The response to anti-TNF-α treatment: gene regulation at the bedside

Approximately 40% of the patients with rheumatoid arthritis do not fulfill the ACR20 response criteria 3 months after initiation of any of the current therapies targeting tumour necrosis factor α (TNF-α) [1–3]. These patients are not only exposed to an expensive treatment strategy but they also risk serious side-effects, notably the increased risk of tuberculosis. It would clearly be useful if we could identify those patients who are not likely to respond satisfactorily to these therapies or who are likely to develop side-effects.

Scientific progress in recent years has offered the promise that indeed such drug effects might be predicted. The elucidation of the complete human genome sequence, the efforts to describe genetic variation in large databases and the introduction of high-throughput screening techniques for genetic polymorphisms have stimulated the development of pharmacogenetic research on drug responses. Clearly, the anti-TNF-α field, characterized by expensive drugs, strong variability in response, dangerous complications and relatively extensive knowledge regarding the molecular target of these therapies, may represent a prime candidate for this niche of research.

Pharmacogenetic studies on anti-TNF-α therapy focus on genetic variability in the production and effector pathways of its targets. While etanercept also binds lymphotoxin α, TNF-α is the target shared by adalimumab, infliximab and etanercept. This is an important pro-inflammatory cytokine synthesized by several cells, notably monocytes and macrophages, that may play a role in leucocyte activation and migration, the acute-phase response and apoptosis [4]. Its production is induced by various bacterial and immunological stimuli. These activate intracellular signalling pathways, leading to the synthesis of several transcription factors that bind to regulatory DNA sequences. The collective binding of the different transcription factors to enhancing and silencing regulatory elements steers the transcription of the gene [5, 6].

Production of TNF-α is also regulated at the post-transcriptional level, e.g. by influencing mRNA stability [7]. TNF-α starts life as a membrane-bound protein but a soluble fragment is cleaved off by the TNF-α converting enzyme (TACE). This soluble fragment binds to its two receptors, the 55-kDa and the 75-kDa TNF-α receptor, resulting in activation of downstream effector pathways. In these different production and effector pathways, several molecules play a key role. Therefore, genetic variation influencing the activity of each of these pathways might help to explain interindividual variation in the effects of anti-TNF-α therapy.

In this journal Kang et al. [8] presented the results of a pharmacogenetic study in Korean patients with rheumatoid arthritis treated with etanercept. They studied genetic variation in that part of the HLA complex on chromosome 6 where the genes for TNF-α and lymphotoxin α are located, hoping to find genetic markers that are associated with increased or decreased TNF-α production, and with worse or better response to TNF-α therapy. To this end they investigated 13 single nucleotide markers (SNPs) in the region and analysed the association of these markers with the response to etanercept therapy. The authors tried to compensate for the limited size of the study (n = 70) by taking an intelligent approach. Based on the fact that response to anti-TNF-α agents follows a normal or skewed but not bimodal distribution [9], the authors decided to primarily compare ACR70 responders with ACR20 non-responders, in order to optimize the chance of finding a major gene effect. Using this approach, the key observation was that a change from cytidine to thymidine at position –857 (C–857T) of the TNF-α promoter is associated with good response to etanercept (odds ratio = 12.95). Uncorrected for the number of tests, a significant association of the –857T allele with response was observed when comparing ACR70 responders with non-responders. Interestingly, the change from the –857C allele has previously been associated with increased transcription efficiency of TNF-α [10]. This suggests that high production of TNF-α is associated with lack of response to anti-TNF-α treatment.

Are the observations of Kang et al. [8] in line with previous results? Data on the association of –857T with response to anti-TNF-α therapy in other populations are lacking. Previous pharmacogenetic studies on anti-TNF-α therapy have been performed in Caucasians and have focused on the role of non-coding substitutions in the promoter region of the TNF-α gene, notably the substitution of guanine at –308 for adenosine (G–308A). French rheumatoid arthritis patients homozygous for the –308G allele showed a favourable response to infliximab [11], a finding confirmed later in 23 Hungarian patients with rheumatoid arthritis or Crohn’s disease [12]. Data from a study among Caucasian patients in the USA demonstrated that, among the HLA-DR1-containing haplotypes, those encoding the –308G allele are significantly associated with a better response to etanercept [13]. Swedish patients responding to etanercept also showed an increased frequency of the G allele [14]. Finally, in Spanish patients, the TNFa2b3 haplotype, linked to –308A, was decreased in responders to infliximab [15]. Thus, current evidence points to an association of the TNF-α –308G allele with the response to infliximab and etanercept in Caucasian populations.

