Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-κB ligand, interleukin (IL) -17, IL-10 and transforming growth factor-β during the progression of chronic periodontitis


Abstract

Aim: T regulatory (Treg) cells have been detected in periodontitis lesions, and forkhead box P3 (Foxp3) expression has been negatively correlated to receptor activator of nuclear factor-κB ligand (RANKL). The aim of this study was to correlate T-helper type 1 (Th1), Th2, Th17 and Treg transcription factor expressions, in gingival tissues from patients undergoing active periodontal tissue destruction, with bone loss-associated cytokines.

Materials and Methods: In 10 chronic periodontitis patients undergoing disease progression, the mRNA expressions of T-bet, GATA-3, Foxp3, RORC2, interleukin (IL)-1β, IL-10, IL-17, RANKL, interferon (IFN)-γ and transforming growth factor (TGF)-β1 were quantified using real-time reverse transcription-polymerase chain reaction. The levels of these markers were compared between active and inactive periodontal lesions.

Results: In active periodontal lesions, Foxp3, T-bet, RANKL, IL-17, IL-1β and IFN-γ were significantly over-expressed compared with inactive lesions. The expression of IFN-γ was the highest within the active periodontal lesions, similar to that of TGF-β1 within the inactive ones. There was a positive correlation between RANKL and IL-17. Additionally, RANKL and IL-17 were positively correlated with RORC2, but no correlation was detected with Foxp3.

Conclusions: These results lead us to speculate that Foxp3+ cells that do not have a regulatory function might have a role in the pathogenesis of active periodontal lesions by down-regulating TGF-β1 and IL-10 synthesis that lead to the over-expression of Th17-associated cytokines RANKL and IL-17.

Key words: CD25; CTLA-4; Foxp3; IL-17; periodontitis; RANKL; regulatory T cells; RORC2; Treg

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Conflict of interest and source of funding statement

The authors declare no competing financial interests.

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The immune system has evolved to protect higher organisms from infection by microorganisms and parasites. It functions via specialized cells and molecules that are able to distinguish between self-antigens and foreign antigens, being tolerant to self-antigens and responding to foreign antigens in order to control and eliminate them. Tolerance to self-antigens is attained initially by eliminating self-reactive T- and B-lymphocytes during their negative selection in the thymus and the bone marrow, respectively. In addition, the immune system has peripheral mechanisms to deal with lymphocytes that escape this central tolerance system. These peripheral mechanisms are based mainly in T regulatory (Treg) cells (Vernal & Garcia-Sanz 2008).

Treg cells control the induction and activity of effector T cells and thereby prevent and control exacerbated immune responses and the development of autoimmune diseases (Green et al. 1983). These regulatory functions are mediated by the expression of the transcription factor forkhead box P3 (Foxp3) and involve the suppression of the activation of T-, B- and natural killer cells through direct cell–cell mechanisms, mediated by surface molecules, such as cytotoxic T-lymphocyte antigen-4 (CTLA-4), or by the synthesis of immuno-suppressive cytokines, such as interleukin (IL)-10 and transforming growth factor (TGF)-β (McGuirk & Mills 2002, Shevach 2002, Mittrucker & Kaufmann 2004, Horwitz et al. 2008, Shevach et al. 2008).

In fact, increased proportions of CD4⁺ CD25⁺ CTLA-4⁺ cells and an elevated expression of Foxp3 have been detected in periodontitis when compared with that in the gingivitis lesions (Nakajima et al. 2005). Furthermore, a majority (≈90%) of T-cell clones derived from gingival tissues with periodontitis lesions expressed Foxp3, which may be induced in CD4⁺ T cells either by polyclonal or by antigen-specific stimulation (Ito et al. 2005, Okui et al. 2008). The expression of Foxp3 has been positively correlated to both IL-10 and TGF-β1 expression in periodontitis lesions; however, only TGF-β1, but not IL-10, was significantly over-expressed in periodontitis when compared with that in the gingivitis lesions (Nakajima et al. 2005). Conversely, on comparing with healthy gingival tissues, Ernst et al. showed that CD25⁺ Foxp3⁺ cells were decreased in periodontitis lesions, while the levels of the receptor activator of nuclear factor-κB ligand (RANKL), a key factor that mediates the osteoclast differentiation and maturation, were negatively correlated to IL-10 and CD25⁺ Foxp3⁺ cells, suggesting that a lower number of Treg cells is associated with over-regulation of the RANKL expression in these affected tissues (Ernst et al. 2007).

