

# Systemic inflammatory host response to periodontopathogenic bacteria in the oral cavity: from experimental to clinical studies

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**Summary:** Periodontal diseases are one of the most common complex infections in humans and many other mammals. Infectious agents causing periodontitis are primarily periodontopathogenic bacteria, but disease pathogenesis and progression are immune-mediated. Local inflammation is well characterized, and experimental animal models of periodontitis have also provided mostly consistent data on systemic inflammatory host response. However, the data from the clinical studies are more variable, especially when obtained from patients post treatment. The variation observed may be a result of the model employed or bacterial species tested with a particular emphasis on mono-infections as compared to co-infections and periodontitis, and individual host response. Experimental animal models also mostly lack the variable extent, severity of periodontitis, and the truly chronic nature of the naturally occurring chronic periodontitis. These shortcomings can at least in part explain some of the discrepancy between experimental and clinical data.

**Keywords:** periodontopathogenic bacteria; periodontal diseases; systemic host response

## 1. Periodontopathogenic bacteria and periodontal diseases

Periodontal diseases are one of the most common complex infections in humans and many other mammals [1]. The general term “periodontal diseases” encompasses inflammatory alterations within the gingiva and the periodontium. Periodontitis progresses from gingivitis and indicates an irreversible stage of the periodontal diseases with loss of the attachment of the tooth [2]. Periodontal diseases result from inflammation of the supporting structures of the teeth in response to chronic infections caused by various periodontopathogenic bacteria and viruses [2-4]. The most prominent putative periodontal pathogens are the Gram-negative anaerobic species *Porphyromonas gingivalis*, *Tannerella forsythia*, *Dialister invisus/pneumosintes*, *Prevotella intermedia*, *Treponema denticola*, *Fusobacterium* species, *Campylobacter rectus*, and *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) [5]. Viruses implied in periodontal diseases in humans include Epstein-Barr virus-1, human cytomegalovirus and other herpesviruses, which may form a pathogenic consortium with subgingival bacteria, although definitive evidence lacks [1]. Periodontal pockets, a unique environment for colonizing microorganisms, contain as many as 400 species of bacteria, which are organized in biofilms [6]. Living in a biofilm community provides many advantages for oral microorganisms [7]. It has long been known, that the subgingival biofilms differ markedly in periodontal health and in periodontitis [8]. The predominant early colonizers of the subgingival plaque biofilms are the *Actinomyces* species and *Streptococci* [9], which develop a complex microbial community within only a few days [10]. A number of the secondary colonizers, in particular *F. nucleatum* and *P. gingivalis*, can bind both to early colonizers and to other, later colonizers [11]. The ability of potential periodontal pathogens to locate and attach to compatible antecedent colonizers may therefore drive the development of pathogenic subgingival plaque biofilms [12]. Odontopathic bacteria exist in saliva before colonizing dental surfaces [13]. There is a close inter-relationship between the planktonic and biofilms phases, as bacteria in saliva adhere to oral surfaces to form biofilms and biofilms release bacteria into saliva by shedding [14]. The severity of periodontal diseases may be ascertained by the salivary level of periodontal pathogens. Saygun *et al.* [15] found that *C. rectus*, *F. nucleatum*, *P. gingivalis*, *P. intermedia* and *T. forsythia* occurred with significantly higher copy-counts in salivary samples from patients with gingivitis, chronic periodontitis and aggressive periodontitis than from periodontally healthy individuals. *A. actinomycetemcomitans* only showed higher salivary copy-counts in subjects with aggressive periodontitis compared to subjects with a healthy periodontium. Therefore, the periodontopathogenic bacteria may be acquired from the infectious saliva of close family members [16-17]. Transmission of periodontal pathogens from person to person depends on the salivary load of pathogens in the donor subject and various ecological factors in the recipient [18]. Human viruses are also frequent inhabitants of the human mouth, and their presence in saliva may be caused by the direct transfer of saliva from infected individuals [13].

The clinical features of chronic periodontitis are gingival inflammation, bleeding on probing from the periodontal pocket area, reduced resistance of the periodontal tissues to probing, loss of clinical attachment and loss of alveolar bone. Chronic periodontitis is variable in that it does not affect all teeth evenly, but has both a patient and site predilection. Local and systemic risk factors may have a bearing on the rate of progression of the disease [19]. The course of the local diseases is very similar in many other mammalian species [20-22], but the bacteria naturally involved

in the initiation and progression of the diseases are much less investigated and bacterial species involved may differ significantly [23].

