

Levels of Interferon-Gamma and Transcription Factor T-Bet in Progressive Periodontal Lesions in Patients With Chronic Periodontitis

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Background: Periodontitis is an infection with an episodic pattern of tissue-support destruction. During the generation of a primary CD4⁺ T helper 1 (Th1) response, interferon-gamma (IFN- γ) acts as a positive regulator by selectively inducing Th1 differentiation through increased transcription of T-bet. The aims of this work were to determine IFN- γ levels in samples of gingival crevicular fluid (GCF) and to determine IFN- γ and transcription factor T-bet expression in gingival tissue from patients undergoing the progression of chronic periodontitis.

Methods: One hundred six patients with moderate or advanced chronic periodontitis were selected. Periodontitis was characterized by at least six sites with probing depth ≥ 5 mm, clinical attachment loss ≥ 3 mm, and radiographic bone loss. Periodontitis progression was determined by the tolerance method. GCF was collected using a paper strip, and enzyme-linked immunosorbent assay was performed to determine the total amount of IFN- γ . Gingival biopsies were obtained from patients for real-time reverse transcription-polymerase chain reaction to determine IFN- γ and T-bet expression. Statistical analysis was performed using statistical software. Data were expressed as subject means \pm SD. The χ^2 and Student *t* tests were used.

Results: The total amount and concentration of cytokine IFN- γ were significantly higher in active sites than in inactive sites (99.90 versus 68.90 pg; $P=0.03$; 106.62 pg/mg versus 75.64 pg/mg, $P=0.04$, respectively). Active sites showed a significantly lower Δ cycle threshold (Ct) of IFN- γ than inactive sites ($P=0.04$), whereas the expression of transcription factor T-bet was increased 1.42-fold in active sites compared to inactive sites.

Conclusion: The total amount and concentration of cytokine IFN- γ in GCF samples and transcription factor T-bet expression were increased in progressive periodontal lesions in patients with chronic periodontitis. *J Periodontol* 2009;80:290-296.

KEY WORDS

CD4⁺; chronic periodontitis; cytokines; interferon-gamma; progressive periodontitis; T cells.

Chronic periodontitis is an inflammatory disease of the supporting tissues of teeth, caused by groups of specific microorganisms, which results in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both.¹ Chronic periodontitis is usually related to polybacterial infection, including, among others, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) and *Tannerella forsythia* (previously *T. forsythensis*).² The inflammatory infiltrate in periodontal diseases consists of mononuclear cells, mainly transmigrated mononuclear phagocytes and lymphocytes.^{3,4}

Based on their pioneer work, Mosmann and Coffman⁵ proposed that T helper cells (Th) could be divided into two distinct subsets, Th1 and Th2, characterized by distinct cytokine profiles and effectors functions. Immunohistology clearly established that a

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T-cell/macrophage lesion identical to a delayed hypersensitivity reaction⁶ occurred within 4 to 8 days of plaque accumulation in an experimental gingivitis study.⁷ This is synonymous with the early lesion described by Page and Schroeder⁸ and with the putative stable lesion.⁹ The striking similarities between this early/stable periodontal lesion and delayed-type hypersensitivity prompted the suggestion that cells with a Th1 cytokine profile are the major mediator. Such a concept is consistent with the proposal that a strong innate immune response leads to the production of interleukin (IL)-12, which, in turn, leads to this Th1 response.¹⁰ The dominance of B cells/plasma cells in the advanced/progressive lesion suggests 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 might occur that may not contain the infection.¹¹

Th1 cells secrete interferon-gamma (IFN- γ) and tumor necrosis factor-alpha, which are critical for the eradication of intracellular pathogens, whereas Th2 cells produce IL-4, -5, -6, and -13, which are essential for optimal antibody production and the elimination of extracellular microorganisms, including helminths and nematodes.¹² IFN- γ has multiple immunoregulatory effects, mediates host defense against infection, and is a potent activator of mononuclear phagocytes.¹³ IFN- γ , released during the early and late stages of the immune response by natural killer cells and activated T cells, respectively, regulates several aspects of the immune response.¹⁴ IFN- γ also acts upon uncommitted myeloid immature dendritic cells (DCs) to polarize them into Th1 cell-promoting effector cells that produce high levels of IL-12 upon stimulation.¹⁵ Data suggest that antigen-presenting cells, including DCs and macrophages, also produce large amounts of IFN- γ .¹⁶ During the generation of a primary Th1 response, IFN- γ acts as a positive regulator by selectively inducing Th1 differentiation through the increased transcription of T-bet, which results in enhanced IL-12 responsiveness, and by suppressing Th2 lineage commitment.¹⁷ T-bet, whose expression is primarily limited to the immune system, is rapidly induced in early developing Th1 cells and is absent in developing Th2 cells, and it correlates with IFN- γ expression in Th1 cells.¹⁸ It was reported that the concentration of IFN- γ was significantly higher in serum samples and gingival tissue biopsies from periodontitis patients than from healthy controls.¹⁹ Therefore, the aims of our study were to determine the total amount and concentration of the cytokine IFN- γ in samples of gingival crevicular fluid (GCF) and to determine IFN- γ and transcription factor T-bet expression in gingival tissue from patients undergoing the progression of chronic periodontitis.