In spite of the experimental evidence for the existence of Treg cells in periodontitis-affected tissues, their role during the initiation and progression of this disease still remains unclear. Whether the number and activity of Treg cells are enhanced or diminished in chronic periodontitis compared with healthy or gingivitis tissues still needs to be better clarified, and their presence should be correlated with the activity of periodontal destruction. With this purpose, we have analysed the expression of Foxp3, CTLA-4, IL-10 and TGF-β1 in gingival tissues from patients undergoing periodontitis progression, and associated these results to the T helper type 1 (Th1), Th2 and Th17 transcription master factors, T-bet, GATA-3 and transcription factor orphan nuclear receptor C2 (RORC2), respectively, as well as RANKL and IL-17 bone destruction-associated cytokines.

Materials and Methods

Patients

Seventy-six patients affected with chronic periodontitis were consecutively enrolled at the Center of Diagnosis and Treatment Dra. Eloisa Díaz in the Northern Metropolitan Health Service, Santiago, Chile. The criteria for patient selection were as follows: (a) a minimum of 14 natural teeth, excluding third molars and including at least 10 posterior teeth, (b) no previous periodontal therapy, (c) absence of relevant systemic diseases and (d) no intake of antibiotics or non-steroid anti-inflammatory drugs in the previous 6 months. The protocol of the study was clearly explained to all the participants who agreed to participate in the study by signing an IRB-approved informed consent form.

Clinical measurements and diagnosis of chronic periodontitis

At baseline, after receiving a supragingival prophylaxis to remove gross calculus, all the selected patients underwent a periodontal clinical examination by a single calibrated examiner (J. G.), who registered probing pocket depths (PD), clinical attachment level (CAL) loss and dichotomous measurements of supragingival bacterial plaque (BP) and bleeding on probing (BOP) at six periodontal sites in all teeth, excluding third molars. The diagnosis of moderate to advanced chronic periodontitis was established by having at least six teeth with PD ≥5 mm and CAL ≥3 mm, together with generalized radiographic bone loss (≥30% of the periodontal sites), according to a classification system of periodontal diseases and conditions (Armitage 1999).

Determination of progressive periodontal destruction

Patients were re-examined every 2 months and progression of chronic periodontitis was determined by the tolerance method and when at least two active periodontal lesions were detected in a patient, as described previously (Haftajee et al. 1983, Hernandez et al. 2006, Silva et al. 2008). A periodontal lesion was defined as an active periodontal lesion when, in a patient undergoing progressive periodontitis, the periodontal site exhibited an increase in CAL ≥2.0 mm during a 2-month interval. In the same patient, an inactive periodontal lesion had severity in terms of CAL, PD and BOP equivalent to an active lesion, but exhibited an increase in CAL <2.0 mm during the same time interval. Ten patients undergoing periodontitis progression were included in this study and, within 1–2 weeks of detecting their disease activity, they were provided with appropriate periodontal therapy.

Gingival tissue biopsies

From each patient, specimens of gingival tissue were collected from one active and one inactive periodontal lesion under appropriate local anaesthesia and following the patient’s periodontal therapy requirements. Samples were minced in approximately 1 mm³ fragments, immediately submerged in 500 μl RNA Safer Stabilizer Reagent (Omega Bio-Tek Inc., GA, USA) and transported at 4°C to be processed for RNA extraction.
Isolation of cytoplasmic RNA

