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Expression of suppressor of cytokine signaling 1 and 3 in ligature-induced periodontitis in rats

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ABSTRACT

Objective: : Evaluate expression of inducible negative regulators of JAK/STAT pathway and their target proteins during the course of ligature-induced experimental periodontal disease in rats.
Design: : Rats were sacrificed 07, 15 and 30 days after disease induction for histological evaluation of periodontal inflammation and macroscopic analysis of alveolar bone loss. SOCS expression and the activation status of STAT1 and STAT3 were evaluated in gingival biopsies by real time PCR and Western blot.
Results: : Ligature-induced model presented significant progressive bone loss from 7 to 30 days. Inflammation was evident and similar for 07 and 15 days; however, a decrease on severity at the end of the experimental period was observed. There was a significant ($p < 0.05$) increase on SOCS1 and SOCS3 gene expression in PD compared to control group at 15 and 30 days. The SOCS1 and SOCS3 protein expression and activation of STAT1 and STAT3 were increased in earlier periods in the ligature model.
Conclusion: : This study suggests that SOCS1 and SOCS3 were directly correlated with regulatory mechanism of the inflammatory process responsible for the periodontal disease destruction.

1. Introduction

Periodontitis is characterised by degradation of connective tissue and alveolar bone, which occurs as a consequence of the host response to the microbial challenge originating from the dental biofilm.[1] According to the current paradigm, disease progression with active degradation of periodontal tissues is a consequence of an unbalanced host–microbial interaction.[2] Even though tissue destruction may be induced directly by toxins and products of microbial metabolism, most of the damage is associated with the host immune/inflammatory response elicited by these microorganisms, usually characterised by the predominance of pro-instead of anti-inflammatory cytokines.[3,4] Therefore, the control of inflammatory mediators by endogenous mechanisms and the balance between pro-inflammatory cytokines and their antagonists will ultimately determine the severity and extent of tissue destruction.[5,6]

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Many cytokines that participate on periodontal destruction such as interleukins and interferons signal through Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway. The activation of this pathway is essential for the signaling of cytokines and other stimuli that regulates inflammatory gene expression. The binding of the cytokine to its specific receptor activates the associated JAK, which phosphorylates the cytoplasmic domain of the receptor to allow the recruitment and tyrosine phosphorylation of STAT. Activated STATs dimerise and translocate to the nucleus, where they work as transcription factors to regulate gene expression.[7]

Inflammatory cytokine gene expression is a process strictly regulated by various mechanisms, including the negative regulation of intracellular signaling. Endogenous proteins are involved in this process, but the mechanisms by which these proteins regulate gene expression are still elusive, especially in periodontal disease. The Suppressor of Cytokine Signaling (SOCS) family of proteins modulates in a fairly specific manner the JAK/STAT pathway, which is critical in signal transduction in inflammation.[8,9]

The SOCS family consists of eight proteins (SOCS1 to 7, and cytokine-inducible SH2-domain-containing protein) that can be induced in response to a wide range of cytokines with pro- and anti-inflammatory activities. They interfere with signaling from the inducing cytokine in a classic negative feedback loop and also regulate signaling downstream of other cytokines in a cross-talk manner.[10] While the mechanisms of cytokine signaling control in periodontal disease remain elusive, SOCS1 and 3 are expressed in established periodontal lesions.[11] SOCS1 and SOCS3 are induced by cytokines that signal through JAK/STAT pathway, including TNF-α, IFN-γ, IL-6, and IL-10 and function and endogenous inhibitors of the activation of JAK/STAT, reducing the cellular effects of these cytokines and also inhibiting their expression.[8,12,13] Therefore, SOCS1 and 3 are supposed to be involved in the negative regulation of inflammatory networks relevant in the periodontal diseases pathogenesis.[11]

However, while *in vivo* studies showed increased SOCS (particularly of SOCS1 and SOCS3) mRNA expression in periodontal lesions the kinetics of SOCS expression during the course of experimental periodontal disease remains unknown. Thus, a better understanding of negative regulatory mechanisms of JAK/STAT pathway during inflammatory response may lead to important information on periodontal disease pathogenesis and also provide a therapeutic perspective based on the modulation of pro-inflammatory gene expression. This study evaluated the kinetics of SOCS1 and SOCS3 expression in ligature-induced model of periodontal disease in rats. We also evaluated the mRNA expression of TNF-α, IL-6 and IL-10 that are direct targets of SOCS proteins and the mRNA expression of RANKL, OPG and that were shown to be relevant for pathogenesis of periodontal disease and may be indirect targets of SOCS proteins.

