

Association of tumour necrosis factor a, b and c microsatellite polymorphisms with clinical disease activity and induction of remission in early rheumatoid arthritis

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Abstract

Objective

To study the associations of tumor necrosis factor (TNF) a, b and c microsatellite markers with 1) the clinical disease activity and 2) the induction of remissions in patients with early rheumatoid arthritis (RA) treated with two treatment strategies.

Methods

In the FIN-RACo (FINnish Rheumatoid Arthritis Combination therapy) trial of two years, 195 patients with recent-onset RA were randomly assigned to receive either a combination (COMBI) (sulphasalazine, methotrexate, hydroxy-chloroquine, and prednisolone) or a single (SINGLE) (initially sulphasalazine with or without prednisolone) disease modifying antirheumatic drug (DMARD) therapy. TNF a, b and c microsatellite and HLA-DRB1 typings were carried out in 165 (79 COMBI; 86 SINGLE) study completers.

Results

At baseline the 28 joint disease activity scores (DAS28) of the patients positive for TNFa2, a13 or b1 microsatellite markers were significantly higher than in the other patients. In the SINGLE patients the DAS28 improved comparably in patients with (n = 31) or without (n = 53) the TNFb1 marker (NS), while the DAS28 of the TNFb1-positive COMBI patients (n = 22) improved significantly more than that of the TNFb1-negative cases (n = 57) (p = 0.014). Respective 31.8% (7/22) and 28.1% (16/57) of the COMBI patients with or without TNFb1 allele achieved remission at one year. The corresponding figure in SINGLE patients were 0% (0/31) and 20.8% (11/53) (p = 0.006). At two years the remission frequencies in the TNFb1+/TNFb1- patients in the COMBI and SINGLE were 50.0%/38.6% and 9.7%/22.6%, respectively.

Conclusion

Early TNFb1+ RA patients have more active disease but respond more favourably to COMBI treatment than the patients without this microsatellite allele. The finding may be of clinical relevance for the choice of DMARDs in early RA.

Key words

Rheumatoid arthritis, tumour necrosis factor, genetics.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease with unknown cause, varying course and no cure (1). The institution of several disease-modifying antirheumatic drugs (DMARDs) simultaneously (combination-strategy) or the rapid institution of a single DMARD in early RA has been shown to increase the proportion of patients achieving remission (2-4). However, still 10-20 percent of patients with early RA do not respond favourably to the treatments but develop a progressive destructive disease (5). Despite increasing treatment options, the tools guiding selection of the patients for optimal treatments are still missing. HLA alleles with the so called shared epitope (SE) and rheumatoid factor (RF) have been found to associate with more severe disease in some studies (6, 7), although the results have been contradictory (8). Nevertheless, the available tools are not good enough to be used in clinical work as predictors of the outcome of each individual patient. TNF α is a potent immunomodulatory cytokine. It has been found to play an important role in the pathogenesis of RA by participating for example to the cartilage and bone destruction in RA joint and amplifying the effects of other proinflammatory cytokines (9, 10). Increasing evidence verifies the dramatic efficacy of the anti-TNF α therapies (11-15).

The gene encoding TNF α is tandemly arranged with the TNF β gene within the major histocompatibility complex (MHC) centromeric to HLA-B and telomeric to the class III genes (16, 17). Five microsatellite loci have been described within the TNF gene region (TNFa-e) (18, 19) of which TNFa and b microsatellite markers are located 3.5 kb upstream (telomeric) to the human TNF β gene while TNFc is found in the intron of the human TNF β gene (17). Interestingly, the production of TNF α has been found to associate with TNF microsatellite alleles and HLA-DR alleles showing strong linkage disequilibrium with those (20-22). Thus, the HLA-DR2 allele is reportedly associated with lower, while -DR3 and -DR4 alleles with higher levels of TNF α pro-

duction (21, 22). On the other hand, Pociot and coworkers showed that TNFa2 and c2 microsatellite alleles associated with higher TNF α production, while TNFa6 and c1 associated with lower TNF α production (20).

In the present study, we analysed TNFa, b and c microsatellite markers in 165 newly diagnosed RA patients randomly assigned to be treated with either a combination- or a single-DMARD treatment strategy for two years. The association of TNF microsatellite polymorphisms with the clinical disease activity and the induction of remission in both treatment groups were defined. In addition, the results were compared to those associated with SEs.

