IL1β+3953 exon 5 and IL-2 -330 promoter polymorphisms in patients with rheumatoid arthritis

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Abstract

Objective

Rheumatoid arthritis (RA) is chronic inflammatory disease in which cytokines play an important role. The aim of present study was to evaluate the exon 5 +3953 IL-1β and IL-2 -330 promoter polymorphisms in patients with RA in association with disease activity and severity.

Methods

In the study 93 patients with rheumatoid arthritis diagnosed according to the criteria of American College of Rheumatology were included. Polymerase chain reaction amplification was used for analysis of the polymorphisms studied.

Results

The distribution of IL-1β and IL-2 genotypes in RA patients did not differ from control subjects. Nevertheless in patients with A2 allele of IL-1β and GG genotype of IL-2, the active form of RA was more frequently diagnosed. Moreover in these patients the measurements of disease activity (DAS 28 score, ESR, number of swollen and tender joints) were significantly increased.

Conclusion

We suggest that exon 5 +3953 IL1β and IL-2 -330 promoter polymorphisms may be a genetic risk factor for RA severity.

Key words
Rheumatoid arthritis, IL-1β, IL-2, polymorphism.

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Introduction

Rheumatoid arthritis (RA) is a multifactorial disease where numerous genetic factors contribute to susceptibility and severity. Cytokines are important mediators of the immune and inflammatory response and play an important role in the pathophysiology of joint inflammation and destruction in RA (1). IL-1 is an important cytokine because it possesses several biological properties resulting in the increased expression of proinflammatory genes. The most relevant property is the ability of IL-1 to initiate and sustain the expression of cyclooxygenase type 2 (COX-2) and inducible nitric oxide synthase. This accounts for the large amount of prostaglandin-E_2 (PGE_2) and nitric oxide (NO) produced by cells exposed to IL-1. Another important proinflammatory property of IL-1 is its ability to increase the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on endothelial and other cell surfaces. This property promotes the infiltration of inflammatory and immunocompetent cells into the extravascular space. Moreover IL-1 increases the production of IL-2 and INF-γ by T lymphocytes, IL-6 and TNF-α by macrophages and INF-β and IL-6 by fibroblasts (2).

IL-2 is the pleiotropic cytokine which acts as a growth and differentiation factor for natural killer (NK) cells, some B lymphocytes, and lymphokine-activated killer (LAK) cells. In addition, IL-2 activates monocytes to produce cytokines, particularly TNF-α (3).

Recently of great interest are cytokine gene variants that have been associated with RA susceptibility and severity. It has been shown, that some allotypes were associated with increased cytokine production contributing to more severe RA. Nevertheless the reports are often controversial. Recent reports suggest that the +3953 exon 5 IL-1β and -330 IL-2 promoter polymorphisms may influence these cytokines expression. The A2 allele of IL-1β and homozygous IL-2 GG allotype are associated with increased cytokines production (4, 5). The aim of the study was to examine the +3953 exon 5 IL-1β and -330 IL-2 promoter polymorphisms in patients with rheumatoid arthritis in relation to disease susceptibility and severity.

Materials and methods

Patients

We examined 93 patients (59 women, 34 men, age 21-74 years, mean 51.9, disease duration 4.2 ± 1.9 years) with rheumatoid arthritis diagnosed according to the criteria of American College of Rheumatology. Patients were recruited from the outpatient and inpatient population of the Department of Rheumatology, Hospital in Szczecin, Poland. All subjects were Caucasian from the Pomeranian region of Poland. Subjects enrolled in the study underwent routine biochemical blood analysis and when required, assays for anticardiolipin antibodies, antinuclear antibodies and immunologic complexes. X-rays of the chest, hands and feet (erosive or non-erosive RA) were obtained in all patients, and when required, radiografts of other joints. These were interpreted by two different expert radiologists. The evaluation of subjects included physical examination with particular focus on the pattern of joint involvement, the presence of nodules and other extra-articular features (such as vasculitis, anemia, sicca syndrome, amyloidosis, organ involvement), and laboratory features such as rheumatoid factor (RF). Amyloidosis was diagnosed by histomorphology (skin- and bowel- or duodenum biopsy), vasculitis by histomorphology (skin biopsy) and angiogram. The extra-articular manifestations in 35 patients were diagnosed, whereas joint erosions in 61 cases. The subcutaneous nodules without other extra-articular manifestations in 12 patients were diagnosed. The group with other extra-articular manifestations included patients: 2 with hematologic abnormalities, 4 with hematologic abnormalities and nodules, 3 with vasculitis and nodules, 5 with vasculitis, 4 with sicca syndrome, 2 with sicca syndrome, amyloidosis and nodules, and 3 with amyloidosis.