MATERIALS AND METHODS

Study Population

This was a longitudinal clinical study in which patients with moderate to severe chronic periodontitis were followed until they developed a clinical attachment loss (AL) >2 mm. One hundred six patients were selected from the Center of Diagnosis and Treatment of Northern Metropolitan Health Service, Santiago, Chile, and consecutively enrolled between March 2005 and January 2006. The criteria for entry were a minimum of 14 natural teeth, excluding third molars, and including ≥ 10 posterior teeth. Patients had at least six teeth with probing depth (PD) ≥ 5 mm with clinical AL ≥ 3 mm and extensive bone loss on radiography (localized, $\leq 30\%$ of sites involved or generalized, $\geq 30\%$ of sites involved)¹ and had received no periodontal treatment at the time of examination. Subjects did not have systemic illness and had not received antibiotics or non-steroid anti-inflammatory therapy during the 6 months prior to the study. Before sampling, all subjects received supragingival prophylaxis to remove gross calculus to allow measurement of PD.

Clinical Measurements and Determination of Progressive Periodontitis

Clinical parameters were evaluated in all teeth, excluding third molars, and included PD, clinical AL, and dichotomous measurements of supragingival plaque accumulation and bleeding on probing (BOP). Six sites were examined for each tooth: mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual. One calibrated examiner monitored the patients and collected the clinical data.

Disease activity was defined by the tolerance method,²⁰ and clinical parameters were measured at baseline and at 2 and 4 months. At the site level, sites were considered active if they exhibited AL ≥ 2.0 mm during the following 2-month period. Inactive sites were defined as those with clinical AL, PD, and BOP equivalent to active sites, but without AL during the same period. At the patient level, at least two active sites were needed to consider the patient as undergoing disease progression. Eleven patients (10.37%) exhibited disease activity during the first 2 months, and 14 patients (13.2%) exhibited disease activity during the following 2 months, for a total of 25 patients (23.57%) who underwent disease progression and were included in this study. Before periodontal therapy, samples of GCF and a gingival biopsy were collected from one active site and one inactive site from each patient undergoing AL.

The protocol was clearly explained to all patients, and Institutional Review Board-approved consent forms were signed. The protocol stated that, within 2 weeks of the detection of disease activity, all

patients would be provided with periodontal treatment. The protocol research stated that if a patient showed one or more teeth with AL at two evaluations or a periodontal abscess during the study period, the tooth or teeth would be treated and excluded from the study. Periodontal therapy consisted of scaling, root planing, and oral hygiene instructions.

Collection of GCF

After isolating the tooth with a cotton roll, supragingival plaque was removed with curets** without touching the marginal gingiva. The crevicular site was dried gently with an air syringe, and GCF was collected on paper strips.†† The strips were placed in the sulcus/pocket until mild resistance was sensed and then left in place for 30 seconds. Strips contaminated with saliva or blood were excluded. After GCF collection, the volume of the sample of each paper strip was measured using a calibrated machine.‡‡ The readings were converted to an actual volume (μl) by reference to the standard curve. After GCF collection, strips were placed in Eppendorf vials and kept at -80°C . GCF was extracted by centrifugation at $18,000 \times g$ for 5 minutes at 4°C in $100 \mu\text{l}$ elution buffer containing 50 mM CaCl_2 and 0.01% Triton X-1000. The elution procedure was repeated twice, and 25 samples from each site were stored at -80°C until further analysis.

Total Protein Measurements

Total protein was measured using a microassay§§ following the manufacturer's instructions, with bovine serum albumin as the standard.