Materials and methods

Selection of patients and study design

One hundred and sixty-five recent-onset Finnish RA patients randomised originally to the FIN-RACo (FINnish Rheumatoid Arthritis Combination therapy) trial were included in this study (2, 5). All the patients were DMARD-naïve, and the other selection criteria were as follows: 1) fulfilment of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for RA (23), 2) age 18-65 years, 3) duration of symptoms < 2 years, and 4) active disease with ≥ 3 swollen joints and at least 3 of the following: (a) erythrocyte sedimentation rate (ESR) of ≥ 28 mm/hour or C-reactive protein (CRP) level of ≥ 20 mg/l, (b) morning stiffness of ≥ 30 minutes, (c) > 5 swollen joints, or (d) > 10 tender joints. Disease duration was defined as the time from the onset of symptoms and was identical to the delay to the institution of the first DMARD therapy in the study.

Study patients were randomly assigned to the two treatments, either the single-DMARD (SINGLE) (79 patients) or combination-DMARD (COMBI) (86 patients) groups. In the SINGLE group, the treatment was started with sulphasalazine 1g twice daily with or without low-dose prednisolone, and in the COMBI group, the treatment was started simultaneously with sul-

phasalazine (1g/day), methotrexate (7.5mg/week), hydroxychloroquine (300mg/day), and prednisolone (5mg/day). The details of the study protocol have been described previously (5).

The patients were assessed clinically at the beginning of the study and at 1, 3, 4, 5, 6, 9, 12, 18, and 24 months. Swollen and tender joint counts, as well as several other variables, including ESR and CRP level, were used to assess clinical disease activity, and the disease activity score (DAS28) was calculated as described by Prevoo and coworkers (24). The primary end point was the induction of remission. At check-up visits the ACR preliminary criteria for remission were applied (25). While the fatigue and duration criteria were excluded, all other criteria had to be fulfilled, including 1) morning stiffness of ≤ 15 minutes, 2) no joint pain (by history), 3) no joint tenderness or pain on motion, 4) no soft-tissue swelling in joint or tendon sheaths, and 5) ESR < 30 mm/hour in women and < 20 mm/hour in men.

HLA and TNF microsatellite typings

The TNFa, b and c microsatellite markers were analysed from DNA by amplifying each microsatellite region using a primer pair with one fluorescent-labelled (6-FAM, HEX or TET) primer. The sequences of the primers have been published earlier (18, 26), and the genotyping was done using ABI 377 (PE Applied Biosystems, Foster City, CA, USA) according to manufacturing instructions. We were not able to type two patients for the TNFa, b and c microsatellite markers and they were omitted. For the definition of SE-positive patients the HLA-DRB1 alleles were determined by sequence-specific polymerase chain reaction (PCR) amplification and DRB1*04 subtyping was made using the sequence-specific PCR and dot-blot hybridization (27).

The study was performed according to the principles of the Declaration of Helsinki. The protocol was approved by the national health authorities and ethics committees in all 18 participating hospitals. All patients gave written informed consent.

Statistical analysis

The descriptive values of variables were expressed as the mean \pm SD and as the median and interquartile range (IQR). Variables with normal distribution were analysed by using Student's t-test. Variables with non-normal or ordinal distribution were analysed using the Mann-Whitney test, Kruskal-Wallis test, or median regression analysis. The 95% confidence interval (CI) between medians difference was calculated using Hodges-Lehman estimation of median difference. Categorical data were analysed by chi-square test or Fisher's exact test. The influence of TNFb1 allele on induction of remission using two different treatment strategies was also analysed by performing logistic regression analysis where sex, age, duration of symptoms, number of ACR RA criteria fulfilled, and RF were used as covariates. No adjustments were made for multiple testing.

Results

The baseline demographic and clinical characteristics of the patients with different treatments were comparable, as shown in Table I. The frequencies of TNFa, b and c microsatellite marker alleles in our study patients did not deviate markedly from the allele frequencies of random Finnish control haplotypes (Table II) (26).

TNF a, b and c microsatellite markers and disease activity

Three TNF microsatellite alleles, TNFa2, a13 and b1, were significantly associated with the disease activity score (DAS28) at baseline. No signifi-

cant associations with the SE were found (Table III). The TNFa2 allele was the most frequent TNFa microsatellite allele among the study patients 75 of 163 (46%). Patients with TNFa2 had a higher DAS28 score compared to the patients without the allele (mean (SD) DAS28 5.8 (1.0) and 5.4 (0.9), respectively, $P = 0.008$). Further, the presence of either the TNFa13 or the TNFb1 allele was associated with more active disease at baseline, and the differences from the patients without the allele were statistically significant ($P = 0.04$ and $P = 0.005$, respectively). On the contrary, the SE did not show any association with the DAS28 at disease baseline.

