Association of Interferon-gamma gene polymorphism (+874 T/A) with systemic sclerosis

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1. Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by abnormalities of three systems (immune, vascular, and mesenchymal extracellular matrix), leading to fibrosis development. Raynaud's phenomenon is usually the first symptom, which is associated with a diffuse small vessel vasculopathy and ischaemia as well as reperfusion injury to skin and organs targeted in this disease. The interaction of immune cells with vascular endothelium, through adhesion molecules and the effect of cytokines, is one of the earlier changes in SSc [1]. Dermal mononuclear cell infiltrates in SSc have been shown to be both CD4 and CD8 activated lymphocytes [2]. The migration of mononuclear cells in the perivascular space and the release of cytokines are responsible for fibroblast activation, excessive collagen and glucosaminoglycan production. Several cytokines that may contribute to the worsening of the disease have been found in the sera and BALF of patients with active disease [3]. Peripheral blood mononuclear cells obtained from SSc patients show spontaneous *in vitro* release of the 'fibrogenic' cytokines tumour necrosis factor (TNF) and IL-1b, and an impairment of mitogen-induced IFN- γ production [4].

IFN- γ is the most potent stimulator of HLA class II antigens on endothelial cells, up-regulating endothelial – leucocyte adhesion; however, it is also a negative regulator of collagen production by fibroblasts [5].

IFN- γ plays a key role in controlling immune response and inflammation, and some polymorphic sites distributed along coding and non-coding regions have been associated with IFN- γ production. The first reported polymorphic site describes a short-tandem CA repeat in the first intron of IFN- γ gene, characterizing a 12 CA repeat, named allele 2, associated with high production of IFN- γ [6]. Next, a single nucleotide polymorphism +874*T/A, at the 5' end of the CA repeat region in the first intron was described. Considering the linkage disequilibrium of the T allele with the allele 2 microsatellite, and considering that the T to A polymorphism coincides with a putative NF-kappa B binding site, an association with high transcription of IFN- γ was reported [7].

Since IFN- γ has a key role in SSc pathogenesis and there is no report of the association between +874 A/T SNP and SSc susceptibility, we investigated the frequency of IFN- γ +874 polymorphism in a series of Brazilian SSc patients.

2. Patients and methods

2.1. Patients

DNA samples were obtained from 177 SSc patients (156 females) followed at the Faculty of Medical Sci-

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Fig. 1. Gel electrophoretic analysis of +874 INF- γ polymorphism (261bp). The PCR products were obtained from two different donors. (1) Homozygous donor TT; (2) Heterozygous donor TA. Primers for human growth hormone gene (428 bp fragment) were used as internal reaction control.

ences, State University of Campinas, UNICAMP, SP, Brazil. All patients fulfilled the American College of Rheumatology classification criteria for SSc [8], and were divided into diffuse and limited SSc according to LeRoy et al. [9]. Hundred nine patients presented limited SSc (100 women) and 68 patients (56 women) presented diffused SSc. All the patients were clinically evaluated based on a standard protocol of investigation of SSc patients of the Unit of Rheumatology of UNICAMP. The extension and severity of skin thickening was evaluated by the modified Rodnan skin score (mRSS) that analyzes 17 anatomical sites, graded from 0 (normal skin) to 3 (intense skin thickening). Esophageal motility, chest radiography, lung functional tests, pulmonary artery systolic pressure and doppler echocardiogram were routinely performed in all patients.

A total of 191 unrelated healthy bone marrow donors (160 females) were also studied. The study protocol was approved by the local Ethics Committee (protocol # 9581/2002).

INF- γ +874 polymorphism was identified using PCR-amplified DNA hybridized with sequence-specific primers, as described by Pravica et al., identifying a 261 bp fragment [7]. Primers for human growth hormone gene (428 bp fragment) were used as internal reaction control (Fig. 1).