Disease activity was determined on the basis of defined parameters and a global physician's assessment. The number of swollen and tender joints, duration

of morning stiffness, ESR, CRP and DAS 28 were measured several times over a one-year period and the mean values were used (6). The patients were divided into two subgroups – with active and non-active disease. The group with non-active disease (24 subjects) included patients in remission for at least 6 months after therapy with methotrexate and glucocorticosteroids. Stable remission was defined as less than 3 swollen joints, morning stiffness < 30 min, ESR < 25 mm/hr. The second group (69 subjects) included patients with active disease despite at least 6 months of therapy with methotrexate and glucocorticosteroids (above 3 swollen joints, morning stiffness > 30 min, ESR > 25 mm/hr) (7).

The control group was selected randomly from the population of Pomeranian region of Poland and consisted of 102 healthy subjects (61 women and 41 men, age 19-72 years, mean 49.5) without history of diseases with immunologic background. The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Methods

Genomic DNA was extracted from 450 μl of whole blood samples using a non-organic and non-enzymatic extraction method (8). Genotyping of each subject for the presence of polymorphisms in studied cytokine genes was performed using previously described PCR-based assays (9, 10) with minor modifications.

We have selected a PCR/RFLP method for detection of T3953C polymorphism in exon 5 of IL-1β. Amplification reaction was performed at total volume of 20 μl, using Eppendorf Mastercycler (Hamburg, Germany). Amplification mix contained 0.4 ρmol/μl of each primer, 150-200 ng of genomic DNA, 1.0 U of RedTaq Polymerase (Sigma) in 1x enzyme-specific buffer (containing magnesium chloride at final concentration of 1.5 μMol) and deoxynucleoside triphosphates (Sigma), 200 μMol of each. PCR reaction was performed with a forward primer IL1B-1 (5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3') and a reverse primer IL1B-2 (5'-GCT TTT TTG CTG TGA GTC CCG-3'). Reaction conditions were as follows: 95˚C for 3 minutes, 7 cycles at 60˚C/72˚C/94˚C (each step for 45 seconds), 28 cycles at 58˚C/72˚C/94˚C (each step for 45 seconds) and finally 72˚C of 5 minutes. PCR product (194 bp) was subsequently digested with *Taq I* restriction endonuclease (New England Biolabs, USA), yielding 3 DNA fragments (97, 85, 12 bp) for 3953T allele or 2 fragments (182 and 12 bp) for 3953C allele. Restrictions fragments were identified in UV light after electrophoresis in 3% agarose gel dyed with ethidium bromide.

An allele-specific PCR method, based on a set of four primers, was used for detection of IL-2 -330 T/G promoter polymorphism. Primer sequences were as follows: G1 5'-TGA AAC AGG AAA CCA ATA CAC T-3'), G2 5'-AAC TCA GAA AAT TTT CTT AGT CC-3', T1 5'-CAA GAC TTA GTG CAA TGC AAG-3' and T2 5'-TCA CAT GTT CAG TGT AGT ATT AT-3'. The composition of amplification mix was the same as for genotyping of IL-1β polymorphism, but the mix contained primers G1, G2 and G3 at concentration of 0.5 ρmol/μl of each and primer G2 at concentration of 1.0 ρmol/μl. Reaction conditions were as follows: 94˚C for 5 minutes, 10 cycles at 63˚C/72˚C/94˚C (each step for 1 minute), 25 cycles at 60˚C/72˚C/94˚C (each step for 1 minute) and finally 72˚C for 5 minutes. In the presence of guanine at the polymorphic site, primers G1 and G2 produced a 143-bp product, whereas in the presence of thymine, primers T1 and T2 produced a product of 447 bp. PCR products were identified in UV light after electrophoresis in 2% agarose gel dyed with ethidium bromide. Method was validated by the amplification of previously genotyped DNA samples kindly provided by Dr. D. Turner from Department of Histocampatibility & Immunogenetics NBS-London.

