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Chapter 6

The Role of Interleukin 10 Promoter Polymorphisms in the Susceptibility of Distal Interphalangeal Osteoarthritis

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Abstract

Objective

The interleukin (IL)-10 single nucleotide promoter polymorphism (SNP) –2849A is associated with decreased IL-10 production as measured by lipopolysaccharide (LPS) stimulated whole blood cultures. A low innate production of IL-10 using the same assay is associated with an increased risk of familial osteoarthritis (OA). We investigated the association of 7 novel SNP located downstream of the IL-10 transcription start site: –2849,–2763, –1330, –1082, –819, and –592, constituting the 4 ancient haplotypes, with distal interphalangeal (DIP) OA.

Methods

The study population comprised consecutive patients with and without radiological DIP OA (Kellgren-Lawrence score of ≥2 in one joint) aged 40–70 years from a cohort of subjects with different types of arthritis in an early stage referred to an Early Arthritis Clinic (EAC). DNA typing for IL-10 SNP as well as radiographs of the hands were performed at clinic enrolment. Patients with rheumatoid arthritis, systemic lupus erythematosus, spondyloarthropathies, and psoriatic arthritis were excluded.

Results

The distribution of DIP OA and IL-10 SNP were comparable to representative samples of the Dutch population. In the cohort of 172 subjects, 57 had DIP OA (33%) and 115 (67%) had no DIP OA. No significant association was found between DIP OA and IL-10 SNP and the 4 common haplotypes IL10.1, IL10.2, IL10.3, and IL10.4.

Conclusion

Our data suggest that IL-10 SNP, including –2849, which is associated with differential production, do not play a major role in the susceptibility of DIP OA.

Introduction

Distal interphalangeal (DIP) osteoarthritis (OA) is one of the most frequent subtypes of OA, causing pain and loss of function in the hands. Familial aggregation and twin studies have shown DIP OA to have a strong familial component(1–3). Despite studies focusing on the genetics of DIP OA, the genes involved have not been identified. Several chromosomal regions are reported to be associated with DIP OA, namely quantitative trait loci (QTL) on chromosomes 2q, 7p, and 11q implicated by linkage studies (4), HLA-DR2 by candidate gene analysis in several populations (5,6), and further, a locus on chromosome 2 containing the matrilin-3 gene, by a genomewide scan in an Icelandic population (7).

Loss of articular cartilage and changes in the subchondral bone and joint margins is a hallmark of OA. An increased matrix catabolism characterized by an upregulation of metalloproteinases (MMP) and the depletion of structural macromolecules such as proteoglycans contributes to the OA disease process (8). Increasing data support the role of cytokines in these processes. Although proinflammatory cytokines have been shown to play a pivotal role in the initiation and development of OA, a shift in the balance between the pro and antiinflammatory cytokines is believed to contribute to the loss of integrity of articular cartilage (9).

Among the antiinflammatory cytokines, interleukin (IL)10 appears to be a crucial factor in inflammatory processes(10). In a mouse model of arthritis, IL-10 administration prevents cartilage destruction by reducing IL-1ß and tumor necrosis factor (TNF)-α mRNA expression in articular chondrocytes, while neutralizing anti-IL-10 antibodies accelerate the onset and enhance the severity of arthritis(11).

The human IL-10 gene is highly polymorphic. Gibson, et al (12) identified 7 novel single nucleotide polymorphisms (SNP) in the distal region of the IL-10 promoter and found that certain haplotypes are significantly associated with high or low IL-10 production. Studies have shown that there are striking differences between healthy individuals in their ability to produce IL-10 following lipopolysaccharide (LPS) stimulation of whole blood cultures ex vivo. Moreover it hasbeen shown that IL-10 haplotypes dictate IL-10 production as measured by this assay (13). Of the IL-10 promoter polymorphisms, –2849A, encoded on haplotype IL-10.01, has been shown to correlate best with protein production (13). Individuals with the AA genotype have a reduced IL-10 production in comparison to AG and GG individuals.

Several studies have reported the clinical relevance of IL10 gene polymorphism in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA)(12,14), and in infectious diseases (15). In an earlier study we found an increased risk of familial OA at multiple sites in subjects with a low innate production of IL-10, as measured by the same ex vivo whole blood assay using LPS stimulation(16) used in all previous studies (12,13). This finding implicating IL-10 in cartilage destruction led us to investigate whether genetic variation in IL-10 contributes to the susceptibility of DIP OA. Our aim was to investigate whether IL-10 SNP –592, –819, –1082, –2763, –2849, and –3575 that constitute the 4 ancient IL-10 haplotypes are a risk factor for DIP OA.

