EXTENDED REPORT

Genetic markers for the efficacy of tumour necrosis factor blocking therapy in rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a genetically complex disease where the response to different treatments varies greatly between different patients. This is the case with the tumour necrosis factor (TNF) blocking agents, where 20–40% of patients have been described as non-responders. No predictive markers exist as yet for the prognosis of response.

Objective: To analyse whether polymorphisms of several cytokine genes are associated with the responsiveness to TNF blockade with etanercept.

Methods: 123 patients with active RA were treated with etanercept and response rates were determined after three months using American College of Rheumatology (ACR)20 and disease activity score (DAS)28 response criteria. Genotyping was done for TNF (–308 TNFA), interleukin (IL)10 (–1087 IL10), transforming growth factor (TGF)β1 (codon 25 TGFB1), and IL1 receptor antagonist (intron 2 IL1RN).

Results: 24 patients (20%) were defined as non-responders owing to their failure to fulfil any of the ACR20 or DAS28 response criteria. None of the recorded alleles was alone significantly associated with responsiveness to treatment. However, a certain combination of alleles (–308 TNF1/TNF1 and –1087 G/G) was associated with good responsiveness to etanercept ($p<0.05$). In addition, a combination of alleles influencing interleukin 1 receptor antagonist (IL1Ra) and TGFβ1 production (A2 allele for IL1RN and rare C allele in codon 25 of TGFB1 gene) was associated with non-responsiveness ($p<0.05$).

Conclusion: Genetic polymorphisms, which may influence the balance of pro- and anti-inflammatory cytokines of relevance for the course of RA, are associated with clinical responsiveness to etanercept treatment.

Rheumatoid arthritis (RA) is a disease defined by well accepted criteria,[1] but its clinical features and the molecular pathways involved are heterogeneous.[2] Thus, also the response to various treatments varies considerably between different patients, which poses problems in drug development and in predicting responsiveness to a given drug. This heterogeneous response to treatment is seen also for the new targeted therapies with tumour necrosis factor (TNF) blockade,[3 4] where there is an increasing need both to understand the molecular basis of this heterogeneity and to predict responsiveness.

So far, important differences between patients with RA have been demonstrated both in their genetics[5–7] and in various molecular features—for example, patterns of molecules present in the inflammatory tissues of affected joints.[8] Thus, patients carrying different HLA-DR haplotypes appear to have different natural courses, and respond differently to conventional disease modifying antirheumatic drug (DMARD) treatment.[9] Patients with different cytokine patterns in joints may also respond differently to treatment with TNF blockers.[10] Considering the critical role that different cytokines are assumed to have in the pathogenesis of RA, and considering the heterogeneity of both the genetic regulation of these cytokines and the actual presence of these cytokines in the joint, it is possible that polymorphisms in regulating cytokine production may not only affect the natural course of the disease but also the response to therapy such as TNF blockade.

A number of polymorphisms with possible functional significance have recently been identified in the promoter regions for several of the cytokines assumed to be of importance for the balance of pro- and anti-inflammatory cytokines (table 1).

Given this background, the goal of the current study was to investigate whether the polymorphisms in genes influencing the production of various cytokines are associated with responsiveness to etanercept. We took advantage of the structured surveillance systems for TNF blockade, including treatment with etanercept, that have been implemented in Sweden, including the Stockholm area.

PATIENTS AND METHODS

Patients

123 patients (100 female, 23 male) with RA diagnosed according to the 1987 revised American College of Rheumatology (ACR) criteria for classification of RA[1] underwent treatment with etanercept in the rheumatology outpatient clinics at the Karolinska Hospital and Huddinge University Hospital during 1998–2000. This evaluation is part of a nationwide surveillance programme for TNF blockade in RA. The mean age of inclusion was 52 years (range 24–76), and the mean duration of disease was 14 years (range 1–51). Among the patients 94 (76%) were rheumatoid factor positive and 74 (60%) were receiving treatment with corticosteroids; the dose was not increased during the treatment with etanercept. No special selection was made, but treatment with at least one DMARD had failed in all patients at trial entry. Patients were

Abbreviations: ACR, American College of Rheumatology; DAS, disease activity score; DMARD, disease modifying antirheumatic drug; IL, interleukin; RA, rheumatoid arthritis; TGF, transforming growth factor; TNF, tumour necrosis factor