Statistical analysis

The distribution of genotypes in RA patients was compared with healthy subjects and statistically evaluated using the chi-square test with Yate's correction for small groups (Epi Info 6 program, version 6.2, World Health Organization, Geneva, Switzerland). The disease activity measurements were statistically evaluated using Mann-Whitney U test.

Results

The distribution of IL-1β and IL-2 genotypes and alleles in patients with RA did not differ significantly from that in healthy control (Table I).

As shown in Table III there was also no significant difference in age at disease onset in relation to IL-1β and IL-2 genotypes.

The active form of RA was diagnosed in 66.0% of patients with IL-1β A1A1 genotype, in 83.3% with A1A2 and in 100% homozygous A2A2 subjects.

The probability of active RA diagnosis

Table I. The distribution of IL-1β and IL-2 genotypes in RA patients and control group.

Genotype	RA patients (n=93)		Control population (n=102)		p
	n	%	n	%	
IL-1b exon 5					
A1A1	53	57.0	55	53.9	NS
A1A2	36	38.7	37	36.3	NS
A2A2	4	4.3	10	9.8	NS
A1 allele	142	76.0	147	72.0	NS
A2 allele	44	24.0	57	28.0	NS
IL-2 promoter					
TT	40	43.0	43	42.2	NS
GT	39	41.9	47	46.1	NS
GG	14	15.1	12	11.1	NS
T allele	119	64.0	133	65.0	NS
G allele	67	36.0	71	35.0	NS

was 2.9 fold greater in carriers of A2 allele than in homozygous A1A1 subjects (OR 2.91, 95% CI 0.94-9.41, p< 0.04). With regard to IL-2 genotypes the active form of RA was diagnosed in 72.5% of patients with TT genotype, in 69.2% with TG and 92.9% with GG. The probability of active RA diagnosis was 5.3 fold greater in homozygous GG patients as compared with carriers of T allele (OR 5.34, 95% CI 0.66 – 43.21, p<0.09).

Erosive RA was diagnosed in 60.3% of IL-1β A1A1 homozygous patients, in 72.2% of patients with A1A2 genotype and 75% with A2A2. The probability of erosive RA diagnosis was 1.73 fold greater in carriers of A2 allele than in A1A1 homozygous subjects but did not reach statistical significance (OR 1.73, 95% CI 0.65 –4.62, p<0.3) (Table II). With regard to IL-2 polymorphism the erosive RA was diagnosed in 62.5% of patients with TT genotype, in 66.7% with GT and 71.4% with GG. The probability of erosive RA diagnosis was 1.37-fold greater in patients with GG genotype as compared with carriers of T allele but was statistically non significant (OR 1.37, 95% CI 0.35–5.58, p< 0.9).

As shown in Table II, the diagnosis of extra-articular manifestations did not differ significantly in relation to genotypes studied.

Rheumatoid factor was diagnosed in 71.7% of subjects with IL-1β A1A1 genotype in 66.7% with A1A2 and 50% with A2A2. With regard to IL-2 genotypes seropositive RA was diagnosed in 67.5% of homozygous TT subjects, 71.8% GT and 64.3% TT. These differences were statistically non significant (Table II).

The parameters of disease activity were significantly increased in carriers of IL-1β A2 allele. The values of DAS 28 score were in A1A1, A1A2 and A2A2 patient as follows: 5.11 ± 1.84, 6.25 ± 1.65, 6.38 ± 1.57 respectively. As shown in Table III, the numbers of swollen and tender joints as well as ESR were significantly increased in patients with A2A2 and A1A2 genotypes in comparison to homozygous A1A1 subjects. When the disease activity parameters with regard to IL-2 genotypes were analyzed, these were significantly increased in homozygous GG patients as compared with carriers of T allele. The DAS 28 score was significantly increased in patients with GG genotype (6.52 ± 1.71) as compared with TT and GT subjects (5.30 ± 1.92, 5.47 ± 1.95 respectively) (Table III). The number of swollen and tender joints, morning stiffness duration and ESR were significantly increased in patients with GG genotype (Table III).

