and exerts a strong pro-inflammatory action by attracting and enhancing phagocyte activity and by inducing expression of pro-inflammatory cytokines and chemokines. Although it has been shown in vitro that IFN-γ inhibits osteoclastogenesis, due to its strong pro-inflammatory action, i.e., elevation of IL-1, TNF-α, RANKL levels, this direct anti-osteoclastogenic effect is overcome in vivo, resulting altogether in alveolar bone loss (Gao et al. 2007; Garlet et al. 2008; Gemmell and Seymour 1994; Ji et al. 2009). Yet, IFN-γ plays also an important role in the defense mechanism against infections by activating cytotoxic CD8$^+$ T cells and natural killer cells; e.g., it has been shown that IFN-γ knockout mice presented severe defense impairment against *A. actinomycetemcomitans* (Garlet et al. 2008). In preclinical and clinical trials, increasing levels of IFN-γ correlated well with increasing severity of periodontal lesions (Garlet et al. 2003; Honda et al. 2006; Isaza-Guzmán et al. 2015; Teng et al. 2005).

12.1.6.7 TGF-ß

Transforming growth factor beta (TGF-ß) is part of the TGF-ß superfamily that represents a variety of proteins, including the bone morphogenetic proteins. TGF-ß is secreted by a variety of cells (e.g., Treg, macrophages, neutrophils) and can regulate the immune response by reducing pro-inflammatory cytokine (e.g., IL-1, TNF-α) and tissue-degrading enzyme (e.g., MMPs) expression; additionally, TGF-ß is considered as an important factor for wound healing. High levels of TGF-ß have been found in periodontally inflamed tissues, which might be indicative of the constant wound healing response of the host (Mize et al. 2015; Steinsvoll et al. 1999).

12.1.6.8 Complement System

The *complement system* consists of more than 40 proteins and can be activated by three different pathways, which converge with cleavage of the complement component 3 (C3) into C3a and C3b. C3a is attracting leukocytes and C3b binds covalently to target surfaces to make the pathogens more susceptible to phagocytosis by leukocytes, i.e., "complement opsonization." Although the complement system supports

the immune system, continuous complement activation can be destructive for the host tissue by indirectly inducing alveolar bone loss (Damgaard et al. 2015). In particular, the membrane attack complex (C5b-9) can lead to activation of phospholipase A2, release of arachidonic acid, and synthesis of prostaglandin E2, which is a very potent osteolytic substance (Klein and Raisz 1970). Further, neutrophils and monocytes express the complement receptor 3, which is strongly activated by *P. gingivalis*; complement receptor 3 activation increases phagocyte recruitment and pro-inflammatory cytokine expression (e.g., IL-1, IL-6, TNF-α) causing increased alveolar bone loss.

12.1.6.9 Lipid Mediators

Arachidonic acid is released by phospholipase A2 from membrane phospholipids and further processed to either pro- or anti-inflammatory mediators. Pro-inflammatory lipid mediators, i.e., *leukotrienes* and *prostaglandins*, induce recruitment of neutrophils (e.g., leukotriene B4) and promote osteoclastogenesis and bone resorption (e.g., prostaglandin E2). Elevated levels of such lipid mediators have been observed in the gingival crevicular fluid of periodontally diseased patients (Offenbacher et al. 1986; Zhong et al. 2007).

The anti-inflammatory lipid mediators, i.e., *lipoxins*, *resolvins*, and *protectins*, are pro-resolving on the inflammation process (Garlet 2010; Graves et al. 2011). Pro-resolution is, in contrast to the passive termination of inflammation, an actively regulated process; e.g., anti-inflammatory lipid mediators were shown to regulate the migration and recruitment of neutrophils and T cells, to attenuate leukotriene B4-dependent pro-inflammatory signals, to reduce the expression of pro-inflammatory cytokines, and to promote T cell apoptosis (Serhan et al. 2008).

