CONCISE REPORT

Transforming growth factor β1 gene polymorphism in rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA) is a chronic inflammatory disease and synovial cells, antigen presenting cells, lymphocytes, and their cytokines might be associated with the disease. Transforming growth factor β1 (TGFβ1) has been reported to have important roles in unresolved inflammation, immune suppression, fibrosing processes, and angiogenesis. TGFβ1 is highly expressed in joints in RA and is considered to be a regulator of anti-inflammation in RA. Polymorphisms of TGFβ1 have been reported to be associated with the production of TGFβ1 protein, and to increase the risk of acquiring several diseases. It was speculated that these polymorphisms might also be involved in RA, and therefore the TGFβ1 codon 10 T869C polymorphism in a series of patients and controls was investigated.
Method: A total of 155 patients with RA and 110 healthy subjects were studied. DNA was extracted from peripheral leucocytes and TGFβ1 codon 10 T869C polymorphism was determined by polymerase chain reaction restriction fragment polymorphism.
Results: A significantly higher proportion of patients with RA with the T allele (CT type or TT type) was found compared with the CC type (p=0.039).
Conclusion: The T allele, previously reported to be linked with production of TGFβ1, may be associated with an increased risk of RA.

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown cause. Typical lesions in the joints are the excessive proliferation of synovial cells, antigen presenting cells, and infiltrating leucocytes, where both T cell and B cell mediated immune responses induce destructive inflammation. This inflammation is mainly seen in the synovial fluid and surrounding synovial tissue. Abundant synovial cells, immunocytes, and macrophages play a major part in the pathogenesis of RA, with increased production of extracellular matrix molecules.[1–3]

Transforming growth factor β (TGFβ) is a 25 kDa disulphide linked homodimer or heterodimer protein with a broad range of biological functions. In mammals, three isoforms, TGFβ1, TGFβ2, and TGFβ3, exist with nearly identical biological properties. TGFβ1 has been reported to have an important role in many diseases, affecting the regulation of tissue repair, unresolved inflammation and immune suppression, fibrosing processes, and angiogenesis.[4 5] Several investigators have reported that TGFβ1 is produced in the synovial fluid of patients with RA, and is considered to be associated with remission of the disease. Moreover, because overexpression of the TGFβ1 gene reduced arthritis in an animal model, TGFβ1 is considered to be an important regulator for anti-inflammation in RA.[6–8]

Recently, polymorphisms have been described for TGFβ1, and TGFβ1 T→C polymorphism at codon 10 869 bp (T869C) was reported to be associated with several diseases.[4 5] Because TGFβ1 may function as a modulator of RA, we investigated this polymorphism in a series of patients with RA and control subjects.

MATERIALS AND METHODS

Subjects

The 155 patients (118 female and 37 male) with RA studied were all inhabitants of central Japan. The diagnosis of RA was based on the criteria of the American college of Rheumatology. They had a mean (SD) age of 59.6 (12.4) years (table 1). To evaluate the correlation between clinical characteristics and polymorphism, age at onset and presence or absence of pulmonary fibrosis, osteoarthritis, and joint replacement were also investigated. The patients had a mean (SD) age at onset of 49.8 (12.5) years. Of the 155 patients, 17 (11%) had pulmonary fibrosis as detected by chest radiography or computed tomography findings.

One hundred and ten unrelated healthy subjects (68 female and 42 male) living in the same area of Japan were selected as controls. They were all volunteers and joined sequentially. Their mean (SD) age was 44.5 (18.9) years (table 1). They had no history of articular disease or any abnormalities on physical examination, chest radiography, electrocardiography, urinary analysis, or routine laboratory blood testing. They were

Abbreviations: PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; TGFβ, transforming growth factor β

Table 1 TGFβ1 genotypic distribution

	Female	Male	Age(SD)	TGFβ1 genotype		
				CC (%)	CT (%)	TT (%)
Healthy controls (n=110)	68	42	44.5 (18.9)	33 (30)	53 (48)	24 (22)
Patients with RA (n=155)	118	37	59.6 (12.4)	29 (19)	92 (59)	34 (22)

There were significant differences in genotype between healthy control subjects and patients with RA when the CC type was compared with other types (grouped CT or TT type) (p=0.039).

not receiving medication at the time of the evaluation. Informed consent was obtained from all the patients and healthy controls.

