T-cell Responses Involved in the Predisposition to Periodontal Disease: Lessons from Immunogenetic Studies of Leprosy

Hideki Ohyama1, Nahoko Kato-Kogoe2, Kazu Takeuchi-Hatanaka3, Koji Yamanegi1, Naoko Yamada1, Keiji Nakasho1, Sho Matsushita3 and Nobuyuki Terada1

1Department of Pathology, Hyogo College of Medicine, Nishinomiya, Japan
2Department of Pharmaceutical Health Care, Himeji Dokkyo University, Himeji, Japan
3Department of Pathophysiology/Periodontal Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
4Department of Allergy and Immunology, Saitama Medical University, Moroyama, Japan

Abstract

Periodontitis, which involves loss of periodontal attachment and resorption of alveolar bone, is initially caused by infection with many kinds of anaerobic, gram-negative bacteria forming a subgingival biofilm. To prevent bacterial invasion, host defense mechanisms need to recruit many kinds of immunoregulatory cells, including helper T (Th) cells which play a central immune-regulatory role against periodontal infection. Similar to many infectious diseases, susceptibility to periodontal disease is partially determined by individual differences in Th cell responsiveness, especially cytokine production, against periodontopathic pathogens. Susceptibility to periodontitis has been associated with gene polymorphisms of several cytokines such as interleukin (IL)-2, IL-4, IL-6 and IL-10, but these correlations are predominantly weak due to their multifactorial nature. Distinct from these studies, we performed immunogenetic studies to investigate associations between periodontal disease susceptibility and hereditary cell-mediated immune responses. In these studies, leprosy patients were used as a human model to understand the susceptibility to periodontitis, as leprosy is considered to be an infectious disease whose pathogenesis is regulated by diverse individually-inherited Th1/Th2 immune responses against the bacterial pathogen. In addition to the results of these studies, the discovery of a distinct Th17 lineage helps us to explain that disease susceptibility to periodontitis appears to be predominantly associated with the IL-23/IL-17 pathway. Therefore, individuals whose immunogenetic background is characterized as having low IL-12/interferon-γ activity may have a tendency to skew their immune system toward the IL-23/IL-17 pathway in periodontal lesions, which results in a predisposition to periodontal diseases. These studies help us to understand the complex immunological factors underlying susceptibility to periodontitis.

Keywords: Periodontitis; Leprosy; Immunogenetics; Disease susceptibility; Th1/Th2/Th17

Introduction

Periodontal disease, which involves loss of periodontal attachment and resorption of alveolar bone, is initially caused by infection with many kinds of anaerobic, gram-negative bacteria forming a subgingival biofilm [1]. To prevent this bacterial invasion, host defense functions are required to recruit many kinds of immunoregulatory cells. Periodontal disease is broadly classified into two types of disease: gingivitis and periodontitis. Gingivitis is clinically characterized by gingival inflammation limited to the gingival tissue, and this stage of gingivitis and periodontitis. Gingivitis is clinically characterized by gingival inflammation limited to the gingival tissue, and this stage of gingivitis and periodontitis is subdivided into two main types: chronic periodontitis (CP), and aggressive periodontitis (AgP). CP is a common form of the disease that is prevalent among adults and seniors with poor oral hygiene. AgP is characterized by an early-onset of the disease with severe and rapid loss of periodontal attachment and alveolar bone destruction, and is found to be aggregated in some families, suggesting that genetic factors are important in the development of AgP, as compared with CP [4-7]. Thus, many studies have tried to identify the important genes responsible for disease susceptibility, however, such genes are yet to be determined [8].