In the Korean population, the –308A allele is practically absent and 100% of patients encode the –308G allele. This explains why Kang et al. [8] did not observe an association of the –308G allele with response among their Korean rheumatoid arthritis patients. If these associations cannot be compared across different populations, are the functional effects of both SNPs pointing in the same direction? The first problem is that there is considerable controversy about the direct functional relevance of the different SNPs in the TNF promoter region. This has focused especially on the G–308A polymorphism itself [16–18]. A recent study suggests a direct functional effect of this –308 SNP [19]. Whether or not there is a direct functional effect, several studies have shown an association of the –308A allele with higher lipopolysaccharide-induced TNF-α production in whole blood cells from healthy individuals and from patients with inflammatory bowel disease [20, 21]. An analogous controversy exists regarding direct functional effects of the –857 SNP, and again studies in healthy (Caucasian) controls have shown higher production of TNF-α in whole-blood cultures stimulated with lipopolysaccharide in individuals homozygous for the –857C allele. This suggests that both alleles associated with good response to anti-TNF-α therapy, the –308G allele and the –857T allele, are associated with lower levels of lipopolysaccharide-induced TNF-α production in whole
blood cell cultures. Clearly, more data are needed to confirm the association of the −857T allele with response to anti-TNF-α therapy. Nevertheless, these data are interesting since they support the notion that individuals predisposed to high TNF production show worse responses to anti-TNF-α therapy.

A second interesting aspect of this paper concerns the interpretation of the results. In this respect, this study may well be one of the early birds in the next wave of genetics, concerning itself with interpretation of gene regulation rather than with polymorphisms of coding sequences or with gene expression itself. It fits perfectly in the current time, when we are recovering from the shock that humans have only 22 500 protein-encoding genes, and from the information that chimpanzees and humans do show differences in the expression of some genes in the brain, but that on the whole these two primates have very similar sets of coding genes [22]. From this information, it becomes clear that much of the evolution and speciation actually takes place by changes in the regulation of genes. Moreover, it indicates that it is on gene regulation that we should focus our attention in the next phase of genetics.

Regulatory DNA sequences are located in the non-coding regions and consist of enhancers, increasing gene expression, and silencers. Regulatory sequences are typically 500 nucleotides in length and are bordered with short DNA sequences (typically 10 nucleotides in length) that bind transcription factors. The presence and arrangement of these sites, the spacing between them, the presence of the different transcription factors in the cell of interest and the interaction between these transcription factors themselves each influence the effect on transcription of genes nearby or further away. Much information needs to be acquired to adequately model these complex processes [6]. In this context, it is readily conceivable that the discrepancies mentioned above between the different reports disputing the direct effects of different SNPs on TNF-α expression might be the result of confounding by such complex interactions between transcription factors which are not adequately modelled in the different gene reporter systems.

Kang et al. [8] support their data on the association of the −857T allele with the response to anti-TNF-α therapy not only by pointing to previous data describing the effects of this polymorphism on TNF-α production. They also discuss data indicating that the −857T allele strongly binds the transcription factor OCT1, while the −857C allele does not. OCT1 binding inhibits binding of the transcription activator NFκB to the nearby site at −873 to −863, and thereby inhibits NFκB stimulated TNF-α transcription. As a result, the −857T allele induces a low TNF-α-producing phenotype, potentially explaining its association with a better response to anti-TNF-α treatment [10]. To illustrate the potential complexity of gene regulation systems, this link is further modified by an effect of another nearby SNP at position −863, substituting adenosine for cytosine. Previous research showed that the competitively inhibiting p50–p50 variant of NFκB only binds to −863C, while the transcriptionally active p65–p50 heterodimer variant binds both to the −863C and the −863A allele [23]. Indeed, the −857C/−863A haplotype, the high TNF-α-producer haplotype in this constellation, is associated with a low anti-TNF-α response, while the −857T/−863C haplotype, the low-producer haplotype, is associated with a good anti-TNF-α response.

Although the sample size of this study is relatively small, taken together the data produced do support the idea that genetic variations in the promoter site of the TNF-α gene that are associated with low inducibility of TNF-α production are also associated with a good response to anti-TNF-α treatment. Furthermore, the observed effects are well explained by the previously described intriguing interactions between the transcription factors OCT1 and the different NFκB variants upon binding to the regulatory DNA sequences upstream of the TNF-α gene. These data indicate that modulation of these interactions might be an interesting goal for future therapies. For doctors currently working at the bedside, the data presented raise the question of whether treatment with higher than standard doses of anti-TNF-α therapy might be needed for patients with high-TNF-α producing genotypes.

The authors have declared no conflicts of interest.

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References