Table 1 Cytokine gene polymorphisms with pro- and anti-inflammatory potential

Gene symbol	Polymorphism position	Alleles	Functional role
TNFA	–308	G=TNF1 A=TNF2	Normal production of TNF Up regulation of TNF production in different type of cells[22] [24]
IL10	–1087	G A	GG genotype associates with up regulation of IL10 production in lymphocytes AA genotype associates with down regulation of IL10 production in lymphocytes[23]
TGFB1	+915, codon 25	G C	Normal production of TGFβ1 Down regulation of secretion of TGFβ1 from the peripheral blood leucocytes[21]
IL1RN	Intron 2	A1=4 repeats A2=2 repeats	"Normal" allele[25] Up regulation of IL1Ra production in sera and down regulation in saliva[19]

treated with etanercept (Immunex Co, Seattle, WA) subcutaneously 25 mg twice a week for three months. Among the 123 patients, 63 patients were treated with etanercept alone, 53 patients received etanercept in combination with methotrexate, one patient received etanercept in combination with methotrexate and cyclosporin, one patient received etanercept in combination with methotrexate and sulfasalazine, two patients received etanercept in combination with sulfasalazine, one patient received etanercept in combination with intramuscular gold, one patient received etanercept in combination with azathioprine, and one patient received etanercept in combination with Reumacon (Conpharm, Uppsala, Sweden); Reumacon is an antirheumatic agent used under licence in Sweden.

Clinical and laboratory assessments

Responders after three months of treatment were defined using both the ACR response criteria[11] and the response criteria based on the modified disease activity score (DAS)28 index.[12] We defined as non-responders those who fulfilled neither the ACR nor the DAS28 response criteria. Blood samples were collected from all patients before treatment (baseline).

Genotyping

DNA was isolated from EDTA blood by a modification of the method described by Aldener-Cannava and Olerup.[13] Primers were synthesised by Scandinavian gene synthesis (SGS, Köping, Sweden). The sequence of primers for amplification of the VNTR region in intron 2 of the IL1RN gene were taken from Tarlow *et al*.[14] The sequences of other primers and the whole procedure were published earlier.[15]

HLA typing was performed by using a DR low resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden) and according to methodology previously described.[16]

Statistical analysis

Fisher's exact test was used to determine whether there was a random association between the observed alleles in the two studied populations or not. All calculations were done in StatView software, version 5.0 (SAS Institute, Inc, Cary, NC).

RESULTS

Clinical response

Clinical response to treatment was determined using both the ACR20 response criteria and response criteria based on the DAS28 index. In our clinical practice treatment is changed for those patients who are defined as non-responders when both these sets of criteria are applied, and thus we chose to define as "non-responders" those patients who failed to fulfil both these response criteria and others as "responders". By these criteria, a total of 99/123 patients were responders (67% responders according to the ACR20; 80% responders according to the DAS28 based criteria) and 24 were non-responders to etanercept therapy.

Association of cytokine gene polymorphisms with response to etanercept therapy

To analyse the association between functionally important gene polymorphisms and clinical response to etanercept therapy, we first analysed whether any of those single polymorphisms were associated with clinical responsiveness. This analysis showed no significant difference in allele or genotype frequencies between responders and non-responders (table 2). Also analysis of HLA-DR polymorphisms failed to show any association between response and HLA-DR alleles known as shared epitope genes (data not shown). We then analysed the combinations of polymorphisms that we considered to be functionally most meaningful, although many others may exist for the genes studied. Nevertheless, several studies have shown the importance of selected genetic markers in predicting the susceptibility and clinical course in other inflammatory conditions.[17–20]

We first analysed the effects of the TNF1/TNF1 genotype at the –308 position of the TNFA gene together with the GG genotype at the –1087 position of the interleukin (IL)10 gene. This combination was chosen to define subjects with a presumed lower propensity for an inflammatory response. Table 3 shows that this genetic combination was significantly more common among responders than among non-responders. Of 23 patients with this composite genotype only

Table 2 Frequency of gene polymorphisms in patients with RA treated with etanercept (Enbrel)

