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INVESTIGATIVE REPORT

IL-6 and IL-10 Promoter Gene Polymorphisms in Psoriasis Vulgaris

Wojciech BARAN[1], Jacek C SZEPIETOWSKI[1,2], Grzegorz MAZUR[3] and Eugeniusz BARAN[1]
Department of [1]Dermatology, Venereology and Allergology and [2]Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, and [3]Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland

Overexpression of IL-6 has been implicated in the pathology of numerous autoimmune and chronic inflammatory diseases, including psoriasis, and relative deficiency of IL-10 in psoriatic patients seems to be important in the development of this disease. The aim of this study was to investigate the association between IL-6 and IL-10 single nucleotide polymorphisms and susceptibility to psoriasis vulgaris. DNA from 78 patients with psoriasis vulgaris and 74 healthy volunteers was investigated. IL-6 promoter gene single nucleotide polymorphisms in position –174, and IL-10 single nucleotide polymorphisms in positions –1082, –819 and –592 were evaluated by polymerase chain reaction using sequence-specific primers. No significant differences were found in the polymorphisms of IL-6 and IL-10 promoter genes between patients with psoriasis and healthy controls. ***Key words: IL-10; IL-6; single nucleotide polymorphism; psoriasis.***

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Wojciech Baran, Department of Dermatology, Venereology and Allergology, Chalubinskiego 1, PL-50-368, Wroclaw, Poland, E-mail: wbaran@derm.am.wroc.pl

Psoriasis is a chronic inflammatory skin disease that affects approximately 2–3% of the Caucasian population (1). This disorder has a multifactorial aetiology, including genetic background, environmental factors, and vascular and immune system disturbances. Current research is dominated by the hypothesis that an immunological disorder with inflammatory reaction, mediated through T-lymphocytes, plays a key role in the pathogenesis of psoriasis (2– 4). The cutaneous and systemic overexpression of several pro-inflammatory cytokines, particularly type-1 cytokines, such as interleukin (IL)-2, IL-6, IL-8, IL-12, interferon (IFN)-γ and tumour necrosis factor (TNF)-α, has been suggested to be responsible for initiation, maintenance and recurrence of skin lesions. On the other hand, relatively low expression of the anti-inflammatory cytokines IL-1, IL-4 and IL-10 suggests an insufficient counter-regulatory capacity of the immunological system in psoriasis (5, 6).

IL-6 is a multifunctional cytokine that plays important roles in host defence, acute phase reactions, immune responses and haematopoiesis. IL-6 is produced by a variety of cell types, including monocytes, macrophages, fibroblasts, T-helper 2 cells and vascular endothelial cells. Overexpression of IL-6 has been implicated in the pathology of numerous autoimmune and chronic inflammatory diseases, including psoriasis (7–11). IL-6 has the ability to induce an acute inflammatory reaction and, in the chronic phase, to support the activation of lymphocytes and myeloid cells (and of keratinocytes in the epidermis), which may increase the serum level of IL-6, leading to increased inflammation. The human IL-6 gene is located on the short arm of chromosome 7. There are 4 polymorphisms in the promoter region of IL-6, at positions –597 (G/A), –572 (G/C), –373 (A/G) and –174 (G/C). The –174 (G/C) polymorphism appears to influence IL-6 production: G at position 174 (transition C→G at position –174) determines increased IL-6 production, C at position 174 (transition G→C at position –174) mediates decreased IL-6 production (12–14).

Relative deficiency of IL-10 in psoriatic patients (cutaneous expression and serum levels) appears to be important in the development of the disease. IL-10 controls inflammatory processes by suppressing the production of pro-inflammatory cytokines, chemokines and antigen-presenting and co-stimulatory molecules on the immune system cells. Various cell populations, including macrophages, T-helper 2 cells, monocytes, B-cells, eosinophils and mast cells, can produce IL-10 (15, 16). The gene encoding IL-10 is located on the long arm of chromosome 1. The promoter region is highly polymorphic, with 3 point mutations: single nucleotide polymorphisms (SNPs) at positions: –1082 (G/A), –819 (C/T) and –592 (C/A). The presence of A in position –1082 (transition G→A at position –1082) is associated with decreased levels of IL-10 and G (transition A→G at position –1082) with higher production of this cytokine. The other 2 polymorphisms probably do not influence the production of IL-10 (17–21). There are also studies claiming adverse relationship between polymorphisms at position –1082 and IL-10 production (22, 23).

