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ABSTRACT

The disruption of host-microbe homeostasis at the site of periodontal disease is considered a key factor for disease initiation and progress. While the downstream mechanisms responsible for the tissue damage *per se* are relatively well-known (involving various patterns of immune response operating toward periodontal tissue destruction), we are only beginning to understand the complexity of host-microbe interactions in the periodontal environment. Unfortunately, most of the research has been focused on the disruption of host-microbe homeostasis instead of focusing on the factors responsible for maintaining homeostasis. In this context, regulatory T-cells (Tregs) comprise a CD4+FOXP3+T-cell subset with a unique ability to regulate other leukocyte functions to avoid excessive immune activation and its pathological consequences. Tregs act as critical determinants of host-microbe homeostasis, as well as determinants of a balanced host response after the disruption of host-microbe homeostasis by pathogens. In periodontitis, Tregs play a protective role, with their natural recruitment being responsible for conversion of active into inactive lesions. With controlled-release technology, it is now possible to achieve a selective chemoattraction of Tregs to periodontal tissues, attenuating experimental periodontitis evolution due to the local control of inflammatory immune response and the generation of a reparative environment.

KEY WORDS: inflammation, bone regeneration, periodontal disease(s)/periodontitis, regulatory T-Lymphocytes, chemokines, drug delivery systems.

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Restoring Host-Microbe Homeostasis via Selective Chemoattraction of Tregs

HOST-MICROBE HOMEOSTASIS IN THE CONTEXT OF PERIODONTITIS

Periodontitis is classically defined as a biofilm-induced chronic inflammatory disease, where micro-organisms trigger a host inflammatory immune response that ultimately leads to tooth-supporting tissue destruction. Indeed, accumulating evidence suggests that the presence of specific periodontopathogens is responsible for a chronic exacerbated host response. The host response involves the chemoattraction and activation of various leukocyte subsets, resulting in a complex cytokine network that ultimately leads to tissue destruction (Garlet, 2010). However, while the mechanisms responsible for the tissue damage are relatively well-known, we are only beginning to understand the complexity of host-microbe interactions in periodontal environment. For instance, the presence of classic periodontal pathogens is not mandatory for the development of periodontal lesions; these same theoretical pathogenic micro-organisms can also be found in healthy sites/individuals. Similarly, host inflammatory mediators responsible for disease progression can be found in inflamed but non-destructive lesions in levels somewhat similar to those found in destructive lesions (Teles *et al.*, 2013). Finally, micro-organisms defined as periodontal commensals and pathogens, because of their respective presence in healthy and diseased sites, appear to have a similar inflammatory potential *in vitro* (Yoshioka *et al.*, 2008). Each of these examples highlights the complexity of the host-microbe interactions that may dictate a diseased or healthy outcome in the periodontium. In this scenario, it is possible to consider that a homeostatic host-microbe interaction is comprised of the presence of microbes (either commensals or pathogens in theory) and a host response that effectively restrains bacterial dissemination without inflicting permanent damage to host periodontal tissues, while a destructive host response elicited by microbes would represent the lack of host-microbe homeostasis that leads to disease progression (Sanchez de Medina *et al.*, 2013).

Recently, periodontitis was categorized as a disease of dysbiosis, where a disruption of the otherwise finely tuned balance of host/microbe interactions is considered the key for disease initiation and progression (Darveau *et al.*, 2012; Hajishengallis, 2014). The dysbiosis model overcomes the former classic infectious disease paradigm, where a single or few pathogens are considered the etiologic agents of the disease. In this new framework, host response is initially subverted by keystone-pathogens and subsequently overstimulated by pathobionts. The keystone-pathogens (such as *P. gingivalis*) are defined as species with a disproportionately large effect on its environment relative to its abundance, while pathobionts are former innocuous commensals that opportunistically emerge upon disrupted homeostasis with potential to cause disease (Hajishengallis, 2014). This model proposes that multiple bacteria with

