Cytokine 60 (2012) 875–881

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Expression of interferon-γ, interferon-α and related genes in individuals with Down syndrome and periodontitis

Marcia H. Tanaka [a,b], Elisa M.A. Giro [a], Lícia B. Cavalcante [a,b], Juliana R. Pires [c], Luciano H. Apponi [d], Sandro R. Valentini [d], Denise M.P. Spolidório [e], Marisa V. Capela [f], Carlos Rossa Jr. [g], Raquel M. Scarel-Caminaga [b,*]

[a] Department of Orthodontics and Pediatric Dentistry, School of Dentistry at Araraquara, UNESP – Univ. Estadual Paulista, São Paulo, Brazil
[b] Department of Morphology, School of Dentistry at Araraquara, UNESP – Univ. Estadual Paulista, São Paulo, Brazil
[c] Department of Periodontics, Educational Foundation of Barretos, Barretos, São Paulo, Brazil
[d] Department of Biological Sciences, School of Pharmaceutical Sciences, UNESP – Univ. Estadual Paulista, São Paulo, Brazil
[e] Department of Physiology and Pathology, School of Dentistry at Araraquara, UNESP – Univ. Estadual Paulista, São Paulo, Brazil
[f] Department of Physical-Chemistry, Institute of Chemistry at Araraquara, UNESP – Univ. Estadual Paulista, São Paulo, Brazil
[g] Department of Diagnosis and Surgery, School of Dentistry at Araraquara, UNESP – Univ. Estadual Paulista, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form 18 August 2012
Accepted 18 August 2012
Available online 18 September 2012

Keywords:
Gene expression
Inflammation
Periodontitis
Cytokines
Down syndrome

ABSTRACT

Background: Recently, attenuation of anti-inflammatory and increase of pro-inflammatory mediators was demonstrated in individuals with Down syndrome (DS) in comparison with euploid patients during periodontal disease (PD), suggesting a shift to a more aggressive inflammation in DS.
Aim: To determine the influence of DS in the modulation of interferons (IFNs) signaling pathway in PD.
Materials and methods: Clinical periodontal assessment was performed and gingival tissue samples obtained from a total of 51 subjects, including 19 DS individuals with PD, 20 euploid individuals with PD and 12 euploid individuals without PD. Expression levels of *interferon-gamma* (*IFNG*) and *interferon-alpha* (*IFNA*), and their receptors *IFNGR1*, *IFNGR2*, *IFNAR1* and *IFNAR2*, the signaling intermediates *Janus kinase 1* (*JAK1*), *signal transducer and activator of transcription 1* (*STAT1*) and *interferon regulatory factor 1* (*IRF1*) were determined using real time quantitative polymerase chain reaction (qPCR).
Results: Clinical signs of periodontal disease were markedly more severe in DS and euploid patients with PD in comparison to euploid and periodontally healthy patients. There was no difference on mRNA levels of *IFNA*, *IFNG*, *INFGR2*, *IFNAR1* and *IFNAR2* between DS and euploid individuals, even though some of these genes are located on chromosome 21. *STAT1* and *IRF1* mRNA levels were significantly lower in DS patients in comparison with euploid individuals with PD. In euploid individuals, PD was associated with an increased expression of *IFNGR1*, *IFNGR2*, *IFNAR1*, *STAT1* and *IRF1*.
Conclusions: Reduced expression of *STAT1* and *IRF1* genes indicate an impaired activation of IFNs signaling in individuals with DS and PD. Expression of *IFNA*, *IFNG* and IFN receptors was not altered in DS patients, indicating that indirect mechanisms are involved in the reduced activation of IFN signaling.

1. Introduction

Down syndrome (DS), or trisomy 21, is a very frequent autosomal chromosomal disorder caused by an error in cell division that results in the presence of all or part of an extra chromosome 21 [1]. The overexpression of specific genes found on chromosome 21 cause a complex condition with more than 30 clinical features [2,3]. Many studies have reported that DS individuals have an increased prevalence of periodontal disease (PD) compared with otherwise chromosomally normal (euploid), age-matched control and other mentally handicapped patients of similar age distribution [4–7]. So, the high susceptibility to PD was not only related to poor oral hygiene, but also associated with the congenital disorder [8,9] in DS individuals [8–10].

