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IFN-γ promoter gene polymorphism in psoriasis vulgaris

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Abstract
This study was performed to investigate the association between interferon (IFN)-γ single nucleotide polymorphism (SNP) and susceptibility for psoriasis vulgaris. DNA from 78 patients with psoriasis vulgaris (54 patients with type I psoriasis, 24 with type II psoriasis) and 74 healthy volunteers was investigated. IFN-γ promoter gene SNP in position 874 was evaluated by polymerase chain reaction with sequence-specific primers (PCR-SSP) and the results were compared between a group of psoriatic patients, divided into early onset of psoriasis (type I) and late onset of psoriasis (type II) subgroups, and healthy control subjects. A significant difference in the genotype frequencies between psoriasis patients and healthy controls was found ($p < 0.02$) and no significant differences were observed analyzing subsets of psoriatic patients (gender, type of disease) also in carriage and allele frequencies. The results suggest that IFN-γ polymorphism is associated with susceptibility to psoriasis vulgaris.

Keywords: *IFN-γ, single nucleotide polymorphism, psoriasis*

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Introduction

Psoriasis is an inflammatory skin disease affecting about 2–3% of the Caucasian population (Bos & DeRie 1999). The aetiology of this chronic disease includes: genetic background, environmental factors and disturbances of the vascular and the immune system. The inflammatory process is associated with characteristic changes in the cutaneous cytokine profile. High levels of tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-2, IL-6, IL-8, IL-12 and leukaemia inhibitory factor (LIF-1), and decreased amounts of IL-1, IL-4, IL-5 and IL-10 are present in psoriatic skin lesions. This pattern of cytokines suggests that mainly Th1 lymphocytes are responsible for the development of skin lesions, and psoriasis is a type 1 immune response disease (Szepietowski et al. 2001, Griffiths 2003, Chamian & Krueger 2004, Ozawa & Aiba 2004, Krueger & Ellis 2005).

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The IFN-γ gene is located on the long arm of chromosome 12 (12q14). Several polymorphisms of this gene have been reported, with two as a main object of intensive research: single nucleotide polymorphism (SNP) in position 874 (adenine/thymine – A/T) and a CA (cytosine, adenine) repeat microsatellite polymorphism in the first intron. The first one lies within a binding site for the nuclear factor (NF) κB (NFκB) transcription factor and the presence of thymine (T) in position +874 determines specific binding of NFκB. The correlation between polymorphism in the human IFN-γ gene and production of this cytokine was described first for microsatellite CA repeat polymorphism by Pravica et al. (1999). The authors found that individuals homozygous for allele 2, which corresponds to 12 CA repeats, produced significantly more IFN-γ than individuals with other allele combination (alleles 1, 3, 4, 5). In their next study, Pravica et al. (2000) reported SNP, T to A, in the first intron of the human IFN-γ gene in position 874. They also showed absolute correlation between the presence of the T allele (thymine in position 874) and previously described high producing microsatellite allele 2. This correlation between SNP and production of IFN-γ was later confirmed by others (Hoffmann et al. 2001, Hutchings et al. 2002) but opposite results were also published (Cartwright et al. 1999). In a meta-analysis concerning cytokine gene polymorphisms and cytokine production published by Warle et al. (2003), the authors concluded that evidence suggests that the relationship between SNP (874 T/A) and IFN-γ production is plausible for healthy individuals but not clear for patients, probably due to differences in the protocols used and patient populations.

IFN-γ is known as a multifunctional cytokine with a multitude of immunoregulatory functions. It is produced mainly by CD4+ and CD8+ T-cell subsets, macrophages, natural killer cells and keratinocytes (McKenzie et al. 2003, Ellis & Beaman 2004). IFN-γ was found in the blood and skin of psoriatic patients and has the ability to provoke psoriasis lesions after injection in the uninvolved skin of psoriatic patients (Fierlbeck et al. 1990, Ozawa & Aiba 2004). IFN-γ stimulates the activation and antigen presentation of keratinocytes by induction of HLA-DR, IL-8 and intercellular adhesion molecule (ICAM)-1 in these cells. IFN-γ also increases expression of vascular cell adhesion molecule (VCAM)-1 and leucocyte adhesion molecules on the endothelial cells, which facilitate immune cell penetration into the skin. Finally, IFN-γ induces the expression of VCAM-1 on dendritic cells and inhibits production of IL-10, which is a potent anti-inflammatory cytokine (Ozawa & Aiba 2004). IFN-γ is known to be potent antiproliferative cytokine which arrests keratinocytes growth *in vitro* (Nickoloff et al. 1984), but also induces Bcl-x in keratinocytes which enhances their survival. These abilities make IFN-γ a key cytokine in the control of proliferation of psoriatic keratinocytes (Bos et al. 2005).