Previously, it has been reported that the proband-wise concordance rate of periodontal disease is higher in monzygotic than dizygotic twin pairs [9]. This etiologic study of a large number of twins from the population-based Virginia Twin Registry has been regarded as one of the studies showing evidence implicating genetic factors in the susceptibility to periodontal disease. Subsequently, extraordinary progress in molecular biological techniques has permitted us to estimate gene polymorphisms in patients with various clinical types.
of periodontal disease. Additionally, we can identify important genes responsible for the disease by studying target genes encoding: 1) cytokines and their receptors; 2) human leukocyte antigens (HLA); 3) Fcγ receptors; 4) Toll-like receptors; and 5) other kinds of molecules regulating bone metabolism, such as vitamin D [10,11]. Many of these epidemiological studies have been performed in patients with AgP since these patients are regarded as susceptible individuals with regard to their genetic factors. Linkage analysis in CP patients has also revealed that severe CP is associated with polymorphisms of the genes regulating immune-cell functions, suggesting that several genetic factors are involved in the pathogenesis of the disease by affecting immune cell activity.

Individual differences in immune responses against periodontopathic bacteria, especially T cell immune responses, are regarded as one of the crucial factors affecting susceptibility to periodontal disease. In particular, cytokines produced by CD4+ helper T (Th) cells play an important role in host defense against periodontal infection. The determination of naïve CD4+ T cells to differentiate into Th1/Th2 cells greatly influences the effectiveness of subsequent immune responses against pathogens, and the progression of the disease [12-15]. Since impairment in Th1/Th2 balance, leading to conditions conducive to periodontal disease, is likely to be dependent on genetic factors, immune responses against various antigens derived from periodontopathic bacteria have been estimated and compared among individuals with different immunogenetic backgrounds to determine important genetic factors responsible for the disease. Nonetheless, it has been postulated that the degree of correlation between each genetic factor regulating immunological responses against periodontopathic bacteria and susceptibility to the disease is not very strong, since there is a great deal of complexity in the immunological responses involved in the pathogenesis of periodontal disease. To overcome this issue, we adopted leprosy patients as a human model to understand the susceptibility to periodontal diseases, as leprosy is considered an infectious disease whose pathogenesis is regulated by diverse individually-inherited Th1/Th2 immune responses.

This article reviews the genetic factors responsible for the susceptibility to periodontal disease with a focus on the cytokines involved in T cell responses, including our immunogenetic studies of periodontal disease and leprosy.

### Immunogenetics of Leprosy

Leprosy, a chronic disease caused by infection with *Mycobacterium leprae* (*M. leprae*), shows a wide spectrum of clinical features [16]. Tuberculoid leprosy (T-lep) is at one end of the spectrum and lepromatous leprosy (L-lep) is at the other end. T-lep patients show Tuberculoid leprosy (T-lep) is at one end of the spectrum and *M. leprae*, shows a wide spectrum of clinical features [16]. L-lep patients, as compared to T-lep patients [23], we investigated gene polymorphisms in the transcriptional regulatory region of IL12RB2 in Japanese subjects with a history of leprosy. In this recent study, we found that several single nucleotide polymorphisms (SNPs) in the 5’ flanking region of IL12RB2, including -1035A>G, -1023A>G, -650delG and -464A>G, whose frequencies are significantly different among the different clinical types of leprosy, are associated with congenital low expression levels of IL-12R β2 molecules [24] and low IFN-γ production from T cells [25]. Thus, it is possible that these SNPs are not only associated with the establishment of certain clinical types of leprosy, but are also associated with susceptibility to other infectious diseases, whose pathogenesis is greatly aggravated by an impaired Th1/Th2 balance against pathogens. Indeed, these SNPs could be one of the

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possible hereditary factors that determine differences in the intensities of CMI responses among individuals.

In analysis that focused on the CA repeat sequence in the IFN-γ gene (IFNG) as a microsatellite marker in Brazilian subjects, polymorphism was significantly more frequent in leprosy patients [26]. In addition, polymorphism of a promoter region that controls the productivity of IL-10, which is a suppressive cytokine in the IL-12/IFN-γ production system, was studied in Brazilians. Differences in polymorphisms and haplotypes at positions -3575, -2849, -2763, and -819 were found to have a possible effect on susceptibility to leprosy [27,28]. These gene polymorphisms, which determine the productivity and responsiveness of cytokines involved in cellular immune responses, are considered to be important genetic factors that may be essential to understanding individual variation in leprosy susceptibility and type.