Periodontal diseases are characterized by a destruction of the periodontium and eventually tooth loss [11] and are caused by local etiologic factors, especially the dental biofilm, but systemic disorders may reduce or change the resistance or the host response to this factor [12]. Additionally, significant correlations were found between the prevalence of bone loss and both the age and the neutrophil chemotactic index in DS individuals. The rate of the periodontal destruction seems to depend on the severity and

* Corresponding author. Address: Department of Morphology, School of Dentistry at Araraquara, UNESP – Univ. Estadual Paulista, Rua Humaitá, 1680, Centro, CP. 331, CEP 14801-903, Araraquara, São Paulo, Brazil. Tel.: +55 16 3301 6504; fax: +55 16 3301 6433.
E-mail address: raquel@foar.unesp.br (R.M. Scarel-Caminaga).

1043-4666
http://dx.doi.org/10.1016/j.cyto.2012.08.020

duration of the chemotaxis impairment in the presence of poor oral hygiene [13]. Abnormalities in the immune response are important contributing factors to the high incidence of PD in individuals with DS, such as production of oxidative radicals [14,15], decreased chemotaxis and phagocytosis of polymorphonuclear leukocytes (PMNs) [15,16], and reduced T lymphocyte counts and immature T lymphocytes [13,17]. This impaired host response characterized by disturbances of the T and B lymphocyte subsets, leads to dysregulation of cytokines, chemokines and prostaglandins [18]. Recently, our research group demonstrated an attenuation of *IL10* and SOCS3 gene expression with a simultaneous increase of *STAT3* mRNA levels in individuals with DS during periodontitis in comparison with euploid individuals with PD [19]. These results indicated and inhibition of endogenous negative regulators of inflammation in DS patients during PD.

A plethora of cytokines has been found in diseased human periodontal tissues, including interferons (IFNs) [12,20–22]. Interferons are a large family of cytokines with broad function, such as antiviral, antitumor, antiproliferative, and immunomodulatory effects [23,24]. Interferon-alpha, also known as type I IFN, is coded by the *IFNA* gene and is produced by fibroblasts or type I helper T cells (Th1) upon stimulation by viral or bacterial infections, as periodontopathogenic bacteria [25–29]. The type II IFN is known as interferon-gamma (coded by the *IFNG* gene) and is a more potent immunomodulator than type I IFN [30]. IFN-γ acts on macrophage activation [31], modulation of leukocyte adhesion by stimulating intercellular adhesion molecule-1 (ICAM-1) expression [32], and upregulates monocytic response to bacterial lipopolysaccharide (LPS), resulting in secretion of proinflammatory molecules, such as prostaglandin E_2 (PGE_2), Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [33]. All of them play important roles in periodontal tissue destruction including alveolar bone loss [21,22,34–36]. The biological effects of IFNs are mediated by their receptors on the cell surface. Therefore, interferons receptors' are important in the inflammatory processes. Whereas the IFNs binds to their receptors, the mechanisms by which signal transduction occurs that trigger specific cellular responses are mediated by phosphorylation of Janus kinase (JAK) enzyme associated with the IFN receptors and transcription factors STAT (Signal Transducer and Activator of Transcription) [23,37,38]. Upon binding of the IFN-γ and IFN-α to their specific receptors, the receptor undergoes oligomerization, with phosphorylation of JAKs associated with the cytoplasmic tails of these receptors. This provides a docking site and subsequent phosphorylation of the transcription factor STATs (Signal Transducers and Activators of Transcription) [23,37,38]. IFN-γ signals induce formation of phosphorylated STAT-1 homodimers, which are translocated to the nucleus, where they activate transcription of IFN-stimulated genes (ISGs) which contain a unique element called IFN-γ activation site (GAS). IFN-α induces the formation of heterodimers STAT-1/STAT-2 which are also translocated to the nucleus and activate promoter regions of ISGs called IFN-stimulated response element (ISRE) [39].

In addition to STATs, another set of transcription factors activated downstream of IFN receptor signaling are the IFN regulatory factors (IRFs). This family of transcription factor plays a critical role in antiviral defense, immune response, cell growth regulation and apoptosis [40,41].

Interestingly, in the long arm of chromosome 21 there is a cluster of genes composed by the Interleukin 10 receptor beta (*IL10RB*), *IFNGR2*, IFN-alpha receptor 1 (*IFNAR1*) and the *IFNAR2* gene, oriented in tandem [24,42]. In addition, considering the importance of IFNs in the immune response, some studies assessed the influence of Trisomy of 21 on the expression of IFN-α receptors on peripheral blood mononuclear cells of individuals with DS [25,26] and in fibroblast cell lines derived from human skin of DS individuals [24].

Despite the involvement of IFNs in the immune response and the relevance of the immune response to PD, to our knowledge there is no information on the expression of IFN signaling pathway genes in individuals with DS during periodontitis. In this study we show a reduced expression of downstream activators of IFN signaling in DS patients with periodontitis, suggesting that this inflammatory pathway does not play a role in increased destruction of periodontal tissues observed in DS patients.