differential roles, and their cooperative combination, are required for the development of periodontitis. However, despite the very elegant experimental demonstration of the dysbiosis hypothesis (Hajishengallis *et al.*, 2011), further confirmations in human disease are required. In addition to keystone-pathogens, other environmental and host-based factors may also be responsible for the host-microbe homeostasis disturbance. Indeed, comorbidities such as diabetes and arthritis can disrupt the homeostatic host-microbe balance, leading to the development of periodontitis in response to commensal oral flora (Trombone *et al.*, 2010; Queiroz-Junior *et al.*, 2011). Therefore, regardless of the factors that disrupt homeostasis, the perturbation of host-microbe evenness appears to be central to the development of periodontitis (Darveau *et al.*, 2012). Once homeostasis is lost, accumulating evidence points to the fact that distinct patterns of immune response (pro-inflammatory, Th1 and Th17 cytokines) have the potential to increase inflammation and mediate osteoclastogenesis *in vivo*, operating in cooperative or independent pathways toward periodontal tissue destruction (Cavalla *et al.*, 2014; Araujo-Pires *et al.*, in press).

Unfortunately, from the host response viewpoint, most of the research in this area to date has been focused on the disease mechanisms (*i.e.*, disrupted host-microbe homeostasis) instead of the factors responsible for maintaining homeostasis (Garlet, 2010). It is important to note that healthy samples are usually considered as those representative of a homeostatic state in disease-focused studies. However, from the host-microbe interaction viewpoint, the healthy state derived from plaque control procedures does not reflect a real host-microbe homeostasis. In other words, the effective microbial control results in a minimal/absent host response, constituting a “pristine” health state, that does not necessarily represent the homeostatic scenario developed along microbial and host co-evolution before the recent increase in systematic plaque control procedures (Garlet *et al.*, 2012; Cavalla *et al.*, 2014).

MUCOSAL IMMUNOLOGY: THE PARALLELS BETWEEN GUT AND PERIODONTAL ENVIRONMENTS

With mucosal tissue and mucosal immunology as a common thread, it could be reasonable to use the gut as a model to propose a better definition for host-microbe homeostasis in the periodontal environment (Sanchez de Medina *et al.*, 2013). In a healthy gut milieu, numerous and diverse microbes coexist homeostatically

Figure 1. Schematic representation of the overall involvement of Tregs in host-microbe homeostasis. In the gut environment, dendritic cells (DCs) sample antigens through epithelial cells (1). In the pro-tolerogenic milieu conferred by the gut lamina propria, the interaction between dendritic cells (DCs) and naïve T-cells results in the preferential (but not exclusive) generation of iTregs (induced regulatory T-cells) (2) instead of classic T-helper effector cells (3), such as Th1, Th17, and Th2 polarized subsets. Still in the gut lamina propria, the recently generated iTregs contribute to local homeostasis by a positive feedback mechanism, since their secretion products contribute to maintain the local environment as a pro-tolerogenic milieu (4). After developing in the gut environment, Tregs can gain access to the circulatory system (5) and are thought to keep recirculating until migrating to lymph nodes (LN) and/or peripheral tissues in response to specific chemotactic signals. In the LNs (6), Tregs shape the interaction between DCs and naïve T-cells (7), allowing for the development of effector Th cells but limiting excessive proliferation to avoid subsequent excessive responses. In the peripheral tissues (8), Tregs also modulate the fate of DC and Th cell interaction (9), similar to its action at LNs, restraining the response to commensals to maintain host-microbe homeostasis (10), but still allowing efficient effector mechanisms to control potentially harmful antigens. Also, the presence of Tregs in a given environment also results in bystander tolerance (11), where Treg responses to a specific antigen can also suppress the responses to a different antigen in the same environment where the specific antigen is present.

with the host, represented by the lamina propria populated by several leukocyte subsets. However, this constitutive “inflammatory” state is not necessarily associated with pathological destruction of host tissues (Sanchez de Medina *et al.*, 2013).

When the parallel is made between the gut and the periodontal environments, it would become apparent that the gut homeostatic scenario is quite different from “pristine” periodontal health described above, which is determined by an exogenous plaque control rather than by the host endogenous response. Instead, the current understanding of gut homeostasis seems to better resemble the condition clinically defined as gingivitis, where the natural accumulation of micro-organisms results in a local host response without permanent damage to the host. This is in accordance with the classic “natural history of periodontitis” studies (Löe *et al.*, 1986), where the regular outcome of the natural host-microbe interaction at the periodontium refers to gingivitis, while destructive periodontal disease is limited to a smaller group of individuals. Therefore, individuals presenting tissue destruction are considered susceptible, while individuals presenting non-progressive gingivitis lesions are considered resistant (Garlet *et al.*, 2012; Hajishengallis, 2014). Therefore, the resistance phenotype, where