**Th1/Th2 Paradigm in the Pathogenesis of Periodontal Diseases and its Immunogenetic Study**

There has long been controversy as to whether the pathogenesis of periodontal disease is explained exclusively by the Th1/Th2 paradigm. Many previous studies which estimated the differences in gene expression profiles of the Th1/Th2 cytokines in periodontal lesions have provided clues for the development of possible hypotheses concerning this issue. Previous studies have demonstrated that Th2 cells are abundant in periodontal lesions [29,30], whereas several other reports have shown that the cytokine profiles produced from cells in periodontal lesions are consistent with those of Th1 or Th0 cells, but not Th2 cells [13,31]. Other studies have suggested that the production of both Th1- and Th2-related cytokines or Th1-dominant cytokine production is induced in response to periodontal micro-organisms and their components [32-34]. Thus, the argument about which immune response, Th1 or Th2, is more dominant, still continues.

In general, Th1 cells are more frequently detected in the early stage of periodontal lesions whereas Th2 cells predominate in the later and advanced period of the disease, suggesting that Th1 cells are associated with a protective response against bacterial infection while Th2 cells have a role in the destruction and progression of periodontal lesions [35-37]. Thus, the predominant Th subset accumulating in the periodontal lesion would change along with the development of the stage of the disease. However, it is difficult to understand whether Th1/Th2 skewing of the Th cells accumulating in periodontal lesions is a result of the progression of the disease, or causes the formation and/or progression of the disease. An immunogenetic approach focusing on the relationship between Th1/Th2 polarization and susceptibility to disease allows us to address this issue.

When investigating other infectious diseases, several studies have used animal models, such as the Th1/Th2 genetic background mouse (C57BL/6 vs. BALB/c strains) or knock-out mice, which is a powerful approach to clarify which subtype of Th cell is implicated in the pathogenesis of a particular disease [38]. In humans, another approach to evaluate the susceptibility to disease is by comparing clinical parameters between individuals with or without SNPs located in the genes related to the regulation of Th1/Th2 immune responses. In studies using individuals with periodontitis, gene polymorphisms of the promoter regions of IL2 [39] IL4 [40-42], IL6 [43] and IL10 [44-47] have been examined, with respect to the establishment of several types of periodontal disease. Distinct from these studies, we used leprosy patients, as human models, to understand the susceptibility to periodontal disease, as leprosy is considered to be an infectious disease whose pathogenesis is regulated by an individually-inherited Th1/Th2 immune response against the pathogen.

In our assessment of the clinical features of periodontal disease in leprosy patients, L-lep patients show more severe periodontitis than T-lep patients or age-matched control subjects, and have lower humoral immune responses against the periodontopathic bacteria, *Porphyromonas gingivalis* [48]. We also previously demonstrated that the frequencies of SNPs in the 5′ flanking region of IL12RB2 including -1035A>G, -1023A>G, -650delIG and -464A>G, which are frequently detected in L-lep patients, are significantly higher in AgP patients, compared with CP patients and healthy controls [49]. Interestingly, when we defined ‘haplotype 1’ as the haplotype consisting of -1035A, -1023A, -650G, and -464A (i.e. without these SNPs), the frequency of individuals carrying haplotype 1 is apparently lower in AgP patients, compared with L-lep patients (Figure 1). Additionally, IgG levels against several periodontal bacteria are significantly higher in patients carrying IL12RB2 SNPs than in patients without the SNPs, suggesting the possibility that the humoral immune responses against periodontal bacteria in patients with IL12RB2 SNPs are hyper-activated as a result of the low CMI response against periodontopathic bacteria. Thus, it is likely that the immune system in patients carrying IL12RB2 SNPs is skewed toward Th2 responses and produces higher amounts of immunoglobulins after infection with periodontal bacteria. Based on the results of these studies, we proposed that low CMI responses or high humoral responses against periodontopathic bacterial infection might constitute a genetic factor that influences susceptibility to this disease. Interestingly, it has also been reported by others that peripheral blood mononuclear cells (PBMCs) in adult periodontitis patients produce low levels of Th1 cytokines, including IFN-γ, in response to mitogenic stimulation [15,50].