## 2. Systemic host response to periodontopathogenic bacteria

Periodontal diseases are infectious diseases, initiated by infectious agents, but their pathogenesis and progression are immune-mediated, and likely also genetically-dependent [2, 24]. Several mechanisms of the host organism are involved in combating bacterial infection in the oral cavity, including physical (e.g., keratinized mucosa) and chemical (e.g., salivary components) barriers, and the highly orchestrated immune system, that consists of cells (e.g., inflammatory cells, tissue resident cells, antigen-presenting cells), soluble mediators (e.g., complement factors, acute-phase proteins, cytokines, chemokines, eicosanoids) and humoral components (e.g., immunoglobulins) involved in innate and acquired immunity [2]. Although innate immunity is considered a first-line and non-specific, more and more data are suggestive of bacteria mediating inflammatory responses that involve specific innate immune pathways in defined host cells, which can impact inflammatory outcomes in chronic inflammation [25]. Polymorphonuclear cells (PMN) generally represent the first line of defense and can be continuously recovered from the gingival sulcus. They are capable of phagocytosis, exocytosis of mediators, and oxidative burst employing active substances such as myeloperoxidase [2]. Soluble effector molecules of the non-specific immunity include acute-phase proteins (APPs) and complement factors, produced mostly by the hepatocytes stimulated directly by bacteria and/or their components (e.g., lipopolysaccharide - LPS) or mediators of inflammation (e.g., TNF-alpha, IL-6) [2, 26-27]. Macrophages are phagocytes and also represent a part of non-specific immune system cellular components, but their antigen presentation capability is an important link to the specific host defense system. Macrophages also produce several bioactive substances, such as proinflammatory cytokines (e.g., TNF-alpha, IL-1, IL-6, IL-8), and oxygen and nitrogen radicals [2]. Long-lived inflammatory cells such as dendritic cells, strategically poised along portals of entry, sample the local microenvironment and interact initially with bacteria in the oral mucosa. Dendritic cells have a critical role in determining the nature of the immune reactions and in fine-tuning the balance between tolerance and the induction of inflammation [25]. Tissue resident cells (e.g., gingival fibroblasts) can also produce several mediators of inflammation if stimulated by oral biofilms [28]. Once T-cells, a part of the second line of defense (specific or acquired immunity), are activated, they also release cytokines of various subclasses to guide further specific immune response, including production of immunoglobulins and memory cells [2].

While locally periodontal diseases affect the gingiva and tooth supporting tissues, bacteremia is also a common finding. Bacteria are also swallowed and/or aspirated from the oral cavity, affecting other sites [29-32]. Remote sites can additionally be affected by bacterial LPS, antigens, other bacterial components, and cytokines absorbed or “spilled-over” at sites of infection and distributed throughout the body [24, 29, 33-34]. Systemic host response to oral exposure to periodontopathogenic bacteria is therefore expected [25] and has been evaluated in experimental animal models and clinical studies.

This review therefore aims at presenting the most recent findings on the most commonly studied systemic (non-specific) inflammatory markers and markers of nitroxidative stress in response to periodontal diseases and periodontopathogenic bacteria. The findings from experimental studies will be compared to those obtained in clinical studies.

### 2.1. Proinflammatory cytokines

#### TNF-alpha, IL-1beta and IL-6

Cytokines are small molecular weight glycopeptides that regulate all important biological processes, including immune response. As noted above, they are produced by a variety of stimulated cells, including monocytes, tissue macrophages, dendritic cells and tissue resident cells [2, 28]. Production of proinflammatory cytokines occurs in part via the Toll-like receptors (TLRs), a family of innate immune recognition receptors that detect conserved microbial patterns and endogenous ligands. These receptors, together with CD14-expressing immune cells [35], play a key role in innate immune signaling [36], leading to inflammatory responses by activating several transcription factors including nuclear factor- $\kappa$ B (NF- $\kappa$ B) [35-36]. Although most cytokines are considered to have local effects, a small group is also effective systemically (TNF-alpha, IL-1, IL-6). They act by binding to specific receptors on the target cells and have numerous roles, including chemotaxis, activation of the release of additional inflammatory mediators (e.g., prostaglandins, PGE) and tissue destructive enzymes (e.g., matrix metalloproteinases, MMPs) involved in tissue degradation in periodontitis patients [2]. They are also responsible for initiation of the acute-phase response [26-27]. During the inflammatory reaction, which is a component of innate immunity, proinflammatory cytokines (e.g., TNF-alpha, IL-1beta, IL-6, IL-8, IFN-gamma) counteract the inhibitory effect of anti-inflammatory molecules that have tissue-protective role (e.g., IL-1ra, IL-10, TGF-beta) [2]. Cytokines are not stored but rather constantly produced when cells are triggered. The production of proinflammatory cytokines is a cascade-like event (with a sequence TNF-alpha, IL-1, IL-6) [2].