Tissue samples were homogenized in 2 ml of 4 M guanidinium thiocyanate in 0.05 M sodium citrate (pH 7.0), supplemented with 1% sarcosyl and 100 mM β-mercaptoethanol (Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Homogenized samples were transferred to an RNase-free tube and 200 μl of 2 M sodium acetate (pH 4.5), 2 ml of water-saturated phenol and 400 μl of chloroform-isoamylalcohol (24:1) (Fluka) were added. After incubation for 15 min. at 4°C and centrifugation at 3000 g for 30 min. at 4°C, the aqueous phase was transferred to a fresh RNase-free tube and mixed with 1 volume of isopropyl alcohol (Merck & Co., Inc., Whitehouse Station, NJ, USA) and precipitated overnight at −20°C. Then, samples were centrifuged at 8000 g for 30 min. at 4°C, lyzed in 400 μl of ice-cold lysis buffer containing 50 mM Tris-HCl (pH 8.0), 1% SDS, 10 mM EDTA pH 8.0 (Fluka) and 25 μg/ml proteinase K (Roche Ltd., Basel, Switzerland) and incubated for 30 min. at 37°C. Subsequently, RNA was extracted with 400 μl of chloroform (phenol:chloroform:isoamylalcohol 25:24:1) and centrifuged for 5 min. at 14,000 g at room temperature (Fluka). 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 μl of glycogen 20 μg/μl (Roche), recovered by centrifugation performed for 30 min. at 14,000 g at 4°C and resuspended in 20 μl RNase-free H2O. RNA quality was determined in a bioanalyzer (Agilent 2100B, Agilent Tech., Palo Alto, CA, USA). Cytoplasmic RNA was quantified using a spectrophotometer (NanoDrop ND-1000, Nanodrop Tech., Wilmington, ND, USA) and stored at −80°C at a final concentration of 1 μg/μl.

First-strand cDNA synthesis

Reverse transcription was performed using a Transcriptor First Strand cDNA synthesis kit (Roche) following the manufacturer’s recommendations. Briefly, 20 μl reaction containing 4 μl 5x RT buffer, 0.5 μl RNase inhibitor (20 U), 2 μl deoxynucleotide mix (1 mM each), 2 μl random hexamer primer (60 μM), 0.5 μl reverse transcriptase (10 U), 10 μl RNA-grade H2O and 1 μl RNA sample (1 μg) were retro-transcribed under the following conditions: 10 min. at 25°C and 1 hour at 50°C (Primus 96 plus, MWG Biotech AG, Ebersberg, Germany). The reverse transcriptase was subsequently inactivated by incubating the sample at 85°C for 5 min.

Quantitative real-time polymerase chain reaction (PCR)

The mRNA expression levels of the cytokines IL-1β, IL-10, IL-17, RANKL, interferon (IFN)-γ and TGF-β1; the surface markers CD4, CD8, CD25 and CTLA-4; and the transcription factors Foxp3, T-bet, GATA-3 and RORC2 were quantified using real-time quantitative PCR. As an endogenous control, the expression of 18S rRNA was determined. Fifty nanogram of cDNA were amplified in 384-well plates using a FastStart Taqman Probe Master (Roche) following the manufacturer’s recommendations. Forward and reverse primers were designed and a specific FAM dye-labelled probe was selected using the Roche website http://www.roche-applied-science.com (Table 1). Ten microlitre reaction containing 0.1 μl PCR probe (250 nM), 0.1 μl forward primer (900 nM), 0.1 μl reverse primer (900 nM), 5 μl FastStart master, 3.7 μl PCR-grade H2O and 1 μl cDNA (50 ng) were amplified under the following conditions: 10 min. at 95°C and 40 cycles of 15 s at 95°C and 1 min. at 60°C, in an ABI PRISM 7900 Sequence Detector System (Applied Biosystems, Foster City, CA, USA).

Data analysis

Clinical and experimental data were analysed using the statistical software SPSS 15.0 (Lead Technologies Inc., Charlotte, NC, USA). For each patient, the mean results from each analysed clinical parameter at the baseline and after the longitudinal analysis were generated and expressed as subject means ± SD (Table 2). The Kolmogorov–Smirnov test demonstrated that these clinical data were normally distributed. Comparisons in age, PD and CAL results were made using the two-tailed unpaired Student’s t-test, while for BP, BOP and gender data the χ2 test was used. Quantitative real-time PCR data were analysed with the ABI PRISM Sequence Detector Systems software (Applied Biosystems) and were expressed as Ct (cycle threshold), ΔCt (Ct mRNA blank – Ct 18S rRNA) and relative quantification (RQ). RQ was obtained using the 2−ΔΔCt method, adjusting the blank mRNA expression to 18S rRNA expression and considering the adjusted expression in inactive periodontal lesions as a reference (RQ = 1) (Livak & Schmittgen 2001). When the RQ levels of IL-10 and TGF-β1 were calculated, the adjusted expression in active periodontal lesions was considered as a reference. The amplification efficiency of each gene reported in inactive periodontal lesions was obtained using the 2−ΔΔCt method.