Gene, polymorphism	Non-responders No (%)	Responders No (%)	p Value
TNFA, –308			
TNF1/TNF1	12 (50)	65 (66)	
TNF1/TNF2	11 (46)	32 (32)	
TNF2/TNF2	1 (4)	2 (2)	0.33
IL10, –1087			
G/G	8 (33)	27 (27)	
A/G	13 (54)	42 (43)	
A/A	3 (13)	30 (30)	0.22
TGFB1, codon 25			
G/G	20 (83)	84 (85)	
G/C	4 (17)	15 (15)	1.00
IL1RN, intron 2 VNTR			
A1/A1	13 (54)	69 (70)	
A1/A2	8 (33)	22 (22)	
A2/A2	1 (4)	6 (6)	
A1/A4	1 (4)	0	
A2/A4	1 (4)	2 (2)	

Table 3 Combined genotype frequencies for IL10 and TNFA genes

	GG, TNF1/TNF1	Other combinations
Non-responders, No (%)	1 (4)	23 (23)
Responders, No (%)	22 (96)	77 (77)

Fisher's exact test $p<0.05$.

Table 4 Combined genotypes for IL1RN and TGFB1 genes

	A2 positive, CG	Other combinations
Non-responders	3	21
Responders	1	98

Fisher's exact test $p<0.05$.

one did not respond to etanercept after three months; the remaining single patient, however, responded after six months. Other combinations of –308 TNFA and –1087 IL10 genotypes did not show any significant association with response to etanercept therapy.

Secondly, we analysed a combination of the C allele in codon 25 of the TGFB1 gene and the A2 allele in intron 2 of the IL1RN gene. These combinations were chosen because of previous reports about their functional role.[19 21] We found that this genetic pattern was associated with a poor response to etanercept therapy (table 4). The relatively rare C allele was found significantly more often in non-responders with A2 positive genotype (homo- or heterozygotic state of A2), in comparison with all other combinations ($p<0.05$). We found no significant association of response to etanercept therapy with other combinations of IL1RN and codon 25 TGFB1 genotypes.

DISCUSSION

This study examined the possibility that functionally important allelic differences in genes controlling cytokine activity might be associated with the response to TNF blockade with etanercept. The data obtained in this first analysis support this concept as a certain combination of allelic forms influencing TNF and IL10 production was associated with a good response to etanercept, whereas another combination of allelic forms influencing transforming growth factor (TGF)β1 and IL1 receptor antagonist (IL1Ra) production was associated with a poorer response.

The methodology chosen to analyse the complex interplay between genetic polymorphisms and clinical response to treatment, in this case to TNF blockade with etanercept, is associated with a problem because both the response to treatment and the genotype may be difficult to define simply, and even more difficult to relate to each other. Mass significance problems are also common and difficult to resolve in this type of analysis. We tried to diminish these problems in two ways. Firstly, response to treatment was dichotomised by combining the ACR20 and DAS28 response criteria in the way that is considered most appropriate in daily clinical practice—that is, by defining as "responders" all those who fulfil either of the two alternative response criteria. Secondly, genetic analyses were performed only for a limited number of alleles or combination of alleles—namely, those we considered functionally most relevant. The choice of alleles to be analysed was made according to published data before analysing the results[19 21–25] (table 1). Previous functional and clinical characteristics associated with the selected alleles and combinations of alleles include TNF1 related to low in vitro production of TNF, and TNF2 related to high production of TNF.[22 24] The influence of these TNF alleles was also investigated in combinations with selected other alleles, including the homozygous state of A at –1087 of IL10; this IL10 genotype together with TNF2 has previously been shown to be associated with high risk for heart transplant rejection.[18] Similarly, an analysis of polymorphisms related to the IL1RN A2 allelic form was included based on the previously shown association between this allele and susceptibility to systemic lupus erythematosus and primary Sjögren's syndrome.[19] The statistical analysis in our study was done in such a way that compensation was made for the mass significance problems associated with these, notably limited, numbers of combinations.

The results generated from analysis of the selected functional polymorphisms show an interesting and not entirely predicted pattern. Thus, on the one hand, the combination of TNFA and IL10 alleles related to low inflammatory reactivity was associated with a good response to etanercept, and, on the other, a combination of IL1RN and TGFB1 alleles related to strong inflammatory reactivity was associated with a poorer response to the same drug. These data suggest that it may be easier to obtain a healthy balance between pro- and anti-inflammatory events in subjects with a genetic predisposition for a low inflammatory reaction than in those with a predisposition for a more vigorous inflammatory response.