Blood TNF-alpha and/or IL-1beta and/or IL-6 were found to be higher in rats with ligature-induced periodontitis [37-40], more so in females [37]. Increased blood TNF-alpha levels were also reported in a rat LPS periodontitis model [41], although LPS models may be difficult to interpret as LPS from different Gram-negative bacteria may elicit different host responses [42]. IL-6 levels were found increased in mice orally infected with *P. gingivalis* and the levels correlated with the antibody titers to *P. gingivalis* [43]. Similarly, IL-6 levels were found to correlate with clinical response to ligation in a pregnant baboon periodontitis model [44].

Human patients with periodontitis also have reportedly higher levels of blood TNF-alpha and/or IL-1beta than healthy subjects [45-47], which seems to be associated with the extent of the local tissue destruction [48-49]. Increased TNF-alpha levels were found to be associated with the presence of periodontopathogenic bacteria (*A. actinomycetemcomitans* and *P. gingivalis*) in dental plaque [46], or serum LPS levels [49], or antibody titers to *P. gingivalis* [50]. However, some studies report on lower systemic TNF-alpha levels in periodontitis patients comparing to healthy controls [51-52], which seems contradictory in view of elevated IL-6 levels found in one of these same studies [52]. There is another report on increased IL-6 levels in humans with extensive periodontal diseases [48], but the other study reports no difference in serum IL-6 levels between periodontitis patients and healthy people [51]. The differences between serum IL-6 levels found in these studies may relate to the severity of the diseases [48].

Interventional clinical trials present even more variable data. Temporary acute increase in IL-6 has been observed after the treatment [53]. Reduction in TNF-alpha and/or IL-6 was reported 3 months after periodontal treatment in healthy [54] and diabetic [55-57] or cardiovascular disease [58] patients, but not in the hemodialysis patients [59], and these observations are not consistent across the studies [52-53, 60-63]. This may reflect the number of individuals tested, study protocol employed (e.g., time until the re-check appointment) or may indicate a variable individual response to the treatment [64].

## 2.2. Acute-phase proteins

Acute-phase proteins (APPs) form part of the systemic acute-phase response, comprised of a large number of systemic manifestations, distant from the site(s) of inflammation, that are considered a cornerstone of innate non-specific immunity [26-27]. The majority of APPs are produced upon stimulation particularly by IL-6 in the liver [26-27], but some (e.g., PTX3) derive from inflammatory and other tissue resident cells [65]. Acute-phase proteins changes are not limited to the acute illness, but persist during chronic inflammatory states as well [26]. Once the initial stimulus is eliminated, the acute-phase response subsides. This phenomenon may include either an active mechanism that down-regulates the over-expressed APPs or down-regulation may result from cessation of continued stimulation. Due to the short circulating half-lives of cytokines and other mediators, blood levels of these molecules decrease rapidly [27]. Acute-phase proteins have been shown to be valuable biomarkers in diagnosis, monitoring of progression, treatment, and prognosis of diseases [26-27]. Also, all animals have demonstrable APPs, but when employing animal models, it is crucial to know that there are significant differences across species in APPs expression [26-27]. C-reactive protein (CRP) has been the most widely studied APP. Detection of this rapidly metabolized APP is considered a precise method of evaluation of acute-phase response as it directly reflects hepatic stimulation and in most inflammatory diseases relates to the extent and severity of the inflammatory process [27].

Ligature-induced periodontitis in rats has been shown to be associated with increased blood CRP [37, 66], but this was related to the female gender [37]. On the contrary, CRP was found to decrease during gingivitis and return to baseline or increase slightly during periodontitis in non-human primates with experimental periodontitis [34].

In a clinical study in dogs, CRP was found not to correlate with periodontal diseases stage at baseline, but 1 month after periodontal treatment reduced CRP levels were noted, which correlated with the severity of the initial attachment loss [67].