12.1.6.10 Nitric Oxide

Nitric oxide (NO), previously named "endothelial-derived relaxing factor," is synthesized during conversion of L-arginine to L-citrulline by NO-synthases. The endothelial and neuronal NO-synthases are constitutively expressed, whereas the inducible NO-synthases (iNOS) are expressed in neutrophils, macrophages, and/or gingival tissue upon pro-inflammatory cytokine expression and/or in the presence of bacterial products. NO has an important role in vascular regulation, platelet aggregation, regulation of mineralized tissue, and the pathogenesis of inflammatory diseases (Alayan et al. 2006; Daghigh et al. 2002; Matejka et al. 1998; Ugar-Cankal and Ozmeric 2006). The role of NO is most likely dual; on the one side, it is part of the defense mechanism and helps to control the bacterial attack, but on the other side, excessive amounts of NO are toxic for various cells (e.g., fibroblasts, epithelial cells) and NO is suspected to increase MMPs and reduce TIMPs (Brennan et al. 2003; Nguyen et al. 1992; Ugar-Cankal and Ozmeric 2006). Indeed, experimental periodontitis in iNOS knockout mice resulted in an elevated amount of neutrophils in the periodontal tissues and more bone, compared to that in wild-type mice (Alayan et al. 2006; Fukada et al. 2008). Further, elevated levels of iNOS have been observed in periodontally diseased tissue (Lappin et al. 2000; Matejka et al. 1998), while reduced amounts of NO_2^-, which is the stable metabolite of NO, have been found in the saliva of periodontally diseased patients (Aurer et al. 2001) (Table 12.1).

Table 12.1 Overview of mediators and enzymes involved in periodontal disease pathogenesis

Mediator/enzyme	Cell source	Main effect
Bone metabolism		
RANKL	Osteoblasts, dendritic cells, B and T cells, periodontal ligament cells, gingival fibroblasts	Induction of osteoclastogenesis
OPG	Osteoblasts	Inhibition of RANKL-RANK binding
Soft tissue metabolism		
MMP	Neutrophils, macrophages	Degradation of periodontal soft tissue
TIMP	Macrophages, fibroblasts, endothelial cells	Inhibition of MMPs
Pro-inflammatory		
IL-1	Various cells of the periodontium and immune system	Pro-inflammatory key cytokine
IL-2	Th1 cells	Activation of T and B cells and natural killer cells
IL-6	Various cells of the periodontium and the immune system	Pro-inflammatory key cytokine
IL-8	Monocytes, macrophages, fibroblasts, keratinocytes, and endothelial cells	Chemotaxis on neutrophils
IL-12	Cells of the immune system	Promotion of a Th1 cell response
IL-17	Th17 cells, neutrophils, dendritic cells, periodontal ligament cells	Pro-inflammatory cytokine and increase in tissue degradation
IL-18	Cells of the immune system, osteoblasts, fibroblasts	Pro-inflammatory cytokine
IL-23	Monocytes, macrophages, and dendritic cells	Promotion of Th17 cell differentiation
IL-33	Intracellular expression in monocytes, and endothelial and epithelial cells	Pro-inflammatory cytokine
TNF-α	Various cells of the periodontium and the immune system	Pro-inflammatory key cytokine
IFN-γ	Th1 cells, natural killer cells	Promotion of phagocyte activity and expression of pro-inflammatory cytokines
Pro-inflammatory lipid mediators	–	Promotion of osteoclastogenesis and recruitment of neutrophils
Anti-inflammatory		
IL-4	Th2 cells	Promotion of Th2 and B cell differentiation and reduction in tissue degradation
IL-10	Cells of the immune system	Anti-inflammatory key cytokine

Table 12.1 continued

Mediator/enzyme	Cell source	Main effect
IL-13	Th2 cells	Anti-inflammatory effect on monocytes and promotion of B cell differentiation and antibody production
TGF-ß	Treg, macrophages, neutrophils	Reduction of pro-inflammatory cytokines and tissue-degrading enzymes
Anti-inflammatory lipid mediators	–	Resolution of inflammatory process
Control of bacterial attack		
Complement system	Monocytes/macrophages, fibroblasts, epithelial and endothelial cells, osteoblasts	C3a – attraction of leukocytes; C3b – "complement opsonization"
NO	Neutrophils, macrophages, gingival tissue	Control of the bacterial attack; toxic effect on various cells and promotion of soft tissue degradation

C3 complement component 3, *IFN* interferon, *IL* interleukin, *MMP* matrix metalloproteinases, *NO* nitric oxide, *OPG* osteoprotegerin, *RANK* receptor activator of NF-kB, *RANKL* receptor activator of nuclear factor "kappa-light-chain-enhancer" of the activated B cell ligand, *Th* T helper cells, *TIMP* tissue inhibitors of metalloproteinase, *TGF* transforming growth factor, *TNF* tumor necrosis factor

12.1.7 Cells Involved in the Pathogenesis of Periodontal Disease

12.1.7.1 Gingival Fibroblasts and Periodontal Ligament Cells

Gingival fibroblasts and *periodontal ligament cells* constitute the main cellular component of the gingiva and periodontal ligament, respectively, and are responsible for maintenance of tissue integrity. Both cell types can be considered as multipotent cells, possessing an *osteogenic potential* (Kook et al. 2009; Lee et al. 2009; Mostafa et al. 2011; Rodrigues et al. 2007), and hence may constitute a potential cellular source during bone wound healing. However, this osteogenic potential is diminished in the presence of tissue-degrading pro-inflammatory proteases, i.e., MMPs, during chronic inflammation (Hayami et al. 2008; Joseph et al. 2010).