2. Methods and materials

2.1. Experimental design

Male adult Wistar (Norvegicus albinus) rats (N = 36) were obtained from the Multidisciplinary Center for Biological Investigation (CEMIB-UNICAMP). The animals, weighing approximately 250 g each, were maintained with food and water ad libitum. The experimental protocol was approved by the Ethical Committee on Animal Experimentation (protocol number 23/2007) of the School of Dentistry at Araraquara – UNESP and performed in accordance with the guidelines from the Brazilian College for Animal Experimentation (COBEA). General anesthesia was induced with intramuscular injections of ketamine and xylazine chloridrate at 0.08 mL/100 g body weight and 0.04 mL/100 g body weight, respectively. The animals were divided into two experimental groups: A – sham-operated group ($n = 9$) – animals were anaesthetised but no ligatures were placed on the lower molars B – experimental group ($n = 27$) – a cotton thread ligature was placed around the cervical area of the lower first molars bilaterally to induce experimental periodontal disease.

After 7, 15, and 30 days of the ligature placement (baseline), 3 animals from the control group and 9 animals from the experimental group were sacrificed per period by anesthetic overdose. The mandibular jaws were hemisected, and half of the block samples including molars with their surrounding tissues were submitted to routine histological processing to be used in the stereometric evaluation. The other half of the blocks had the gingival tissue around the first molars carefully dissected for extraction of total RNA and protein for RT-qPCR and western blot analysis. After dissection of the gingival tissues, the samples were immersed in 3% hydrogen peroxide for 24 h to remove remaining soft tissues. Subsequently, these samples were stored in 70% ethanol and used for the macroscopic assessment of bone resorption.

2.2. Macroscopic analysis of bone resorption

The area of bone resorption in the lingual surface of the first molars was measured macroscopically. Briefly, the pieces were removed from alcohol, dried, immersed for 5 min in a solution containing 0.7 g/L of methylene blue and washed with tap water to remove the excess dye.

Digital images of the lingual surfaces of stained first molars were obtained at a standardised 90° angle with the mid-lingual aspect of the first molars with a stereomicroscope (Leica MZ6 – 20× magnification). The total area of exposed root (CEJ-bone crest) surfaces stained in blue (the crown enamel is not stained) on the images was measured by an examiner blind to experimental groups using software Image tool 3.0. An increase on the area of exposed roots in comparison to control, non-ligated, teeth indicates alveolar bone resorption.

2.3. Stereometry

Tissue blocks were fixed in 4% buffered formalin for 48 h, decalcified in EDTA (0.5 M, pH 8.0) for 3 months at room temperature and embedded in paraffin. Semi-serial 5 μm sections were obtained in the frontal plane (buccal–lingual orientation), and stained with hematoxylin and eosin (H/E). Three different sections, spaced 300 μm apart, representing the mesial, mid and distal areas of the teeth were examined from each specimen and images were captured using a digital camera (Leica DFC 300 FX) on an optical microscope (Diastar-Cambridge Instruments) under 200× magnification. A

32400 μm^2 grid with 9 × 4 squares of 30 μm was constructed using an image managing/editor software (Adobe Photoshop CS5) and overlayed on the digital images obtained from the histological sections. The region of interest for the analysis was represented by the whole grid, which was positioned in a submarginal area of the buccal and lingual surfaces, representing the connective tissue subjacent to the gingival sulcus (the apical border of the junctional epithelium and tooth structure were used as upper and lateral limits of the grid, respectively). A single examiner, who was previously trained and calibrated (data not shown) and blind to the purpose of the experiment, performed the stereometric analysis using a point-counting technique. The following structures observed on each intersection point of the grid were recorded: fibroblastic cells, extracellular matrix, vascular structures and inflammatory cells. This procedure allows the quantitative assessment of inflammatory reaction in the vicinity of the aggression. For each specimen, the values obtained from the measurements from each surfaces were combined and averages and standard deviations were calculated. The presence of each structure was expressed as a percentage of the total area analyzed in accordance with Odze et al.[14]

2.4. *Reverse transcription-real-time PCR*

Total RNA was extracted from tissue samples using RNAqueous 4PCR kit, according to the manufacturer's protocol (Ambion). The quantity and purity of total RNA were determined on a Biomate 3 (Thermo Electron Corporation) spectrophotometer by evaluating the absorbance at 260 nm and the 260/280 nm ratio, respectively. The integrity of total RNA was confirmed by electrophoresis of 0.5 μg of total RNA in 1% formaldehyde–agarose gels, followed by visualisation of the bands corresponding to 18S and 28S ribosomal RNA in the appropriate ratio (1:2) under UV transillumination. Complementary DNA was synthesised by reverse transcription of 400 ng of total RNA in the presence of Oligo (dT), reverse transcriptase enzime, $MgCl_2$, dNTPs, RNAse inhibitor, according to the manufacturer's protocol (Applied Biosystems). Real-time polymerase chain reaction (qPCR) was performed using a StepOne thermocycler (Applied Biosystems). The reaction included 1 μL of the RT reaction product in a 20 μL total volume PCR reaction mix that included: 8 μL of nuclease-free water, 10 μL of TaqMan qPCR master mix and 1 μL of TaqMan gene expression assays, including forward, reverse primers and fluorophore-conjugated probe (Applied Biosystems) for rat genes (see Table 1). The cycling conditions used for all primers were pre-optimised: 50 °C for 2 min and 40 cycles of: 95 °C for 15 s and 60 °C for 1 min. The determination of the relative levels of gene expression was performed using the cycle threshold method and normalised to the housekeeping gene GAPDH. Results are represented as the mean mRNA expression from duplicate measurements normalised by internal control GAPDH and expressed as fold change over the levels determined in cDNA samples prepared from healthy (non-ligated) control gingival tissues.