Human clinical studies reveal mostly consistent data - periodontal diseases are in most (although not all [48, 68]) studies reported to be associated with an increase in CRP, indicative of systemic inflammation [51-52, 69-75]. Although it would seem logical that serum CRP levels correlate with periodontal diseases severity and extent, this has only been confirmed in some [49, 76-78], but not all studies [35, 79], which is suggestive of individual host response. Some studies actually suggest that a threshold of periodontal diseases severity is required for elevating CRP in humans and non-human primates [80]. Similar to TNF-alpha levels, in humans with periodontitis, increased CRP levels were reported to be associated with the presence of periodontopathogenic bacteria (*A. actinomycetemcomitans* and *P. gingivalis*) in subgingival samples [48, 78], but independently of antibody titers to *P. gingivalis* [81].

Similar to studies on proinflammatory cytokines, interventional clinical studies reveal variable data. A temporary acute (1 day) increase in CRP is observed after the treatment, with a greater magnitude after the non-surgical than surgical phase [53, 82]. Reduction in CRP is observed 1-3 months after the treatment in healthy [52, 54, 79], diabetic [55, 62], cardiovascular disease [58, 83], and hemodialysis [84] patients, but these observations are not consistent across the studies [53, 60, 63, 82].

Fibrinogen, another APP, is also increased in rats [85] and non-human primates [34] with ligature-induced periodontitis, and humans with periodontitis [70]. In humans, this may be associated with a specific genotype [86].

Fibrinogen is not reduced 6 weeks after the treatment in one study [79], but is reported to be reduced 2-3 months after the treatment in diabetic [56] and cardiovascular disease patients [58, 83].

Among other APPs, plasma levels of serum amyloid-A (SAA) vary usually with those of CRP. Similar to CRP (both are “short” members of the pentraxin (PTX) superfamily [65]), SAA is normally present in only trace amounts, but may exhibit rapid dramatic increase in inflammation in humans [27]. Data on systemic SAA evaluation in experimental animal models with oral infection lack, however SAA was found to be increased in some periodontitis patients, but not in correlation with the disease parameters [87].

Serum amyloid-A was found to drastically increase 1 day after periodontal treatment [82], with the levels observed to be reduced 3 months after extensive periodontal treatment [87].

Changes in other APPs occur at different rates and to different degrees. With some of the APPs (i.e., negative APPs), such as albumin, transferrin and several others, a decrease is typically observed during the acute-phase response [26-27]. However, data on systemic levels in periodontal diseases are scarce in experimental and clinical studies, and include evaluation of complement components [88], coagulation and fibrinolytic system members [64], transport proteins (e.g., albumin [59], haptoglobin [34, 74, 80]), ceruloplasmin [89]), “long” PTX (e.g., PTX3) [85, 90], soluble CD14 (sCD14) [35], and LPS-binding protein [44, 53] with varying outcomes.

Erythrocyte sedimentation rate (ESR) that measures the rate at which erythrocytes fall through plasma is an indicator of the acute phase response as it depends on plasma concentration of fibrinogen and selected other APPs. It is a simple yet robust indirect method of assessing acute-phase changes [27] and has rarely been employed in studies on systemic effects of periodontal diseases, but shares similarities with CRP values before [75] and after the treatment [91].

### 2.3. Oxidative and nitroxidative stress

Reactive oxygen (ROS) and nitrogen (RNS) species are powerful oxidants produced as a response to stimulation by bacterial components or cytokines mostly, but not solely, by immune cells (e.g., PMN, macrophages) to eliminate bacteria [92-94]. Peripheral blood leukocytes were found to be increased in rats [95] and humans [75] with periodontitis, mostly due to increased number of PMN [35, 72]. Small amounts of ROS and RNS are physiological and necessary for cell function, but large amounts lead to cell damage, therefore for protection cells have developed anti-oxidant mechanisms (e.g., superoxide dismutase, glutathione peroxidase) [96]. However, during inflammation, antioxidant mechanisms may become overwhelmed or exhausted, which may lead to cells and tissue damage. Reactive oxygen species and RNS as well as antioxidants and products of nitroxidative stress (e.g., modified proteins) can be measured to provide an insight into the effects of diseases [96].