TNF microsatellite alleles and clinical improvement

The reduction of DAS28 during two years was similar in the RA patients with or without TNFb1 marker in the SINGLE arm (mean (SD); 2.5 (1.6) v 2.6 (1.5), NS), but in the COMBI patients with TNFb1, the improvement was significantly higher than in those without the allele (3.8 (1.6) v 2.8 (1.5), $P = 0.014$). The same analysis using the SE did not show any statistically significant differences (data not shown).

TNF microsatellite alleles and induction of remission

The induction of remissions was significantly associated with the presence of the TNFb1 allele. Furthermore, the association depended on the treatment strategy used. The remission rate (%) of RA patients with and without the TNFb1 allele one year after the institu-

Table I. Baseline demographic and clinical characteristics of 163 patients with early RA classified according to the treatment strategy.

Variable	Combination treatment n = 79	Single treatment n = 84
Women, no (%)	46 (58)	55 (66)
Age in years, mean \pm SD	47 \pm 10	48 \pm 11
Duration of symptoms (in months) mean \pm SD	7 \pm 5	9 \pm 6
Number of ACR RA criteria fulfilled, median (IQR)	5 (5, 6)	5 (4, 6)
RF positive, no (%)	58 (73)	59 (70)
Larsen score, median (IQR)	0 (0, 4)	2 (0, 8)
DAS28, mean \pm SD	5.4 \pm 0.9	5.7 \pm 1.1

RA: rheumatoid arthritis; ACR: American College of Rheumatology; IQR: interquartile range; RF: rheumatoid factor; DAS: disease activity score.

Table II. Tumor necrosis factor (TNF) a, b and c microsatellite allele frequencies of 163 RA patients and 285 control haplotypes*.

TNFa					TNFb					TNFc				
allele	Patients		Control haplotypes		allele	Patients		Control haplotypes		allele	Patients		Control haplotypes	
	n	%	n	%		n	%	n	%		n	%	n	%
1	1	0.3	2	0.7	1	57	17.5	42	14.7	1	205	62.9	195	68.4
2	86	26.4	69	24.2	2	0	0	0	0	2	121	37.1	90	31.6
3	1	0.3	3	1.1	3	35	10.7	28	9.8					
4	17	5.2	15	5.3	4	95	29.1	90	31.6					
5	55	16.9	42	14.7	5	139	42.6	125	43.9					
6	67	20.6	59	20.7	6	0	0	0	0					
7	8	2.5	9	3.2	7	0	0	0	0					
8	0	0	0	0										
9	6	1.8	5	1.8										
10	27	8.3	21	7.4										
11	35	10.7	45	15.8										
12	0	0	0	0										
13	23	7.1	15	5.3										
Total	326		285			326		285			326		285	

*Ref. 26.

Table III. TNF a, b and c alleles showing association with disease activity score (DAS28) at baseline and the same analysis of shared epitopes (SE).

Presence of allele	n (%)	DAS28 ± SD	P
TNF			
a2 +	75 (46)	5.8 ± 1.0	0.008
a2 -	88 (54)	5.4 ± 0.9	
al3 +	23 (14)	5.9 ± 0.9	0.04
al3 -	140 (86)	5.5 ± 1.0	
bl +	53 (33)	5.9 ± 0.9	0.005
bl -	110 (67)	5.4 ± 1.0	
c2 +	100 (61)	5.7 ± 0.9	0.053
c2 -	63 (39)	5.4 ± 1.0	
SE			
SE +	130 (80)	5.6 ± 0.9	NS
SE -	33 (20)	5.5 ± 1.1	

Table IV. Crude remission rate (%) of RA patients with TNFb1 (n = 53) compared to patients without TNFb1 (n = 110) one year after the institution of two different treatment strategies.