Determination of TGFβ1 T869C genotypes

DNA was extracted from peripheral leucocytes using standard techniques. Genotypes were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) based on the method previously described by Wood *et al*.[5] Specific oligonucleotide primers 5′-TTCCCTCGAGGC CCTCCTA-3′ and 5′-GCCGCAGCTTGGACAGGATC-3′ were used in the PCR to amplify a fragment of the TGFβ gene, with denaturation at 96°C for 10 minutes, followed by 35 cycles at 96°C for 75 seconds, 62°C for 75 seconds, 73°C for 75 seconds, and a final extension at 73°C for five minutes (DNA Thermal Cycler 2400, Perkin Elmer-Cetus, Norwalk, CT, USA). Aliquots of the PCR products were analysed on 2% agarose gels stained with ethidium bromide before digestion to control for correct amplification of the 294 bp fragments. MspA1I (New England Biolabs, Hitchin, UK) digestion of the 294 bp fragments at 37°C for 180 minutes resulted in fragments of the T allele of 161, 67, 40, and 26 bp, and the C allele of 149, 67, 40, 26, and 12 bp. The samples were then analysed by electrophoresis on a 4% agarose gel stained with ethidium bromide and the genotypes were determined. There were three genotypes, homozygous for the alleles TT, CC, and heterozygous CT.

Statistical analysis

Genotypic distributions in the patients and healthy control subjects were analysed with Fisher's exact test. Bonferroni's test was used for evaluation of the age at onset. A p value<0.05 was considered significant.

RESULTS

Genotypic distribution of TGFβ1 T869C

Of the 110 healthy subjects, 33 (30%) were CC type, 53 (48%) were CT type, and 24 (22%) were TT type. Of the 155 patients with RA, 29 (19%) were CC type, 92 (59%) were CT, and 34 (22%) were TT type. A significantly higher proportion of patients with RA carrying the T allele (grouped CT heterozygous or TT homozygous) was found compared with those carrying the CC homozygous allele ($p=0.039$) (table 1), although this difference in genotypic distribution between the controls and patients was not significant when we compared all three genotypes.

Genotypes and clinical characteristics of patients with RA

For CC, CT, and TT genotypes, the mean (SD) ages at onset of patients with RA were 48.2 (14.3), 50.3 (12.6), and 49.9 (11.1), respectively. No significant differences in age of onset between genotypes was found. Of the 155 patients, 17 (11%) had pulmonary fibrosis. The genotypic distribution of these 17 patients was four CC type, seven CT type, and six TT type. No difference in genotypic distribution was found between the patients with or without pulmonary fibrosis. We also investigated patients with osteoarthritis and genotype and those with joint replacement and genotype. No associations were found (data not shown).

DISCUSSION

In this study, significantly more patients carried the T allele (CT or TT type) than the CC type. The genotype distribution found for the healthy subjects in our study was similar to that reported earlier in a larger population of Japanese.[9] From these results, we speculate that this polymorphism is related to the risk of developing RA.

Previous studies have shown that TGFβ1 is produced in large amounts in synovial fluid and is strongly expressed in the joints of patients with RA. Synovial cells have also been reported to be able to produce TGFβ1 in vitro and in vivo.[6 7] TGFβ1 is related to immunosuppression and anti-inflammatory reactions, and is considered to be associated with remission of arthritis. Furthermore, recent investigations have reported that TGFβ1 gene therapy in an animal model showed improvement of arthritis of the junction. Thus, TGFβ1 is considered to be a modulator of reduced inflammation in RA.[7 8]

In several previous studies the T allele of T869C polymorphism has been reported to be associated with reduced production of TGFβ1 proteins.[9 10] These findings were supported by a study of another polymorphism, C-509T, which is linked to T869C polymorphism.[11] Therefore, we speculate that the T alleles of T869C polymorphism might be associated with relatively low production of TGFβ1, and may correlate with the rate of progression of the disease, as reduced TGFβ1 may result in increased inflammation in RA. On the other hand, Awad *et al* reported that the T allele of T869C was linked to higher production of TGFβ1.[12] The association between TGFβ1 T869C polymorphisms and their protein concentrations remains unclear and we have no data of our own to show TGFβ1 concentrations by genotype. Thus, care must be taken in interpretation of our results. However, we speculate that our data and those of several other investigations are in agreement in indicating lower production of TGFβ1 in patients with the T allele of T869C.

TGFβ1 is also considered to be a regulator of fibrosing processes, and TGFβ1 polymorphism was associated with the risk of lung fibrosis in pulmonary transplant recipients, or deteriorated lung function in cystic fibrosis.[12 13] In our patient group there were no significant correlations between TGFβ1 genotypes and the presence or absence of pulmonary fibrosis. However, as there were only 17 patients with RA and pulmonary fibrosis, we speculate that the small population size may have an effect on comparisons. Further study is necessary to evaluate the association between pulmonary fibrosis in RA and TGFβ1 polymorphisms.

Although RA is an inflammatory disease of unknown cause, several investigators have suggested that there may be several genetic risk factors that affect the susceptibility for this disease.[14] The details of the mechanisms remain unclear, but our results suggest that in TGFβ1 gene polymorphism the T allele might be one of the genetic risk factors for RA. Limitations of this study were its small size, and the fact that it only considered Japanese patients, as racial differences may be important. Furthermore, recent investigations showed that there are other polymorphic sites of the TGFβ1 gene and TGFβ type III receptor gene.[5 15] Further investigation of the association between the TGFβ related genetic variation and RA seems warranted.

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