A single oral inoculation with *P. gingivalis* was reported to reduce acute systemic host response as evaluated by nitric oxide formation in mice within a day after inoculation [97]. This may be an important way for the bacterium to be initially tolerated by the host [98]. Chronic oral exposure to periodontopathogenic bacteria, even without causing periodontal disease, results, however, in an elevation of nitroxidative stress markers in rat and murine experimental models [41, 99-104].

As for CRP [67], no association was found between periodontal diseases stage and markers of nitroxidative stress in dogs [105] and cats [22] with naturally occurring periodontal diseases. However, periodontal treatment seemed to cause an increase in nitroxidative stress early (2 weeks) after the treatment in the most severely affected dogs, but the response varied greatly among individuals [105]. Treatment, however, had no impact on plasma malonyldialdehyde, a marker of oxidation, in cats [22]. Another study in dogs found significantly lower serum total antioxidant capacity levels in animals with more severe periodontitis, which could indicate consumption/sequestration of the antioxidants due to local oxidative stress during inflammation [106].

In humans, the presence and extent of periodontitis have been associated with systemic increase in markers of oxidation/oxidative stress [72-73, 107-110], while the data on serum antioxidants/antioxidant capacity vary from increased [108, 111] to not associated [109] to reduced [72-73, 112], which seems to depend also on the method used (e.g., specific antioxidant measured vs. total antioxidant potential of plasma). After causing a transitory acute increase in oxidative stress [73], periodontal treatment has been mostly reported to improve oxidative status in the medium-term (1-4 months) [108, 110-112].

## 3. Conclusion

Other molecules [44, 48, 54, 113-117] have been occasionally evaluated as markers of systemic inflammation in animals and humans with periodontal diseases, but are not focused on in this review. Also, despite being excellent for studying specific mechanisms and physiologic processes, the *in vitro* and *ex vivo* models are not included here as they are lacking complex host response [20, 118]. Periodontal diseases may increase the risk of chronic, inflammatory-based systemic diseases such as atherosclerotic and coronary disease, stroke, chronic obstructive pulmonary disease, diabetes, and hypertension [19]. While chronic low-grade inflammation may therefore be tightly associated with organ function and dysfunction and disease [24], the discussion on systemic markers of tissue damage and organ function in periodontal diseases is beyond the scope of this review.

As has been documented, a systemic inflammatory response occurs in animals and humans with periodontal diseases, although the cause-effect relationship is still poorly understood. Experimental animal models of periodontal diseases have provided important and in majority consistent data on systemic effects of periodontal diseases. However, it is sometimes difficult to directly apply the data from experimental animal models to other animal species and humans and to a clinical setting [20, 118]. Relatively little information on systemic inflammatory host response is available from experimental animal models using peroral route of infection or experimentally induced periodontal diseases. On the other hand, clinical studies, that have been employed more and more in the research on systemic effects of periodontal diseases in the recent years, seem to provide very variable data. Data from the clinical studies likely vary from those obtained in experimental animal models as a result of a model employed or bacterial species tested with a particular note on mono-infections as compared to coinfections and periodontitis, and individual host response [20, 119-121]. Experimental animal models also mostly lack the variety of extent and severity of clinical periodontal diseases, and the host response seems to vary with different stages of periodontal diseases. The truly chronic nature of the naturally occurring periodontal diseases may lead to exhaustion of the body's capacities to respond, which could be one of the reasons for lower systemic levels of inflammatory markers reported in some studies [122]. In most experimental animal models the treatment evaluation phase also lacks [41], and this is where the variety of systemic host response among individuals is even more evident.

With periodontal diseases being the most common chronic inflammatory diseases of dogs, relatively fast (given the shorter life span comparing to humans) development of the diseases in this species, shared environmental etiological factors with humans, raised awareness about periodontal diseases in animals among owners, and availability of the high-standard veterinary dentistry, the canine naturally occurring periodontal diseases model is becoming very attractive [21]. At this point, however, the rare data on systemic inflammation associated with periodontal diseases are inconsistent and characterization of infectious agents causing periodontal diseases in this species is limited. However, further investigation into this model is warranted as it also brings the potential of investigating a more personalized approach to diagnosis, treatment and follow-up of the diseases.

**Acknowledgements;** The studies by the authors were supported by the Slovenian Research Agency - Young Researchers' grant and grant No. P4-0053, and the University of California - Davis Center for Companion Animal Health - grant No. 2009-51-R. The authors would like to thank Prof. Frank J.M. Verstraete for proofreading the manuscript.

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