2. Materials and methods

2.1. Subject population

All patients enrolled in the present study were also included in a recent study of our group which investigated mRNA levels of the interleukin-10 signaling pathway genes to reach potential influence of DS in modulation of host immune response caused by periodontitis [19]. This study was approved by the local Ethical Committee (process number 79/04 – FOAr-UNESP). All patients with DS and their parents as well as chromosomally normal (euploid) individuals were informed about the study and informed consent forms were signed. A total of 150 patients with DS and 100 chromosomally normal patients were initially recruited into the study. Their medical history was taken and they underwent a clinical periodontal examination in the School of Dentistry of Araraquara (UNESP) and in 12 of the Associations of Parents and Friends of Disabled Patients (APAEs) of Araraquara and the surrounding cities (Bocaina, Jaú, Ibitinga, Itápolis, Dourado, Taquaritinga, Matão, Jaboticabal, Ibaté, São Carlos and Guaíra) from March 2005 to December 2007.

All DS patients were referred to a cardiologist before clinical analysis. Patients diagnosed with cardiac abnormalities were prescribed an appropriate antibiotic prophylaxis to avoid the risk of complications such as endocarditis.

To be enrolled in the study, the participants were selected on a three step process: (1) non-institutionalized DS adult subjects (i.e., older than 19 years, according to WHO [43]) with a karyotype of simple 21st trisomy (mosaics were excluded) and chromosomally normal (euploid) adult individuals. (2) None of the following exclusion criteria: diabetes mellitus or any other chronic inflammatory disease or infections; pregnancy or lactation; current smoker status or cessation of smoking for less than 5 years; use systemic and/or topical steroidal and non-steroidal anti-inflammatory drugs or antibiotics during the last 3 months before the experimental phase. (3) The subjects had to have areas with indication for oral surgery treatment (tooth extractions and/or periodontal surgery) to enable obtaining gingival biopsy.

2.2. Clinical parameters

All subjects underwent an intra-oral clinical examination by a single trained and calibrated examiner. For calibration, a total of 252 sites were randomly evaluated in six subjects (one quadrant *per* patient). The examiner evaluated PPD on two occasions, 48 h apart, and data were submitted to Student's *t*-tests. This analysis was repeated throughout the period of data collection because the patient selection was spread over many months. Calibration was made with the aim of standardizing the evaluation criteria and was approved because evaluation differences between the two periods were not statistically significant (83% of sites showed 1 mm of maximum variation between tests; $p > 0.05$; kappa test). Full-mouth clinical measurements (except for third molars) including visible plaque index (VPI) [44]; gingival bleeding index (GBI) [44]; bleeding on probing (BOP), probing pocket depth (PPD) and clinic attachment loss (CAL) were conducted using a manual

periodontal probe from the University of North Carolina (Hu-Friedy®, Chicago, IL, USA). Six sites per tooth were evaluated for PPD, BOP and CAL (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual). Patients were classified as periodontally diseased when exhibiting more than three non-adjacent sites with CAL and PPD $\geqslant$4 mm and BOP. Patients were classified without periodontitis when exhibiting a maximum of three non-adjacent sites with PPD $\geqslant$4 mm and 15% of sites with BOP [45]. Subjects were divided into the following groups based on their genetic condition and periodontal status:

- DS+PD: Down syndrome individuals with periodontal disease (n = 19).
- E+PD: Euploid individuals with periodontal disease (n = 20).
- EH: Euploid individuals periodontally healthy (n = 12).

2.3. Gingival tissue collection

During the surgical procedure, a gingival biopsy of about 3 mm^3 was obtained from each individual. From individuals with periodontitis, an area of inflamed gingiva with CAL and PPD $\geqslant$4 mm was chosen, whereas from those without periodontitis, a non-inflamed area of gingival tissue was selected. Biopsied gingival specimens (containing epithelium and connective tissue) were immediately immersed in a microtube containing 500 μL of RNA extraction solution (Invitrogen, Carlsbad, CA, USA) and stored at −80 °C until extraction of the total RNA.