2.5. *Western blot*

Activation of STAT1 and STAT3 as well as the global expression of SOCS1 and SOCS3 was assessed using samples of total protein extracted from gingival tissues collected from rats sacrificed in the different experimental periods (7, 15 and 30 days after ligature placement). A detergent-based extraction buffer (T-PER, Tissue Protein Extraction Reagent – Pierce) containing a protease inhibitor cocktail (Protein Stabilizing Cocktail – Santa Cruz Biotechnology) was used for protein extraction. The tissue samples were macerated in 30 μL of ice-cold buffer, centrifuged for 5 min at 13,000 RPM at 4 °C and the supernatant was collected. Concentration of total proteins was determined with a Bradford-based assay (Bio-Rad Lab.) and 30 μg of total protein were added to a sample buffer containing 2% SDS, 10 mM of DTT as a reducing agent, glycerol and bromophenol blue dye (Cell Signaling), heated-denaturated at 97 °C for 5 min and chilled on ice of 5 min before loading on 10% SDS–polyacrylamide gels. Electrophoresis on discontinuous acrylamide gels was carried out at constant 100 V for 90 min and subsequently electrotransfered to 0.4 μm nitrocellulose membranes using a 300 mA constant current for 1 h. The membranes were blocked for 1 h in Tris-buffered saline containing 5% non-fat dry milk and 0.1% Tween-20 and subsequently washed for 10 min (three times) with TBS–0.1% Tween-20. The membranes were then incubated with pre-optimised dilutions of the primary antibodies overnight at 4 °C with mild agitation. Membranes were washed in TBS-T buffer three times for 10 min each and incubated with secondary antibodies conjugated to horseradish peroxidase (1:5000 dilution in the blocking buffer) for 1 h at room temperature and washed again three times for 10 min with TBS-T buffer. The presence of target proteins (GAPDH, SOCS1, SOCS3 and total and phosphorylated STAT1 and STAT3) on the nitrocellulose membranes was detected by using a chemiluminescence system (Pierce ECL Western Blotting Substrate) and exposing the membranes to radiographic films.

Table 1 – Inventoried TaqMan Primers and probe (TaqMan Gene Expression Assays, Applied Biosystems).

Target gene	Assay ID	Acession #	Amplicon length (bp)
GAPDH	Rn99999916_s1	NM_017008.3	87
SOCS1	Rn00595838_s1	NM_145879.1	76
SOCS3	Rn00585674_s1	NM_053565.1	73
RANKL	Rn00589289_m1	NM_057149.1	69
OPG	Rn00563499_m1	NM_012870.2	75
TNF-α	Rn01525859_g1	NM_012675.2	92
IL-6	Rn99999011_m1	NM_012589.1	90
IL-10	Rn00563409_m1	NM_012854.2	70