12.1.7.2 Osteoblasts, Osteoclasts, and Osteocytes

Osteoblasts are derived from mesenchymal cells of the bone marrow, are lining the bone surfaces, and are responsible for bone formation. *Osteoclasts* are multinucleated monocyte/macrophage lineage cells of hematopoietic origin with the capacity to resorb bone and are found close to resorption lacunae (called Howship's lacunae). *Osteocytes* are previous osteoblasts that became entrapped within the bone matrix and reside in lacunae connecting to each other via long cytoplasmic extensions. Osteocytes can regulate the activity of osteoclasts and osteoblasts, resulting in bone loss, through expression of RANKL and sclerostin; specifically, sclerostin expression reduces osteoblast differentiation (Kim et al. 2014, 2015).

Osteoblast and osteoclasts are controlling together bone remodeling and homeostasis, i.e., the lifelong constant coupling of bone formation and resorption (Fuller et al. 1998; Lacey et al. 1998; Quinn et al. 1998; Yasuda et al. 1998). As mentioned earlier, differentiation and activation of osteoclasts are regulated by the RANKL/RANK/OPG system in combination with M-CSF. In periodontitis, osteoclastogenesis is constantly upregulated due to a high RANKL expression by resident and immune cells. Indeed, RANKL levels have been shown to correlate well with the cathepsin K levels; cathepsin K is a protease expressed by osteoclasts and is responsible for degradation of the organic matrix of bone. In addition, LPS from periodontal pathogens seems to impair osteoblastic cell differentiation partially due to TNF-α upregulation (Kadono et al. 1999; Roberts et al. 2008; Tomomatsu et al. 2009; Wang et al. 2010).

12.1.7.3 Neutrophils

Neutrophils (syn. polymorphonuclear leukocytes) differentiate in the bone marrow before entering the blood flow and are the most abundant leukocytes in the blood, accounting for about two thirds of all blood leukocytes. This constant mature state of neutrophils in the blood circulation allows an immediate immune response whenever and wherever necessary; if bacterial invasion occurs, neutrophils can exit the blood flow and migrate into the affected tissue following a chemical gradient, i.e., chemotaxis. In the periodontium, neutrophils enter the tissues constantly from the terminal blood circulation and exit into the gingival crevicular fluid, where they form a "defense wall" against potential invaders.

Neutrophil involvement in the pathogenesis of periodontal disease may be considered as either *hyporesponsive* or as *hyperresponsive*. Hyporesponsive neutrophils (e.g., due to defects in chemotaxis, transendothelial migration, or phagocytosis) weaken the first host defense mechanism and the bacterial attack might overwhelm host immunity. In contrast, an intense response of hyperresponsive neutrophils not only degrades the microbial invaders but also causes collateral tissue damage. A hyperresponsive neutrophil response is characterized by excessive release of toxic products, an elevated oxidative burst, and increased secretion of degrading enzymes (e.g., MMPs) (Gustafsson et al. 2006; Nussbaum and Shapira 2011; Ryder 2010; Shaddox et al. 2010).

Further, neutrophils can interfere directly and indirectly with the bone metabolism, being a source of pro-inflammatory cytokines (e.g., IL-1, TNF-α) that further upregulate the immune response and cause an increased degradation of soft and mineralized periodontal tissue. Neutrophils also promote the adaptive immune response via chemotactic effects on Th17 cells, which in turn release high amounts of the pro-inflammatory cytokine IL-17 (Pelletier et al. 2010). In addition, activated neutrophils can express – but not secrete – on their cell surface RANKL; hence, in close proximity to osteoclast precursors, they can directly activate and promote osteoclastogenesis (Bloemen et al. 2010; Chakravarti et al. 2009).