2.4. RNA extraction and reverse transcription

Total RNA was extracted from each gingival tissue biopsy by macerating the tissue with phenol and guanidine isothiocyanate and following manufacturer's (Invitrogen, Carlsbad, CA, USA) instructions. Digestion of contaminating genomic DNA was performed using RNAse-free DNAse (Promega, Madison, WI, USA) according to the manufacturer's instructions. The quality of RNA was assessed by agarose gel electrophoresis, and its concentration and purity were assayed by measuring the A260/280 ratio using a spectrophotometer (Biophotometer, Eppendorf, Germany) and the ratio was always >1.8. At least 350 ng RNA was used for the reverse transcription (RT) reaction in a final volume of 20 μL, using a SuperScript III First Strand Synthesis Super Mix (Invitrogen, Carlsband, CA, USA). The obtained cDNA was stored at −80 °C until the polymerase chain reaction (PCR) amplifications were carried out.

2.5. Quantitative real-time PCR (qPCR)

Expression of *IFNG*, *IFNA* and their receptors (*IFNGR1*, *IFNGR2*, *IFNAR1* and *IFNAR2*), *JAK-1*, *STAT-1* and *IRF-1* mRNA were determined by quantitative PCR (qPCR) performed in a Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using the SYBR Green chemistry for detection (Applied Biosystems, Warrington, UK).

Oligonucleotide primers for the target genes were designed from sequences in the GenBank database using the Primer Express 3 software (Applied Biosystems, Foster City, CA, USA). The primer sequences, the product region, the predicted amplicon sizes and the annealing temperatures are depicted in Table 1. PCR reaction conditions for all primers were optimized and all the reactions included a melting curve to assess the formation of primer-dimers.

All qPCR reactions in a final volume of 25 μL were assayed in 96-well optical plates (Applied Biosystems, Singapore, SG) using the following cycling parameters: 50 °C for 2 min and 95 °C for 10 min; PCR cycling for 40 cycles at 95 °C for 15 s, 60 °C for 1 min and a dissociation cycle (95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s and 60 °C for 15 s). Negative controls with nuclease-free water (Ambion, Life Technology, CA, USA) instead of cDNA were performed for all reactions.

Calculations for determining the level of gene expression were made by reference to the housekeeping gene, *ACTB* (β-Actin), in the sample using the cycle threshold (Ct) method. The mean Ct values from duplicate measurements were used to calculate expression of the target gene, with normalization to the reference gene using specific quantification software Relative Quantification (ddCt) Study software (Applied Biosystems, Foster City, CA, USA).

2.6. Statistical analysis

The Shapiro–Wilk test was utilized to assess the normality of the data. Thereafter, Mann–Whitney *U* tests were used for pairwise comparisons between groups, as appropriate. Spearman's correla-

Table 1
Primer information: accession number of deposited mRNA sequences for the target gene, primer sequences, product size and annealing temperature (Ta).

Target gene	Accession number	Type	Primer sequence	Product amplicon size	Ta (°C)
ACTB	NM_001101.3	F	5′ AAGCCACCCCACTTCTCTCTAA 3′	88	58
		R	5′ AATTTACACGAAAGCAATGCTATCA 3′		
IFNA	NM_000619	F	5′ GAAACGAGATGACTTCGAAAAGC 3′	113	58
		R	5′ GCTGCTGGCGACAGTTCA 3′		
IFNGR1	NM_000416	F	5′ GGTCTGTGAAGAGCCGTTGTC 3′	142	58
		R	5′ CGGGACCACGTCAGGAATAT 3′		
IFNGR2	NM_005534	F	5′ GGAAAAGGAGCAAGAAGATGTTCT 3′	93	58
		R	5′ AGCTCCGATGGCTTGATCTC 3′		
IFNA	NM_002169	F	5′ GAAGAATCTCTCCTTTCTCCTGCC 3′	110	59
		R	5′ ATGGAGGACAGAGATGGCTTG 3′		
IFNAR1	NM_000629	F	5′ CACTTCTTCATGGTATGAGGTTGACT 3′	96	59
		R	5′ ATTGCCTTATCTTCAGCTTCTAAATGT 3′		
IFNAR2	NM_207584	F	5′ TCATATGATTCGCCTGATTACACA 3′	108	58
		R	5′ TGGTACAATGGAGTGGTTTTTTAATT 3′		
JAK1	NM_002227	F	5′ GTCACAACCTCTTTGCCCTGTAT 3′	91	58
		R	5′ CGGAGGGACATCTTGTCATCA 3′		
STAT1	NM_139266	F	5′ GTGTTATGGGACCGCACCTT 3′	107	58
		R	5′ AAGACCAGCGGCCTCTGA 3′		
IRF1	NM_002198	F	5′ GCATGGCTGGGACATCAAC 3′	97	58
		R	5′ CTTGGGATCTGGCTCCTTTTC 3′		

tions (ρ) were used to investigate associations among the clinical parameters and gene expression of the gingival biopsy of each group. Statistical analysis was performed using Statistica 8.0 for Windows. For all of the tests used, values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Demographic and clinical findings

Age, gender distribution and number of teeth were similar in all individuals, indicating the homogeneity of these data in the total sample ($p > 0.05$). Because the data distribution was not normal, median (minimum and maximum) values are presented for DS+PD, E+PD and EH, respectively (Table 2).