IFN-γ polymorphism has been examined in many diseases, e.g. chronic hepatitis C (Dai et al. 2006), Hashimoto's disease (Ito et al. 2006), pulmonary tuberculosis (Lopez-Maderuelo et al. 2003) and Wegener's granulomatosis (Spriewald et al. 2005), but no studies concerning SNP of the IFN-γ gene in psoriasis vulgaris were found in the available literature.

The aim of this study was to identify whether or not SNP of IFN-γ, which influences its production (genotype T/T – high, T/A – intermediate and A/A low producer) is a risk factor for the development of psoriasis.

Materials and methods

Patients and controls

Seventy-eight patients with psoriasis vulgaris (37 females (47.43%) and 41 males (52.57%)) were included in the study. Two subsets of patients were established – early-onset psoriasis (type I – onset not later than the age of 40 years with a positive family history of psoriasis) and type II psoriasis (onset after the age of 40 years with negative family history of the disease). The type I group included 28 women (51.85%) and 26 men (48.15%) with a mean age of 44.12 ± 11.80 years (range 19–67). The type II group had a mean age of 61.37 ± 11.21 years and consisted of 9 women (37.5%) and 15 men (62.5%). Due to the differences in the age of onset of type I and type II psoriasis it was not possible to perform the comparisons with age-matched patient groups. The healthy control group consisted of 74 unrelated subjects (33 women and 41 men, mean age 34.82 ± 14.18 years, range 21–86) with no family history of psoriasis. The study was approved by the Commission of Bioethics at Wroclaw Medical University (KB 359/2003).

IFN-γ genotyping

DNA was isolated from the whole peripheral blood taken on EDTA with the use of Qiagen DNA Isolation Kit (Qiagen GmbH, Hilden, Germany). Biallelic polymorphism within the promoter region of the IFN-γ gene promoter polymorphism +874 A/T was determined by the polymerase chain reaction and sequence-specific primers (PCR-SSP) technique employing commercial primers (One Lambda, Inc., Canoga Park, CA, USA). The use of this kit (due to the number of primer mix combinations) allows assessment of the presence of particular genotypes of IFN-γ (IFN-γ – high – T/T, intermediate – T/A and low producers – A/A). For each polymorphic site one PCR reaction was carried out on a DNA template with a pair of specific primers (5′-TTCTTACAACACAAAATCAAATCT-3′; 5′-TTCTTACAACACAAAATCAA ATCA-3′), the additional control primers, reaction mix (provided by a manufacturer), and Taq polymerase (Invitrogen, San Diego, CA, USA) in a total volume of 10 μl. Amplifications were performed in MJ Research Apparatus (Watertown, MA, USA). PCR cycling conditions were as follows: 96°C for 130 s, 63°C for 60 s, followed by nine cycles of 96°C for 10 s, 63°C for 60 s, and followed by 20 cycles of 96°C for 10 s, 59°C for 50 s, 72°C for 30 s, ending with 4°C. PCR products were analyzed electrophoretically in 2% agarose gel and visualized under UV.

Evaluation and statistical analysis

Genotype and allele frequencies were compared between the study groups by the χ^2-test with Yates correction or Fisher's exact test when necessary. Multiple comparisons were made in this study, so the results were finally corrected with Bonferroni's adjustment, which lowered the alpha for each test to 0.02. Therefore, p values less than 0.02 were considered statistically significant.

Results

Frequencies in genotypes were significantly different between psoriatic patients and the control group ($p < 0.02$). The intermediate producer genotype (T/A) was

markedly more common in the control group of healthy people; high (T/T) and low (A/A) producer genotypes were slightly more frequent in psoriatic patients (Table I); similar differences were found between type I psoriasis and the control group ($p = 0.01$). No significant differences in genotypes between type I and type II psoriatic patients were present. No significant differences were observed in frequencies of carriage and alleles between psoriasis individuals and controls, as well, as between subsets of psoriasis (Table II).