Table 1. Forward primers, reverse primers and PCR probes used for cytokine, surface marker and transcription factor amplifications by quantitative real-time PCR

<table>
<thead>
<tr>
<th>Forward primers</th>
<th>Reverse primers</th>
<th>Probes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>ctgctcgcgtgctgaaaga</td>
<td>tgggttaatttttggtgtcaga</td>
</tr>
<tr>
<td>IL-10</td>
<td>tgggggagaaccttgaaac</td>
<td>ccttgccttgtttcacag</td>
</tr>
<tr>
<td>RANKL</td>
<td>ggtggaactctagggttgg</td>
<td>ggatttcgtgggattgtgat</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>ggcaatgtggaatgggaagaa</td>
<td>ttgagagctttgctactt</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>caacgtggagttcagaga</td>
<td>cagccggttgctgaggta</td>
</tr>
<tr>
<td>CD4</td>
<td>gaggctgtgctgtgtag</td>
<td>gacatggtagcggcag</td>
</tr>
<tr>
<td>CD8</td>
<td>cgctctgtatcatcttgtg</td>
<td>ggcttcatctgcatt</td>
</tr>
<tr>
<td>CD25</td>
<td>caaggagccacccctgg</td>
<td>ggcctgctttgcagt</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>ttctcctctccttgca</td>
<td>cacacaacgctgggag</td>
</tr>
<tr>
<td>Foxp3</td>
<td>acctacgccaacgtcatc</td>
<td>tcatggtgcctgcgc</td>
</tr>
<tr>
<td>T-bet</td>
<td>tcaagaatccctagacagca</td>
<td>tgggcacatggggaag</td>
</tr>
<tr>
<td>GATA-3</td>
<td>ctcattataaccccagcag</td>
<td>ttgagctgtgctcggag</td>
</tr>
<tr>
<td>RORC2</td>
<td>aagggagacggagggagag</td>
<td>caagggatatctcatag</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>ctcaacaggaggaacacteac</td>
<td>cgcctcacaactgaagct</td>
</tr>
</tbody>
</table>

*Number of the specific FAM dye-labelled probes selected from the quantitative real-time PCR system of Roche.

IL, interleukin; PCR, polymerase chain reaction; RANKL, receptor activator of nuclear factor κB ligand; IFN, interferon; TGF, transforming growth factor; CTLA-4, cytotoxic T-lymphocyte antigen-4; Foxp3, transcription factor forkhead box P3; RORC2, transcription factor orphan nuclear receptor C2.
Table 2. Clinical characteristics of the chronic periodontitis patients at baseline and of the active and inactive periodontal lesions sampled from patients who underwent progression of chronic periodontitis

<table>
<thead>
<tr>
<th></th>
<th>Total subjects (n = 76)</th>
<th>Patients with progressive periodontitis (n = 10)</th>
<th>Inactive lesions (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD (mm)</td>
<td>3.50 ± 0.68±</td>
<td>4.42 ± 0.46</td>
<td>4.64 ± 0.62</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>3.90 ± 0.48</td>
<td>6.20 ± 0.76</td>
<td>6.42 ± 0.68</td>
</tr>
<tr>
<td>BP (%)</td>
<td>74.1±</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>57.9±</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Females (%)</td>
<td>56.6±</td>
<td>72.2</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.1±</td>
<td>45.6 ± 6.6</td>
<td></td>
</tr>
</tbody>
</table>

*PD, total subjects versus active and inactive lesions: p-value < 0.05.
*cAL, total subjects versus active and inactive lesions: p-value < 0.05.
*BP, total subjects versus active and inactive lesions: p-value < 0.05.
*bBOP, total subjects versus active and inactive lesions: p-value < 0.05.

Clinical data are expressed as subject mean ± standard deviation.

PD, probing depth; CAL, clinical attachment level loss; BP, periodontal sites with supragingival bacterial plaque; BOP, periodontal sites with bleeding on probing.