microbes coexist with the host without inflicting pathological permanent tissue damage, fits the host-microbe homeostasis concept previously mentioned (Sanchez de Medina *et al.*, 2013). While one may argue that gingivitis constitutes a form of periodontal disease and conceptually cannot be defined as a “homeostatic” situation, from a functional host-microbe interaction viewpoint, gingivitis provides a compelling parallel with the better-characterized homeostatic host-microbe interaction in the gut. Indeed, gut homeostasis/health can be regularly achieved without specific procedures of microbial control such as toothbrushing. Importantly, this homeostatic state in the gut can be disrupted by environmental and host-based factors (similar to those described for periodontitis), leading to pathological inflammation (again, likewise periodontitis) (Hooper and Macpherson, 2010).

Indeed, new studies focused on host-microbe homeostasis at mucosal sites could provide helpful clues to the better understanding of periodontitis (Indriolo *et al.*, 2011). Similarly to the oral cavity, the contiguous gastrointestinal tract constitutes a mucosal firewall in close contact with a highly diverse and abundant microbiota (Sanchez de Medina *et al.*, 2013). Also, as a parallel to the oral cavity, gut host-microbe homeostasis disruption due to dysbiosis results in severe inflammatory pathologies (Sanchez de Medina *et al.*, 2013). While mammals have formed a partnership with microbiota during evolutionary development, the establishment of a homeostatic host-microbe interaction depends on the host’s ability to discriminate between harmful and harmless antigens. The type of immune response that ensures such homeostatic interaction is defined as immunological tolerance (Castro-Sanchez and Martin-Villa, 2013). While the term “tolerance” may suggest that the host simply disregards harmless commensal antigens, and consequently does not respond to them, the immunological tolerance process entails active responses that preserve physiological/functional tissue homeostasis while simultaneously retaining the capacity to respond to pathogenic antigens (Chaudhry and Rudensky, 2013). The tolerance response achieved by a series of simultaneous and interconnected immunological events, characterized by antigen-specific hyporesponsiveness, is largely dependent upon a subset of immune cells called regulatory T-cells (Tregs) (Chaudhry and Rudensky, 2013).

TREGS AS DETERMINANTS OF HOST-MICROBE HOMEOSTASIS

Tregs are a subtype of CD4⁺ T-cells, representing barely < 5% of CD4⁺ T-cells, which regulate other leukocyte functions to avoid excessive immune activation and its pathological consequences (Josefowicz *et al.*, 2012a) (Fig. 1). In parallel with the definition of keystone pathogens, Tregs can be considered “keystone immunoregulatory elements”, since they represent a low-abundance leukocyte subset with a wide impact on the immune system.

Several types of Treg cells have been described on the basis of their origin, generation, and mechanism of action. In a simplistic manner, the major Tregs subsets are comprised of natural Treg cells (nTreg) that originated in the thymus (nTreg are so-called tTregs by virtue of its thymic origin) and express the transcription factor Foxp3, being implicated in the control of the

reactivity against auto-antigens, and inducible Treg cells (iTreg cells), also positive for FOXP3, generated in certain peripheral tissues in response to non-self (such as microbial) antigens *via* many different mechanisms, which act as critical determinants of host-microbe homeostasis (Belkaid and Chen, 2010; Josefowicz *et al.*, 2012a,b) (Fig. 1). Despite the absence of definitive specific markers for Treg subsets, in a general way Tregs are characterized by the expression of phenotypic/functional markers such as FOXP3, GITR, CD45RO, CD103, CTLA-4, IL-10, and TGF- β . Although the precise mechanisms by which these cells function to maintain the balance between immunity and tolerance remain to be fully understood, Tregs’ immunosuppressive arsenal includes the release of cytokines such as IL-10, TGF- β , and IL-35 or cell-surface molecules such as CTLA-4, CD39, and Nrp-1, which can directly suppress effector Th subsets (Belkaid and Chen, 2010). Also, Tregs can manipulate antigen-presenting cells in a way that renders these cells less efficient at inducing effector cells (Belkaid and Chen, 2010).

