Interleukin-10 Promoter Polymorphism in Psoriasis

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Beneficial effects of interleukin-10 therapy and lower endogenous interleukin-10 formation compared with atopic dermatitis and cutaneous T cell lymphomas indicated that interleukin-10 is a key cytokine in psoriasis. The interleukin-10 promoter is highly polymorphic, with two informative microsatellites, interleukin-10.G and interleukin-10.R. In order to understand whether interleukin-10 itself is a predisposing gene for the psoriasis susceptibility we analyzed interleukin-10 promotor polymorphism in patients. The distribution of interleukin-10.G and interleukin10.R microsatellite alleles did not vary between patients (n = 78) and healthy controls (n = 80). In addition, when the psoriasis patients were stratified according to age of onset (younger than 40 y of age, or age 40 and older), no difference in allele distribution was observed; however, a clear differential distribution was revealed at the interleukin10.G locus when patients were stratified according to whether they had a positive family history of psoriasis (p = 0.04). This difference was due to an over-representation of the interleukin10.G13 allele in those patients with familial disease (40.4% *vs* 19.6%, Chi-square = 7.292, p = 0.007). The positive association of allele interleukin10.G13 with familial psoriasis was especially true when patients with an early onset (< 40 y of age) of the disease were compared with those patients with early onset against a nonfamilial background (39.6% *vs* 14.5%, Chi-square = 8.959, p = 0.003). Patients with age-of-onset of less than 40 were 4-fold [odds ratio = 3.85 (1.55–9.62)] more likely to have a psoriatic family background if they carried this interleukin10.G13 allele. These data suggest that the interleukin-10 locus contributes to the heritability of psoriasis susceptibility. ***Key words: cytokines/genetic/interleukins/polymorphism/psoriasis. J Invest Dermatol 116:975–978, 2001***

Psoriasis is considered to be a T cell-dependent (auto)-immune disease (Valdimarsson *et al*, 1986). The demonstrated overexpression of several proinflammatory cytokines, in particular type 1, is considered to be responsible for the initiation, maintenance, and reoccurrence of the skin lesions (reviewed in Bos and De Rie, 1999). In contrast, we recently found that the cutaneous mRNA expression of the anti-inflammatory cytokine interleukin (IL) -10 is significantly lower in psoriasis than in other inflammatory dermatoses, such as atopic dermatitis and cutaneous T cell lymphomas (Asadullah *et al*, 1998). Our results correspond well with findings regarding low cutaneous IL-10 protein expression, as determined by immunohistochemistry (Nickoloff *et al*, 1994) and by measurement in suction blister fluid (Mussi *et al*, 1994) in psoriatic patients.

Several lines of evidence support the hypothesis that relative IL-10 deficiency might be a central phenomenon and that IL-10 is a key cytokine in psoriasis (reviewed in Asadullah *et al*, 1999c): (i) Various effective antipsoriatic treatment modalities, e.g., ultraviolet radiation or calcitriol therapy, increase IL-10 production by several cell types. (ii) Cyclic adenosine monophosphate-elevating drugs upregulate IL-10 expression. Interestingly, there are sporadic reports that these substances improved psoriasis. (iii) Inhibitors of the β-adrenergic receptor pathway or cyclooxygenase may lead to an exacerbation of psoriasis. These drugs diminish intracellular cyclic adenosine monophosphate formation, presumably associated with decreased IL-10 release. (iv) Direct support of a type 1 cytokine pattern (i.e., inhibition of IL-10 secretion) by interferon-α or -γ application as well as interferon-elevating drugs, such as lithium are also known to provoke or to exacerbate psoriasis. (v) Pregnancy often leads to the depression of type 1 cytokines, and the majority of psoriatic women who become pregnant experience an improvement in their disease activity. (vi) Calcipotriol and glucocorticoids increase IL-10 receptor expression. It might be speculated that this leads to an enhancement of the IL-10 susceptibility contributing to the antipsoriatic activity of these compounds. The key role of IL-10 in psoriasis is further supported by the beneficial effects of IL-10 therapy recently reported by us and others. So one pilot and two phase-2 trials with subcutaneous IL-10 administration over 3–7 wk in patients with moderate to severe psoriasis showed very promising results (Asadullah *et al*, 1998, 1999a; Reich *et al*, 1998).