The aim of this study was to identify whether polymorphisms of IL-6 and IL-10 are risk factors for the development of psoriasis. To the best of our knowledge, only a few studies concerning IL-10 single nucleotide polymorphism in psoriasis have been published, and the results are conflicting (17, 19, 24). We found no papers

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Table I. *Distribution of IL-6 (–174 G/C) genotypes among 78 patients with psoriasis and 74 controls*

	Psoriasis		Controls		Type I psoriasis[a]		Type II psoriasis[a]	
IL-6 genotypes	*n*	f	*n*	f	*n*	f	*n*	f
CC	16	0.205	13	0.176	10	0.185	6	0.250
CG	38	0.487	38	0.514	25	0.463	13	0.542
GG	24	0.308	23	0.311	19	0.352	5	0.208
p		> 0.79				> 0.93		

[a]For explanantion of type II psoriasis see Material and Methods. n: number; f: relative frequency; G: guanine; C: cytosine.

concerning IL-6 polymorphism in psoriasis vulgaris, and only one for psoriatic arthritis (25).

MATERIALS AND METHODS

Patients and controls

Seventy-eight patients with psoriasis vulgaris (37 women (47%) and 41 men (53%)) were included in the study. All patients gave their informed consent. Two subsets of patients were established: early onset psoriasis (type I: onset not later than 40 years of age with a positive family history of psoriasis) and type II psoriasis (onset after 40 years of age with a negative family history of the disease). The type I group included 28 women (52%) and 26 men (48%) with mean age of 44.1 ± 11.8 years (range 19–67 years). The type II group had a mean age of 61.4 ± 11.2 years and comprised 9 women (37.5%) and 15 men (62.5%). The healthy control group comprised 74 unrelated subjects (33 women and 41 men) with no family history of psoriasis. The control group was recruited from among students of the Faculty of Medicine Wroclaw Medical University. The study was approved by the Commission of Bioethics at Wroclaw Medical University (KB 359/2003).

IL-6 and IL-10 genotyping

DNA was isolated from the whole peripheral blood taken into ethylenediaminetetraacetic acid (EDTA) tubes with the use of Qiagen DNA Isolation Kit (Qiagen GmbH, Hilden, Germany). Bi-allelic polymorphism within the promoter region of the IL-6 gene (promoter polymorphism –174 G/C) and the IL-10 gene (at positions –1082 A/G, –819C/T and –592A/C) was determined by polymerase chain reaction using sequence-specific primers (PCR-SSP), employing commercial primers (One Lambda, Inc., Canoga Park, CA, USA). The use of this kit (due to number of primer mix combinations) allows assessment of the presence of particular IL-6 and IL-10 genotypes (IL-6 – high – G/G,G/C and low producers – C/C; IL-10 – high – GCC/GCC, intermediate – GCC/ACC, GCC/ATA and low producers – ACC/ACC,ACC/ATA, ATA/ATA). For each polymorphic site one PCR reaction was carried out on a DNA template with a pair of specific primers, the additional control primers, reaction mix (provided by the manufacturer), and Taq polymerase (Invitrogen, Carlsbad, California, USA) in a total volume of 10 µl. Amplifications were performed using MJ Research Apparatus (Watertown, MA, USA). PCR cycling conditions were as follows: 96ºC for 130 s, 63ºC for 60 s, followed by 9 cycles of 96ºC for 10 s, 63ºC for 60 s, followed by 20 cycles of 96ºC for 10 s, 59ºC for 50 s, 72ºC for 30 s, and ending at 4ºC. PCR products were analysed electrophoretically in 2% agarose gel and visualized under ultraviolet (UV) light.

Evaluation and statistical analysis

Genotype and allele frequencies were compared between the study groups using a χ^2-test with Yates correction or Fisher's exact test when necessary. *p*-values considered as statistically significant ($p < 0.05$) were corrected by Bonferroni adjustment.