Quantification of Cytokine IFN- γ

Aliquots of each GCF sample were assayed by enzyme-linked immunosorbent assay¶¶ to determine the levels of IFN- γ according to the manufacturer's recommendations. Briefly, $50 \mu\text{l}$ standard and GCF samples were added to wells in duplicate. Fifty microliters of detection antibody was added to the wells, mixed gently, covered with strips of plastic film, and incubated for 90 minutes at room temperature (RT; 18°C to 26°C). Plates were washed four times and incubated with $100 \mu\text{l}$ conjugate for 45 minutes at RT. The plates were washed again, and $100 \mu\text{l}$ substrate were added and incubated for 30 minutes at RT in the dark. The reaction was stopped by the addition of $50 \mu\text{l}$ stop solution, and color was measured using an automated microplate spectrophotometer.¶¶¶ The amount of IFN- γ was expressed as picograms. Cytokine levels in the GCF were calculated with a standard curve (3.9 to 500 pg) obtained with recombinant cytokines. Values lower than the detection limit ($<3.0 \text{ pg}$ in our assay) were considered undetectable. Cytokine concentrations ($\text{pg}/\mu\text{l}$ or pg/mg) were calculated from the volume of GCF estimated from the calibration unit reading: total cytokine (pg)/volume GCF

(μl) and according to the amount of total protein: total cytokine (pg)/amount total protein (mg).

Real-Time Polymerase Chain Reaction (PCR)

Five gingival samples were minced into $\sim 1 \text{ mm}^3$ fragments, incubated in $500 \mu\text{l}$ reagent,### and transported at 4°C to be processed for RNA extraction.

Cytoplasmic RNA Isolation

Tissue samples were homogenized in 2 ml 4 M guanidinium thiocyanate in 0.05 M sodium citrate, pH 7.0 , supplemented with 1% sarcosyl and 100 mM β -mercaptoethanol.*** Homogenized samples were transferred to plastic RNase-free tubes, and $200 \mu\text{l}$ 2 M sodium acetate, pH 4.5 , 2 ml water-saturated phenol, and $400 \mu\text{l}$ chloroform-isoamylalcohol ($24:1$)††† were added. After incubation for 15 minutes at 4°C and centrifugation at $3,600$ revolutions per minute (rpm) for 30 minutes at 4°C , the aqueous phase was transferred to a fresh RNase-free tube, mixed with one volume of isopropyl alcohol†††† and incubated overnight at -20°C . Then samples were centrifuged at $8,000 \text{ rpm}$ for 30 minutes at 4°C ; lysed in $400 \mu\text{l}$ ice-cold lysis buffer containing 50 mM Tris-HCl, pH 7.5 , 1% sodium dodecyl sulfate, 10 mM EDTA, pH 8.0 ,§§§§ and $25 \mu\text{g}/\text{ml}$ proteinase K;¶¶¶¶ and incubated for 30 minutes at 37°C . Subsequently, RNA was extracted with $400 \mu\text{l}$ chloroform (phenol:chloroform:isoamylalcohol $25:24:1$)¶¶¶¶¶ and centrifuged for 5 minutes at $14,000 \text{ rpm}$ at RT. The RNA present in the aqueous solution was precipitated overnight at -20°C with 0.3 M Na-acetate, 2.5 volumes of 100% ethanol, and $1 \mu\text{l}$ glycogen $20 \mu\text{g}/\mu\text{l}$;### recovered by centrifugation for 30 minutes at $14,000 \text{ rpm}$ at 4°C ; and resuspended in $20 \mu\text{l}$ H_2O RNase-free. RNA quality was determined in a bioanalyzer.**** Cytoplasmic RNA was quantified using a spectrophotometer††††† and stored at -80°C at a final concentration of $1 \mu\text{g}/\mu\text{l}$.

First-Strand cDNA Synthesis

Reverse transcription was performed††††† following the manufacturer's recommendations. A $20\text{-}\mu\text{l}$ volume reaction containing $4 \mu\text{l}$ $5\times$ RT buffer, $0.5 \mu\text{l}$ RNase inhibitor (20 U), $2 \mu\text{l}$ deoxynucleotide mix (1 mM each), $2 \mu\text{l}$ random hexamer primer ($60 \mu\text{M}$),

** Hu-Friedy, Chicago, IL.

†† ProFlow, Amityville, NY.

‡‡ ProFlow.

§§ Bio-Rad Laboratories, Hercules, CA.

¶¶ BioSource International, Camarillo, CA.

¶¶¶ ELx 800, BioTek Instruments, Winooski, VT.