Treatment strategy	with TNFb1 allele		without TNFb1 allele		P
	% (n)	95% CI	% (n)	95% CI	
Single-DMARD	0* (0)	0 to 11.2	20.8 (11)	3.1 to 27.7	0.006
Combination-DMARD	31.8* (7)	12.6 to 51.1	28.1 (16)	17.5 to 41.6	1.0

*P = 0.001 for the comparison between single- and combination-DMARD group in patients positive for TNFb1.

tion of both treatment strategies is shown in Table IV. None of the RA patients in the SINGLE group with the TNFb1 allele had reached remission,

while 20.8% (11/53) of patients without TNFb1 were in remission (p = 0.006). No difference in the remission rate was, on the other hand, observed

in the COMBI group.

Table V presents the corresponding figures after two years of follow-up. The differences in the frequencies of the patients in remission with respect to the TNFb1 status inside each treatment group were no longer statistically significant. However, both at one and two years' treatment, a difference in obtained remissions between SINGLE and COMBI groups was seen only in TNFb1 allele-positive groups (Tables IV and V).

The influence of the TNFb1 allele on the patients' chance of achieving remission by the two different treatment strategies was confirmed by using regression analysis where gender, age, duration of symptoms, number of ACR RA criteria fulfilled and RF were used as covariates. In this model, the difference in the adjusted probabilities of achieving remission by the SINGLE therapy with and without the TNFb1 allele at one year was even more pronounced 0% (95% CI 0 to 11.2), v 10.0% (95% CI 3.1 to 27.7), P = 0.001.

TNF microsatellite alleles and the development of joint damage

None of the studied TNF microsatellite alleles (including TNFb1) was found to impose a statistically significant effect on the development of joint destructions (Larsen score (28)) at two years.

Table V. Crude remission rate (%) of RA patients with TNFb1 (n = 53) compared to patients without TNFb1 (n = 110) two years after the institution of two different treatment strategies.

Treatment strategy	with TNFb1 allele		without TNFb1 allele		P
	% (n)	95% CI	% (n)	95% CI	
Single-DMARD	9.7* (3)	2.0 to 25.8	22.6 (12)	12.3 to 36.2	0.15
Combination-DMARD	50.0* (11)	28.2 to 71.8	38.6 (22)	26.0 to 52.4	0.50

*P = 0.002 for the comparison between single- and combination-DMARD group in patients positive for TNFb1.

The figures regarding the TNFb1 allele are shown in Table VI. The SE did not impose any significant effect to the changes in the Larsen score (data not shown).

Discussion

Treatment of RA should aim at early clinical remission. Long disease duration (2), any prior DMARD use, female sex and poor functional class according to the Steinbrocker criteria, have been found to associate with decreased response to DMARD treatment (29). Today, new therapeutic agents are available, but tools for guiding the selection of appropriate drugs for individual patients are missing. Earlier some evidence has been presented that in the HLA complex there exist genetic determinants of response to infliximab therapy (30, 31). Our present study aimed to clarify the association of TNFa, b and c microsatellite marker polymorphisms with clinical remission, taking into account the two separate DMARD treatment strategies. Previously we found that the SE does not associate with remission in the patients included in the present study

(2). On the other hand, persistent remission of RA is not associated with any particular HLA alleles (32). In the present study, we showed that the TNFb1 allele associates with clinical activity of the disease at baseline. Further, the TNFb1 allele was able to divide the patients into groups achieving remission in different proportions. The TNFb1-positive patients in the SINGLE group reached remission very seldom (9.7%), and those in the COMBI group more frequently (50%) than the patients without the allele (22.6% and 38.6%, respectively). The results indicate that the TNFb1 allele – in addition to the treatment strategy – imposes an effect on RA outcome. In our study protocol we were not able to evaluate whether the statistically significant difference between TNFb1-positive COMBI and SINGLE group patients in improvement of the disease activity might be related in particular to one of the other DMARDs (MTX, HCQ) or prednisone. However, we suggest that the improvement of the disease activity only in those TNFb1-positive patients taking the combination treatment might be more the sum-

mary effect of many drugs together than the effect of some single drug only. In our study the TNFb1 allele was associated with increased disease activity at baseline. Therefore combination treatment inhibiting several factors of inflammation was more effective than treatment with one agent only. Whether our results on specific TNF microsatellite alleles in terms of induction of remission in Finnish patients can be applicable to other populations is still unknown. Further, it is noteworthy to find that our results apply only to the patients with RA fulfilling our inclusion criteria.