12.1.7.4 Monocytes

Monocytes are myeloid cells of the hematopoietic system, which migrate from the bone marrow with the blood stream in a quite immature state that enables them to further differentiate according to given requirements. For example, monocytes can enter the tissue and differentiate into *macrophages*, which are typical phagocytosing

cells. Macrophages can kill pathogens by producing antimicrobial substances (e.g., myeloperoxidase, reactive oxygen species, reactive nitrogen species) and can ingest, process, and present antigens to T cells. Further, macrophages are contributing to inflammatory tissue damage by secretion of pro-inflammatory cytokines (e.g., IL-1, TNF-α). Another possible differentiation pathway of monocytes – in the presence of M-CSF and RANKL – is into osteoclasts, and thereby they are also contributing to bone resorption (Bar-Shavit 2008; Faust et al. 1999; Massey and Flanagan 1999).

Interestingly, monocytes from periodontally diseased patients may present distinct differences comparing to mononuclear cells from periodontally healthy individuals. Specifically, in vitro studies suggest that a *hyper-reactive phenotype* may exist in periodontitis patients, which upon stimulation with bacteria or LPS releases increased amounts of pro-inflammatory cytokines and generates more oxygen radicals comparing to monocytes from healthy subjects (Gustafsson et al. 2006). Further, discrepancies in *peripheral osteoclastogenesis* have been reported, with monocytes from periodontitis patients spontaneously forming osteoclasts without stimulation with M-CSF and/or RANKL. Hence, it has been suggested that the priming of the osteoclast precursors in periodontally diseased patients may already take place – at least partly – in the peripheral blood (Brunetti et al. 2005; Tjoa et al. 2008).

12.1.7.5 Dendritic Cells

Peripheral *dendritic cells* are professional antigen processing and presenting cells; after antigen incorporation, they prime naive T cells in the lymph nodes (Wolff 1972), and this naive T cell stimulation is promoted in the presence of RANKL (Anderson et al. 1997). In the presence of RANKL and M-CSF, dendritic cells differentiate into mature osteoclasts, while in the absence of RANKL, they differentiate into mature dendritic cells and support the adaptive immunity by processing and presenting antigens. However, once differentiated into mature dendritic cells, they do not posses any longer their osteoclastogenic potential (Liu et al. 2010).

Langerhans cells are dendritic cells located above the basal layer of epithelial cells, e.g., in the skin and oral mucosa. Increased numbers of Langerhans cells have occasionally been observed in the gingiva of periodontitis patients, comparing to what observed in healthy individuals (Ford et al. 2010).

12.1.7.6 Natural Killer Cells

Natural killer cells are a subset of lymphocytes and play an important role in the innate immunity. Natural killer cells express primarily IFN-γ but exhibit additional antimicrobial action by expression of defensins, which are specific antimicrobial peptides. Interestingly, tissue infiltration with activated natural killer cells has been observed in AgP but not CP lesions (Nowak et al. 2013). It has been suggested that this difference is due to the differences among the infecting periodontal pathogens. Specifically, infection with *A. actinomycetemcomitans*, which is characteristically present in localized AgP, induces via dendritic cells activation of natural killer cells and subsequent IFN-γ expression, while infection with *P. gingivalis*, which is a key periodontal pathogen in CP, does not (Chalifour et al. 2004; Gonzales 2015; Kikuchi et al. 2004; Nowak et al. 2013).

12.1.7.7　T Cells

T cells are part of the adaptive immunity, derive from the hematopoietic stem cells in the bone marrow, but migrate and mature in the thymus. They can be subdivided based on the expression of the co-receptors CD8 or CD4. *CD8+ T cells*, also called cytotoxic T cells, control intracellular antigens (e.g., viruses) and may play a role in periodontitis in the presence of coinfection with herpes virus; recognition of herpes virus by the immune system induces a strong CD8^{+} T cell activation and mobilization (Gonzales 2015; Slots 2010).