RNA Safer Stabilizer Reagent, Omega Bio-tek, Norcross, GA.

*** Fluka, Sigma-Aldrich Chemie, Buchs, Switzerland.

†††† Sigma-Aldrich Chemie.

††††† Merck & Co., Whitehouse Station, NJ.

§§§§ Sigma-Aldrich Chemie.

¶¶¶¶ Roche, Basel, Switzerland.

¶¶¶¶¶ Sigma-Aldrich Chemie.

Roche.

**** Agilent 2100B, Agilent Technologies, Palo Alto, CA.

††††† Nanodrop ND-1000, Nanodrop Technologies, Wilmington, DE.

††††† Transcriptor First Strand cDNA Synthesis Kit, Roche.

0.5 μ l reverse transcriptase (10 U), 10 μ l RNA-grade H₂O, and 1 μ l RNA sample (1 μ g) were retrotranscribed for 10 minutes at 25°C and for 1 hour at 50°C.^{§§§§} The reverse transcriptase activity was subsequently inactivated by incubating the sample at 85°C for 5 minutes.

Quantitative Real-Time PCR

The mRNA expression levels of IFN- γ were quantified using real-time quantitative PCR and an endogenous control assay in which 18S rRNA levels were determined. Fifty nanograms cDNA were amplified following the manufacturer's recommendations.^{|||||} Forward and reverse primers were designed, and a specific 6-carboxyfluorescein dye-labeled probe was selected: IFN- γ : 5'-GGCATTGGAAGAATTG-GAAAG, 5'-TTTGGATGCTCTGGTCATCTT, probe #21; T-bet: 5'-TCCAAGTTTAATCAGCACCAGA, 5'-TGACAGGAATGGGAACATCC, probe #9; and 18S: 5'-CTCAACACGGGAAACCTCAC, 5'-CGCTCCACCAACTAAGAACG, probe #77. Twenty microliters volume reaction containing 0.2 μ l PCR probe (250 nM), 0.2 μ l forward primer (900 nM), 0.2 μ l reverse primer (900 nM), 10 μ l master mix,^{¶¶¶¶} 8.4 μ l PCR-grade H₂O, and 1 μ l cDNA were analyzed under the following conditions: 10 minutes at 95°C and 40 cycles of 15 seconds at 95°C and 1 minute at 60°C, in a real-time PCR system.^{####}

Data Analysis

Clinical and experimental data were analyzed using statistical software^{*****} and expressed as subject means \pm SD. The Kolmogorov-Smirnov normality and Shapiro-Wilk normality tests showed a normal distribution of the data. The χ^2 and Student *t* tests were used to compare parameters between groups because of normal data distribution. Cytokines values at each site were recorded, and the paired Student *t* test was used to analyze differences in levels between active and inactive sites in the same patient. Statistical significance was considered when $P < 0.05$. Data were obtained with software^{†††††} and were expressed as cycle threshold (Ct), Δ Ct (Ct IFN- γ or T-bet – Ct 18S rRNA), and relative quantification (RQ). The amplification efficiency of IFN- γ or T-bet reported to 18S rRNA expression (internal control) was evaluated analyzing the Δ Ct variation, with template dilutions having a 100-fold range. RQ was obtained using the $2^{-\Delta\Delta Ct}$ method, adjusting mRNA expression of IFN- γ or T-bet to 18S rRNA expression, and considering the adjusted expression in the control group as the reference (RQ = 1).²¹

RESULTS

Table 1 summarizes the clinical characteristic of the patient population; no statistically significant differences in age or gender were observed between the

groups. The mean plaque, BOP, PD, and AL were significantly lower in the main study cohort than in patients experiencing progressive chronic periodontitis ($P < 0.05$). The clinical characteristics of 25 active sites from periodontitis patients experiencing progressive chronic periodontitis are grouped in Table 1. No statistically significant difference existed between active and inactive sites with regard to the clinical parameters ($P > 0.05$).

The mean amounts and concentrations of cytokine IFN- γ in the periodontitis patients are shown in Table 2. IFN- γ in GCF was analyzed in 18 samples from 18 patients experiencing progressive chronic periodontitis. The total amount of cytokine IFN- γ was significantly higher in active sites than in inactive sites (99.90 pg versus 68.90 pg; $P = 0.03$). When the cytokine concentration was calculated from the volume of GCF estimated from the calibration unit reading, levels were higher in active sites than in inactive sites (106.68 pg/ μ l versus 75.64 pg/ μ l; $P = 0.04$). When the cytokine concentration was calculated based on the protein content of the samples, active sites showed significantly higher concentrations than inactive sites (408.75 pg/mg versus 223.58 pg/mg; $P = 0.02$).