In contrast, several studies have established that cytokines associated with Th1 cells, especially IFN-γ, play central roles in the progression of inflammatory responses and bone resorption in rodent periodontal disease models [38,51-53]. Since IFN-γ has a strong inhibitory effect on receptor activator of NF-κB ligand (RANKL)-associated osteoclastogenesis due to degradation of tumor necrosis factor receptor-associated factor 6 from osteoclast precursor cells [54], Th1 cells may be associated with resistance against disease. Meanwhile,
another study using a general mouse model demonstrates that the net effect of IFN-γ is skewed toward bone resorption due to the greater secretion of osteoclastogenic factors such as RANKL and TNF-α from activated T cells and proinflammatory cytokines, whereas IFN-γ actually inhibits osteostal formation [55]. These studies support the relevance of Th1 cells in alveolar bone resorption in periodontitis and the progression of the disease. However, it is likely that the net balance of the two opposing effects of IFN-γ on osteostalogenesis may vary among different animal species and/or among different strains and individuals according to their different genetic backgrounds.

**Th cell Paradigm Shift from Th1/Th2 toward Th1/Th2/Th17**

As mentioned above, there is controversy as to whether periodontal pathogenesis can be explained within the limitations of the Th1/Th2 paradigm. However, a third distinct Th cell lineage, Th17, has been identified, and is composed of IL-17-producing T cells which expand in the presence of IL-23 following the priming of IFN-γ in naïve T cells [56-57]. IL-23 is a cytokine belonging to the IL-12 family which is secreted as a heterodimer composed of a p40 subunit, identical to the p40 subunit of IL-12, and a unique p19 subunit, similar to the p35 subunit of IL-12 [58]. The ligands of these molecules, IL-12Rβ1 and IL-12 receptor (IL-23R), are expressed on the surface of Th17 cells, while IL-12Rβ1 and β2 molecules are expressed on the surface of Th1 cells. The Th17 cell lineages is thought to play an important role in the pathogenesis of cell-mediated tissue damage caused by either autoimmunity or immune responses against microbial infection [59].

The IL-23 and Th17 pathways have recently been implicated in the pathogenesis of rheumatoid arthritis (RA) [60-61], a disease classically regarded as a good model for periodontal tissue destruction [62]. To date, a number of studies have reported the involvement of IL-17 and Th17 cells in the pathogenesis of RA [63]. Since the Th17 cell lineage has a high capacity to induce osteostalogenesis caused by IL-17-mediated induction of RANKL on osteoblastic cells [64], expansion of the Th17 lineage, which is augmented by the IL-23/IL-17 pathway, is considered to play an important role in inflammatory tissue destruction in RA. Moreover, a recent study revealed that osteostalogenesis by IL-17 was induced from monocytes alone in the absence of osteoblasts, resulting from TNF-β production and constitutive expression of RANKL on monocytes by IL-17 [65]. Based on these RA research reports, a number of studies have also been performed implicating IL-17 and Th17 cells in the pathogenesis of periodontal disease. IL-17 and Th17-related cytokine production is elevated in the inflamed tissues and gingival cervical fluids (GCFs) of diseased sites in periodontitis [66-71]. Th17 cells are also highly detected in diseased tissues from periodontitis patients [72]. Our previous study demonstrated that gene expressions of IL23R and IL17A, but not IL12RB2 and IFNG, were elevated in periodontal lesions compared to periododontally healthy sites which were almost free from apparent inflammation and tissue destruction, if any, with only mild gingivitis apparent [70]. In addition, IL17A expression especially, appears to be frequently detected at the sites adjacent to bone destruction [70]. These findings suggest that the IL-23/IL-17 pathway, rather than the IL-12/IFN-γ pathway, is stimulated in periodontal lesions involving tissue and alveolar bone destruction.