The IL-10 deficiency in psoriasis is particular remarkable as the IL-10-inducing cytokine tumor necrosis factor-α was found to be significantly overexpressed in psoriasis (Kristensen *et al*, 1993; Bonifati *et al*, 1994; Ettehadi *et al*, 1994; Asadullah *et al*, 1999b), arguing against an insufficient stimulation, and might rather be the result of a genetic predisposition to ''low IL-10 secretion''.

Genetic factors are likely to be of fundamental importance in the expression of the disease. This is supported by numerous family, twin, and HLA allotype studies, which gave clear evidence of a heritable component (reviewed in Bos and De Rie, 1999). In fact a

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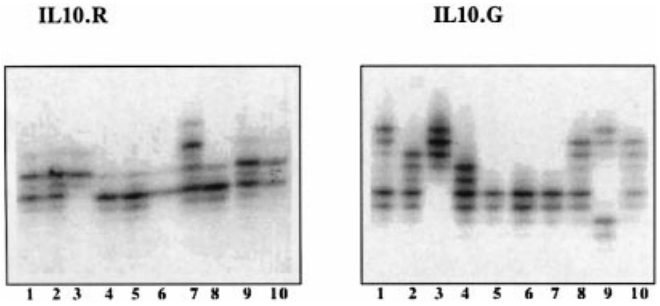

Figure 1. Typical gels showing the IL10.R and IL10.G microsatellite alleles. Typical sequencing gels for IL10.R (*left panel*) and IL10.G (*right panel*). For IL10.R, *lanes 1–10* typed as: 2/3, 2/3, 3/3, 2/2, 2/2, 2/2, 2/4, 2/2, 2/3, 2/3, respectively. For IL10.G, *lanes 1–10* typed as: 9/14, 9/12, 13/14, 9/11, 9/9, 9/9, 9/9, 9/13, 7/14, 9/13, respectively.

linkage between certain HLA antigens and psoriasis has been reported. A high proportion of patients with type 1 psoriasis (onset before the age of 40 y) are carriers of HLA Cw6, B57, and DR7 and show a positive family history. Moreover, it has been recently reported that a tumor necrosis factor-α promotor polymorphism is associated with juvenile onset of psoriasis (Höhler *et al*, 1997; Reich *et al*, 1999), although this was not confirmed in another study (Jacob *et al*, 1999).

The IL-10 promoter is highly polymorphic, with two informative microsatellites, IL10.G and IL10.R (Eskdale and Gallagher, 1995; Eskdale *et al*, 1996) and three commonly used point mutations (Eskdale *et al*, 1997a; Turner *et al*, 1997). Given the importance of IL-10 in the immunopathology and treatment of psoriasis, we felt it was important to understand whether there was evidence to suggest that IL-10 itself is a predisposing gene for psoriasis susceptibility. A preliminary study of the IL-10 locus in psoriasis (Reich *et al*, 1999) examined one particular promoter point mutation, but found no association with disease. In the present report, we have used the highly informative microsatellite markers to more efficiently address the question of whether the IL-10 locus contributes to susceptibility to psoriasis.

SUBJECTS AND METHODS

Patient samples Peripheral blood was obtained from patients and controls, and germline DNA was extracted by standard means. German Caucasian patients (n = 80), unrelated to one another and with a confirmed diagnosis of psoriasis vulgaris, were examined. Patients were considered to have ''early onset'' psoriasis if disease onset was at age younger than 40 y (n = 55) and ''late onset'' psoriasis if age of onset was after their fortieth birthday (n = 19); in six cases, age of onset could not be confirmed. In addition, patients were considered to have ''familial'' psoriasis if they have one or more first- or second-degree relative afflicted with the condition (n = 26), or to have ''sporadic'' psoriasis if this was not the case (n = 46); in eight cases this information could not be confirmed. Of those patients with ''early onset'' psoriasis, some were also ''familial'' (n = 24, i.e., typical type 1 psoriasis) and some were sporadic (n = 31). German Caucasian healthy volunteers (n = 90) served as a control group. Control individuals were healthy and unrelated to one another. They did not have a first- or second-degree relative with psoriasis.