Materials & Methods

Study population

The study population consists of subjects from the outpatient clinic of the Department of Rheumatology between the ages of 40 to 70 years with and without radiological DIP OA. This population, enrolled between 1993 and 2000, is part of an ongoing project, the Early Arthritis Clinic (EAC). Consecutive patients with arthritis in at least one joint with a short history of complaints are admitted to this clinic by general practitioners. Each patient subsequently undergoes full clinical, biochemical, and radiographic assessment. Diagnoses in the EAC are made according to international classification and if necessary revised up to 1 year of followup (17). Patients with a definitive EAC diagnosis after one year of followup were included in this study. Patients with RA, SLE, and spondyloarthropathies were excluded because these diseases have been associated with IL-10. Patients with psoriatic arthritis were also excluded since DIP involvement is common in psoriatic arthritis and could thus interfere with our readings.

Reference populations

Prevalence of radiological DIP OA and distribution of IL-10 promoter polymorphisms in the study population were compared to 2 reference populations representative of the general Dutch population. As a reference for radiological DIP OA, data were used from the Zoetermeer population, a population survey consisting of 3109 men and 3476 women (18) as described (5). A comparison between the frequency of radiological DIP OA in the present population in reference to the Zoetermeer population gave an observed-to-expected ratio of 0.96 (0.7–1.2). Distribution of IL-10 promoter polymorphisms was compared to its distribution in a random panel of the southwest region of the Netherlands (n = 321)(19).

Radiographs and radiographic scoring

Plain dorsovolar hand radiographs were taken routinely in each patient during the period of the first visit to the EAC. Only radiographs obtained at a maximum of 3 months before till 3 months after the first visit were included. Each of the radiographs was independently graded using the Kellgren-Lawrence scale for DIP OA by 2 out of 3 observers blinded to the underlying EAC diagnosis. This overall score distinguishes 5 degrees of severity of OA according to the presence of radiological features: osteophytes, joint space narrowing (JSN), subchondral sclerosis, cysts, and deformity. A patient was diagnosed with DIP OA if a Kellgren score of 2 or more was observed in at least one DIP joint. The inter-rater agreement for the presence or absence of DIP OA was 0.7 (Cohen's kappa). In case of disagreement radiographs were re-evaluated until consensus was reached.

Determination of IL-10 promoter polymorphisms

Peripheral blood cells were lysed using sodium dodecyl sulfate and treated with proteinase K. DNA was isolated by phenol-chloroform extraction. A Perkin-Elmer thermal cycler Gene Amp 9600 (PE-Cetus, Norwalk, CA, USA) was used to amplify the IL-10 promoter region by polymerase chain reaction. The primer combination and methods used have been described (20).

Statistical analysis

Means were compared using an independent sample Student's t test. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated to determine whether the distribution of IL-10 promoter polymorphisms was comparable to the random panel by comparing the distribution of the minor allele grouped with the heterozygote [11 + 12] versus the major allele [22] in both sets of populations. OR were also used to assess the association between DIP OA and the different genotypes of IL10 promoter polymorphisms. Multiple locus haplotype frequencies (SNPHAP: a program for estimating frequencies of haplotypes of large numbers of diallelic markers from unphased genotype data from unrelated subjects (21) and the measures of pairwise linkage disequilibrium (LD) were determined using the HAPLO program (22). It uses an expectation-maximization algorithm to calculate maximum likelihood estimates of haplotype frequencies, given genotype measurements. The estimator of LD (where $D = h_{pq} - pq$) indicates the difference in the observed (h_{pq}) and expected (pq) frequencies of haplotypes. Its maximum value depends on the allele frequencies and whether the rare alleles are associated together on a haplotype (positive value of D) or whether the common allele is associated with the rare allele (negative value of D). |D| (Lewontins D') is the fraction of D of its maximum ($D_{max} = p - pq$) or minimum ($D_{min} = -pq$) possible value. Power analysis was based on achieving 5% significance in order to detect a difference if IL-10 promoter polymorphisms or IL-10 haplotypes were associated with an almost 3-fold increased risk of DIP OA (OR = 2.7). This analysis was based on the frequency of the IL-10 A carriage rate in the SNP –2849 associated with differential IL-10 production.

Results

Six hundred and one consecutive patients aged between 40 to 70 years visited the EAC. Sixty-nine patients were excluded because no definitive diagnosis was made within one year, 281 patients were excluded based on diagnosis of RA, systemic diseases, spondyloarthropathies, and psoriatic arthritis and 79 patients were excluded due to missing data. Of the remaining 172 patients included in the study, 3 were not genotyped for the –3575, –2763, –1082, and –819 SNP due to poor DNA quality and an additional 18 samples were missing at the time of genotyping the –3575 SNP.