3. Statistical analysis

The Hardy – Weinberg equilibrium (HWE) was determined by Pearson's χ^2 goodness-of-fit test. Allele and genotype frequencies were compared between groups using the Fisher's exact test. A p value ≤ 0.05 was considered to be significant. Odds ratios (OR) with 95% confidence interval (CI) were calculated using the statistical program GraphPad Instat.

Table 1
Genotype and allele frequencies for the +874 at the first intron of the INF- γ gene in healthy controls and SSc patients

	Controls ($n = 191$)	SSc limited patients ($n = 109$)	SSc diffused patients ($n = 68$)
Genotype frequencies			
A/A	60 (31%)	40 (37%)	32 (47%)
T/T	34 (18%)	32 (29%)	7 (10%)
T/A	97 (51%)	37 (34%)	29 (43%)
Allele frequencies			
A	214 (56%)	116 (54%)	92 (68%)
T	168 (44%)	100 (46%)	44 (32%)

4. Results

Genotype and allele frequencies for the +874 INF- γ polymorphism in patients and controls are shown in Table 1. Genotype frequencies of healthy individuals and patients were in accordance with HWE. The +874 A allele was overrepresented in patients presenting Rodman modified skin score greater than 12 ($p = 0.034$, $OR = 1.57$ 95% IC: 1.044–2.360). The A/A genotype was also associated with reduced ($\leq 70\%$) vital force capacity ($P = 0.01$, $OR = 1.79$; 95% IC: 1,235–2,562).

When patients were compared according to SSc clinical variants, the T/T genotype was significantly increased in patients presenting limited SSc ($P = 0.002$; $OR = 0.27$; 95% IC: 0,1140–0,6686). Compared to non-Caucasians, the T/T genotype was significantly increased in Caucasians, irrespective of the disease variant ($P = 0.009$; $OR = 0.8$; 95%IC: 0,6976–0,9207).

5. Discussion

INF- γ exhibits inhibitory effects on collagen synthesis, being considered an immunomodulatory cytokine in SSc, especially in early stages of the disease [3,10]. Both serum levels and peripheral blood mononuclear

Table 2

Genotype and allele frequencies for the +874 at the first intron of the INF- γ gene in clinical manifestations that presented allelic or genotypic significant differentiation

	1 (n = 69)	2 (n = 34)	3 Caucasian/AB
Genotype frequencies			
A/A	34 (49%)	19 (56%)	50 (28%) / 19 (11%)
T/T	11 (16%)	6 (18%)	48 (27%) / 5 (3%)
T/A	24 (35%)	9 (26%)	40 (23%) / 15 (8%)
Allele frequencies			
A	92 (67%)	47 (72%)	140 (39%) / 53 (15%)
T	46 (33%)	18 (28%)	136 (38%) / 25 (8%)

(1) Patients presenting Rodman modified skin score greater than 12 ($p = 0.034$, $OR = 1.57$ 95% IC: 1.044–2.360). (2) Patients presenting reduced ($\leq 70\%$) vital force capacity ($P = 0.01$, $OR = 1.79$; 95% IC: 1.235–2.562). (3) Race (Caucasian/African-Brazilians)- ($P = 0.009$; $OR = 0.8$; 95%IC: 0.6976–0.9207).

cell IFN- γ production are described to be decreased in SSc patients with clinically active disease compared to stable disease, suggesting that high levels of IFN- γ may play a protective role in clinically stable patients [11].

In the present study, the IFN- γ T/T genotype, which has been associated with high levels of cytokine production, was also associated with the limited SSc variant, particularly among Caucasians, in whom the disease has a better clinical evolution [8]. Although no study on IFN- γ polymorphism has been reported for SSc, the +874 A/A genotype has been associated with the presence of arthritis in systemic lupus erythematosus patients [12].

Our results suggest that the genotype T/T could be associated with the better prognostic of SSc, because this polymorphism seems to favor the INF- γ gene transcription and consequently the higher production of the cytokine, which has immunomodulatory effect in collagen production.

SSc is a complex disease and several genes may contribute to its pathogenesis. Ours results support that genetic variation in the IFN- γ gene might influence the disease course of SSc.

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