　　CD4+ T cells, also called Th cells, primarily assist other cells of the immune system in their functions; for example, they assist in B cell maturation. CD4^{+} T cells differentiate further depending on the presence of specific cytokines (Mosmann et al. 1991); while high IL-12 levels favor *Th1 cell* development, high IL-4 levels promote *Th2 cell* differentiation. Th1 and Th2 cells can, in turn, be distinguished by differences in their cytokine expression pattern. Th1 cells secrete mainly IFN-γ, IL-2, IL-12, and TNF-α and initiate a cellular and pro-inflammatory immune response characterized by presence of phagocytosing cells (Gonzales 2015), while Th2 cells appear to have a more protective function by expressing IL-4, IL-5, IL-9, IL-10, and IL-13 and promoting B cell activation, differentiation, and antibody production (Appay et al. 2008; Cronstein 2007; Gonzales 2015; Murphy and Reiner 2002). In addition to Th1 and Th2 cells, *Th17* and *Treg cells* have been detected in periodontally diseased tissues (Cardoso et al. 2008; Gaffen and Hajishengallis 2008; Gonzales 2015; Teng 2006b). Th17 cells differentiate in the presence of IL-23 and express IL-6 and mainly IL-17, which – as already described – exerts a strong pro-inflammatory immune response (Cardoso et al. 2008; Cheng et al. 2014; Ford et al. 2010; Kotake et al. 1999; Sato et al. 2006; Yu et al. 2007). In contrast, *Treg* develops in the presence of TGF-ß and IL-2 and they have a protective role by decreasing periodontal disease progression through expression of IL-10 and TGF-ß (Cardoso et al. 2008; Gonzales 2015).

12.1.7.8　B Cells

B cells belong to the adaptive immunity and they have two main functions: (a) they recognize antigens by using a high-affinity receptor and then process and present them to CD4^{+} and CD8^{+} T cells (Gonzales 2015) and (b) they produce and secret antibodies for opsonization of invading pathogens.

　　In the gingival tissue in periodontitis lesions, B and T cells are the predominant mononuclear cell types, with the number of B cells regularly exceeding that of T cells. B cells exert a dual role in periodontal disease progression; they have a major protective role through opsonization of periodontal pathogens, thus facilitating phagocytosis by neutrophils and macrophages, and through activation of the complement system (Guentsch et al. 2009; Teng 2006a). For example, *A. actinomycetemcomitans* can only be controlled by neutrophils after opsonization by IgG, while other opsonizing factors (e.g., C3b, LPS-binding proteins, other Ig isotypes) appear not effective against it (Guentsch et al. 2009; Teng 2006a). In a longitudinal human trial, patients with active periodontal lesions presented lower serum levels of IgG against *A. actinomycetemcomitans*

and *P. gingivalis* compared to patients that were periodontally stable within a regular maintenance program (Rams et al. 2006). However, B cells appear to play also a major role in alveolar bone loss in periodontitis by expression of RANKL after stimulation by periodontal pathogens (Cochran 2008; Han et al. 2009; Han et al. 2006; Kawai et al. 2006); indeed, B cell deletion resulted in remarkably reduced alveolar bone loss (Baker et al. 2009). In addition, autoreactive B cells, that produce antibodies against host tissue components (e.g., collagen, keratin), have also been detected in tissue samples from periodontitis sites (Donati et al. 2009; Koutouzis et al. 2009).

Interestingly, mice severely affected by a combined immunodeficiency resulting in complete deletion of B and T cells experience less bone loss after challenge with *P. gingivalis*, comparing with non-immunodeficient controls, which indicates that lymphocytes may not be indispensable in infection control, but they play a major role in alveolar bone loss (Baker et al. 1999) (Table 12.2).

12.1.8 Periodontal Treatment

12.1.8.1 Standard Treatment

Cause-related periodontal treatment consists of *oral hygiene instructions* aiming at the efficient daily removal of the accumulating biofilm from the teeth surfaces and of the *mechanical removal of the infectious agents* already accumulated on the root surface, i.e., scaling and root planing. The mechanical removal of the biofilm can be combined with the administration of local or systemic use of chemotherapeutic agents (e.g., rinsing solutions based on chlorhexidine, hyaluronan, essential oils; antibiotics). In cases where nonsurgical treatment does not result in disease resolution, periodontal surgery is performed to achieve better access to the root surfaces for efficient mechanical instrumentation. After efficient control of the infection and establishment of inflammation-free tissues, regular maintenance appointments, including mechanical biofilm removal and reinforcement of oral hygiene attitudes, are essential for long-term stability of treatment and for prevention of disease recurrence (Bertl et al. 2015; Deas and Mealey 2010; Zandbergen et al. 2013).

12.1.8.2 "Osteoimmunological Targets" in Periodontal Treatment

Although it is nowadays recognized that the host immune response is the major cause for periodontal tissue destruction, routine periodontal treatment aims primarily to control the bacterial invasion. In periodontitis, unlike in systemic conditions like osteoporosis or rheumatoid arthritis, treatment aiming at *interfering with the host's immune system* (e.g., by applying anti-inflammatory or antiresorptive agents) is associated with two major concerns: firstly, any (severe) side effects of systemically administered medications might not justify the possible – most likely small – additional benefit compared to what achieved with standard periodontal treatment alone and secondly any (partial) blocking of the immune system may result in inefficient immune response against the bacterial attack.