Discussion

In the present study we examined the relation between IL-1β and IL2 promoter polymorphisms and disease susceptibility, severity and activity in patients with RA. We have not found the differences in distribution of these genotypes between control group and RA patients. It might suggest, that IL-1β and IL2 promoter polymorphisms are not the factors predisposing for RA

Table II. The diagnosis of active, erosive, seropositive RA and extra-articular manifestations in relation to IL-1β and IL-2 genotypes.

	A1A1 (n = 53)		A1A2 (n = 36)		A1A2 (n = 4)		p*	TT (n = 40)		GT (n = 39)		GG (n = 14)		p**
Patients	no.	%	no.	%	no.	%		no.	%	no.	%	no.	%	
Active RA	35	66.0	30	83.3	4	100.0	<0.04	29	72.5	27	69.2	13	92.9	<0.09
Erosive RA	32	60.3	26	72.2	3	75.0	NS	25	62.5	26	66.7	10	71.4	NS
Extra-articular manifestations	20	37.7	13	36.1	2	50.0	NS	15	37.5	14	35.9	6	42.9	NS
Seropositive RA	38	71.7	24	66.7	2	50.0	NS	27	67.5	28	71.8	9	64.3	NS

*p: A1A1 vs A1A2 + A2A2 genotypes; **p: GG vs TT +GT genotypes.

Table III. The disease activity parameters in relation to IL-1β and IL-2 genotypes.

	IL-1β				IL-2			
Parameter (mean value ± SD)	A1A1 (n = 53)	A1A2 (n = 36)	A1A2 (n = 4)	p*	TT (n = 40)	GT (n = 39)	GG (n = 14)	p**
Age at disease onset (years)	42.7 ± 14.1	41.2 ± 12.3	43.8 ± 10.4	NS	41.3 ± 13.4	43.2 ± 10.9	41.8 ± 12.4	NS
DAS 28	5.11 ± 1.84	6.25 ± 1.65	6.38 ± 1.57	<0.05	5.30± 1.92	5.47 ± 1.95	6.52± 1.71	<0.05
ESR (mm/hr)	53.1 ± 29.7	70.2 ± 31.4	74.9 ± 18.4	<0.05	51.8 ± 29.5	59.8 ± 23.9	88.7 ± 20.4	<0.05
CRP (mg/l)	67.9 ± 35.9	69.2 ± 38.4	82.7 ± 27.3	NS	64.2 ± 35.8	71.4 ± 29.4	75.9 ± 30.4	NS
Number of swollen joints	6.2 ± 1.7	7.5 ± 2.2	7.6 ± 1.8	<0.05	6.3 ± 2.1	6.5 ± 2.4	9.2 ± 1.8	<0.05
Number of tender joints	7.4 ± 4.2	8.7 ± 3.7	9.3 ± 1.9	<0.05	7.5 ± 4.1	7.3 ± 3.5	11.4 ± 3.1	<0.05
Morning stiffness duration (hr)	1.3 ± 0.35	1.6 ± 0.38	1.7 ± 0.21	NS	1.2 ± 0.22	1.5 ± 0.25	2.0 ± 0.38	<0.05

*p: A1A1 vs A1A2 + A2A2 genotypes; **p: GG vs TT + GT genotypes.

development. This is not completely in accordance with the previous reports; however the differences in cytokine gene distribution in various populations may be of ethnic origin (11, 12). The genotypes encoding high cytokines expression were associated with increased parameters of disease activity, whereas the correlation with disease severity (erosions, RF positivity) did not reach statistical significance.