Mean \pm SD of Δ Ct for IFN- γ and T-bet in active and inactive sites are shown in Table 3. Active sites showed a significantly lower Δ Ct for IFN- γ than inactive sites (16.12 ± 1.73 versus 18.00 ± 1.66 ; $P = 0.04$). Using the $2^{-\Delta\Delta Ct}$ method, it was determined that IFN- γ expression in active sites was augmented 2.70-fold (range: 1.84 to 23.86) over inactive sites, whereas the expression of T-bet in active sites was increased 1.42-fold (range: 2.02 to 0.92) over inactive sites.

DISCUSSION

In this study, we determined and compared the total amount and concentration of IFN- γ in samples of GCF, together with the expression of IFN- γ and T-bet levels in gingival tissue obtained from active and inactive sites in patients with chronic periodontitis experiencing episodic AL. Our data demonstrated that the total amount of cytokine IFN- γ in the GCF of active sites in patients with the progression of periodontitis was significantly higher than in inactive sites. Longitudinal studies^{22,23} in periodontitis progression demonstrated that the rate of periodontal tissue destruction is low, and advanced forms of the disease occur in comparatively few individuals and few tooth

§§§§ Primus 96 plus, MWG Biotech, Ebersberg, Germany.

||||| Roche.

¶¶¶¶ FastStart Master, Roche.

Applied Biosystems, Foster City, CA.

***** Stata 8.0, StataCorp, College Station, TX.

††††† ABI PRISM Sequence Detector Systems software, Applied Biosystems.

Table 1.
Clinical Characteristics of Periodontitis Patients and Active and Inactive Sites

Clinical Parameters	Total Subjects (N = 106)	Active Sites (n = 25)	Inactive Sites (n = 25)
Mean PD (mm; mean ± SD)*	3.06 ± 0.50	4.42 ± 0.46	4.64 ± 0.62
Attachment level (mm; mean ± SD)*	3.46 ± 0.48	6.20 ± 0.76	6.42 ± 0.68
Sites with plaque (%)*	72	100	100
Sites with BOP (%)*	45	100	100
Age (years; mean ± SD [range])	43.22 ± 7.00 (35 to 62)	45.66 ± 6.67 (35 to 56)	
Females (%)	74.52	72.22	

* Total subjects versus active sites and inactive sites: *P* < 0.05.

Table 2.
Cytokine IFN-γ Levels in GCF From Active and Inactive Sites

	Active Sites (n = 25)		Inactive Sites (n = 25)	
	Mean ± SD	Range	Mean ± SD	Range
IFN-γ (pg)*	99.90 ± 55.93	42.91 to 278.40	68.90 ± 27.53	32.11 to 126.40
IFN-γ (pg/μl)†	106.62 ± 59.19	51.78 to 296.10	75.64 ± 35.61	34.16 to 181.20
IFN-γ (pg/mg)‡	408.83 ± 279.91	155.30 to 1050.01	223.63 ± 71.67	125.01 to 345.74

* In active sites versus inactive sites: *P* = 0.03.
 † In active sites versus inactive sites: *P* = 0.04.
 ‡ In active sites versus inactive sites: *P* = 0.02.

Table 3.
ΔCt and Fold Change of IFN-γ and T-Bet From Active and Inactive Sites Using the 2^{-ΔΔCt} Method

	ΔCt IFN-γ (mean ± SD)	2 ^{-ΔΔCt} IFN-γ (fold change)	ΔCt T-Bet (mean ± SD)	2 ^{-ΔΔCt} T-Bet (fold change)
Active sites	16.12 ± 1.73*	2.70 (1.84 to 23.86)	16.89 ± 0.61†	1.42 (0.92 to 2.02)
Inactive sites	18.00 ± 1.66*	1.00 (0.32 to 3.15)	17.37 ± 0.74†	1 (0.70 to 1.20)

* Active versus inactive sites: *P* = 0.04.
 † Active versus inactive sites: *P* = 0.08.