3.2. Gene expression analysis by RT-qPCR

The mRNA levels for each investigated gene after the normalization by the β-Actin gene expression are shown in Fig. 1. Individuals with DS+PD demonstrated significantly lower expression of *STAT1* (Fig. 1H) ($p < 0.02$) and *IRF1* (Fig. 1I) ($p < 0.01$) in comparison with E+PD. Considering the euploid individuals, the mRNA expression levels of *IFNGR1* (Fig. 1C), *IFNGR2* (Fig. 1E), *IFNAR1* (Fig. 1D), *STAT1* (Fig. 1H) and *IRF1* (Fig. 1I) was significantly higher in the presence of PD.

3.3. Correlation analysis of the clinical periodontal and gene expression data

The correlation analysis among the clinical periodontal parameters of the gingival biopsied sites and gene expression is shown in Table 3. DS+PD demonstrated a strong positive correlation between *IFNA* mRNA levels and PPD ⩾4 mm (0.74). For E+PD, strong negative correlation (−0.74) were observed between CAL (mean) and *IFNGR2* mRNA levels. Regarding EH was observed strong negative correlation (−0.84) between *IFNAR2* and *STAT1* mRNA levels and both PPD (mean) and CAL (mean) indices.

4. Discussion

Mechanisms responsible for the beginning and progression of PD are not well understood in DS individuals. While the role of the IFN-γ and the IFN-α in PD have been investigated in euploid individuals, to our knowledge, this is the first study which assessed the participation of these mediators and other important related genes in the diseased tissue (gingival biopsies) from DS individuals affected by PD, and also, compared with euploid individuals.

Table 2
Demographic and clinical periodontal findings of the studied subjects.

Variables	DS+PD	E+PD	EH
Gender (M/F)	14/5	9/11	3/9
Age	31.00 (22–48)	42.00 (21–66)	33.50 (20–49)
Number of teeth	23.00 (14–32)	24.50 (10–36)	29.00 (18–31)
BOP (%)	45.90 (25–100)[a]	45.00 (24–100)[a]	5.00 (0–11)[b]
PPD ⩽ 3 mm (%)	61.00 (33–71)[a]	58.50 (22–61)[a]	80.00 (70–97)[b]
PPD ⩾4 mm (%)	39.00 (10–40)[a]	41.50 (22–76)[a]	20.00 (6–22)[b]
CAL ⩽ 3 mm (%)	58.00 (30–64)[a]	51.50 (22–55)[a]	80.00 (70–97)[b]
CAL ⩾ 4 mm (%)	42.00 (9–47)[a]	48.50 (4–76)[a]	20.00 (6–22)[b]

Median (minimum–maximum). Different letters in rows mean statistically significant difference between groups (Mann–Whitney test). DS+PD: Down syndrome individuals with periodontal disease; E+PD: Euploid individuals with periodontal disease; EH: Euploid individuals periodontally healthy (modified from Cavalcante et al. [19]).

The importance of IFN-γ in euploid individuals with PD has been well documented [21,22,35,46,47]. Dutzan et al. [21] showed that in gingival tissue of patients with chronic periodontitis the *IFNG* gene expression at sites of active disease was 7.8 times greater than the expression at inactive sites (without PD). Surprisingly, we did not find a significant difference on *IFNG* mRNA levels between euploid individuals with PD and periodontally healthy individuals. There was also no significant difference in the *IFNG* expression between individuals with DS with PD and euploid individuals with PD (Fig. 1A). This is particularly intriguing, since, all the tissue samples from individuals with PD were harvested from sites considered clinically active (PD ⩾4 mm and presence of bleeding on probing). Although unexpected, these results are consistent with the reports of Roberts et al. [48] and Bickel et al. [20] who also did not observe differences on IFNG mRNA levels in inflamed and healthy gingival tissues. These authors hypothesized that the lack of difference may be related with the low levels of *IFNG* gene expression in these tissues. It is important to note that we only assessed gene expression at the mRNA level and since *IFNG* is also regulated at the post-transcriptional level and has a short half-life [49,50] the protein levels may not correspond directly to the mRNA.