Importantly, Tregs play a role in the prevention of exaggerated responses to foreign harmful antigens, such as from pathogens, acting as central regulators of host inflammatory immune response (Garlet *et al.*, 2010a). Indeed, the presence of Tregs is essential to ensure a balanced response with efficient effector mechanisms to control infectious agents, while minimizing the damage to the environment where the response takes place (Josefowicz *et al.*, 2012a). Indeed, Tregs have been used as “exogenous” cell therapy to restore immune homeostasis (Riley *et al.*, 2009). Therefore, it is possible to hypothesize that Tregs could play 2 major roles in the periodontal environment: as determinants of host-microbe homeostasis (in “pristine” health or stable lesions, depending on the semantic or functional homeostasis definition), and as determinants of a balanced host response after the disruption of host-microbe homeostasis.

THE PROTECTIVE ROLE OF TREGS IN PERIODONTITIS

In periodontal tissues, the presence of cells with a phenotype characteristic of Tregs is associated with the expression of the regulatory cytokines IL-10 and TGF- β (Nakajima *et al.*, 2005; Cardoso *et al.*, 2008; Garlet *et al.*, 2010b), reinforcing its potential pro-homeostatic role. In a broad analysis of T-helper subset marker expression in periapical lesions (which share several immunopathogenic aspects with periodontitis), the presence of Tregs and IL-10 is identified as a major determinant of lesion inactivity (Araujo-Pires *et al.*, in press). Analysis of experimental periodontitis data also supports a protective role for Tregs in periodontitis scenario. Along the course of periodontitis after oral infection of C57Bl/6 mice, the appearance of cells with a Treg phenotype in periodontal tissues is temporally associated with a significant attenuation of disease progression and host responses that characteristically result in tissue damage (Garlet *et al.*, 2010a). The protective role of Tregs was confirmed in a cause-and-effect manner, when disabling Tregs function resulted in increased experimental periodontitis severity accompanied by lower IL-10, TGF- β , and CTLA-4 levels (Garlet *et al.*, 2010a). Indeed, the absence of Tregs impairs the conversion of active

into inactive lesions, demonstrating the central role of these cells in restoring the host-microbe homeostasis disrupted by a virulent pathogen (Garlet *et al.*, 2010a). Unfortunately, the experimental conditions to date have not permitted the analysis of the role of Tregs in maintaining periodontal homeostasis to commensals, since the long-term absence of functional Tregs (required for evidence of the possible pathological effects of commensals) results in massive systemic inflammatory and auto-immune responses (Ramsdell and Ziegler, 2014).

An immediate concern related to a Treg-mediated “attenuated host response” would be diminished protective immunity against infecting bacteria. Indeed, cytokines such as TNF- α and IFN- γ control the bacterial burden at the periodontium, avoiding a systemic acute phase response and (a sometimes lethal) bacterial dissemination (Garlet *et al.*, 2007, 2008). However, even a Treg-attenuated response effectively controls infection without increasing the systemic response to infection (Garlet *et al.*, 2010a; Glowacki *et al.*, 2013). Accordingly, since host response trespasses onto a minimal threshold that confers protection, the increase in intensity of host responsiveness does not provide additional protection and also results in increased tissue damage (Trombone *et al.*, 2009). Also, the arrest of disease by chemoattraction of Tregs, even in the presence of bacterial levels that usually result in the development of periodontitis in mice, reinforces the role of Tregs in restoring host-microbe homeostasis, even in unfavorable conditions (Garlet *et al.*, 2010a; Glowacki *et al.*, 2013). Interestingly, this description of the role of Tregs fits into the resistance phenotype previously discussed, where microbes (even potential pathogens) coexist with the host without causing immunopathological damage.

It is important to consider that the influx of Tregs, and not their local development, is the most likely mechanism of their increased prevalence in diseased periodontal tissues. Notably, iTreg differentiation requires environments that meet specific prerequisites (high-affinity TCR interaction with CD103⁺ dendritic cells under suboptimal co-stimulation and TGF- β and IL-2 signaling), as provided by the gut lamina propria (Josefowicz *et al.*, 2012a; Castro-Sanchez and Martin-Villa, 2013) (Fig. 1). However, considering that oral bacteria are constantly swallowed and abundant in the gastrointestinal system, it is reasonable to hypothesize that the oral-bacteria-specific iTregs generated in the gut are similar to those specific to gut flora. While this hypothesis remains to be confirmed, its supposition is supported by the fact that the delivery of antigens *via* the oral route results in marked Treg-mediated tolerance, a strategy currently used to avoid/control exacerbated immune responses (Weiner *et al.*, 2011). Indeed, the immunosuppressive activity of gut-derived iTregs is not necessarily restricted