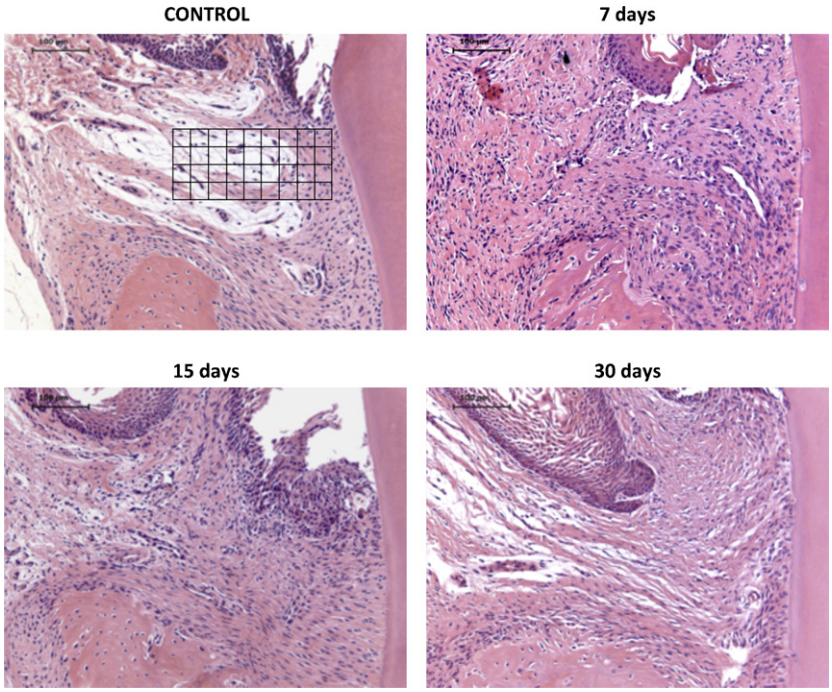

Fig. 1 – Photomicrographs illustrating histological sections of each experimental period (control, 7, 15 and 30 days) are shown. Inflammation associated with ligatures decreases its severity in the submarginal area towards the end of the experimental period. The general characteristics of the inflammatory reaction include increase in cell densities and vascular structures, and decrease in the number of fibroblasts and extracellular matrix. The image of a control site depicts the placement of the 32,400 μm^2 grids on the submarginal area limited coronally by the apical border of the junctional epithelium and laterally by the tooth structure.

2.6. *Statistical analysis*

The purpose of the statistical analyses was to determine if there were significant variations on the histological structures observed and on the levels of expression of target genes according to presence of experimental periodontal disease in each period. Comparison of the results in each experimental period according to the control group was performed using unpaired Student's *t*-test.

Moreover, we also wanted to determine if the area of bone resorption in the lingual surface varied within the experimental periods. One-way Analysis of Variance test (ANOVA) followed by the Tukey post hoc test was used to evaluate significant differences among experimental periods.

Significance level was set to 5%. All calculations were performed using GraphPad Prism 5 software (GraphPad, Inc., San Diego, CA, USA).

3. Results

3.1. *Severity of inflammation associated with ligature-induced periodontitis peaked at 7 days*

There was a significant increase on the number of inflammatory cells and vascular structures already at 7 days post-ligature placement. The overall changes on the composition of the connective tissue, including a decrease on the number of fibroblasts and on the density of collagen is a common finding in periodontal disease. The severity of inflammation was significantly higher in comparison to the control group throughout the 30-day experimental period; but a decrease in inflammatory cell density is observed after 15 days, as well as a trend of increasing number of fibroblasts and extracellular matrix at 15 and 30 days (Figs. 1 and 2).

Cytokine gene expression in the gingival tissues corroborate these findings, with a maximum increase of mRNA expression for bone-related cytokines RANKL and OPG and pro-inflammatory cytokines TNF-α and IL-6 at 7 days, followed by a decrease at 15 and 30 days. These results also agree with the finding that anti-inflammatory cytokine IL-10 tended to increase over the 30 day-experimental period (Fig. 4).

3.2. *SOCS1 and SOCS3 protein expression in ligature-induced periodontal disease is correlated with bone resorption and inflammatory status*

Expression of SOCS1 and 3 proteins were significantly increased already at 7 days in the disease-induced group, followed by a significant decrease on remaining experimental periods, although their expression remained higher than in the control group (Fig. 5). These results mirror those of the macroscopic analysis of bone resorption and of the stereometry indicating a strong correlation of the inflammatory status and the expression of SOCS (Figs. 1–3). It is well documented that SOCS is expressed at low levels in healthy periodontal tissues.[11] Our results are in accordance with these findings.