Interestingly, in the only reported genetic study of the efficacy of TNF blockade an association was found between the relatively rare haplotype in the lymphotoxin A gene and a lower response to infliximab in a group of patients with Crohn's disease.[26]

Whereas the results from this study indicate that certain functionally important cytokine gene polymorphisms may be associated with clinical response to etanercept, caution is obviously warranted in transferring those into algorithms that can be useful for predicting responses to etanercept in the clinic. The polymorphisms that were investigated comprise only a very minor part of the existing polymorphisms, and were chosen only because of previously documented functional and/or clinical significance. It is also well established that linkage disequilibrium is an important factor to account for interpretation of genetic data. The associations found in our study should thus be mainly taken as a first indication of the potentials of a pharmacogenomic approach to understanding and predicting response to TNF blockade. Studies in larger patient groups are needed to verify their eventual practical usefulness in clinical practice.

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REFERENCES

1 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.

2 **Choy EH**, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344:907–16.
3 **Bathon JM**, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, *et al*. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000;343:1586–93.
4 **Maini R**, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, *et al*. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999;354:1932–9.
5 **Weyand CM**, Hicok KC, Conn DL, Goronzy JJ. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. Ann Intern Med 1992;117:801–6.
6 **Gough A**, Faint J, Salmon M, Hassell A, Wordsworth P, Pilling D, *et al*. Genetic typing of patients with inflammatory arthritis at presentation can be used to predict outcome. Arthritis Rheum 1994;37:1166–70.
7 **Thomson W**, Harrison B, Ollier B, Wiles N, Payton T, Barrett J, *et al*. Quantifying the exact role of HLA-DRB1 alleles in susceptibility to inflammatory polyarthritis: results from a large, population-based study. Arthritis Rheum 1999;42:757–62.
8 **Ulfgren AK**, Grondal L, Lindblad S, Khademi M, Johnell O, Klareskog L, *et al*. Interindividual and intra-articular variation of proinflammatory cytokines in patients with rheumatoid arthritis: potential implications for treatment. Ann Rheum Dis 2000;59:439–47.
9 **Lard LR**, Vos KK, Zwinderman AH, Hazes JMW, Breedveld FC, Schreuder G, *et al*. Influence of HLA class II and autoantibodies on treatment effects of newly diagnosed rheumatoid arthritis patients [abstract]. Arthritis Rheum 2000;43(suppl):S402.
10 **Ulfgren AK**, Andersson U, Engstrom M, Klareskog L, Maini RN, Taylor PC. Systemic anti-tumor necrosis factor alpha therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor alpha synthesis. Arthritis Rheum 2000;43:2391–6.
11 **Felson DT**, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, *et al*. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727–35.
12 **Prevoo ML**, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
13 **Aldener-Cannava A**, Olerup O. HLA-DPA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) and distribution of DPA1 alleles in Caucasian, African and Oriental populations. Tissue Antigens 1996;48:153–60.
14 **Tarlow JK**, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, *et al*. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993;91:403–4.
15 **Padyukov L**, Hahn-Zoric M, Lau YL, Hanson LÅ. Different allelic frequencies of several cytokine genes in Hong Kong Chinese and Swedish Caucasians. Genes Immunity 2001;2:280–3.
16 **Olerup O**, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR- SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992;39:225–35.
17 **McGuire W**, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature 1994;371:508–10.
18 **Turner D**, Grant S, Yonan N, Sheldon S, Dyer P, Sinnot P, *et al*. Cytokine gene polymorphism and heart transplant rejection. Transplantation 1997;64:776–9.
19 **Perrier S**, Coussediere C, Dubost JJ, Albuisson E, Sauvezie B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjögren's syndrome and rheumatoid arthritis. Clin Immunol Immunopathol 1998;87:309–13.
20 **Sankaran D**, Asderakis A, Ashraf S, Roberts IS, Short CD, Dyer PA, *et al*. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. Kidney Int 1999;56:281–8.
21 **Awad MR**, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation 1998;66:1014–20.
22 **Kroeger KM**, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997;34:391–9.
23 **Turner DM**, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1–8.
24 **Wilson AG**, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997;94:3195–9.
25 **Danis VA**, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 1995;99:303–10.
26 **Taylor KD**, Plevy SE, Yang H, Landers CJ, Barry MJ, Rotter JI, *et al*. ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. Gastroenterology 2001;120:1347–55.