Discussion

The expression of many cytokines is thought to be influenced by polymorphism in their gene loci and this may contribute to the development of inflammatory diseases. IFN-γ polymorphism was examined in many disorders and was significant for liver cirrhosis in the course of chronic hepatitis C (Dai et al. 2006), severity of Hashimoto's disease (Ito et al. 2006), and susceptibility to pulmonary tuberculosis (Lopez-Maderuelo et al. 2003) and Wegener's granulomatosis (Spriewald et al. 2005). Increased amounts of IFN-γ were found by many authors in the skin of psoriatic patients (suction blister fluids of involved and uninvolved skin), serum (Bjerke et al. 1983, Livden et al. 1989, Gomi et al. 1991) and *in vitro* in T-cell lines cultured from lesional psoriatic skin (Brown et al. 2000). SNP of the cytokine genes in psoriasis is the object of intensive research. Reich et al. (1999), found no significant differences in IL-10 polymorphism in position −1082 between psoriasis patients ($n = 151$) and controls ($n = 123$) and early and late onset of psoriasis groups, but 2 years later Craven with his group (2001) in their study found significant differences in the genotype distributions between patients with late-onset psoriasis ($n = 35$) and healthy controls ($n = 84$; $p = 0.02$). In the last paper the authors also evaluated polymorphisms of IL-4 and TNF- α genes but no significant differences were found. Other studies concerning TNF-α polymorphism in psoriatic patients have been published (Reich et al. 1999, Nishibu et al. 2002, Kim et al. 2003, Tsunemi et al. 2003), but none of them showed significant differences, apart from one in which significant differences in polymorph-

Table I. Distribution of interferon (IFN)-γ (+874 A/T) genotypes among 78 psoriasis patients and 74 controls.

	IFN-γ genotypes				
	Psoriasis		Controls		
Genotype	*n*	f	*n*	f	*p*-Value
AA	22	0.282	10	0.135	
TA	37	0.474	49	0.662	<0.02
TT	19	0.244	15	0.203	
	Type I psoriasis		Type II psoriasis		
Genotype	*n*	f	*n*	f	
AA	17	0.315	5	0.208	
TA	23	0.426	14	0.583	0.36
TT	14	0.259	5	0.208	

f, relative frequency; A, adenine; T, thymine.

Table II. Distribution of interferon (IFN)-γ (+874 A/T) carriage and alleles among psoriasis patients, type I and type II subgroups and healthy controls.

	Psoriasis		Controls	
Carriage	*n*	f	*n*	f
A	59	0.514	59	0.480
T	56	0.486	64	0.520
Alleles	*n*	f	*n*	f
A	81	0.519	69	0.466
T	75	0.481	79	0.534
	Type I psoriasis		Type II psoriasis	
Carriage	*n*	f	*n*	f
A	40	0.519	19	0.500
T	37	0.481	19	0.500
Alleles	*n*	f	*n*	f
A	57	0.528	24	0.500
T	51	0.472	24	0.500

F, relative frequency; A, adenine; C, thymine.

ism of TNF-α in position −238 were observed between the early- and late-onset psoriatic patient group (Reich et al. 1999).

Results of the present study suggest a significant association between IFN-γ promoter polymorphism (+874) and psoriasis vulgaris; however, our results should be confirmed by other authors in larger groups of patients. Currently we are not able to compare these results with other studies because no other paper concerning SNP of the IFN-γ gene in psoriasis vulgaris was found in the available literature. In 2001, Craven et al. in their study concerning the IFN-γ microsatellite repeats polymorphism in psoriatic patients found no significant differences, but these results could not be compared with ours (Craven et al. 2001).

In conclusion, the results based on the examination of a +874 promoter polymorphism of the IFN-γ gene suggest that there is an association between this SNP and susceptibility for psoriasis vulgaris, but not with the type of disease. Further studies concerning this phenomenon seem to be required to confirm our findings in different populations. To the best of our knowledge this study presents for the first time the difference in SNP of IFN-γ between psoriatic patients and a healthy population.

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