RESULTS

Distribution of IL-6 alleles and genotypes

There were no significant differences in the distribution of the polymorphism of IL-6 genotypes between patients with psoriasis and healthy controls. Frequencies in genotypes were similar between patients and control group ($p > 0.79$). Analysing subgroups of patients with psoriasis separately reveals no statistically significant differences between type I and type II psoriasis ($p > 0.93$) (Table I). Other differences, including gender groups, were also not significant (data not shown).

Distribution of IL-10 alleles and genotypes

Three polymorphic positions within the IL-10 promoter region were analysed (–1082 A/G, –819 C/T and –592 A/C). Their frequencies in genotypes were similar between patients and controls ($p > 0.51$). No statistically

Table II. *Distribution of IL-10 (-1082 A/G,-819C/T, -592A/C) genotypes among patients with psoriasis, type I and type II subgroups and 74 controls.*

	IL-10 genotypes				
	Psoriasis		Controls		
Genotype	*n*	f	*n*	f	*p*
ACC/ACC	9	0,115	6	0,081	$p > 0.51$
ACC/ATA	10	0,128	8	0,108	
ATA/ATA	10	0,128	7	0,095	
GCC/ACC	21	0,269	20	0,270	
GCC/ATA	16	0,205	18	0,243	
GCC/GCC	12	0,154	15	0,203	
Genotype	Type I psoriasis		Type II psoriasis		$p < 0.015$*
	n	f	*n*	f	
ACC/ACC	7	0,130	2	0,083	
ACC/ATA	10	0,185	0	0	
ATA/ATA	4	0,074	6	0,250	
GCC/ACC	15	0,278	6	0,250	
GCC/ATA	13	0,241	3	0,125	
GCC/GCC	5	0,093	7	0,292	

*Considered statistically non-significant after Bonferoni's correction for multiple testing

n: number; f: relative frequency; G: guanine; A: adenine; T: thymine; C: cytosine

significant differences were found when subgroups of patients were analysed separately (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, including psoriasis. The results of the present study showed no association between IL-6 promoter polymorphism (–174) and psoriasis vulgaris in a Polish population. We could not compare these results with other studies because no other paper concerning IL-6 polymorphism in psoriasis vulgaris was found. In 2003, Balding et al. (25) published research concerning gene polymorphism in a few cytokines, including IL-6, and found no significant differences in IL-6 cytokine polymorphism in patients with psoriatic arthritis. Due to the different patient population, the results of that study cannot be compared with ours. IL-10 promoter gene single nucleotide polymorphism was the subject of a few earlier studies. First, Reich et al. (24), showed no significant difference in IL-10 polymorphism in position –1082 between patients with psoriasis ($n = 151$) and controls ($n = 123$) and early and late onset of psoriasis groups. Two years later, Craven et al. (17) in their study concerning IL-10 –1082 polymorphism found significant differences in genotype distributions between patients with late-onset psoriasis (n=35) and healthy controls ($n = 84$) ($p = 0.02$). This late onset group of patients had a higher frequency of G/A genotype (intermediate producer) but lower G/G (high producer) and A/A (low producer) than the control group. Significant differences were also found by Kingo et al. (19) in haplotype distributions for 3 IL-10 single nucleotide polymorphisms (–1082, –819 and –592). Patients with psoriasis with persistent eruption more often had the ATA haplotype than patients with intermittent course of the disease ($p < 0.01$), lower Psoriasis Area Score Index (PASI) score ($PASI \leq 20$) patients and the group of patients with limited eruption (extent ≤ 10%) more frequently had the ACC haplotype than did controls ($p < 0.05$). The authors concluded that the ACC haplotype should be protective in the development of the advanced disease and referred to previous studies suggesting that not GCC (transition A→G in position –1082) but ACC haplotype (transition G→A in position –1082) is associated with higher IL-10 production (22, 23). Our results showed no significant association between IL-10 gene promoter polymorphism and susceptibility to psoriasis vulgaris, but the difference between type I and type II patients with psoriasis subgroups may be a good subject for further studies.

In conclusion, the results based on examination of a –174 promoter polymorphism of the IL-6 gene suggest that there is no association between this single nucleotide polymorphism and susceptibility to psoriasis vulgaris. Further studies concerning IL-10 gene polymorphism and susceptibility to psoriasis vulgaris should be performed, especially on larger populations, in order to define its importance in the pathogenesis of psoriasis.