Figure 2. Schematic representation of the CCL22-releasing formulation that restores host-microbe homeostasis via selective chemoattraction of Tregs to control inflammation and promote repair. The injection of a polymeric controlled-release system in gingival tissue results in a highly stable and steady CCL22 gradient (1), effectively recruiting Tregs to the site of injection in mice (2). The influx of Tregs in the periodontal tissues results in a local increase of IL-10 and TGF- β levels (3), associated with a significant decrease in the influx of inflammatory leukocytes and decreased production of pro-inflammatory, Th1- and Th17-type cytokines (4), without compromising antimicrobial protection (5). The influx of Tregs also restores the MMPs/TIMPs and RANKL/OPG ratio at physiological non-catabolic levels (6), as well increases the levels of pro-reparative markers (7), which collectively are supposed to not only limit tissue destruction but also promote repair.

to the local environment, since these cells access different tissues after migrating in response to specific chemotactic signals (Josefowicz *et al.*, 2012a). Tregs are thought to migrate to mucosal tissues (including periodontal tissues) in response to ligands of chemokine receptor CCR4 (Garlet *et al.*, 2005, 2010a; Glowacki *et al.*, 2013), preferentially present in Tregs rather than in effector T-cell subsets (Griffith *et al.*, 2014). Accordingly, the presence of cells with a Treg phenotype in periodontal tissue was previously described in parallel with the expression of CCR4 ligands CCL17 and CCL22 (Cardoso *et al.*, 2008). While a previous study demonstrated that macrophages from periodontitis patients are able to produce CCL22 (Fokkema *et al.*, 2003), the exact source of CCL22 in periodontal tissues remains to be determined.

EXPLOITING TREGS AS A THERAPEUTIC TOOL

Recently, our research groups (Garlet, Little, and Sfeir labs) decided to utilize controlled-release technology to exploit the potential of endogenous Tregs for restoring periodontal tissue homeostasis in a natural way (Fig. 2). As opposed to *ex vivo* manipulation and re-administration of Tregs, we hypothesized that recruitment of a patient's own Tregs could exert a significant effect on the local inflammatory environment while being far more easily translatable from a clinical perspective. While bolus administration of CCL22 in the periodontal tissue can recruit Tregs for a limited period (unpublished observations), the constant/long-term presence of soluble CCL22 and Tregs in the tissues is required to arrest disease progression. This could

be accomplished by sustaining a chemokine gradient using safe and biocompatible polymeric controlled-release systems that have outstanding track records of clinical approval (Jhunjunwala *et al.*, 2012). It is important to mention that while PLGA has been used only topically in the periodontal pocket to deliver small-molecule antimicrobials in humans, PLGA has been successfully approved in the past in even more invasive applications (injection and implantation) for delivery of recombinant proteins via PLGA delivery systems (Silverman *et al.*, 2002).

Using this strategy, we were able to maintain a highly stable and steady gradient from a controlled-release point-source, effectively recruiting Tregs to the site of injection (Jhunjunwala *et al.*, 2012). After demonstration of *in vitro/in vivo* effectiveness, we then explored the promise of this cell-recruiting controlled-release system as a potential treatment for periodontitis (Glowacki *et al.*, 2013). We found that Treg recruitment to the local site of disease significantly arrested disease progression, despite the similarities in bacterial loads of treated vs. untreated animals. Furthermore, in a canine pre-clinical model, well-established for acute inflammation-induced periodontitis, the administration of Treg-recruiting formulations in the periodontal pockets significantly reduced clinical signs of periodontitis in response to a multi-microbial challenge. Notably, ablation of Tregs by anti-GITR abrogated the protective effect, as would be expected if Tregs were responsible for the therapeutic effect. Also, the molecular changes observed upon Treg recruitment point to the hallmark Treg-effectors IL-10 and TGF- β as probable mediators of its regulatory effects. It is essential to mention that the release of CCL22 may also result in the chemoattraction of CCR4+ T-cell subsets other than Tregs to periodontal tissues, as suggested by the previous association between CCR4 and IL-17A mRNA levels in periodontal lesions (Honda *et al.*, 2008). However, unpublished observations from our group demonstrate an increased severity of experimental periodontitis in CCR4KO mice, associated with decreased migration of Tregs, reinforcing the potential protective potential of CCL22/CCR4 axis. However, since the knowledge regarding the exact nature of CCR4+ cells in periodontitis lesions is scarce, further studies in this field are required to determine which lymphocyte subsets actually migrate in response to CCR4 ligands.