Patient characteristics

Because the patients in this study were included in the EAC, they had a broad variety of diagnoses independent of the presence or absence of DIP OA. Fifty-seven (33%) patients had DIP OA. As expected, the average age of patients with DIP OA was higher than patients with no DIP OA [58 yrs (range 43–70) vs 51 yrs (40–70); mean difference: 6.8 (4.1–9.1)]. The clinical characteristics of the study population are summarized in Table 1.

Distribution of IL-10 promoter polymorphisms in comparison to controls

In Table 2, the distribution of the minor allele grouped with the heterozygote is shown versus the major allele of the IL-10 promoter polymorphisms –3575, –2849, –2763, –1082, and –819 in the study population and in the random panel. No difference was observed in the distribution of these alleles in the 2 groups. The IL-10 promoter polymorphisms –1330 and –592 are in complete linkage disequilibrium with –1082 and –819 respectively; therefore, these variables were excluded from further analyses of single promoter polymorphisms. Genotypes did not show deviations from the Hardy-Weinberg equilibrium (data not shown) (23).

Association of IL-10 promoter polymorphisms and haplotypes with DIP OA

The association between DIP OA and genotypes of IL-10 SNP –3575, –2849, –2763, –1082, and –819 is shown in Table 3. No association was found between these polymorphisms and DIP OA. Since the interaction of 2 or more SNP in haplotypes may be more informative than single polymorphisms, a haplotype analysis was performed. No difference was observed in the distribution of the 4 extended haplotypes, IL-10.1, IL10.2, IL-10.3, and IL-10.4 in patients with and without DIP OA ($p = 0.67$). Data are presented in Table 4. In a separate analysis of the distal haplotype frequency (A–3575T, A–2849G, and A–2763C) and the proximal haplotype frequency (A-1330G, G-1082 A, T-819 C, and A-592 C) no difference was seen in the distribution in patients with and without DIP OA (data not shown).

Table 1. Clinical characteristics of the study population: patients with and without distal interphalangeal (DIP) osteoarthritis (OA).

	DIP OA (n = 57)	No DIP OA (n = 115)
Mean age (range)	58 (43–70)	51 (40–70)
Women, n (%)	33 (58)	55 (47)
EAC diagnoses, n		
Septic arthritis	1	2
Reactive arthritis	3	7
Crystal arthropathy	8	18
Post-traumatic	1	4
Osteoarthritis	17	10
Unclassified arthritis	23	51
Malignancy related arthritis	1	5
Sarcoidosis	1	5
Other	2	11
Unknown	0	2

Table 2. Distribution of the minor allele grouped with the heterozygote (11 + 12) versus the major allele (22) in single nucleotide promoter polymorphisms (SNP) of the interleukin (IL)-10 gene in the study population compared to a random panel in the south-west region of the Netherlands expressed as odds ratios (OR) with 95% confidence intervals (95% CI).

SNP	11 + 12	22	OR (95% CI)
IL-10 A-3575T			
Study population	0.71	0.29	
Random panel	0.64	0.36	1.4 (0.7–2.7)
IL-10 A-2849G			
Study population	0.55	0.45	
Random panel	0.43	0.57	1.6 (0.9–3.0)
IL-10 A-2763C			
Study population	0.65	0.35	
Random panel	0.58	0.42	1.3 (0.7–2.5)
IL-10 G-1082A			
Study population	0.75	0.25	
Random panel	0.76	0.24	1.0 (0.5–1.9)
IL-10 T-819C			
Study population	0.40	0.60	
Random panel	0.40	0.60	1.0 (0.6–1.8)

Table 3. Summary of the association between DIP OA (n = 57) and the genotypes of the IL-10 promoter polymorphisms within the study population (n = 172) expressed as OR (95% CI).

SNP	Genotype distribution			11 versus 12 + 22	11 + 12 versus 22
	11	12	22	OR (95% CI)	OR (95% CI)
IL-10 A-3575T*					
DIP OA	9	27	14		
No DIP OA	13	57	30	1.5 (0.5–4.1)	1.1 (0.5–2.5)
IL-10 A-2849G					
DIP OA	4	30	23		
No DIP OA	8	52	55	1.0 (0.2–3.9)	1.4 (0.7–2.7)
IL-10 A-2763C*					
DIP OA	7	33	16		
No DIP OA	12	58	43	1.3 (0.4–3.8)	1.5 (0.7–3.3)
IL-10 G-1082A*					
DIP OA	16	26	15		
No DIP OA	22	62	28	1.6 (0.7–3.6)	0.93 (0.4–2.1)
IL-10 T-819C*					
DIP OA	3	18	36		
No DIP OA	6	41	65	0.98 (0.2–4.7)	0.81 (0.4–1.6)

Table 4. Haplotype frequencies in patients with DIP OA (n = 57) and patients without DIP OA (n = 116).

