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TNF-α gene polymorphism does not affect the clinical and radiological outcome of rheumatoid arthritis

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Abstract The present study was undertaken in order to investigate the relationship between tumor necrosis factor-α (TNF-α) gene polymorphism and the radiological progression of rheumatoid arthritis (RA) within the first 3-years of the disease.
Sixty-eight RA patients (59 women and nine men) were observed for 3-years. TNF-α polymorphism analysis was performed in all patients. Radiographs of the hands were taken at the onset of study and after 3-years of follow-up. Radiographs were assessed according to the Larsen index (damage score and progression of damage score). We did not observe any correlation between TNF gene polymorphism and damage score or progression of damage score. The obtained data suggests that TNF-308 polymorphism cannot serve as an indicator of the disease course in RA patients.

Key words Rheumatoid arthritis · Radiological progression · TNF-α gene polymorphism

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the synovium. Like other autoimmune disease, RA is thought to be caused by an interaction between environmental and genetic factors. Genetic factors not only play a role in susceptibility but probably also affect the outcome of the disease [1]. An association with genes located within the major histocompatibility complex (MHC) region has been much investigated since the demonstration of an increased prevalence of human leukocyte antigen (HLA)-DR4 in RA patients [2]. Besides the association with class II genes (DRBI*0401, DRBI*0404, DRBI*0405, DRBI*0408), the association with some class III genes, such as complement gene C4b (30 kb), has been described.

The gene encoding the proinflammatory cytokine TNF-α lies within 7 kb stretch of DNA in the class III region of the MHC [3]. The TNF-α locus lies approximately 1 Mb telomeric of the HLA-DR locus and 300 kb (250 kb) centromeric of the HLA-B locus [4]. Recently, polymorphism in the TNF region was reported [5].

One of the most important features of RA is cytokine network dysregulation. TNF-α expresses a broad range of proinflammatory, catabolic, and immunostimulatory activities [6]. It has been suggested that chronic inflammation with tissue destruction may be the result of a strong local expression of TNF-α or TNF-β [7].

An important practical issue is whether a genetic factor, such as TNF-α gene polymorphism may predict severity of the disease in terms of radiological deterioration. Therefore, in the present study we investigated the relationship between TNF-α, polymorphism, and the radiological progression of RA within the first 3 years of the disease.

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Materials and Methods

Patients

Sixty-eight RA patients (59 women and nine men) were observed for 3 years. All patients met the revised criteria of the American College of Rheumatology for the diagnosis of rheumatoid arthritis [8]. Their mean age was 48.4-years (standard deviation, SD = 11.9), with a range of 27–70 years. The control group consisted of 28 healthy subjects (14 women, 14 men) with an age range of 22–52-years (mean 38 ± 8-years).

Clinical and laboratory assessments

Clinical examination was performed twice: at the most onset of the study and after 3-years of observation. A modified Mallya–Mace index [9] was used to evaluate disease activity. This index includes erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) serum level, grip strength, duration of morning stiffness, hemoglobin level, and Ritchie joint index. Serum samples were taken when clinical examination was performed and stored at −20 °C. Enzyme-linked immuno-sorbent assay (ELISA) techniques (Cambridge Life Science, England) were developed to measure rheumatoid factors (RF) of IgM, IgG, and IgA isotypes. The plates were read with a spectrophotometer at 492 nm. RF titeres were expressed as U/ml. Sera, in which RF exceeded 15 U/ml for IgM RF, 50 U/ml for IgG RF, or 20 U/ml for IgA, were considered as positive.

Radiographic assessments

Hand radiographs were obtained at the baseline visit and after 3-years. Twenty joints were evaluated: ten metacarpophalangeal, eight proximal interphalangeal, and two interphalangeal joints of the thumbs. Each joint was graded on a 0–5 point scale (0, normal conditions; 1, slight abnormality; 2, definite abnormality; 3, marked abnormality; 4, severe abnormality; 5, mutilating abnormality) according to Larsen [10]. The individual gradings were summed to form a damage score (DS) with a range from 0 to 100. Radiological progression was referred to as the progression of damage score (PDS) and was estimated by subtracting the scores recorded at entry from those recorded after 3 years of follow-up.