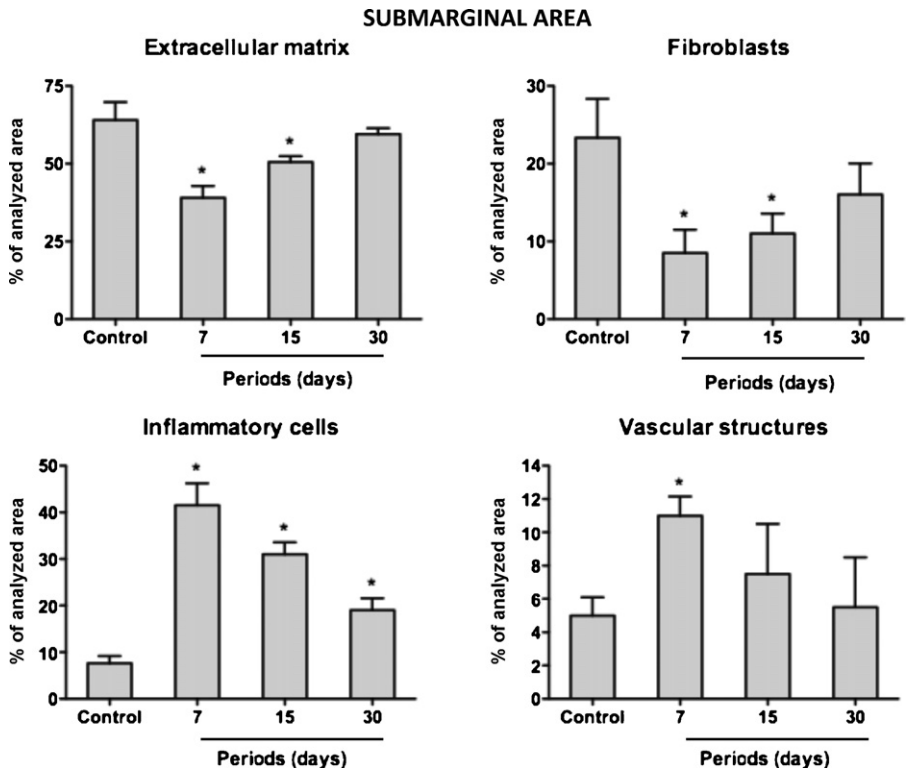

Fig. 2 – Stereometric analysis indicates that ligature is associated with a sustained inflammatory reaction. Inflammation was assessed in 4 sections per period by the relative presence of inflammatory cells, vascular structures, fibroblasts and extracellular matrix in 32,400 μm^2 area (schematically shown in Fig. 1). The severity of inflammation was significantly higher in comparison to the control group throughout the 30-day experimental period, but a decrease in inflammatory cell density is observed after 15 days, as well as a trend of increasing number of fibroblasts and extracellular matrix at 15 and 30 days. *Significant differences compared with control groups ($p < 0.05$).

Interestingly, activation of STAT1 and STAT3 in both total and phosphorylated forms followed the expression of SOCS1 and SOCS3 proteins, respectively. A significant activation of STAT1 and STAT3 was observed already at 7 days in animals with ligature-induced periodontal disease. Another interesting finding is the decrease on the activation of these transcription factors on ligature sites after 15 and 30 days (Fig. 5). The stereometric analysis supports these results, as the density of inflammatory cells decreased at days 15 and 30 (Figs. 1 and 2).

3.3. *Lack of correspondence between SOCS1 mRNA expression and protein levels in ligature-induced periodontitis*

The results show that there was an increase on SOCS gene expression in ligature-induced periodontitis compared to control group at 7, 15 and 30 days (Fig. 4). Interestingly, the kinetics of SOCS3 expression at the mRNA level was directly correlated to the expression at the protein level. Surprisingly, for SOCS1 there was a lack of transcription–translation coupling, as mRNA levels did not correlate to protein expression. Considering the fact that RNA and protein samples were harvested simultaneously from the same wells, this may suggest the influence of post-transcriptional regulation, which has been shown to play a role for SOCS1. Alternatively, the lack of correspondence between mRNA and protein levels may merely reflect an increased efficiency of translation or a longer half-life of the protein. The mechanism of regulation of SOCS expression by periodontal disease will be explored in future studies.

4. Discussion

Human *in vivo* studies suggest the involvement of SOCS1 and SOCS3 in the negative regulation of immune inflammatory networks in diseased periodontal tissues.[13] However, such data from cross-sectional studies does not allow the analysis of the kinetics of SOCS expression throughout disease onset, neither its possible association with inflammatory cell migration and with the pathological changes of the gingival tissues. In this scenario, experimental animal models of periodontitis are widely used for a better understanding of periodontal disease pathogenesis and the information derived from these models may be useful to other chronic inflammatory conditions.

The ligature model of experimental periodontitis has been commonly used and considered by some authors to be more representative of periodontitis in humans than other models. The justification for this preference is the participation of live microorganisms naturally present in the animal species (in contrast to monoinfection models with microorganisms