Table 12.2 Overview of the dual role of resident and immune cells in periodontal disease pathogenesis

Cell type	Protective aspect	Destructive aspect
Resident cells		
Gingival fibroblasts	Osteogenic potential	Source of pro-inflammatory cytokines and RANKL
Periodontal ligament cells	Osteogenic potential	Source of pro-inflammatory cytokines and RANKL
Osteoblasts	Bone formation	Source of RANKL
Osteoclasts	–	Bone resorption
Osteocytes	Regulator of bone homeostasis	Source of RANKL and sclerostin
Innate immunity		
Neutrophils	"Defense wall"	Source of pro-inflammatory cytokines and RANKL; causing collateral tissue damage
Monocytes	Differentiation into macrophages	Differentiation into osteoclasts
Macrophages	Phagocytosis of pathogens and antigen presentation to T cells	Source of pro-inflammatory cytokines; causing collateral tissue damage
Dendritic cells	Antigen presentation	Differentiation into osteoclasts
Natural killer cells	Expression of antimicrobial defensins	IFN-γ expression
Adaptive immunity		
Th1 cells	Increase in phagocyte activity and in vitro inhibition of osteoclastogenesis via IFN-γ	Expression of IFN-γ, IL-2, IL-12, and TNF-α and promotion of expression of other pro-inflammatory cytokines
Th2 cells	Expression of IL-4, IL-5, IL-9, IL-10, and IL-13 and promotion of B cell activation	Strong Th2 response favors RANKL expressing B cells
Th17 cells	Activation of neutrophils	Expression of IL-6 and IL-17 and promotion of expression of other pro-inflammatory cytokines
Treg cells	Expression of IL-10 and TGF-ß	–
B cells	Production of antibodies	Source of RANKL and possibility of auoantibodies

IFN interferon, *IL* interleukin, *RANKL* receptor activator of nuclear factor "kappa-light-chain-enhancer" of the activated B cell ligand, *Treg* regulatory T cells, *Th* T helper cells, *TGF* transforming growth factor, *TNF* tumor necrosis factor

In the following, a brief overview of major new therapeutic targets considered in periodontitis treatment is discussed (for summary, see Table 12.3).

Doxycycline

Doxycycline delivered in *subantimicrobial doses* (e.g., 20 mg doxycycline twice daily for 3 months) has been shown to downregulate MMP activity and, more recently, also pro-inflammatory cytokine production, i.e., IL-1, IL-17, and TNF-α. A systematic review of the literature and meta-analysis have confirmed that there

Table 12.3 Overview of potential osteoimmunological targets for periodontitis treatment, evaluated in preclinical and/or clinical studies

Target	Agent	Mode of action
Preclinical trials		
RANKL/RANK/OPG system	OPG/OPG analogues	Blockade of RANKL-RANK binding
–	Lipid mediators	Pro-resolving and anti-inflammatory
Sclerostin	Sclerostin antibody	Improved bone formation
Osteoclasts	Bisphosphonates	Reduced osteoclast activity and bone turnover
Cathepsin K	Cathepsin K inhibitors	Reduced bone loss
iNOS	iNOS inhibitors	Reduced NO production
Mitogen-activated protein kinases	Mitogen-activated protein kinases inhibitors	Anti-inflammatory and antiresorptive
–	Melatonin	Antioxidant and free radical scavenger
Angiotensin II receptor	Angiotensin II receptor blocker	Anti-inflammatory
Clinical trials		
MMPs	Doxycycline	Inhibition of MMPs
TNF-α	TNF-α-antagonists	Anti-inflammatory and antiresorptive
–	Statins	Anti-inflammatory and antiresorptive
–	Vitamin D and calcium	Regulation of bone homeostasis and immune system

iNOS inducible NO-synthases, *MMP* matrix metalloproteinases, *NO* nitric oxide, *OPG* osteoprotegerin, *RANK* receptor activator of NF-kB, *RANKL* receptor activator of nuclear factor "kappa-light-chain-enhancer" of the activated B cell ligand, *TNF* tumor necrosis factor

seems to be a significant improvement of clinical periodontal parameters after non-surgical periodontal treatment with adjunctive delivery of doxycycline in subantimicrobial doses compared to nonsurgical treatment alone (Castro et al. 2016; Sgolastra et al. 2011).