It is worth mentioning that the Treg-recruiting formulation also enhances the expression of a wide variety of pro-regenerative markers in the periodontium (Glowacki *et al.*, 2013). Indeed, in the pro-reparative context, Tregs attenuate but do not fully terminate the inflammatory process, allowing for a moderate response described as fundamental to proper tissue regeneration/repair (Haertel *et al.*, 2014). The generation of such a pro-reparative environment would be a clear advantage of Treg-attractive systems if compared with traditional anti-inflammatory approaches, which have a tendency to limit/delay the healing process (Geusens *et al.*, 2013).

The chemoattraction of Tregs also presents significant advantages when compared with traditional antimicrobial approaches. While the antimicrobial treatments may be effective (despite the growing restrictions to antibiotics use), the eradication of microbes is clinically utopic and the subsequent recolonization highly probable. Instructing the host to tolerate the presence of (putative) pathogens results in a virtually long-term protective

effect. Also, analysis of recent data points to a potential protective role of the formerly bystander commensal flora in the context of periodontitis. Indeed, LTA, a common product of oral Gram-positive commensals, is a characteristic ligand of TLR2 receptor, recently described to boost Treg function (Liu *et al.*, 2006). Interestingly, the absence of TLR2 results in increased experimental periodontitis severity (Gelani *et al.*, 2009). It is important to mention that TLA binding to TLR2 is not exclusive, and even classic periodontopathogen products (such as *P. gingivalis* LPS) could enhance Treg function locally; however, this hypothesis remains elusive and requires further confirmation. Also, Treg responses directed to commensal bacterial antigens may also suppress the local response regardless of the antigen specificity in the view of the bystander tolerance phenomenon (Weiner *et al.*, 2011) (Fig. 1). The bystander tolerance concept states that Treg induced by a given antigen will secrete cytokines that then will suppress inflammation in the environment where the antigen is present, with this spatial effect not being limited to the antigen that generated/triggered the Treg response (Josefowicz *et al.*, 2012a; Molloy *et al.*, 2012). Therefore, in view of the extensive exposition of oral microbes to the gut environment, a broad commensal-targeting Treg receptor repertoire is supposed to reach periodontal tissues and suppress the response to pathogens in the vicinity. Nevertheless, the antigen-specificity of Tregs acting in the periodontal environment remains to be elucidated.

CONCLUDING REMARKS

In summary, the understanding of host-microbe homeostasis in periodontal tissues, while still incomplete, supports the selective chemoattraction of Tregs as a promising strategy to control inflammation and promote repair, with a clear translational potential (Fig. 2). From the clinical viewpoint, the recent history of clinical approval for polymeric controlled-release systems, associated with the effective biological effect generated by tiny (ng/kg) amounts of chemokine administered in a simple way, reinforces the safety and affordability of the proposed strategy. From the biological viewpoint, the re-establishment of local host-microbe homeostasis by the body's own chemoattractants and cells avoids the tremendous hurdles of *ex vivo* cell therapy, as well as the issues associated with classic drugs. It is also mandatory to consider that the full picture of the role(s) of Tregs in the context of periodontitis remains to be completely elucidated. Further studies are under way to complement the Treg chemoattraction strategy with a subsequent local boost of its function, as well as to direct and anticipate Treg development into pathogenic antigens as a preventive tool. Finally, it is important to mention that the rationale for homeostasis restoration is not limited to periodontal diseases, since virtually all conditions resulting from a host-microbe imbalance can potentially benefit from the strategy discussed here.