Extraction of DNA from whole blood samples and PCR analysis

DNA was extracted from whole blood samples according to the previously described method [11]. To detect the G to A transition polymorphism at position −308 of the human TNF-α gene, a 107 bp fragment was PCR amplified using the oligonucleotide A1 (5′AGGCAATAGGTTTTGAGGGCCCAT3′), which contained a single base exchange at the 3′ end to form an NcoI recognition site and A2 (5′TCCTCCCTGCTCCGATTCCG3′). A total of 100 ng of genomic DNA was amplified using 0.2 μM concentrations of the primer A1 and A2 in a total volume of 50 μl containing 1.5 units Taq DNA polymerase, 200 μM of each dNTP, and PCR reaction buffer (Perkin Elmer, Rotkreuz. Switzerland) containing 10 mM Tris-HCl, 1.5 mM MgCl, 50 mM KCl and 0.1% Triton X-100. Amplification was performed at 94 °C for 4 min, 60 °C for 1 min, and 72 °C for 1 min. This is followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min. and 72 °C for 1 min, and a final cycle of 94 °C for 1 min, 60 °C for 1 min. and 72 °C for 5 min. After amplification, 10 μl of PCR product was digested with 2 μl NcoI restriction endonuclease and 2 μl buffer D (Promega, Switzerland) at 37 °C for 2 h. Both, the digested and undigested products were analyzed on a 9% polyacrylamide gel.

Statistical analysis

Data were analyzed with the software package SAS (version 6.03) employing z statistics. Paired data were compared with Wilcoxon's signed rank test, non-paired data with Wilcoxon's rank-sum test for two groups. To test for associations between variables within groups, Spearman's rank correlation coefficients was used.

Results

In RA patients, the allele frequency of TNF1 and TNF2 did not differ significantly from the control group (TNF1, 0.82 vs 0.84; TNF2, 0.18 vs 0.16). TNF1/TNF1 homozygosity was seen in 45 patients (66.2%), TNF1/TNF2 heterozygosity was seen in 21 patients (30.9%), whereas TNF2/TNF2 homozygosity was seen only in 2 patients (2.9%).

Disease activity assessed according to the Mallya–Mace index at the beginning of the study as well as after 3-years of disease did not differ in patient with TNF1 and/or TNF2 (Table 1). Similarly, we did not find any relationship between the presence of TNF1 and/or TNF2 alleles and ESR and CRP levels (Table 2). The presence of IgG, IgM, and IgA rheumatoid factors showed no association with TNF1 and/or TNF2 (Tables 3, 4). Finally, we did not observe any association between TNF1 and/or TNF2 alleles and damage

Table 1 Changes in disease activity according to Mallya–Mace index (*MMI*) in rheumatoid arthritis patients with TNF1 and/or TNF2 alleles. MMI (3–0), difference between MMI after 3-years and at the onset of disease. Difference between TNF1 and TNF1/TNF2 groups were not statistically significant. The TNF2 group was not statistically analyzed due to a low number of cases

	TNF1 $n = 45$	TNF1/TNF2 $n = 21$	TNF2 $n = 2$
MMI at the onset	2.4 ± 0.5	2.5 ± 0.3	2.2 ± 0.2
MMI after 3 years	2.4 ± 0.6	2.4 ± 0.7	2.1 ± 0.6
MMI (3–0)	0.1 ± 0.6	−0.1 ± 0.8	−0.1 ± 0.4

Table 2 Changes in acute phase markers in rheumatoid arthritis patients with TNF1 and/or TNF2 alleles. Erythrocyte sedimentation rate, (*ESR*) (3–0), the difference between ESR after 3-years and at the onset of disease; C-reactive protein, (*CRP*) (3–0), the difference between CRP after 3-years and at the onset of disease. Differences between TNF1 and TNF1/TNF2 groups were not statistically significant. The TNF2 group was not statistically analyzed due to a low number of cases. (*NA* not analyzed)

	TNF1 $n = 45$	TNF1/TNF2 $n = 21$	TNF2 $n = 2$
ESR at the onset (mm/h)	55 ± 26	59 ± 29	36 ± 20
ESR after 3-years (mm/h)	36 ± 26	45 ± 34	16 ± 13
ESR (3–0) (mm/h)	−18 ± 27	−14 ± 40	−20 ± 7
CRP at the onset (mg/l)	27 ± 22	35 ± 21	NA
CRP after 3-years (mg/l)	30 ± 21	32 ± 28	NA
CRP (3–0) (mg/l)	3 ± 24	−3 ± 35	NA