Our early DMARD-naïve RA patients had higher disease activity if they were positive for TNFa2, a13 or b1 alleles compared with the other patients. In contrast to our results, Matvey and coworkers found no significant associations between any single TNFa microsatellite polymorphisms and disease severity. Instead, they reported that the presence of SE and TNFa6 among female patients worsened outcome. Further, in the absence of SE, TNFa6 was associated with a more favourable outcome of the disease (33). However, in that study the effects of the therapy were not taken into account. Moreover, the mean (\pm SD) disease duration in their data set was 11.3 (\pm 5.1) years in contrast to our newly diagnosed, DMARD-naïve cohort.

The results concerning the association between SEs and clinical disease activity of RA are contradictory. The studies showing an association between SEs and RA disease activity or erosiveness

Table VI. The development of joint damage measured by Larsen score in RA patients with TNFb1 as compared to those patients without TNFb1.

Treatment strategy	with TNFb1 allele n = 53		without TNFb1 allele n = 110		P [#]
	Larsen score in baseline Median (IQR)	Change in Larsen score at 2 years Median (95% CI)*	Larsen score in baseline Median (IQR)	Change in Larsen score at 2 years Median (95% CI)*	
Single-DMARD	0 (0, 8)	12.0 (8.0 to 15.5)	2 (0, 8)	10 (7.0 to 13.0)	0.18
Combination-DMARD	2 (0, 11)	6.5 (1.0 to 12.0)	0 (0, 2)	7.0 (3.0 to 10.0)	0.82

*Hodges-Lehman estimation of median difference.

[#]Baseline adjusted P-value calculated using median regression analysis.

of the disease have been mainly cross-sectional and have studied patients with long-standing disease (34-36). On the other hand, the studies finding no associations mostly include patients with early RA, and have been prospective (37-42). As an exception, there are two prospective studies which have shown an association between DR4-positive SEs and development of erosions or more severe disease (43, 44). In these two prospective, as well as in most of the cross-sectional and retrospective studies, the effects of different treatment strategies have not been considered. Discrepancies between the results of various studies concerning the effect of SE on disease progression also suggest that not only SE but other loci within the haplotype are important for disease progression. This is also indicated by variable disease-risk associated with different SE-positive haplotypes (8, 45, 46). Much more studies characterizing haplotypes for both HLA class II alleles and gene polymorphisms within TNF and class III region are needed to solve which all loci are important in susceptibility, disease progression and responsiveness to different therapies.

In the present study, we found no statistically significant association of either the SEs or the TNF marker alleles with either the baseline amount of erosions or their development during the two-year follow-up. This may at least partly be explained by uncoupling between clinical inflammation and radiologically detected joint destruction (47, 48). Nevertheless, the patients positive for TNFb1 allele with single-DMARD treatment were those with the most prominent increase in radiographic joint destruction (Larsen score), while the patients positive for TNFb1 but treated more aggressively by combination of DMARDs were those with the smallest increase in Larsen scores. It is possible that a longer follow-period, for example 5 years, might account for differences in terms of TNF microsatellite allele associations with joint damage. Interestingly, TNFa2 has been found to be associated with erosive disease in RA patients from Northwest Spain (49). In that retrospective study

the association was independent of the HLA-DRB1*0401 and SE status. Based on that and our present study, it is possible, that regardless of the genetic background, TNFa2 allele may be a marker of severe disease and its presence may be associated with the development of erosive disease.

The variation in the microsatellite length by itself apparently does not affect gene structure or regulation, but is solely a convenient marker of polymorphism (17). However, it is noteworthy that the TNFb1 allele was found, in all except one of 53 patients positive for it, in combination with TNFa2 and c2 alleles which have been earlier shown to associate with increased TNFa secretion (20). The TNFb1 allele is most probably a marker characterising a haplotype in which an additional locus within the TNF or class III region is affecting the disease activity and responsiveness to specific treatments. More detailed mapping of this locus is a task for further studies.

The results of our present study emphasize the need for homogenous groups of patients when studying the prognostic factors of RA. This applies especially to the patient groups using different treatment strategies. If our material had been analysed as one group, the different response according to TNF microsatellite alleles would not have been detected. The main results became obvious only when the COMBI and SINGLE groups were analysed separately.

In summary, we found 1) the association between TNFa2, a13 and b1 microsatellite marker alleles and increased clinical disease activity of early RA and 2) the TNFb1 allele to be a marker of patients who especially benefit from the DMARD combination treatment. It is possible that individuals with TNFb1 have a more active disease with several different mediators of inflammation involved. Therefore combination treatment inhibiting several factors of inflammation is more effective than treatment with one agent only.

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