Genotyping at the IL-10 microsatellites DNA was prepared from white-cell pellets using the ''DNA-Fast'' kit from Epicentre Technologies (Cambridge, U.K.). Genotyping at the IL10.G and IL10.R microsatellites was performed as previously described in detail (Eskdale and Gallagher, 1995; Eskdale *et al*, 1996, 1997a). In addition, full details of primers and reaction conditions are present in the Genome Data Base entry for the human IL-10 gene. Examples of IL10.G and IL10.R genotyping gels are shown in **Fig 1**.

Statistical analysis The allelic distribution between two whole populations (for example, between psoriasis patients and controls, or between those patients with familial psoriasis or not) was compared using the Monte Carlo simulation, and the freely available ''CLUMP'' software of Sham and Curtis (1995). This technique has been developed specifically to examine disease associations at multiallelic loci. It uses a Monte Carlo approach by measuring the difference in allele frequencies between the two groups and then generates multiple simulated datasets to see how many times the observed difference might be generated by chance if the frequencies were in fact the same. In so doing, it generates the ''T4'' statistic, which measures the overall difference in frequencies of all alleles simultaneously. Although this is generated in the same way as conventional chi-square values, the use of the Monte Carlo approach to assess empirical significance avoids the need for a Bonferonni correction and the problems of dealing with rare alleles (D.Curtis, personal communication). $p < 0.05$ was taken as significant. In some cases, individual alleles were compared by 2×2 Chi-square analysis using Minitab software. Odds ratios and 95% confidence intervals were calculated on the ''Simple Interactive Statistical Analysis (SISA) homepage: http://home.clara.net/sisa/index.htm

Table I. Distribution of IL10.G and IL10.R alleles in psoriasis patients and normal individuals[a]

Allele	Normal controls	Psoriasis patients
	(n = 90)	(n = 80)
IL10.G1	0 (0.00)	0 (0.00)
IL10.G2	0 (0.00)	0 (0.00)
IL10.G3	0 (0.00)	0 (0.00)
IL10.G4	0 (0.00)	0 (0.00)
IL10.G5	0 (0.00)	0 (0.00)
IL10.G6	0 (0.00)	0 (0.00)
IL10.G7	7 (3.89)	4 (2.56)
IL10.G8	8 (4.44)	10 (6.41)
IL10.G9	61 (33.89)	44 (28.21)
IL10.G10	13 (7.22)	11 (7.05)
IL10.G11	18 (10.00)	12 (7.69)
IL10.G12	9 (5.00)	10 (6.41)
IL10.G13	49 (27.22)	42 (26.92)
IL10.G14	15 (8.33)	19 (12.18)
IL10.G15	0 (0.00)	4 (2.65)
	(n = 88)	(n = 77)
IL10.R1	0 (0.00)	0 (0.00)
IL10.R2	130 (73.86)	129 (83.77)
IL10.R3	43 (24.43)	24 (15.58)
IL10.R4	2 (1.14)	1 (0.65)
IL10.R5	1 (0.57)	0 (0.00)

[a]IL10.G and IL10.R alleles were visualized and defined as described in the text, assuming no null alleles. The numbers of alleles observed are shown, with the proportion in parenthases. A comparison of these two populations using the Monte Carlo simulation for alleles 7–15 showed that the two populations did not vary in their allelic distribution. Alleles IL10.G1 to IL10.G6 were not represented in any populations tested here. Similarly, comparison of the IL10.R alleles showed no difference between patients and controls.