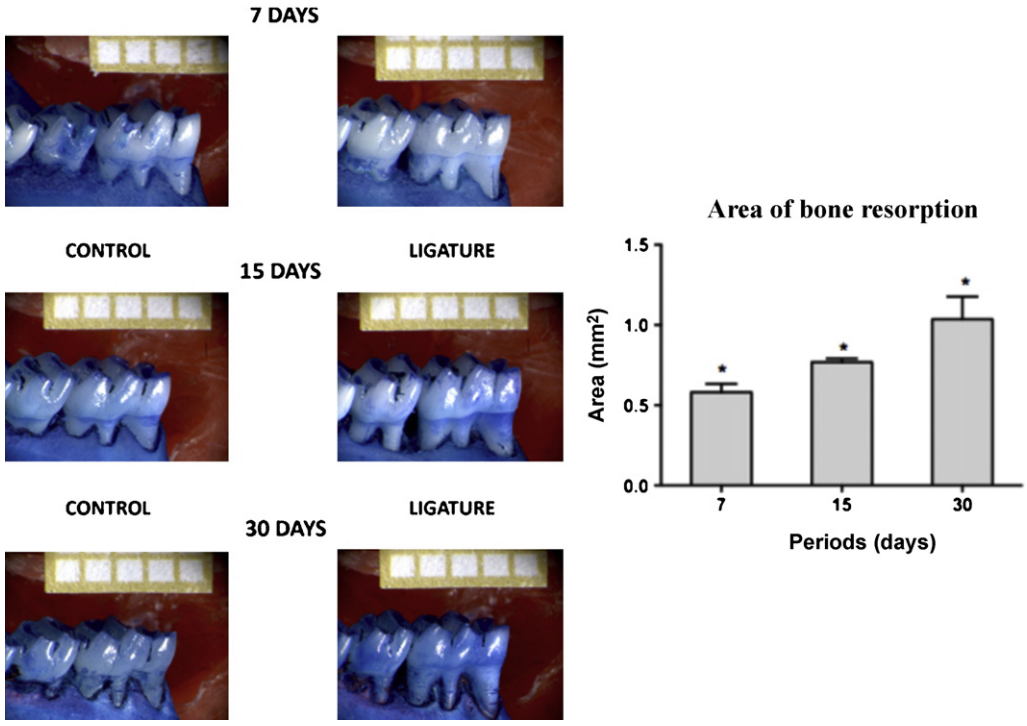

Fig. 3 – Bone resorption area in the lingual surface of the first molars was measured macroscopically. Digital images were obtained at a standardised 90° angle with the mid-lingual aspect of methylene blue stained first molars with a stereomicroscope. The total area of exposed root surfaces stained in blue (the crown enamel is not stained) on the images was measured by an examiner blind to the experimental groups using software Image tool 3.0. An increase in the area of exposed roots in comparison to control, non-ligated teeth, indicates alveolar bone resorption. *Significant differences ($p < 0.05$) among experimental periods. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

present in humans but not in rodents) with diverse virulence factors, known as pathogen-associated molecular patterns (PAMPs), including toxins, microbial metabolism products, CpG DNA and peptidoglycan. This greater diversity of antigens may result in a more complex host response; which may have an effect on the profile of cytokine and inflammatory mediators in the gingival tissues. However, the ligature model has limited usefulness in studying natural mechanisms of infectivity since periodontal disease is facilitated by the ligature.

In this study we show increased expression of SOCS1 and SOCS3, at the mRNA and protein level, in diseased gingival tissues when compared with levels in healthy gingival tissues from control animals. SOCS are produced in response to a variety of stimuli, including PAMPs such as bacterial lipopolysaccharide, characteristically present in the biofilm accumulated around ligatures, and pro-inflammatory cytokines, produced in response to the bacterial challenge. After the inducing-stimuli and its production, SOCS proteins act as endogenous negative regulators of inflammatory attenuating cytokine-induced signal transduction affecting primarily the JAK-STAT pathway, as part of a negative feedback loop to suppress the downstream effects of cytokines. Therefore, in accordance with our findings, SOCS is usually absent or minimally expressed in healthy tissues, and their up-regulation and differential expression in inflamed tissues is an important regulatory mechanism that may influence the outcome of inflammatory reaction.[12,15] The increased levels of SOCS proteins in the experimental group are consistent with data from literature showing that SOCS expression can be induced by inflammatory cytokines present in diseased periodontal tissues such as IL-6, INF-γ and TNF-α.[2,16,17] Furthermore, biopsies of inflamed/diseased gingival tissues show higher SOCS1 and -3 mRNA expression when compared with control group without disease.[11] In addition to the host-derived cytokines, the increased microbial burden associated with the transition from periodontal health to disease can also induce expression of SOCS proteins.[18,19]

Since several inflammatory mediators may regulate SOCS expression,[20] the nature of inflammatory process in periodontal tissues can influence SOCS production by different cell types. Our results show that the expression of SOCS protein mirror inflammation degree/intensity and bone loss during periodontal disease progression. In diseased tissues, already at 7 days, SOCS protein expression had a significant increase, followed by a significant decrease on remaining experimental periods. These results indicate a strong association of SOCS expression and the inflammatory status and density of inflammatory cells, suggesting the kinetic involvement of these cells, or its products/cytokines, and SOCS expression.

Studies show that the function of SOCS is to prevent transduction of the cytokine signal by binding to specific receptor sites and ultimately preventing activation of STATs.[12,21] Through a negative feedback regulatory mecha-