Regarding the *IFNGR1* and *IFNGR2* gene expression, a previous study showed that IFN-γ receptor 2 was highly expressed in cell culture of fibroblasts derived from DS in comparison with control fibroblast cells, but not the IFN-γ receptor 1 [24]. In the present study, euploid individuals with PD showed higher expression of both *IFNGR1* and *IFNGR2* when compared with euploid individuals without PD, and there was no difference in the expression of these receptors between DS and euploid individuals both affected by PD. These results indicate that PD is associated with an increased expression of *IFNGR1* and *IFNGR2* in the affected tissues. Importantly, in spite of DS individuals having an extra copy of the *IFNGR2* gene this did not result in increased expression of this gene in gingival tissue of DS patients. On the other hand, it is tempting to speculate on an increased activity of the IFN signaling pathway in PD as a result of a higher sensitivity of the cells to interferons due to the increased expression of their receptors, even without modulation of *IFNA* and *IFNG* genes. This hypothesis will be investigated in subsequent studies assessing expression of these receptors at the protein level directly on immune cells.

Controversial results have been reported on the differential expression of IFN-α in patients with and without PD [27–29,51,52]. Prabhu et al. [51] found *IFNA* mRNA levels significantly higher in gingival tissue of patients with PD in comparison with periodontally healthy individuals. In agreement, Wright et al. [29] observed that IFN-α protein levels were twofold higher in peripheral blood of patients with periodontitis in comparison with periodontally healthy patients ($p = 0.0045$), and periodontal treatment reduced IFN-α concentration to levels similar to those of periodontally healthy individuals ($p = 0.603$). Kajita et al. [52] found that the *IFNA* mRNA expression was higher in patients with chronic periodontitis compared to patients with gingivitis ($p = 0.033$), although low expression levels were detected in both groups. Conversely, a study conducted by Mathur et al. [27] showed no difference in the IFN-α protein concentration in gingival crevicular fluid of patients with chronic periodontitis in comparison to healthy subjects. Similar results regarding to *IFNA* protein levels were observed by Wolff et al. [28] in gingival crevicular fluid. We did not find a significant difference on *IFNA* mRNA expression in individuals with PD (independently of the presence of DS) compared to individuals without PD (Fig. 1B). However, in the DS with PD there was a positive significant correlation between *IFNA* mRNA levels and the PPD ⩾4 mm (0.74), which may be related to a higher sensitivity of the responsive cells associated with a higher expression of the receptors.

