The TH17 vs. TREG Imbalance in the Pathogenesis of Periodontitis: New Approach for Dichotomy TH1 vs. TH2

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ABSTRACT

For decades several authors have directed their efforts to elucidate the nature of the immune response in periodontitis. The subject has been matter of controversy, and answers remain unclear. The present review intends to summarize the character of the inflammatory response in periodontitis, with emphasis on the T helper imbalance produced during the development of the disease.
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Key words: **Periodontitis, immune response, Th1, Th2, Th17, Treg.**

Nature of the inflammatory response in periodontitis

Periodontitis encompass multifactorial diseases involving bacterial biofilms and the generation of an inflammatory response[(1)]. Periodontitis, chronic and aggressive subtypes, is usually related to polybacterial infection and three specific pathogens have been repeatedly identified as etiologic agents in the periodontal destruction: *Aggregatibacter* (Actinobacillus) *actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) y *Tannerella forsythia* (Tf)[(2)]. Bacterial biofilms have been shown to be the primary aetiological factor in the initiation of gingival inflammation and subsequent destruction of periodontal tissues[(3)]. Although chronic bacterial and endotoxin exposure is a prerequisite for gingival inflammation and periodontal tissue destruction to occur, its presence alone accounts for a relatively small proportion (i.e. 20%) of the variance in disease expression[(4)]. According to a novel model of pathogenesis of periodontitis, this is not sufficient to explain disease initiation and progression[(5,6,7)]. The major component of soft- and hard-tissue destruction associated with periodontitis is the result of activation of the host's immune-inflammatory response to the bacterial challenge, it is the nature of the inflammatory responses which determines the destructive character of the disease[(8)].

Based upon histopathological features, inflammatory processes developed in the periodontitis may be divided into three phases: an acute phase, an immune response and a chronic phase[(5)]. The transition process from gingival health to early inflammatory changes is characterized by a local increase in vascular permeability, redness, swelling and by the recruitment and activation of polymorphonuclear granulocytes (PMNs)[(9)]. In the course of this acute phase, several products modulate vasodilatation (e.g. bradykinin and prostaglandins), vascular permeability (e.g. histamine and leukotriene) and additional recruitment of inflammatory cells through chemotaxis (e.g. complements products and chemokines)[(5)]. The subsequent immune response starts when antigen-presenting cells become involved presenting the foreign microorganisms or antigens to immunocompetent cells such as T lymphocytes. This leads to the expansion of antibody-secreting plasma cells and the development of a chronic lesion[(10)].

Response TH1 vs. TH2 in periodontitis

Based on their pioneer work, Mosmann and Coffman proposed that T helper cells could be divided into two distinct subsets, T helper type 1 (TH1) and TH2, characterized by distinct cytokine profiles and effectors functions [(11)]. TH1 cells secrete interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), which are critical for the eradication of intracellular pathogens, while Th2 cells produce interleukin-4, -5, -6, and -13, which are essential for optimal antibody production and elimination of extracellular microorganisms, including helminths and nematodes[(12)].

Immunohistological studies have clearly established that a T-cell/macrophage lesion identical to a delayed hypersensitivity reaction[(13)] occurs within 4 to 8 days of plaque accumulation in an experimental gingivitis study[(14)]. This synonymous with the early lesion described by Page and Schroeder[(15)] and with the putative stable lesion[(16)]. The striking similarities between this early/stable periodontal lesions and delayed-type hypersensitivity prompted the suggestion that cells with a TH1 cytokine profile are the major mediators. Such a concept is consistent with the proposal that a strong innate immune response leads to the production of IL-12, which in turn leads to this TH1 response[(8)]. The dominance of B-cells/plasma cells in the advanced/progressive lesion would suggest a role for TH2 cells. Clearly, if the innate response is poor, low levels of IL-12 would be produced and a poor TH1 response may occur which may not then contain the infection[(17)].