After determining the normality of the data distribution using the Kolmogorov–Smirnov test, the differences among the mRNA expressions of the analysed transcription factors and cytokines were determined analysing the ΔCt data and using either the Wilcoxon test or the paired Student’s t-test. Correlation coefficients were obtained analysing the RQ data and using the Pearson test. A statistical significance was considered when p-value < 0.05.

Results

Table 2 summarizes the clinical characteristics of all the chronic periodontitis patients evaluated at the beginning of the investigation. Similarly, the clinical characteristics of the active and inactive periodontal lesions sampled for real-time quantitative PCR analysis from patients who underwent progression of chronic periodontitis are given. From a total of 76 patients diagnosed with chronic periodontitis, 10 patients undergoing disease progression were included in this investigation. At the time of surgical therapy and when the biopsy was taken, there were no significant differences in the clinical parameters between active and inactive periodontal lesions.

The expression of mRNA for Foxp3, T-bet, GATA-3, RORC2, IL-1β, IL-10, IL-17, RANKL, IFN-γ, TGF-β1, CD4, CD8, CD25 and CTLA-4 was detected in all the tissue samples of both active and inactive periodontal lesions. The fold-change of RQ for each studied cytokine, cell marker or transcription factor was expressed as the mean and standard deviation (Fig. 1). In active periodontal lesions, Foxp3 was significantly over-expressed (3.4-fold) compared with inactive periodontal lesions. Similarly, CD25 (3.8-fold) and T-bet (2.8-fold) were also significantly over-expressed in active periodontal lesions. CTLA-4, GATA-3 and RORC2 (~ 1.5-fold) over-expression, however, was not statistically significant.

IFN-γ was the highest over-expressed cytokine detected within active periodontal lesions, with an RQ of 7.8 (Fig. 1). Similarly, in active periodontal lesions, RANKL (3.4-fold), IL-17 (2.8-fold) and IL-1β (3.7-fold) were also significantly over-expressed compared with inactive periodontal lesions. IL-10 and TGF-β1 were, however, down-regulated in the active periodontal lesions (RQ of 0.52 and 0.08, respectively). When the RQ was re-calculated considering the adjusted IL-10 and TGF-β1 expression in the active lesions as RQ = 1, the expression of IL-10 and TGF-β1 in the inactive periodontal lesions was 3.1- and 27.3-fold higher, respectively, than the levels detected in the active periodontal lesions (Fig. 1).

The analyses of correlations between the different genes yielded significant positive correlations between Foxp3 and CD25, Foxp3 and CD4 and CD4 and CD25 (p < 0.001); however, Foxp3 and CTLA-4, Foxp3 and CD8 and CD4 and CD8 did not show any positive correlation (Fig. 2). In the analysis of cytokine expression, positive correlations were detected between RANKL and IL-1β (p < 0.001), and IFN-γ (p = 0.004) and IL-17 (p < 0.001). In contrast, RANKL was negatively correlated with TGF-β1 (p = 0.042) and did not correlate with IL-10 (Fig. 3).
The analysis of correlations between RANKL and IL-17 with the different transcription factors studied yielded a positive correlation with RORC2 ($p < 0.001$), but not with Foxp3 (Fig. 4). A positive correlation was also detected between RANKL and T-bet ($p = 0.015$) and between T-bet and RORC2 ($p = 0.015$). Foxp3 was not correlated with any of the other transcription factors (Fig. 5); however, there was a negative correlation between T-bet and GATA-3 ($p = 0.002$).

**Discussion**

The existence, among the T-lymphocyte population, of cells harbouring regulatory functions, able to modulate the immune response during the course of an infection, has been established (Bluestone & Abbas 2003, Mittrucker & Kaufmann 2004). These Treg cells are characterized by the expression of the surface antigen CD25 and the transcription factor Foxp3, which represents a T-cell lineage-specific marker (Bluestone & Abbas 2003, Hori & Sakaguchi 2004, Mittrucker & Kaufmann 2004, Roncarolo & Gregori 2008). Immuno-histological analyses have shown higher numbers of CD4+CD25+Foxp3+ T cells in samples from periodontitis-affected patients as compared with gingivitis controls. Furthermore, increased Foxp3 mRNA levels were also detected in these samples (Nakajima et al. 2005).