sites. In the past, attention was focused on the possibility that periodontal disease may not be a continuous process but could be characterized by episodes of activity, followed by periods of relative quiescence.²⁴ Episodic periodontal clinical AL could be associated with variations in supracrestal inflammatory cell populations, where significantly higher numbers of mast cells, monocytes/macrophages, and plasma cells

are present in active sites compared to inactive sites.⁴ However, the contribution of acquired immune cells in the progression of periodontal disease has long been controversial; their exact role in the protection versus destruction of the host's periodontium remains unclear.^{25,26} One of the main concerns in chronic periodontitis is the definition of active sites, at which tissue destruction and probably, the secretion of certain cytokines, are occurring. We showed higher levels of CD4⁺ Th cells in the context of periodontal disease progression, which could directly trigger osteoclastogenesis and alveolar bone loss, associated with periodontitis in vivo.²⁷ Considering the levels of IFN-γ found in active sites, we speculate that this cytokine is involved in the development of the gingival inflammatory response by mediating its activation.

Our findings demonstrated that the total amount and concentration of IFN-γ are significantly higher in active sites versus inactive sites. Disease activity is generally accepted to be the loss of soft or hard tissue attachment to the tooth; nevertheless, changes in clinical attachment level may represent true changes in attachment level, changes in tissue texture, or a combination of both.²⁸ The results of the present study are consistent with the findings of Teng et al.,²⁹ who described the role of

IFN-γ (in humans and mice) in modulating receptor activator of nuclear factor-kappa B ligand (RANKL)⁺ Th cell-mediated alveolar bone loss under postmicrobial challenge inflammatory conditions in vivo. IFN-γ, a cytokine with multiple immunoregulatory effects, mediates the host defense against infection and is a potent activator of mononuclear phagocytes.¹³ The effect of T cells on osteoclastogenesis depends

on the balance between RANKL and IFN- γ .³⁰ In some studies,^{31,32} IFN- γ seemed to be the predominant cytokine produced by T cells in periodontal diseases, and an enhancement of IFN- γ -producing cells was correlated with the progression of disease.

Using real-time PCR analysis of gingival tissues, we demonstrated that the Ct and Δ Ct of IFN- γ in active sites were lower than in inactive sites. Because Ct values decrease linearly with increasing input-target quantity,³³ IFN- γ mRNA levels were higher in active sites. Additionally, using the $2^{-\Delta\Delta$ Ct method,²¹ we observed 2.70 mean-fold (range: 1.84 to 23.86) and 1.42-fold (range: 0.92 to 2.02) increases in IFN- γ and T-bet expression, respectively, in active sites compared to inactive sites, indicating that the IFN- γ and T-bet gene are highly overexpressed in periodontal tissue associated with progressive destruction. Based on these data, higher levels of IFN expression found in active sites in chronic periodontitis could result in an increased severity of disease. We found that IFN- γ and T-bet levels were also higher in active periodontal pockets, suggesting a destructive role for the Th1 response in the periodontium; IFN- γ and IL-2 levels were associated with Th1 patterns of immune response.³⁴

Studies³⁵⁻³⁸ regarding the role of cytokines in bone tissue associated Th1 and Th2 cytokines with the activation and suppression of bone resorption, respectively. Recently, characterization of the Th1 response was improved by the discovery that T-bet transcription factor was induced rapidly in T cells upon Th1 differentiation.¹⁸ Retroviral T-bet expression strongly augmented IFN- γ production. It was initially believed that T-bet might act downstream of IL-12 and Stat4 and mediate their effects on Th1 differentiation.¹⁸ Based on this theory, it was believed that Th1 and Th2 development shared symmetric internal mechanisms, where IL-12, Stat 4, and T-bet represented Th1 equivalents of IL-4, Stat6, and GATA-3 from Th2 responses. One of the difficulties in sorting out the molecular mechanism of Th1 and Th2 development has been the large number of indirect effects that cytokines (or other factors, such as antigen concentration and types of antigen-presenting cells) can exert on Th cell development in vivo or in vitro.³⁹ IFN- γ has a central role in controlling all stages of Th1 differentiation and memory formation.⁴⁰ During the generation of a primary Th1 response, IFN- γ acts as a positive regulator by selectively inducing Th1 differentiation through the increased transcription of T-bet, which results in enhanced IL-12 responsiveness and suppressed Th2 lineage commitment.¹⁷ In addition, IFN- γ enhances IL-12 transcription, which further optimizes the generation of Th1 cells.⁴¹

CONCLUSION

Our data showed higher cytokine IFN- γ levels in the GCF and greater IFN- γ and transcription factor T-bet expression in gingival tissue from progressive periodontal lesions in patients with chronic periodontitis.

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