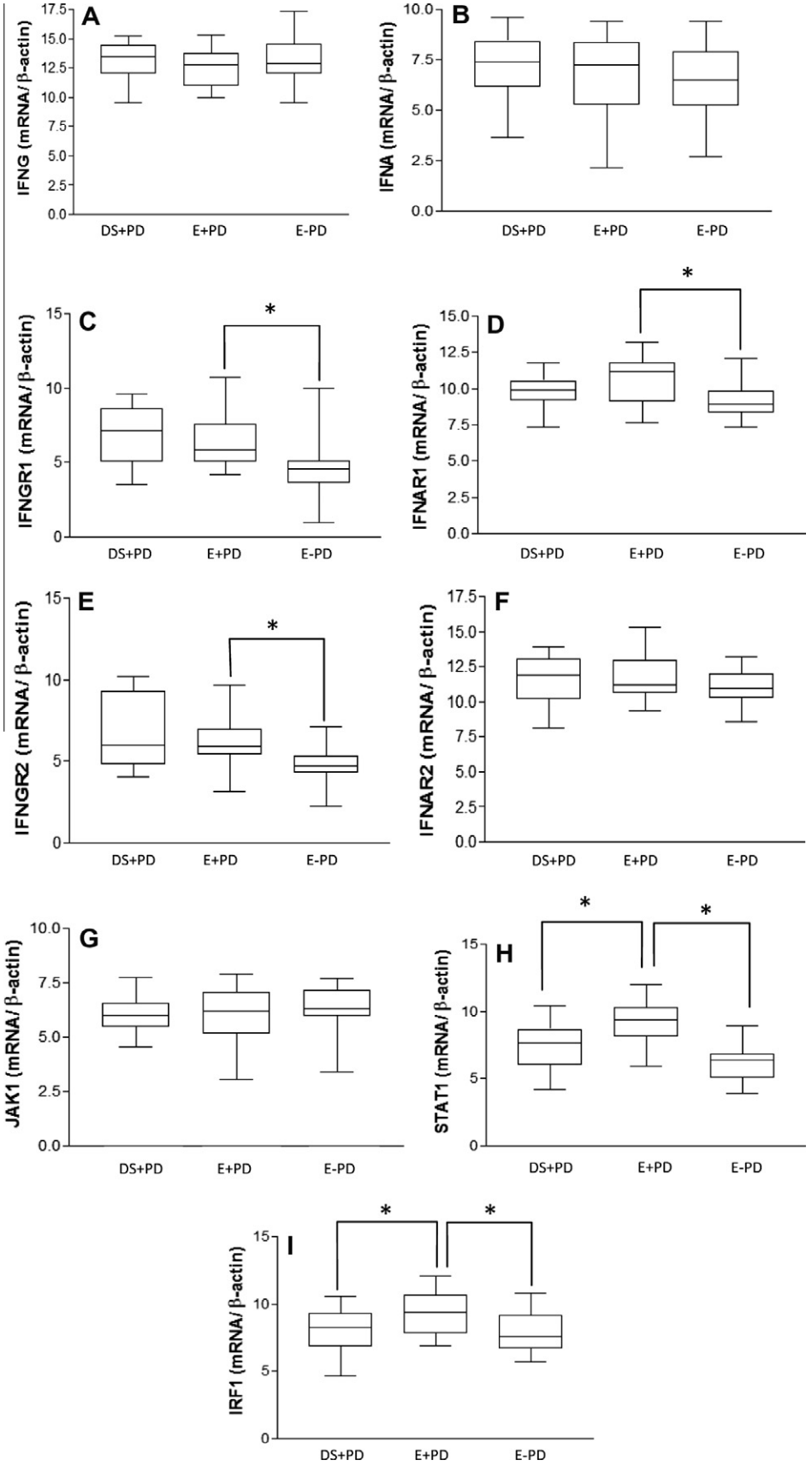

Fig. 1. Expression of *IFNG*, *IFNGR1*, *IFNGR2*, *IFNA*, *IFNAR1*, *IFNAR2*, *JAK1*, *STAT1* and *IRF1* in all individuals. Differences in the comparisons of gene expression between the DS+PD and E+PD, and between E+PD and EH were evaluated by Mann–Whitney U test ($^{*}p < 0.03$).

Table 3
Correlation coefficients between the clinical data and mRNA expression levels for *IFNG*, *IFNGR1*, *IFNGR2*, *IFNA*, *IFNAR1*, *IFNAR2*, *JAK1*, *STAT1* and *IRF1* in all individuals.

	BOP (%)	PPD (mean)	CAL (mean)	PPD ⩾4 mm	CAL ⩾ 4 mm
DS+PD					
IFNG	0.15	0.39	0.52	0.24	0.48
IFNGR1	−0.09	0.42	0.44	0.29	0.40
IFNGR2	−0.03	0.19	0.25	0.26	0.32
IFNA	0.30	0.60	0.39	**0.74**	0.41
IFNAR1	−0.01	−0.19	0.17	−0.06	0.31
IFNAR2	−0.08	−0.33	−0.02	−0.53	−0.02
JAK1	−0.12	−0.40	−0.42	−0.39	−0.42
STAT1	−0.02	0.09	0.46	0.09	0.58
IRF1	0.08	−0.34	0.01	−0.29	0.14
IFNG	−0.21	−0.01	0.33	0.00	0.11
IFNGR1	0.12	−0.30	−0.16	0.07	−0.13
IFNGR2	0.03	−0.42	**−0.74**	−0.03	0.15
IFNA	−0.19	0.00	−0.03	−0.15	0.00
E+PD					
IFNAR1	−0.25	0.19	0.15	0.23	0.15
IFNAR2	0.04	−0.38	0.18	0.10	0.34
JAK1	−0.02	−0.14	−0.58	0.11	0.07
STAT1	0.01	−0.11	−0.43	−0.10	0.00
IRF1	−0.26	0.11	0.66	0.14	0.07
IFNG	−0.15	−0.35	−0.35		
IFNGR1	−0.20	−0.67	−0.67		
IFNGR2	−0.37	−0.49	−0.49		
IFNA	−0.15	0.16	0.16		
EH					
IFNAR1	−0.25	−0.24	−0.24		
IFNAR2	−0.13	**−0.84**	**−0.84**		
JAK1	0.16	−0.18	−0.18		
STAT1	0.16	**−0.84**	**−0.84**		
IRF1	−0.11	−0.41	−0.41		

Statistically significant values are presented in bold. DS+PD: Down syndrome individuals with periodontal disease; E+PD: Euploid individuals with periodontal disease; EH: Euploid individuals periodontally healthy.