To test this hypothesis, several investigators have attempted to delineate the TH1/TH2 profile in periodontal disease. Pilon et al.[(18)] demonstrated lower levels of IL-2 in the gingival crevicular fluid of periodontitis sites compared with healthy sites, and Fujihashi et al[(19)] have shown that gingival mononuclear cells from adult periodontitis patients produce IL-4 and IL-5 but not IL-2. Peripheral blood mononuclear cells from periodontitis patients stimulated with mitogens resulted in reduced IFN-γ secretion and mRNA expression of IFN-γ and IL-2. At the same time, significantly higher levels of IL-5 and granulocyte/macrophage-colony stimulating factor (GM-CSF) were observed[(20)]. Significantly less IL-2 activity was also found in peripheral blood mononuclear cell cultures stimulated with *P. gingivalis* and *F. nucleatum*[(21)]. Increased Th2 response in periodontitis has also been reported. Memory T-cells from the peripheral blood of adult periodontitis patients with high anti- *P. gingivalis* titers stimulated *in vitro* with *P. gingivalis* have been shown to produce higher amounts of IL-4 than cells from healthy subjects[(22)]. Yamazaki et al.[(23)] demonstrated an increase percentage of IL-4+ cells proportional to an increasing B-cell/T-cell ratio. Another study suggested a role for IL-4 and TH2 responses in periodontitis lesions by the demonstration of concentrations of IgG4 many times higher in sites of active periodontitis than in serum, as well as significantly elevated concentrations compared with stable lesions[(24)]. Seymour et al.[(25)] proposed that due to the shift in lymphocyte populations in the inflammatory infiltrate from predominantly T-cells in gingivitis to an increased proportion of B-cells in periodontitis, susceptibility to periodontal disease progression may involve a predominantly TH2-like response. In contrast to these studies, Ebersole and Taubman[(26)] found that IFN-γ message was prominently expressed by disease gingival tissue cells. Cytokine profiles of cells extracted from six patients were consistent with TH1 cells in that they were IL-2 and IFN-γ-positive but negative for IL-4 and IL-5.

Another study comparing the local and systemic responses in periodontitis patients with so-called terminal dentition periodontitis dem-

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onstrated reduced TH2 responses. Levels of prostaglandin E2 (PGE2), IL-1β and IL-2 in the gingival crevicular fluid samples were highest, followed by lower levels of TNF-α and IFN-γ and even lower of IL-4 and IL-6[27].

TH17 cells and IL-17

In light of recent data, the TH1/TH2 dichotomy is now being revisited. The discovery of the IL-17 family of cytokines and the analysis of IL-23-mediated effector functions on T cells have suggested the existence of an additional subset of CD4+ T cells that produce IL-17[28,29]. During the past few years, it has become apparent that IL-12, STAT4 and IFN-γ, and therefore Th^1 cells, are not the primary instigators of model inflammatory diseases[30]. Rather, the pathogenic cells in these models of disease are induced in response to signals provided *in vivo* by the IL-12-like cytokine IL-23, and characterized by production of a suite of cytokines, chemokines, effector molecules and transcription factors distinct from that of TH1 cells[29,30,31]. One of the genes most highly expressed in these effector cells is the proinflammatory cytokine IL-1[29]. Consequently, they have been termed TH17 cells. These TH17 cells have been recognized as key mediators of inflammation and tissue damage in several animal models of human diseases[29,30,31]. Analogous to the central roles played by T-bet and GATA3 in generating TH1 and TH2 cells, respectively[32], RORγt is considered to be the transcription factor responsible for guiding the development of TH17 cells[33], Transforming growth factor β (TGF-β) and IL-6 are cytokines that also guiding the development of TH17 cells[34]. The role of TH17 cells is very important for responses against specific pathogens that are poor eliminated by TH1 and TH2 responses (Korn et al. 2007).

Recent studies showed that IL-21 also controls the generation of TH17 cells[35,36,37]. They showed that IL-21, in combination with TGF-β, induces IL-17 production from naive CD4+ T cells. These papers also demonstrated that IL-6 or IL-21 could induce TH17 cells themselves to produce IL-21. Such endogenous production of IL-21 by TH17 cells appeared to be biologically significant, because the number of IL-17-producing cells generated by TGF-β and IL-6 was reduced in the absence of IL-21/IL-21R signalling[35,36]. Thus, IL-6 can elicit IL-21 production by CD4+ cells, which then functions in an autocrine loop to amplify the TH17 response in a similar way to IL-4 for Th2 and IFN-γ for TH1. It is possible that TH17 cells induced by TGF-β and either IL-6 or IL-21 make qualitatively different contributions to the development of inflammation depending on the pathogen and affected tissue[38].

IL-17, or IL-17A, is the founding member of a six-member family of cytokines (IL-17 A-F)[39,40]. IL-17A and IL-17F are proinflammatory cytokines, and have important action over different cells, inducing expression of cytokines (e.g. IL-6, GM-CSF, G-CSF, IL-1β, TNF), chemokines (e.g. IL-8, MCP-1) and MMPs (41). IL-17 plays a particularly significant role in regulating neutrophil recruitment and granulopoiesis[39,42].