In our investigation, Foxp3 and CD25 mRNAs were significantly over-expressed in active when compared to inactive periodontal lesions. In addition, these over-expressed Foxp3 levels positively correlated with both CD25 and CD4 mRNA expression. These data confirm previous reports indicating that Foxp3+ T cells may be involved in the pathogenesis of chronic periodontitis (Ito et al. 2005, Nakajima et al. 2005, Ernst et al. 2007, Okui et al. 2008). On the other hand, it has been shown that low levels of Foxp3 expression were detected in CD8+ T cells (Hori et al. 2003, Okui et al. 2008), and Th1 and Th2 cells generated from CD4+CD25− cells failed to express Foxp3 (Hori & Sakaguchi 2004). These findings are consistent with our results in the chronic periodontitis specimens, where Foxp3 expression was not correlated with CD8, T-bet and GATA-3 expression in active periodontal lesions.

Treg cells function through CTLA-4-mediated cell–cell interactions with dendritic cells (DCs) and effector T cells (McGuirk & Mills 2002, Shevach 2002). CTLA-4-expressing Treg cells induce the expression of the enzyme indoleamine 2,3-dioxygenase, which degrades tryptophan, and in fact, the lack of this essential amino acid inhibits the T-cell activation and promotes T-cell apoptosis (Fallarino et al. 2003). TGF-β1 and IL-10 are also important mediators of these regulatory activities (Mittrucker & Kaufmann 2004, Horwitz et al. 2008, Shevach et al. 2008). TGF-β1-synthesizing Treg cells directly target T cells to ensure immune tolerance to self-antigens, whereas IL-10 inhibits the function of DCs by inhibiting the MHC class II and co-stimulatory-molecule expression (Moore et al. 2001, Li & Flavell 2008). In this investigation, we have shown that CTLA-4 was not significantly over-expressed in active periodontal lesions and this expression was not correlated with Foxp3 over-expression. Furthermore, TGF-β1 and IL-10 expression was down-regulated in active periodontal lesions when compared with their expression in in active ones. Hence, Foxp3-expressing cells do not probably fulfill their regulatory functions through the expression of CTLA-4, TGF-β1 or IL-10 in periodontal lesions showing clinical evidence of periodontal tissue destruction.

It has been previously described that Foxp3+ gingival T cells are functionally different from natural Treg cells (Okui et al. 2002).
et al. 2008). In this regard, a transient wave of Foxp3 expression is not sufficient to confer regulatory activity (Allan et al. 2007); rather, a high and sustained Foxp3 expression induced by TCR-stimulation is required to generate functional Treg cells (Passerini et al. 2007). In fact, a combination of strong anti-inflammatory signals to TCR and maximal co-stimulation is required to generate functional Treg cells (Passerini et al. 2008). Naïve T cells exposed to TGF-β up-regulate Foxp3 and differentiate to Treg cells; however, when cultured with TGF-β and IL-6, naïve T cells transform into Th17 cells (Bettelli et al. 2006, Yamazaki et al. 2007). Thus, when the immune response is not activated, TGF-β favours the generation of Treg cells, which suppress inflammation; however, when an infection is established, IL-6 is synthesized during the innate immune response, inhibiting the generation of Treg cells and inducing the differentiation of proinflamma-
double-positive T regulatory cells are associated with the increased nuclear factor-kappaB ligand (RANKL+) T cells in bone resorption lesion of periodontal disease. Clinical and Experimental Immunology 148, 271–280.


Silva, N., Dutzan, N., Hernandez, M., Dezerega, A., Rivera, O., Aguillon, J. C., Aravena, O.,
Clinical Relevance

*Scientific rationale for the study:* Although periodontopathogens are the primary aetiological agents of periodontitis, the ultimate determinant of disease progression and clinical outcome is the host’s immune response. Foxp3<sup>+</sup> cells are the key element in the regulatory network that controls immune response during an infection.

*Principal findings:* A Foxp3 overexpression was associated with active periodontal lesions; however, Foxp3<sup>−</sup> T-cells that do not bear regulatory functions may have a role in periodontal destruction due to the down-regulation of IL-10 and TGF-β1.

*Practical implications:* Patient susceptibility to periodontal destruction is determined by the balance between effector and regulatory mechanisms developed during the immune response against periodontopathogens. Understanding these regulatory mechanisms should provide a greater insight into the pathogenesis, and this may lead to the development of new immunotherapeutic strategies.