Sufficient IL-17 production, but not IFN-γ, is detected in the synovial tissues and fluids of RA patients, even though a number of T cells accumulate and are activated there [73,74]. Since the neutralization of IL-17 in a murine collagen-induced arthritis model reduces joint inflammation, cartilage destruction and bone erosion, IL-17 is considered to be one of the possible cytokines playing an important role in the pathogenesis of RA [75]. Neutralizing IL-23 activity ameliorates synovial tissue inflammation and bone destruction in a rat collagen-induced arthritis model, suggesting that IL-23 is also involved in the progression of the disease by inducing IL-17 production [76]. Actually, the ratio of IL-17 to IFN-γ production from activated human T cells is elevated by IL-23 at 1-10 ng/mL in cultured PBMCs [63]. Thus, these RA results suggest that the predominance of the IL-23/IL-17 pathway over the IL-12/IFN-γ pathway in periodontal lesions may be explained by our previous results, in which the relative expression level was higher for IL23A than IL21A at each site in each patient, whereas the expression levels of both IL-13 and IL-12 were also higher in periodontal lesions than in control sites. While it is controversial whether differentiation of the Th1/Th2/Th17 lineage could be regulated by the local environmental cytokine profiles of the periodontal lesions, local IL-23 production may induce the expansion of the Th17 cell lineage in periodontal tissues, since IL-23 acts on memory Th cells primed by IL-6 and TGF-β, but not naïve T cells, to induce proliferation and terminal differentiation [77].

Meanwhile, an IL-17 receptor A (IL17RA) knockout mouse study, using oral infection with Porphyromonas gingivalis, showed enhancement of alveolar bone loss in the knockout mice [78]. This result contradicts the RA studies but is explained by decreased chemokine expression levels in the knockout mice, resulting in a reduction in neutrophils migrating into the alveolar bone of periodontal lesions. This mouse model suggests that IL-17 plays an important role in host defense functions by the recruitment of neutrophils into the periodontal lesions. Inherited dysfunctions of neutrophils such as chemotaxis, phagocytosis and adhesiveness have been well studied to show an association with susceptibility to AgP [79].