In support to this assumption, *IFNAR1* and *IFNAR2* genes are located on chromosome 21 and there is evidence demonstrating higher expression in the peripheral blood of individuals with DS in comparison with euploid individuals [24,25,53]. However, in vitro responsivity of PBMC of DS individuals to IFN-α is reduced, which was associated with the increased prevalence of infectious and malignant diseases in those individuals [25]. Expression of IFN-α receptor 1 was higher in fibroblasts cell lines from DS individuals in comparison with fibroblasts cell lines from euploid individuals [24]. We did not find significant difference on *IFNAR1* mRNA expression in gingival tissues affected by PD, both in DS and euploid individuals (Fig. 1D). However, the *IFNAR1* expression was significantly higher in euploid individuals with PD compared to individuals without PD (Fig. 1D). *IFNAR2* gene expression in the gingival tissues was not affected by PD or DS (Fig. 1F). Therefore, even though IFN-α receptor genes are located on chromosome 21, its expression was similar in DS and euploid individuals.

The present study also investigated the expression of *JAK1* and -*STAT1* genes, since these molecules are classical downstream targets of IFN-γ and IFN-α signaling [54–56]. Gene expression of *JAK1* and *STAT1* were previously demonstrated to be greatly increased in response to stimulation of blood cells with either -IFN-γ or IFN-α [57]. In the Fig. 1G, we show that there were no statistical differences in *JAK1* expression in the gingival tissues, regardless of the genetic and clinical conditions. On the other hand, *STAT1* expression was significantly reduced (1.2-fold) in DS with PD in comparison with euploid individuals with PD (Fig. 1H). This finding appears to support the hypothesis of a deficiency in the immune response of DS individuals. This is support by the finding that PD was associated with increased expression of *STAT1* mRNA in the gingival tissues of euploid patients. However, in the same DS patients, we recently reported on the reduced expression of *IL10* coupled with an increase of *STAT3* and reduction of *SOCS3* genes, which rather interestingly suggests an increased severity of inflammation [19]. In fact, low doses of IFN-γ cause an increased production of IL-6 by fibroblasts from DS patients [24]. STAT-3 is activated by IL-6 signaling, which we reported in our previous study, as well as reduced expression of *IL10* and *SOCS3*, the endogenous negative regulator of *STAT3* [19] . This suggests a complex regulation of the immune response in the periodontal microenvironment in DS patients, which may present a shift to Th1-type responses (based on reduced *IL10* mRNA and increased expression of *STAT3*, *IFNGR1* and *IFNGR2*). However, we cannot derive the status of activation of the downstream signaling intermediates of IFN signaling directly from their mRNA expression; and this will require assessment of the phosphorylation status and subcellular localization in subsequent studies.

Importantly, *STAT1* gene is under direct transcriptional regulation by IFNs, but it may also be regulated by *IRF1* positive feedback mechanisms, which further increase *STAT1* gene expression [58–60]. In agreement, it is interesting to note that there was a similar pattern of gene expression of *STAT1* and *IRF1* genes among the studied groups (Fig. 1H and I), which can be illustrated by the strong positive correlation ($\rho = 0.75$) between *STAT1* and *IRF1* in DS with PD (Supplemental data). Therefore, the reduced expression of *STAT1* in the gingival tissues of DS patients may be associated with the reduced *IRF1* mRNA levels that are also significantly lower in DS patients in comparison with euploid individuals with PD. Therefore, taking into account the present results, we suppose that, even DS individuals having an extra 21 chromosome that carries an extra copy of *IFNGR2*, *IFNAR1* and *IFNAR2* genes, in the gingival tissue affected by PD, the difference between them and euploid individuals regarding to the IFNs related genes resides on the expression of the transcriptional factors *STAT1* and *IRF1*.

Overall, the results of this study suggest that individuals with DS exhibit a less efficient interferon-mediated immune response against microbial stimulus in the periodontal microenvironment. This finding, coupled with previous information on increased severity of inflammation in DS and higher prevalence of PD in these patients suggests a complex regulation of the immune response in the host-microbial interactions in the periodontal microenvironment. In conclusion, the expression of *STAT1* and *IRF1* genes were reduced in DS patients in comparison with euploids during the same inflammatory stimulus of PD. This result corroborates the literature's knowledge of the DS impaired immune response. In addition, this contributes to the observed increased prevalence and severity of periodontitis in DS patients.

Acknowledgments

The authors thank the DS patients for their participation in this study, as well as the collaboration of their families and the Associations of Parents and Friends of Disabled Patients (APAEs). This study was supported by grants from the Fundação de Amparo à Pesquisa do Estado de Sao Paulo (FAPESP) (2005/00588-1, 2005/03175-0 and 2006/04936-7).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cyto.2012.08.020.

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