REFERENCES

1. Hensen P, Asadullah K, Windemuth C, Ruschendorf F, Huffmeier U, Stander M, et al. Interleukin-10 promoter polymorphism IL10.G and familial early onset psoriasis. Br J Dermatol 2003; 149: 381–385.
2. Bos JD, De Rie MA. The pathogenesis of psoriasis: immunological facts and speculations. Immunol Today 1999; 20: 40–46.
3. Griffiths GEM. The immunological basis of psoriasis. J Eur Acad Dermatol Venereol 2003; 17: 1–5.
4. Chamian F, Krueger G. Psoriasis vulgaris: an interplay of T lymphocytes, dendritic cells, and inflammatory cytokines in pathogenesis. Curr Opin Rheumatol 2004; 16: 331–337.
5. Krueger G, Ellis CN. Psoriasis – recent advances in understanding its pathogenesis and treatment. J Am Acad Dermatol 2005; 53: 94–100.
6. Ozawa M, Aiba S. Immunopathogenesis of psoriasis. Curr Drug Targets Inflamm Allergy 2004; 3: 137–144.
7. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, et al. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 1989; 86: 6367–6371.
8. Neuner P, Urbanski A, Trautinger F, Moller A, Kirnbauer R, Kapp A, et al. Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 1991; 97: 27–33.
9. Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. Mediators Inflamm 2005; 5: 273–279.
10. Abanmi A, Al Harthi F, Al Agla R, Khan HA, Tariq M. Serum levels of proinflammatory cytokines in psoriasis patients from Saudi Arabia. Int J Dermatol 2005; 44: 82–83.
11. Mizutami H, Ohmoto Y, Mizutani T, Murata M, Shimizu M. Role of increased production of monocytes TNF-α, IL-lβ and IL-6 in psoriasis: relation to focal infection, disease activity and responses to treatments. J Dermatol Sci 1997; 14: 145–153.
12. Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002; 13: 357–368.
13. Simpson RJ, Hammacher A, Smith DK, Matthews JM, Ward LD. Interleukin-6: structure-function relationships. Protein Sci 1997; 6: 929–955.
14. Sehgal PB. Interleukin-6: molecular pathophysiology. J Invest Dermatol 1990; 94: 2–6.
15. Asadullah K, Sabat R, Friedrich M, Volk HD, Sterry W. Interleukin-10: an important immunoregulatory cytokine with major impact on psoriasis. Curr Drug Targets Inflamm Allergy 2004; 3: 185–192.
16. Roussaki-Schulze AV, Kouskoukis C, Petinaki E, Klimi E, Zafiriou E, Galanos A, Rallis E. Evaluation of cytokine serum levels in patients with plaque-type psoriasis. Int J Clin Pharmacol Res 2005; 25: 169–173.
17. Craven NM, Jackson CW, Kirby B, Perrey C, Pravica V,

Hutchinson IV, Griffiths CE. Cytokine gene polymorphisms in psoriasis. Br J Dermatol 2001; 144: 849–853.
18. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24: 1–8.
19. Kingo K, Koks S, Silm H, Vasar E. IL-10 promoter polymorphisms influence disease severity and course in psoriasis. Genes Immun 2003; 4: 455–457.
20. D'Alfonso S, Rampi M, Rolando V, Giordano M, Momigliano-Richiardi P. New polymorphisms in the IL-10 promoter region. Genes Immun 2000; 1: 231–233.
21. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy-review of a new approach. Pharmacol Rev 2003; 55: 241–269.
22. Eskdale J, Keijsers V, Huizinga T, Gallagher G. Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. Genes Immun 1999; 1: 151–155.
23. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 1999; 42: 1101–1108.
24. Reich K, Westphal G, Schulz T, Muller M, Zipprich S, Fuchs T, et al. Combined analysis of polymorphisms of the tumor necrosis factor-alpha and interleukin-10 promoter regions and polymorphic xenobiotic metabolizing enzymes in psoriasis. J Invest Dermatol 1999; 113: 214–220.
25. Balding J, Kane D, Livingstone W, Mynett-Johnson L, Bresnihan B, Smith O, FitzGerald O. Cytokine gene polymorphisms: association with psoriatic arthritis susceptibility and severity. Arthritis Rheum 2003; 48:1408–1413.