RESULTS

IL-10 microsatellite alleles do not contribute to overall psoriasis susceptibility The distribution of IL10.R and IL10.G alleles in psoriasis patients and normal controls is shown in **Table I**. No difference in allele distribution was observed overall at either locus (Monte Carlo simulation), nor was any individual allele differentially represented between the patient and control groups. In addition, when we examined the distribution of IL10.G and IL10.R alleles in relation to age of onset, there was no difference between the ''early'' and ''late'' onset groups.

IL10.G13 microsatellite allele is over-represented in familial psoriasis In addition to comparing the distribution of IL-10 locus alleles between patients and controls, we also considered the question of whether the IL-10 locus was important when patients were considered as having ''sporadic'' disease (i.e., no family history of psoriasis) or ''familial'' disease (i.e., a clear family history of psoriasis). As shown in **Table II**, this was the case. Patients with

Table II. Distribution of IL10.G alleles in subgroups of psoriasis patients[a]

IL10.G allele	Familial patients (total) (n = 26)	Sporadic patients (total) (n = 56)	Familial patients (onset < 40 y) (n = 24)	Sporadic patients (onset < 40 y) (n = 31)
IL10.G7	2 (3.85)	0 (2.56)	2 (4.17)	0 (0.00)
IL10.G8	2 (3.85)	7 (7.61)	1 (2.08)	4 (6.45)
IL10.G9	12 (23.08)	30 (32.61)	11 (22.92)	20 (32.26)
IL10.G10	3 (5.77)	8 (8.70)	3 (6.25)	7 (11.29)
IL10.G11	4 (7.69)	6 (6.52)	4 (8.33)	4 (6.45)
IL10.G12	4 (7.69)	5 (5.43)	4 (8.33)	5 (8.06)
IL10.G13	**21 (40.38)**	**18 (19.57)**	**19 (39.58)**	**9 (14.52)**
IL10.G14	3 (5.77)	15 (16.30)	3 (6.25)	10 (16.13)
IL10.G15	1 (1.92)	3 (3.26)	1 (2.08)	3 (4.84)

[a]IL10.G alleles were visualized and defined as described in the text, assuming no null alleles. The numbers of alleles observed are shown, with the proportion in parentheses. A comparison of all patients with familial disease and those with sporadic disease using the Monte Carlo simulation for alleles 7–15 gave an T4 statistic of 10.788, indicating that the two populations vary significantly in their allelic distribution, p = 0.04. Similarly, comparing patients with early onset disease according to whether or not they had a family background of psoriasis again showed these two populations to vary significantly in their allelic distribution (T4 = 11.567, p = 0.025). Alleles in bold (IL10.G13) varied significantly between the populations, as described in the text.

familial psoriasis had a different distribution of IL10.G alleles from those with sporadic psoriasis (Monte Carlo simulation, p = 0.04), such that the IL10.G13 allele was over-represented in those with inherited disease (Chi-square = 7.292, p = 0.0069). The carriage of IL10.G13 (i.e., the presence of one or more copies of the allele) was correspondingly elevated in patients with familial psoriasis; 17 of 26 familial patients were IL10.G13$^+$, whereas only 17 of 46 sporadic psoriasis patients carried this allele [odds ratio = 3.22 (1.17–8.81), p = 0.02]. IL10.G allele distribution in the sporadic disease group was not different from that seen in the controls, thereby confirming that it is indeed the patients with inherited psoriasis who demonstrate skewed IL10.G allele distribution (not shown). IL10.R locus alleles were not different between the two subgroups of psoriasis patients.

The differential distribution of IL10.G alleles was emphasized when the ''early'' psoriasis patients were analyzed with respect to whether they had ''familial'' or ''sporadic'' disease. These two groups varied in their distribution of IL10.G alleles (p = 0.025, Monte Carlo simulation), with an enhanced over-representation of the IL10.G13 allele in the familial group (39.6% *vs* 14.5%, Chi-square = 8.959, p = 0.0028). These patients also showed a similarly elevated carriage of the IL10.G13 allele, with enhanced significance; 15 of 24 familial patients were IL10.G13$^+$, whereas only eight of 31 sporadic psoriasis patients carried this allele [odds ratio = 2.82 (1.51–15.18), p = 0.006].