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REFERENCES

- Araujo-Pires AC, Francisconi CF, Bigueti CC, Cavalla F, Aranha A, Letra A, *et al.* (2014). Simultaneous analysis of T helper subsets (Th1, Th2, Th9, Th17, Th22, Tfh, Tr1 and Tregs) markers expression in periapical lesions reveals multiple cytokine clusters accountable for lesions activity and inactivity status. *J Appl Oral Sci* (in press).
- Belkaid Y, Chen W (2010). Regulatory ripples. *Nat Immunol* 11:1077-1078.
- Cardoso CR, Garlet GP, Moreira AP, Júnior WM, Rossi MA, Silva JS (2008). Characterization of CD4+CD25+ natural regulatory T cells in the inflammatory infiltrate of human chronic periodontitis. *J Leukoc Biol* 84:311-318.
- Castro-Sanchez P, Martin-Villa JM (2013). Gut immune system and oral tolerance. *Br J Nutr* 109(Suppl 2):3-11.
- Cavalla FA, Araujo-Pires AC, Bigueti CC, Garlet GP (2014). Cytokine networks regulating inflammation and immune defense in the oral cavity. *Curr Oral Health Rep* 1:104-113.
- Chaudhry A, Rudensky AY (2013). Control of inflammation by integration of environmental cues by regulatory T cells. *J Clin Invest* 123:939-944.
- Darveau RP, Hajishengallis G, Curtis MA (2012). *Porphyromonas gingivalis* as a potential community activist for disease. *J Dent Res* 91:816-820.
- Fokkema SJ, Loos BG, van der Velden U (2003). Monocyte-derived RANTES is intrinsically elevated in periodontal disease while MCP-1 levels are related to inflammation and are inversely correlated with IL-12 levels. *Clin Exp Immunol* 131:477-483.
- Garlet GP (2010). Destructive and protective roles of cytokines in periodontitis: host defense and tissue destruction viewpoints. *J Dent Res* 89:1349-1363.
- Garlet GP, Avila-Campos MJ, Milanezi CM, Ferreira BR, Silva JS (2005). *Actinobacillus actinomycetemcomitans*-induced periodontal disease in mice: patterns of cytokine, chemokine, and chemokine receptor expression and leukocyte migration. *Microbes Infect* 7:738-747.
- Garlet GP, Cardoso CR, Campanelli AP, Ferreira BR, Avila-Campos MJ, Cunha FQ, *et al.* (2007). The dual role of p55 tumour necrosis factor- α receptor in *Actinobacillus actinomycetemcomitans*-induced experimental periodontitis: host protection and tissue destruction. *Clin Exp Immunol* 147:128-138.
- Garlet GP, Cardoso CR, Campanelli AP, Garlet TP, Avila-Campos MJ, Cunha FQ, *et al.* (2008). The essential role of IFN- γ in the control of lethal *Aggregatibacter actinomycetemcomitans* infection in mice. *Microbes Infect* 10:489-496.
- Garlet GP, Cardoso CR, Mariano FS, Claudino M, de Assis GF, Campanelli AP, *et al.* (2010a). Regulatory T cells attenuate experimental periodontitis progression in mice. *J Clin Periodontol* 37:591-600.
- Garlet GP, Giazza SP, Silveira EM, Claudino M, Santos SB, Avila-Campos MJ, *et al.* (2010b). Association of human T lymphotropic virus 1 amplification of periodontitis severity with altered cytokine expression in response to a standard periodontopathogen infection. *Clin Infect Dis* 50:e11-e18.
- Garlet GP, Trombone AP, Menezes R, Letra A, Repeke CE, Vieira AE, *et al.* (2012). The use of chronic gingivitis as reference status increases the power and odds of periodontitis genetic studies - a proposal based in the exposure concept and clearer resistance and susceptibility phenotypes definition. *J Clin Periodontol* 39:323-332.
- Gelani V, Fernandes AP, Gasparoto TH, Garlet TP, Cestari TM, Lima HR, *et al.* (2009). The role of Toll-like receptor 2 in the recognition of *Aggregatibacter actinomycetemcomitans*. *J Periodontol* 80:2010-2019.
- Geusens P, Emans PJ, de Jong JJ, van den Bergh J (2013). NSAIDs and fracture healing. *Curr Opin Rheumatol* 25:524-531.
- Glowacki AJ, Yoshizawa S, Jhunjunwala S, Vieira AE, Garlet GP, Sfeir C, *et al.* (2013). Prevention of inflammation-mediated bone loss in murine and canine periodontal disease via recruitment of regulatory lymphocytes. *Proc Natl Acad Sci USA* 110:18525-18530.