IL10 HAP	-3575	-2849	-2763	-1330	-1082	-819	-592	DIP OA	No DIP OA
IL10.1	A	A	A	A	G	C	C	0.30	0.23
IL10.2	T	G	C	G	A	C	C	0.28	0.27
IL10.3	A	G	A	A	G	C	C	0.19	0.23
IL10.4	T	G	C	G	A	T	A	0.07	0.09

Discussion

We are the first to investigate the relationship between a highly genetic form of OA, namely DIP OA and promoter polymorphisms located downstream of the IL-10 transcription start site: –2849, –2763, –1330, –1082, –819, and –592 constituting the 4 ancient IL-10 haplotypes. These SNP as well as the 4 haplotypes were not associated with a higher risk of radiological DIP OA. Association of DIP OA with IL-10 promoter polymorphisms in our investigation was studied in patients with a variety of underlying forms of arthritis. The consecutive patient population was collected in a prospective manner. Because the study population consisted of patients included in an EAC, the existing correlation between certain rheumatic diseases and the genetic variables under study was taken into consideration while selecting patients. All patients with diseases reported in the literature to be associated with the polymorphisms under study were excluded. Furthermore, patients with diseases that can lead to radiological damage of the DIP joints, such as psoriatic arthritis, were also excluded. However, radiological damage would not have been very likely since patients are seen in the EAC at a very early stage. Although patient selection in the EAC was outside our research question, we did find a patient population with a well-documented phenotype, i.e., radiological DIP OA with IL-10 and DIP OA distributions comparable to the general population.

Among the IL-10 promoter polymorphisms under study, the SNP –2849A has been associated with low IL-10 production. We found an OR of one when comparing the –2849 AA versus –2849AG and –2849GG. These data strongly suggest that the association is absent; although a type II error may have occurred given the power of the current study. We conclude that it is unlikely that the SNP –2849A has a large effect on genetic susceptibility to radiological DIP OA.

Although the antiinflammatory role of IL-10 is recognized in arthritis, its role in OA is still undefined. Based on its biologic activity it is, however, conceivable that low IL10 production may contribute to a catabolic state in OA. IL10 is an important immunoregulatory cytokine in man (10) and plays a crucial role in inflammation and tissue destruction. In an arthritis model, mice lacking the gene for IL-10 experienced higher rates of clinical signs and more severe knee and paw injury compared to IL-10 wild-type controls. Furthermore, plasma levels of TNF-a, IL-1ß, and IL-6 were also enhanced in knockout compared to wild-type mice (24).

IL-10 has been reported in vitro to be synthesized in increased amounts either spontaneously by synovial membrane and cartilage (25) or after stimulation of chondrocytes with IL-1ß or TNF-α (8). IL-10 contributes to cartilage homeostasis through several pathways. In experiments on joint tissue, it has been shown that a lack of IL-10 can lead to joint destruction as a result of an increased expression of metalloproteinases (26). Upregulation of IL-1 receptor antagonist production by isolated monocytes has been found for IL-10 by human monocytes and neutrophils (27). Besides exerting antiinflammatory activity, IL-10 has been shown to directly stimulate proteoglycan synthesis by human chondrocytes in vitro (28).

In an earlier study (16), we investigated whether genetic variation in cytokine production contributes to OA susceptibility. The ex vivo production of cytokines in whole blood assays was used to classify subjects as “high” or “low” producers based on the distribution in controls. We observed that a low innate production of IL-10 using LPS production was associated with an increased risk of familial OA at multiple sites (defined as multiple sites in the hands or at 2 or more joint sites including the hands, spine, knees, and hips). The low innate IL-10 production in these patients is assumed to be partly caused by genetic variation at the IL10 locus (13,15), thus implicating that genetic variation of ex-vivo cytokine production upon LPS stimulation contributes to OA susceptibility. Therefore, we investigated whether IL10 SNP that constitute the 4 ancient IL-10 haplotypes, some associated with IL-10 production, are a risk factor for a predominant subtype of OA specifically in the DIP joints. Our results indicate that this is not the case. The discrepancy between findings in our earlier study and the present study may be due to different OA phenotypes: familial OA at multiple joint sites versus radiographically defined OA in DIP joints. Alternatively, not all of the genetic variation in IL-10 production is dictated by IL-10 haplotypes (13). It may be that genetic factors that dictate inter-individual IL-10 production differences that are not located in the IL-10 locus are those relevant for DIP OA.

In summary our current work suggests that the currently known IL-10 SNP do not exhibit a major effect on genetic susceptibility to DIP OA.

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