Statins
Statins (e.g., simvastatin, atorvastatin) are widely used to control lipid metabolism by reducing serum cholesterol levels. Additionally, they appear to have anti-inflammatory and antiresorptive properties. Clinical trials have reported a significant positive effect of locally or systemically administered statins as adjunct to nonsurgical periodontal treatment, primarily in terms of posttreatment alveolar bone levels but also regarding the clinical periodontal parameters (Fajardo et al. 2010; Pradeep et al. 2012; Pradeep et al. 2013; Pradeep et al. 2015).

TNF-α Antagonists
TNF-α antagonists are used in the treatment of rheumatoid arthritis. Preclinical trials evaluating the effect of TNF-α antagonists in experimental periodontitis have shown

reduced neutrophil infiltration and periodontal inflammation levels (Di Paola et al. 2007; Gonçalves et al. 2014). However, reduced neutrophil infiltration may impair pathogen clearance. For example, increased *A. actinomycetemcomitans* load and inflammatory marker levels have been detected in a TNF-α knockout mouse model; in particular, knockout mice presented reduced activation, migration, and phagocytosing activity of neutrophils and macrophages in periodontal lesions (Garlet et al. 2007). In a case-control study, however, patients with rheumatoid arthritis taking TNF-α-antagonists presented better periodontal indices and lower gingival crevicular fluid levels of TNF-α, compared to patients taking another medication (Mayer et al. 2009).

Lipid Mediators

Pro-resolving *lipid mediators*, i.e., lipoxins, resolvins, and protectins, as adjunct to periodontal therapy, would theoretically reduce the amount of tissue degradation by actively promoting inflammation resolution. Indeed, the extent of tissue breakdown during experimental periodontitis and disease resolution was significantly improved in preclinical trials involving local application of resolvins (Hasturk et al. 2007; Van Dyke et al. 2015).

OPG

Systemic and local administrations of *OPG* or *OPG analogues* in experimental periodontitis models have shown significant alveolar bone loss prevention, due to blocking RANKL binding to RANK and, hence, interfering with the process of osteoclastogenesis (Jin et al. 2007; Lin et al. 2011; Tang et al. 2015; Teng et al. 2000). However, systemic long-term administration of a RANKL inhibitor may entail unwanted systemic side effects on physiologic bone turnover and on the immune system, considering the fact that RANK is not only expressed on osteoclasts and their precursors but also on monocytes/macrophages and dendritic cells (Ferrari-Lacraz and Ferrari 2011; Kong et al. 1999).

Sclerostin Antibody

In a preclinical trial in rats, administration of a *sclerostin antibody* significantly improved bone healing in periodontal defects, by blocking the inhibitory effect of sclerostin on osteogenesis (Taut et al. 2013).

Bisphosphonates

Bisphosphonates reduce osteoclast activity and thereby the amount of bone resorption and are regularly used in the treatment of osteoporosis or tumor-associated osteolysis. Systemic application of bisphosphonates appears not applicable in periodontitis treatment, due to the interference with the physiologic bone turnover, but also because it might cause severe side effects, i.e., bisphosphonate-related osteonecrosis of the jaw; in fact, periodontitis itself appears as risk factor for developing bisphosphonate-related osteonecrosis of the jaw. Yet, it has been demonstrated in preclinical trials of experimental periodontitis models that local administration of bisphosphonates results in reduced alveolar bone destruction and in enhanced alveolar bone regeneration (De Almeida et al. 2015; Furlaneto et al. 2014; Thumbigere-Math et al. 2014).

Vitamin D and Calcium

Vitamin D plays important role in bone homeostasis and also exerts additional immune regulatory properties; for instance, vitamin D deficiency is associated with reduced calcium levels in the bone and bone volume loss. Indeed, a moderate positive effect of *vitamin D* and *calcium* supplement intake has been described in cross-sectional and cohort studies (Garcia et al. 2011; Miley et al. 2009). However, there are currently no randomized controlled clinical trials on vitamin D and calcium supplementation during nonsurgical periodontal treatment.

Cathepsin K Inhibitors

Cathepsin K inhibitors are considered as promising drugs in osteoporosis treatment. Recently, *inhibition of cathepsin K* was shown to reduce the extent of the immune response and of alveolar bone loss in experimental periodontitis in mice (Bartold et al. 2010; Hao et al. 2015).

iNOS Inhibitors

iNOS inhibitors (e.g., mercaptoethyl guanidine, aminoguanidine) are shown in vitro to reduce NO production by human gingival fibroblasts, while, in an experimental periodontitis model, they decreased the level of periodontal inflammation and bone loss (Chang et al. 2014; Daghigh et al. 2002; Di Paola et al. 2004; Lohinai et al. 1998).