- Griffith JW, Sokol CL, Luster AD (2014). Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol* 32:659-702.
- Haertel E, Werner S, Schafer M (2014). Transcriptional regulation of wound inflammation. *Semin Immunol*. [Epub ahead of print 2/17/2014]
- Hajishengallis G (2014). Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends Immunol* 35:3-11.
- Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, *et al.* (2011). Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* 10:497-506.
- Honda T, Aoki Y, Takahashi N, Maekawa T, Nakajima T, Ito H, *et al.* (2008). Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease. *Clin Chim Acta* 395:137-141.
- Hooper LV, Macpherson AJ (2010). Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* 10:159-169.
- Indriolo A, Greco S, Ravelli P, Fagioli S (2011). What can we learn about biofilm/host interactions from the study of inflammatory bowel disease. *J Clin Periodontol* 38(Suppl 11):36-43.
- Jhunjunwala S, Raimondi G, Glowacki AJ, Hall SJ, Maskarinec D, Thorne SH, *et al.* (2012). Bioinspired controlled release of CCL22 recruits regulatory T cells in vivo. *Adv Mater* 24:4735-4738.
- Josefowicz SZ, Lu LF, Rudensky AY (2012a). Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol* 30:531-564.
- Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, *et al.* (2012b). Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 482:395-399.
- Liu H, Komai-Koma M, Xu D, Liew FY (2006). Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. *Proc Natl Acad Sci USA* 103:7048-7053.
- Löe H, Anerud A, Boysen H, Morrison E (1986). Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. *J Clin Periodontol* 13:431-445.
- Molloy MJ, Bouladoux N, Belkaid Y (2012). Intestinal microbiota: shaping local and systemic immune responses. *Semin Immunol* 24:58-66.
- Nakajima T, Ueki-Maruyama K, Oda T, Ohsawa Y, Ito H, Seymour GJ, *et al.* (2005). Regulatory T-cells infiltrate periodontal disease tissues. *J Dent Res* 84:639-643.
- Queiroz-Junior CM, Madeira MF, Coelho FM, Costa VV, Bessoni RL, Sousa LF, *et al.* (2011). Experimental arthritis triggers periodontal disease in mice: involvement of TNF- α and the oral microbiota. *J Immunol* 187:3821-3830.
- Ramsdell F, Ziegler SF (2014). FOXP3 and scurfy: how it all began. *Nat Rev Immunol* 14:343-349.
- Riley JL, June CH, Blazar BR (2009). Human T regulatory cell therapy: take a billion or so and call me in the morning. *Immunity* 30:656-665.
- Sanchez de Medina F, Ortega-Gonzalez M, Gonzalez-Perez R, Capitan-Canadas F, Martinez-Augustin O (2013). Host-microbe interactions: the difficult yet peaceful coexistence of the microbiota and the intestinal mucosa. *Br J Nutr* 109(Suppl 2):12-20.
- Silverman BL, Blethen SL, Reiter EO, Attie KM, Neuwirth RB, Ford KM (2002). A long-acting human growth hormone (Nutropin Depot): efficacy and safety following two years of treatment in children with growth hormone deficiency. *J Pediatr Endocrinol Metab* 15(Suppl 2):715-722.
- Teles R, Teles F, Frias-Lopez J, Paster B, Haffajee A (2013). Lessons learned and unlearned in periodontal microbiology. *Periodontol* 2000 62:95-162.
- Trombone AP, Ferreira SB Jr, Raimundo FM, de Moura KC, Avila-Campos MJ, Silva JS, *et al.* (2009). Experimental periodontitis in mice selected for maximal or minimal inflammatory reactions: increased inflammatory immune responsiveness drives increased alveolar bone loss without enhancing the control of periodontal infection. *J Periodontol Res* 44:443-451.
- Trombone AP, Claudino M, Colavite P, de Assis GF, Avila-Campos MJ, Silva JS, *et al.* (2010). Periodontitis and arthritis interaction in mice involves a shared hyper-inflammatory genotype and functional immunological interferences. *Genes Immun* 11:479-489.
- Weiner HL, da Cunha AP, Quintana F, Wu H (2011). Oral tolerance. *Immunol Rev* 241:241-259.
- Yoshioka H, Yoshimura A, Kaneko T, Golenbock DT, Hara Y (2008). Analysis of the activity to induce Toll-like receptor (TLR)2- and TLR4-mediated stimulation of supragingival plaque. *J Periodontol* 79:920-928.