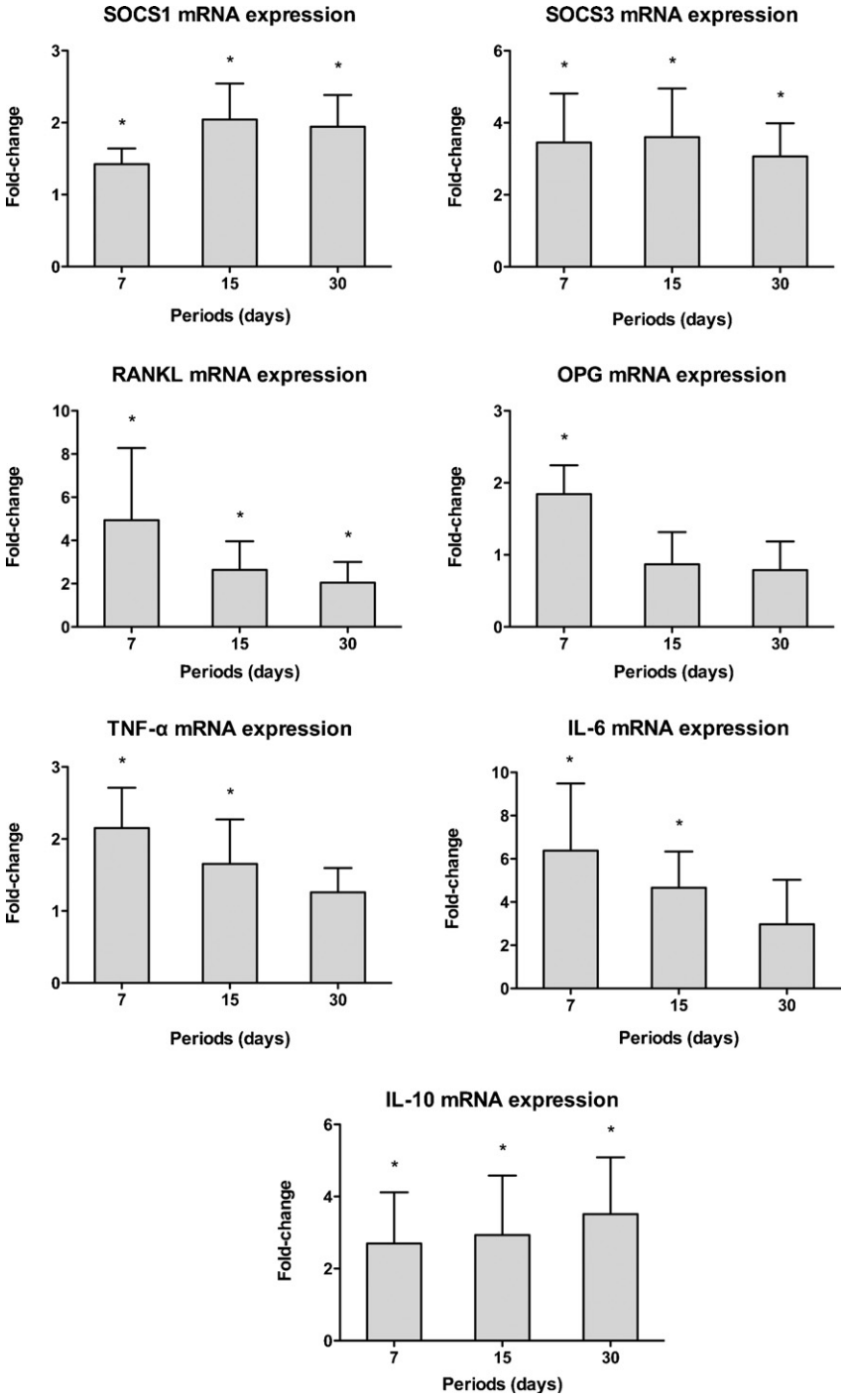

Fig. 4 – Expression of SOCS, bone-related cytokines RANKL and OPG, pro-inflammatory cytokines TNF-α and IL-6 and anti-inflammatory cytokine IL-10 profile during the course of ligature-induced periodontal disease. Reverse transcription-real-time PCR shows that SOCS expression increases significantly already at 7 days, maintaining elevated levels at 15 and 30 days. mRNA expression of bone-related cytokines RANKL and OPG and pro-inflammatory cytokines TNF-α and IL-6 increase at 7 days, followed by a decrease at 15 and 30 days, and anti-inflammatory cytokine IL-10 tended to increase over the 30 day-experimental period. mRNA expression was normalised to the expression of the house-keeping gene GAPDH. The bars represent mean fold changes and the vertical lines the standard deviation of the mean fold change of six animals in each experimental group in comparison to untreated control. *Significant differences ($p < 0.05$) compared with control sites.