Table 3 Changes in the number of rheumatoid factor positive patients with TNF1 and/or TNF2 alleles. Differences between TNF1 and TNF1/TNF2 groups were not statistically significant. The TNF2 group was not statistically analyzed due to a low number of cases

	TNF1/TNF1 $n = 45$	TNF1/TNF2 $n = 21$	TNF2/TNF2 $n = 2$
IgM at the onset	31 (68.9%)	14 (66.7%)	1
IgM after 3-years	32 (71.1%)	16 (76.2%)	2
IgG at the onset	31 (68.9%)	14 (66.7%)	1
IgG after 3-years	32 (71.1%)	13 (61.9%)	1
IgA at the onset	35 (77.8%)	17 (81.0%)	0
IgA after 3-years	31 (68.9%)	15 (71.4%)	2

score. There was also no relation to the progression of damage score (Table 5).

Discussion

The outcome of RA is more severe than previously considered. Mortality among RA patients is higher than in control groups, and the quality of life is significantly decreased. It has already been suggested that the progression of RA is most rapid during the early phase of the disease [12–15]. Therefore, an optimal prognostic monitoring factor is sought.

The role of TNF-α in pathogenesis of rheumatic inflammations as well as in joint exudates has already been reported. Although TNF-α gene polymorphism is situated in the promoter region, it has been demonstrated that it many affect TNF-α production [16, 17]. There are many examples of single base changes in the promoter regions of genes having major effects on the gene transcription rate [18]. The TNF2 allele is strongly associated with HLA-A1, -B8, and -DR3 [19]. The association is even stronger when the three alleles are analyzed together [20]: it seems to be of great value in lupus erythematosus, but in rheumatoid arthritis it still remains under discussion.

The findings in the present study show a lack of association between TNF-α polymorphism and susceptibility to RA [21, 22]. Other studies found that there was a slight increase in the rare TNF2 allele among RA patients [23, 24], but the association did not reach statistical significance. The polymorphism was not associated with disease activity as defined by the Mallya–Mace index. This observation is in accordance with studies reported by Wilson et al. [21] and Brinkman et al. [22], but Vinasco [24] noted a slight association of the TNF alleles with more severe disease. The present study demonstrated that the polymorphism does not correlate with radiological progression of the disease. This is in agreement with Brinkman et al. [22] who additionally found that the −238GA genotype was associated with a lower number of hand affected by erosions within the first years of disease than the −238GG genotype. The obtained data may suggest that TNF −308 polymorphism cannot serve as an indicator of the disease course in patients with RA.

Table 4 Changes in rheumatoid factors serum concentration in rheumatoid arthritis patients with TNF1 and/or TNF2 alleles. Rheumatoid factor (*RF*) (3–0), the difference between RF after 3-years and at the onset of disease. Difference between TNF1 and TNF1/TNF2 groups were not statistically significant. The TNF2 group was not statistically analyzed due to a low number of cases

	TNF1 $n = 45$	TNF1/TNF2 $n = 21$	TNF2 $n = 2$
RF IgG at the onset (U/ml)	270 ± 652	266 ± 391	61 ± 58
RF IgG after 3 years (U/ml)	167 ± 252	96 ± 95	66 ± 41
RF IgG (3–0) (U/ml)	−102 ± 671	−170 ± 373	5 ± 100
RF IgM at the onset (U/ml)	74 ± 92	113 ± 198	12 ± 12
RF IgM after 3 years (U/ml)	130 ± 203	94 ± 189	55 ± 23
RF IgM (3–0) (U/ml)	57 ± 176	−20 ± 283	43 ± 35
RF IgA at the onset (u/ml)	91 ± 123	127 ± 197	18 ± 1
RF IgA after 3 years (U/ml)	70 ± 121	141 ± 376	21 ± 1
RF IgA (3–0)	−21 ± 135	14 ± 357	3 ± 1

Table 5 Changes in damage score (*DS*) and progression in damage score (*PDS*) in rheumatoid arthritis patients with TNF1 and/or TNF2 alleles. Differences between TNF1 and TNF1/TNF2 groups were not statistically significant. The TNF2 group was not statistically analyzed due to a low number of cases

	TNF1 $n = 45$	TNF1/TNF2 $n = 21$	TNF2 $n = 2$
DS at the onset	4.3 ± 5.2	4.3 ± 4.3	1.5 ± 2.1
DS after 3-years	32 ± 16	31 ± 17	8 ± 7
PDS	27.3 ± 14.9	26.3 ± 16.1	6.5 ± 9.2

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