In periodontal lesions, Lester et al. described that gingival concentrations of IL-12 and IFN-γ were significantly lower at severe clinical attachment loss sites than at moderate clinical attachment loss sites, and that the gingival concentration of IL-12 and IFN-γ were negatively correlated with IL-23, whereas IL-17 showed a positive correlation with IL-23 [69]. Actually, IFN-γ strongly inhibits the differentiation of T cells into Th17 cells [56]. A recent study has revealed that IFN-γ regulates macrophages to attenuate Th17 responses through inhibition of IL-23 production [80]. On the basis of these studies, Th17 development is likely restricted by IFN-γ production. However, as mentioned above, it is unclear whether Th cell differentiation could be affected by the cytokine profile of the local environment of periodontal lesions, but not the secondary lymphoid tissues. Another recent study demonstrated that IL-21 production is enhanced in GCF and periodontal tissues of CP patients, which induces Th17 differentiation in periodontal lesions [81,82]; however, the effect of local IL-21 production on Th17 polarization is still unclear. Meanwhile, a report by Zhao et al. shows that the GCF level of IFN-γ does not significantly change after periodontal treatment [71], which provides corroborating evidence for our result of no difference between periodontal lesions and periodontally healthy sites [70]. Taken together, these studies suggest that susceptibility to periodontitis appears to be principally and directly associated with the IL-23/IL-17 pathway rather than the IL-12/IFN-γ pathway. Moreover, from an immunogenetic viewpoint, we can speculate that individuals with low IL-12/IFN-γ activity, including those who possess genetic factors such as gene polymorphisms in IL12RB2, recovered from our immunogenetic studies in leprosy patients, may have a tendency to skew their immune system toward the IL-23/IL-17 pathway in periodontal lesions, which results in a predisposition to periodontal diseases.
IL-22 has been implicated in protection from cell damage, enhancement of regeneration, and the repair process [83-85]. In keratinocytes, IL-22 plays a role in host defense responses by inducing the production of various antimicrobial proteins including β-defensins [86]. Th17 cells are reported as one of the most predominant subset lineages that produce IL-22 [87]. However, IL-22 expression levels remain low even though the IL-23-induced Th17 pathway is stimulated in inflammatory periodontal lesions, suggesting that the IL-17-producing Th cells that accumulate in these lesions have no potential to produce IL-22 [88]. Indeed, recent criteria for classifying functional T-cell subsets place Th cells into several subsets according to their production of IL-17, IFN-γ, and IL-22. These subsets include Th cells producing only IL-17, but not IL-22, Th cells producing only IL-22 (Th22 cells) [89], and Th cells producing both IL-17 and IFN-γ (Th17/Th1 cells) [90]. This Th17/Th1 cell lineage is suggested to be implicated in the pathogenesis of periodontal diseases [71]. Since IL-22 plays a role in host defense responses by inducing the production of various antimicrobial proteins and enhancing the mineralized matrix-forming activity of periodontal ligament cells [88], we speculate that low or no expression of IL-22 could be implicated in protracted inflammation of periodontal disease with severe tissue destruction. It is unclear why Th cell clones, having the potential to produce IL-22 in response to periodontal pathogens, are frequently detected in PBMCs, even though IL-22 expression is low in each individual (our personal communication). The involvement of IL-22 is also supported by a recent study suggesting that IL-22 deficiency may be involved in chronic inflammatory disorders such as acne inversa [91].

Concluding Remarks

Elucidating the pathogenesis of periodontal diseases is extremely complicated because numerous biological, genetic, and environmental factors are involved. In this paper, we have reviewed the implication for Th cell responses in periodontal biology, especially focusing on the immune system regulated by cytokines, from an immunogenetic viewpoint along with our previous studies. Although T cell responses against periodontopathic bacteria play central roles in the host defensive functions to eliminate foreign pathogens, they also coincidentally induce destructive reactions in periodontal tissues. These two opposing T cell responses create a so-called ‘double-edged sword’ in the pathogenesis of periodontal disease. It is very important to analyze these responses, both the protective and destructive roles. Several studies have provided results implicating Th1 cells in tissue destruction in periodontal disease, and we agree with this issue to a certain extent since there are some reports showing similar mechanisms in other diseases. However, we and several other researchers think that Th1 responses contribute greatly to protective function against microbial infections. Meanwhile, many studies have similarly provided evidence that Th17 cells are involved in the tissue destruction in periodontitis lesions. However, given there is an immunological difference between healthy humans and mice in the presence of IL-17-producing cells in their PBMCs [92], it is controversial whether Th1 cells play a central role in periodontal tissue destruction in humans, since the studies implicating Th1 cells in bone destruction were performed using a Th1-slanted C57BL/6 mouse model [38,51-53]. Moreover, it is important to consider that the net effect of various immune responses on clinical manifestations such as bone resorption in periodontal lesions would vary between different animal species, such as humans and mice. Therefore, it may be valuable to evaluate the net effect of Th cell responses on the pathogenesis of periodontal disease in individuals with infectious diseases, such as leprosy, whose pathogenesis is regulated by an individually-inherited Th1/Th2 immune responsiveness against infected pathogens.

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