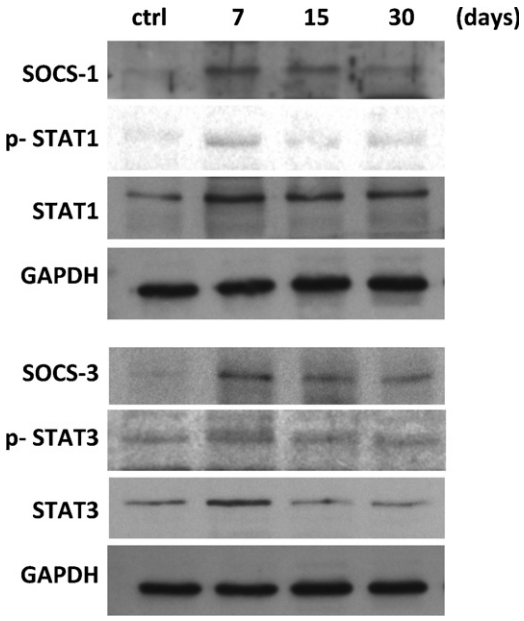

Fig. 5 – Total protein was extracted from gingival tissue samples obtained from ligature and control sites at 7, 15 and 30 day periods. 30 μg of total protein was separated by electrophoresis in 10% acrylamide gels under denaturing conditions and transferred to nitrocellulose membranes. Activation of STAT1 and STAT3 as well as the global expression of SOCS1 and SOCS3 was determined by incubating the membranes with the specific primary antibodies and revealed with HRP-conjugated secondary antibodies and an ECL system. Expression of SOCS1 and 3 proteins and activation of STAT1 and STAT3 in both total and phosphorylated forms were significantly increased already at day 7 followed by a significant decrease in the remaining experimental periods. The images are representative of the results obtained using samples from three different animals per period.

nism, increasing STAT activity leads to increased expression of SOCS in an attempt to decrease the very activation status of the JAK/STAT pathway and, consequently, reduce the consequences of prolonged activation of STAT, such as increased expression of inflammatory cytokines (e.g. IL-1β, IL-6 and TNF-α) associated with periodontal tissue destruction.[8,22] Interestingly and in accordance with the literature, in the diseased periodontium the SOCS1 and SOCS3 proteins expression levels were correlated with the levels of total and phosphorylated (activated) STAT1 and STAT3, respectively. In fact, the increased production of inflammatory mediators by resident and inflammatory cells in the response to stimuli leads to an increase in the intracellular signaling and in the activation of these transcription factors.[23] These data suggest that increased expression of SOCS1 and SOCS3 may represent a mechanism of negative regulation in response to activity of STAT1 and STAT3, and may be an important mechanism in regulating expression of genes associated with degradation of connective tissue and alveolar bone resorption.

Even though deletion of SOCS1 and SOCS3 genes in mice is lethal,[24] it is tempting to speculate that in the absence of this endogenous regulatory mechanism the host response would be exacerbated in terms of severity and duration, with a major increase on the activation of STATs. In these conditions, inflammatory cytokine expression and tissue destruction associated with periodontal diseases and other conditions associated with chronic inflammation, would be severely aggravated. Experiments in transgenic animals with tissue-specific deletion of these genes will define their relevance for the immune response.

In addition to directly modulating tissue destruction, SOCS could also impact periodontitis outcome through the modulation of healing process. Indeed, *in vivo* studies demonstrate the importance of SOCS3 in negative modulation of gp130/STAT3 signaling pathway in wound healing. The absence of SOCS3 leads to an increased activity of STAT3 causing delay in healing.[25,26] In our study, we found that even after 30 days of ligature placement, mRNA and protein levels of SOCS3 remain elevated in spite of the decrease on the severity of inflammation. In fact, the apical migration of the junctional epithelium increasing the distance to the site of aggression located on the gingival margin reduced the aggression and consequently decreased the severity of the inflammatory infiltrate. This may be followed by an attempt to repair the damaged tissues, which is characterised by the tendency to increase the number of fibroblasts and extracellular matrix verified by stereometry. This interpretation is supported by the fact that once placed, ligatures were kept throughout the 30-day experimental period; however they were not pushed further apically even if the gingival margin receded. This suggests that SOCS3 may also participate in the healing of periodontal tissues.

To our knowledge, this is the first study to describe the kinetic profile of SOCS1 and SOCS3 expression during experimental periodontal disease, and its association with STAT activation profile. Additional studies will include gain and loss of function experiments to determine the role of these proteins in the modulation of host response associated with chronic inflammation and also to verify possible novel targets of SOCS proteins for direct protein–protein interactions.

In summary, our study shows the kinetics of SOCS1 and SOCS3 mRNA and protein expression in the experimental model of ligature-induced periodontal disease. The kinetics of SOCS1 and SOCS3 expression was directly correlated with the severity of inflammation, of alveolar bone resorption and the level of pro-inflammatory cytokine expression, as well as with the activation status of STAT1 and STAT3 transcription factors during the course of experimental periodontal disease. This suggests that these proteins play an important role in the modulation of host response.

Understanding the role of SOCS proteins in the negative regulation of cytokine signaling, especially in the JAK/STAT pathway, may provide novel information on the susceptibility to periodontal diseases and also for therapeutic strategies based on the modulation of the inflammatory process.

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Conflict of interest: None.

Ethical approval: By the Ethical Committee on Animal Experimentation of the School of Dentistry at Araraquara – UNESP (protocol number 23/2007), where the in vivo part of the study was conducted.

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