A common polymorphism of the transforming growth factor-β1 gene and coronary artery disease

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Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine involved in many physiological and pathological processes. Its actions are complex and in vitro experiments and clinical findings suggest that under different circumstances it may have opposing biological effects [1]. There are in vitro experimental data indicating that it may be both anti-atherogenic [2] and pro-atherogenic [3]. With regard to clinical studies, Grainger et al. [4] reported that serum active TGF-β1 levels were depressed in 31 patients with triple-vessel coronary disease, whereas we showed that levels were higher in patients with triple vessel disease (n = 72) than in those with two vessel one vessel disease or in normal coronary arteries (n = 197) [5]. These contradictory observations could result from a real difference in biological effects at different stages of the disease or be an artifact, perhaps related to a difference in the sample sizes of the two studies. Also, there is a high intra-individual variation in active TGF-β1 levels and a single active TGF-β1 measurement may not provide a reliable estimate of the true TGF-β1 status. This could be particularly relevant to atherogenesis – a chronic process occurring over decades. However, DNA variants at the TGF-β1 gene, if functional or in linkage with functional changes, could be reliable markers for TGF-β1 action and reflect a true estimate of its relationship with atherogenesis.

Since TGF-β1 is a highly conserved protein, mutations at coding regions, if functional, are likely to be fatal. Common variants at non-coding regions, particularly the promoter region on the other hand, could have quantitative but non-fatal effects. Such DNA variants could be particularly relevant to genetically determined susceptibility to common diseases such as coronary artery disease (CAD). Recently, Cambien et al. [6] identified a number of DNA variants at the TGF-β1 locus in both the promoter and coding regions. All markers in that study were found to be negative, or at the most weakly positive, in associations with myocardial infarction and hypertension [6]. Given the importance of the promoter region in gene transcription, we explored a common polymorphic marker (C → T) at the promoter region of the TGF-β1 gene (−509 bp) in 371 angiographically defined patients for associations with TGF-β1 levels and severity of CAD [6]. The severity of CAD was measured as the number of significantly diseased coronary arteries (≥50% luminal obstruction demonstrated angiographically). The study was approved by the Ethics Committee of the University of New South Wales. The polymorphic marker was identified using the polymerase chain reaction to amplify the promoter region with the primers described by Cambien et al. [6]. The polymerase chain reaction products (265 bp) were digested with Bsu361 for the detection of the C → T change at −509 bp. The rare T allele eliminated the Bsu361 restriction site whereas when the common C allele was cut it resulted in 194-bp and 71-bp fragments after digestion.

The allele frequency for T_{−509} was 0.326 in 371 patients and similar to that reported for the French population (0.343) [6]. The genotype distribution was in Hardy–Weinberg equilibrium and not different between males and females \( \chi^2 = 0.921, \text{degrees of freedom (df)} = 2, P = 0.631 \). However, there was no significant difference in either active \( (F = 0.546, P = 0.579) \) or total \( (F = 0.66, P = 0.517) \) TGF-β1 levels among patients with different genotypes using one-way ANOVA analyses. The total TGF-β1 levels for patients with CC, TC and TT genotypes were \( (\text{means} \pm \text{S.E.M.}) 58.3 \pm 2.1 \text{ng/ml} (n = 161), 55.0 \pm 2.0 \text{ng/ml} (n = 167) \) and \( 55.9 \pm 4.4 \text{ng/ml} (n = 35) \) respectively; and for active TGF-β1, logarithmically converted because of skewed distribution.
they were $3.77 \pm 0.16$ ng/ml, $3.78 \pm 0.16$ ng/ml and $3.51 \pm 0.35$ ng/ml respectively.

There was also a lack of association between the polymorphic marker and the number of significantly diseased vessels in this patient population ($\chi^2 = 4.225$, df = 8, $P = 0.646$). The distribution of the rare TT homozygote among patients with 0, 1, 2 and 3 significantly diseased vessels was 7.7%, 8.4%, 6.4% and 12.2% respectively, and the T allele frequencies were 0.336, 0.306, 0.314 and 0.361 respectively. The $T_{-509}$ polymorphism was also not associated with the presence of hypertension. The frequencies of TT homozygotes and the T allele in hypertensive patients (9.1% and 0.313) were not different from those in non-hypertensive patients (8.4% and 0.344) ($\chi^2 = 2.824$, df = 2, $P = 0.244$). In the patients with or without a history of documented myocardial infarction there were also no differences in numbers of TT homozygotes (7.8% versus 10%) or the T allele frequencies (0.321 versus 0.340).

In summary, our study showed that the C $\rightarrow$ T$_{-509}$ polymorphism was not associated with levels of active or total TGF-$\beta$1, or with the severity of CAD, the occurrence of myocardial infarction or hypertension. We conclude that the polymorphism is unlikely to be functional or in linkage with other functional changes.

REFERENCES

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