TH17 and Treg cells

Recently, CD4+ CD25+ regulatory T cells (Treg) have been described as one distinct subset from T cells[43]. Pathogenic autoimmunity is controlled in healthy individuals by a specialized subset of T cells named Treg cells[44]. Treg cell differentiation and function are driven by the transcription factor Foxp3 and they have an anti-inflammatory role in maintaining tolerance to self components by contact-dependent suppression or releasing anti-inflammatory cytokines such as IL-10 and TGF-β[43]. Treg cell development is closely related to the generation of TH17 cells. TFG-β induces the differentiation of Treg cells[45], whereas TFG- β in combination with IL-6[46] or IL-21[35] results in the differentiation of TH17 cells.

The participation of TGF-β, secreted by Treg cells, in the differentiation of TH17 cells has been recently evaluated[35]. Subsequently, it was shown that IL-17 production by naïve CD4+ T cells could be driven by TGF-β and IL-6[47]. It is clear that TGF-β is readily available in mucosa associated lymphoid tissue, while in secondary lymphoid organs, TGF-β may be provided by T cells themselves as suggested by recent findings[48]. The role of TGF-β in the development of these cells is particularly interesting, as TFG-β is also important for driving the generation of Treg cell. Thus, TFG-β will promote the differentiation of inhibitory Treg; however, in the presence of additional inflammatory signals such as IL-6, TH17 cells will be generated. Thus, IL-6 represent a crucial switch for controlling the differentiation of CD4+ T cells to the TH17 or Treg lineages[34].

The relationship between TH17 and Treg cells, suggest that there is not only a functional antagonism but there is a dichotomy in their generation as well[34]. Therefore, Treg cells and TH17 effector arise in a mutually exclusive fashion, depending on whether they are activated in the presence of TGF-β or TGF-β plus IL-6. At the steady-state level or in the absence of any inflammatory insult, TGF-β produced by the immune system will suppress the generation of effector T cells and will induce Foxp3+ regulatory T cells thereby maintaining self-tolerance. However, on infection or inflammation, IL-6 induced by the activated innate immune system will suppress the generation of TGF-β-induced Treg cells and will induce a pro-inflammatory T-cell response predominated by TH17 cells. This is consistent with the in vivo observation that destructive arthritis in IFN-γ-deficient mice can be treated with anti-IL-17, and neutralization of IL-17 results in the generation of CD24+CD25+ regulatory T cells[49]. So the balance between TH17 and Treg may be important in the development/prevention of inflammatory and autoimmune diseases[50].

TH17, Treg cells and IL-17 in periodontitis

The infiltrate present in periodontal diseases contains mononuclear cells, mainly transmigrated mononuclear phagocytes and lymphocytes[51]. Previous studies demonstrated the presence and the role of IL-17 in the pathogenesis of periodontitis[52,53,54]. Vernal et al.[55] studied the levels of IL-17 in patients with chronic periodontitis and found that they were significantly increased in both gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue, compared with the control group. Johnson et al.[52] demonstrated a positive correlation between levels of IL-17 within solubilised gingival biopsies and the progression of gingivitis to periodontitis. IL-17 treatment of human gingival fibroblast has also been observed to lead to IL-6 production, which may contribute to local inflammation[55]. Takahashi et al.[53] suggest that tissue destruction in periodontitis lesion results from major secretion of IL-6, induced by IL-17. The above findings have led to the suggestion that IL-17 may play a pivotal role in the initiation or maintenance of an inflammatory response.

Natural Treg are key regulators of immune responses, express CD4 and CD25 surface markers, and regulate the activation, proliferation, and effector function of activated conventional T cells. Previous studies found that many of the CD4+ T-cell clones derived from either gingival tissue or peripheral blood express the Foxp3 gene[56,57]. Okui et al. [58] showed that Foxp3+ cells were also identified in periodontitis lesions. The cells were restricted to the CD4+ populations.

Final Considerations

Several investigators have tried to establish the predominant T helper profile during periodontitis. Although there has not been consensus about it, in the majority of the literature it was accepted that a Th1 profile could be mediator of the early/stable lesion, and a Th2 profile could be responsible of the progression of the disease. With the discovery of the Th17 response, new insights have been made in the pathogenesis of the periodontitis, suggesting that Th17 population may play a major role in the initiation and maintenance of the inflammatory response typical of this disease.

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