DISCUSSION

IL-10 is considered to be a key cytokine in the pathogenesis of psoriasis. When we examined known polymorphic microsatellite markers in the human IL-10 promoter in psoriasis patients, no difference was noted in comparison with the control group. This was true of both markers utilized, IL10.G and IL10.R. In addition, when the psoriasis patients were stratified according to age of onset no difference in allele distribution was observed; however, a clear differential distribution was revealed when patients were stratified according to whether they had a positive family history of psoriasis. In this case, allele IL10.G13 was positively associated with familial psoriasis and this was also true when younger patients were considered – patients with age-of-onset of less than 40 were 3-fold more likely to have a psoriatic family background if they carried this allele. Thus, it appears that the IL-10 locus contributes to the genetic background in familial psoriasis.

Genetic markers in cytokine genes are becoming widely used in studies of immune-mediated disease and it is becoming apparent that they can be markers of overt susceptibility and of disease severity (reviewed in Bidwell *et al*, 1999). In this study we have demonstrated that an important immunologic gene (IL-10), whose product is known to be dysregulated in psoriasis, is in fact genetically marked within a subpopulation of psoriasis patients. This situation is reminiscent of that found in systemic lupus erythematosus, where the same locus (IL10.G microsatellite) shows significant allele skewing in patients *vs* controls. This has been observed in patients from the U.K. (Eskdale *et al*, 1997b), Mexico (Mehrian *et al*, 1998), and Italy (D'Alfonso *et al*, 2000). Indeed, in the U.K. and Italian studies, it was the same allele that was associated with systemic lupus erythematosus, namely IL10.G13. It is, therefore, unlikely to be a coincidence that it is this same IL10.G13 allele that has been highlighted by this study on psoriasis.

Our recent investigation of the relationship between IL10.G alleles and IL-10 secretion from lipopolysaccharide-stimulated peripheral blood (Eskdale *et al*, 1998), however, failed to demonstrate a role for the IL10.G13 allele in this context. In agreement with this we observed only a tendency of diminished IL-10 formation capacity in psoriatic patients, but not a statistical significant difference (Asadullah *et al*, 1998). Although it has been shown that genetic relationships between cytokine alleles and secretion may vary in patient groups compared with normal groups (Koss *et al*, 1999), the possibility must be considered that the dysregulation of IL-10 seen in psoriasis patients (and from which this study was launched), and the association between an IL-10 locus gene marker and the disease reported here are complementary rather than related. Of course, this allele may well be of importance in alternative pathways of IL-10 regulation, but if IL10.G13 is not associated with differential IL-10 secretion then it is likely that this allele is associated with both psoriasis and systemic lupus erythematosus, through linkage disequilibrium with a novel functional element yet to be defined. Recently, several new single-nucleotide polymorphisms have been defined in the human IL-10 locus (D'Alfonso *et al*, 1999), but none of these was specifically linked to IL10.G13. Furthermore, the complex haplotypic nature of the IL-10 locus (Eskdale *et al*, 1999) placed the IL10.G13 on at least two frequent haplotypes. Both these observations support the hypothesis that any functional element linked to this allele may in fact lie outside the IL-10 locus *per se*. If true, this would implicate IL-10 as a disease modifying gene in psoriasis rather than a disease-causing gene.

While interesting, the studies reported here represent a first step in investigating whether a genetic relationship truly exists between the human IL-10 gene and psoriasis susceptibility and/or heritability. Further investigations necessary to explore fully the impact of IL-10 polymorphism on psoriasis susceptibility should include analyses of psoriatic families (Jacob *et al*, 1999) and correlation analyses between the IL-10 secretion capacity and the IL-10 promoter genotype in psoriatic (as opposed to normal) individuals.

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