Mitogen-Activated Protein Kinase

Mitogen-activated protein kinases are intracellular molecules involved in signal transduction during inflammation and production of pro-inflammatory cytokines. Administration of *mitogen-activated protein kinases inhibitors* in an experimental periodontitis model reduced the inflammatory response and thereby also the alveolar bone loss (Kirkwood et al. 2007; Rogers et al. 2007).

Melatonin

Melatonin, a hormone primary involved in the control of the circadian rhythm, acts also as an antioxidant, immune modulator, and free radical scavenger. It has been demonstrated that the levels of salivary melatonin are decreased in periodontal disease, while systemic melatonin administration during experimental periodontitis reduced the level of pro-inflammatory cytokines and the extent of alveolar bone destruction (Arabacı et al. 2015; Bertl et al. 2013; Kara et al. 2013).

Angiotensin II Receptor Blockers

Angiotensin II receptor blockers (e.g., azilsartan, olmesartan, telmisartan) exert an anti-inflammatory effect by suppressing TNF-α-induced IL-6 gene promoter activity. In an experimental periodontitis model, angiotensin II receptor blocker administration resulted in reduced tissue degradation, including reduced levels of MMPs and RANKL, increased levels of OPG, reduced levels of IL-1, TNF-α, and increased levels IL-10 cytokines (Araújo et al. 2013a, b, 2014) compared to controls (Table 12.3).

12.1.9 Summary

Periodontal disease is initiated by oral pathogens that provoke a response from the immune system, which exerts a two-sided effect: on one side, it controls the infection and protects the organism from bacterial invasion, but on the other side, it causes collateral tissue destruction within the periodontium. This dual effect is reflected in the often two-sided duties of cells and mediators involved in periodontitis pathogenesis. Interestingly, it appears that the intensity of the immune response is not the main relevant factor for periodontal infection control but is decisive for the extent of periodontal tissue destruction. Thus, the stronger the immune response is, the larger the damage caused in the periodontal tissues (Trombone et al. 2009). In contrast, a deficient immune response also leads to increased tissue damage due to failure in controlling the infection. The increased knowledge on the interactions between cells of the immune system and the resident cells in the periodontal tissues and on the involved enzymes and cytokines offers new exciting targets for the treatment of periodontal disease; yet, evaluation of most of these targets is still on the preclinical level (Fig. 12.6).
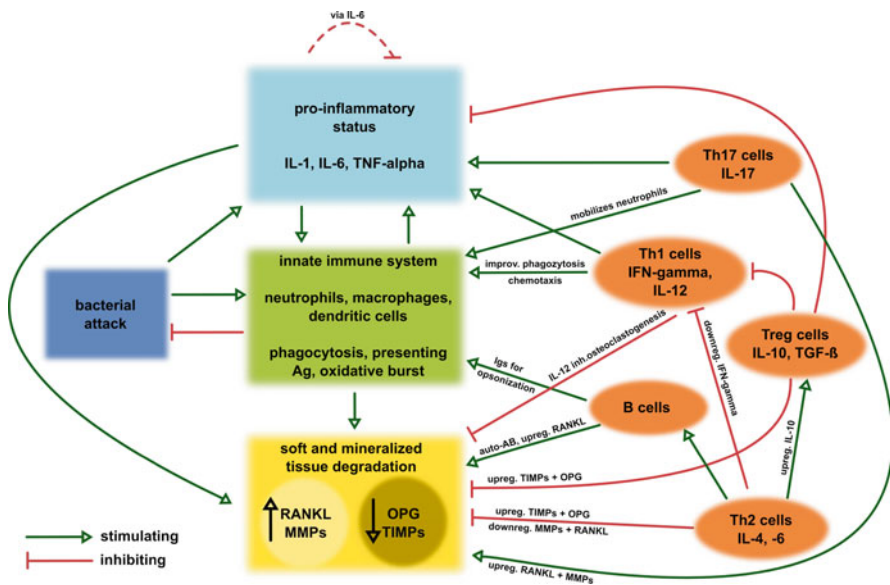

Fig. 12.6 Graphic presentation of the enzymes, mediators, and cells involved in periodontitis pathogenesis, including their interactions and their effects on the soft and mineralized periodontal tissues

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