All patients included in the study were treated with low doses of methotrexate and prednisone. The antiinflammatory properties of these drugs are associated with decrease in cytokine production, such as IL-1β , IL-2, IL-6 (13, 14). One potential explanation for the effects of MTX treatment on cellular and humoral immunity and cytokine secretion is that MTX, via its effects on the purine and pyrimidine synthesis required for cell division, is cytotoxic for the cells that generate cytokines or incite other cells to generate cytokines (15, 16). Another mechanism by which MTX may diminish cytokine expression is associated with the release of adenosine (17). The antiinflammatory effects of adenosine result from the interaction of adenosine with specific receptors on the cell surface. Adenosine inhibits lymphocyte proliferation and induces suppressor phenotype and function. Moreover, adenosine acting at its receptors on macrophages and monocytes inhibits the production of cytokines (18). It is possible that in subjects with genetically determined high cytokine production rate, cytokine levels remain increased despite treatment. Therefore, the studied alleles may be associated with response to methotrexate and prednisone therapy rather than with disease severity.

Several studies have examined the possible association of IL-1β and IL2 polymorphisms with RA. Nevertheless the results are inconsistent. Cantagrel *et al*. suggested a role for the IL1β +3953 A2 allele in the prediction of erosive disease, but did not find the association of this allele with occurrence of sustained remission (19). Buchs *et al*. studied the IL-1β polymorphism as a factor influencing the RA severity. Carriage of the IL-1β A2 allele was increased in destructive RA. Patients carrying this allele had a more severe disease than homozygous A1A1 subjects (increased Larsen wrist radiological index, Steinbrocker functional index, Ritchie articular index and ESR) (20). In study of Cvetkovic *et al*. patients with A2A2 genotype had higher accumulated disease score than patients with A1A1 and A1A2 (21). These authors did not find the association between IL1β polymorphism and age of disease onset and cardiovascular complications. Kaijzel *et al*. demonstrated that extent of joint destruction was higher in heterozygous A1A2 patients compared to homozygous A1A1 and A2A2 subjects, although differences did not reach statistical significance (22). Genevay *et al*. studied the association of IL1β polymorphism with Larsen score progression in RA (23). These authors did not shown significant correlation between IL1β genotypes and progression of joint destruction. Moreover Huang *et al*. did not demonstrate the IL1-β polymorphism as a genetic marker of RA susceptibility or severity (24).

Whereas Fedetz *et al*. studied the -384 and +114 IL-2 gene polymorphisms in RA patients. These authors did not detect the association of IL-2 genotypes with RA susceptibility and activity (25).

Numerous studies have implicated IL-1β and IL-2 in the pathogenesis of rheumatoid arthritis. Various animal models of inflammatory joint disease have also implicated IL-1β and IL-2 as a key factor in the disease. Overexpression of IL-lβ in the knee joints of rabbits has been observed to result in an arthritis analogous to that observed in chronic rheumatoid arthritis in humans, characterized by leukocytic infiltration, synovial hyperplasia, invasive pannus and erosion of the articular cartilage, and periarticular bone formation (26).

The strongest case for a pivotal role for IL-1β in rheumatoid arthritis is that the administration of anti-IL-lβ or IL-IRa, both highly specific in blocking IL-1β, has reduced the intensity and destructive nature of the disease in animals and humans (27). Administration of IL-1Ra to rats with adjuvant arthritis has reduced bone erosions and joint destruction (28).

Several studies in humans support a role for IL-2 in the etiology of the disease. Although the amount of IL-2 present in the synovial fluid of patients with rheumatoid arthritis is low compared to that of other cytokines, IL-2 is present in nearly all rheumatoid arthritis fluids but absent in fluids from patients with osteoarthritis or reactive arthritis (29). Spontaneous production of IL-2 by peripheral blood mononuclear cells (PBMC) has also been consistently observed in rheumatoid arthritis but not in other forms of arthritis. IL-2-expressing CD4+ T cells are found in the perivascular area, whereas IL-2-expressing CD8+ T cells are present in the synovium. Sera from patients with rheumatoid arthritis have elevated levels of IL-2 and soluble IL-2Ra, but this is rarely observed in non-rheumatoid arthritides (30).

Previous studies have examined the possible genetic linkage of IL-2 production to rheumatoid arthritis. Two hundred rheumatoid arthritis-affected sibling-pair families were investigated for IL-2 microsatellite markers. A significant association for IL-2 in sibling pairs was found (31).

The results of present study suggest also that subjects with genetically determined high IL-lβ and IL-2 expression may have more active disease course and poor response to therapy with methotrexate and glucocorticosteroids.

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