Tumour necrosis factor α (TNFα), a potent immunomodulator and pro-inflammatory cytokine, has been implicated in many pathological processes. In addition to autoimmune and infectious diseases, it also plays a significant role in obesity, insulin resistance, endothelial dysfunction, oxidative stress and atherosclerosis. Elevated adipose tissue expression of TNFα occurs in obesity and falls with weight loss [1]. TNFα acts as a mediator for obesity-related metabolic disorders, including insulin resistance, dyslipoproteinaemia, hypercoagulability and atherosclerosis [2,3]. An inflammatory process is an essential part of the initiation and progression of atherosclerosis [4]. TNFα as a marker of monocyte/macrophage activation is elevated in vascular wall injury [5]. It stimulates the endothelial expression of adhesion molecules for mononuclear cells and induces endothelial apoptosis [6]. It also down-regulates endothelial nitric oxide synthase [7] and increases oxidative stress in endothelial cells [8,9]. These processes are highly relevant to the initiation and progression of atherosclerosis. While many factors can affect TNFα production (e.g. infection), genetic regulation also plays a significant role. Among many DNA variants in the TNFα gene, a G→A transition at the −308 bp position (NcoI polymorphism) was shown to be associated with increased promoter activity [10,11] and elevated plasma TNFα levels [12,13].

In the present study, we explored the relevance of the TNFα G−308 → A polymorphism to obesity, coronary atherosclerosis and biochemical markers related to oxidative stress in 641 Caucasians (age 55.7 ± 0.6 years for men and 57.6 ± 0.9 years for women). The subjects were consecutively referred to the Eastern Heart Clinic at the Prince of Wales Hospital for coronary angiography, as described previously [14]. The study was approved by the local Ethics Committee, and informed consent was obtained from all subjects. The G−308 → A polymorphism in the promoter region of the TNFα gene was determined by a PCR method, as detailed by Fernandez-Real et al. [15].

The frequencies of the TNFα G−308 → A genotypes were 69.9% (n = 448), 25.4% (n = 163) and 4.7% (n = 30) for GG, GA and AA respectively, and the rare ‘A’ allele frequency was 0.174. The genotype distribution was not statistically different between men (70.3%, 24.1% and 5.6% respectively) and women (68.5%, 29.6%, 1.9% respectively; χ² = 5.25, P = 0.027). There was no association between the TNFα polymorphism and body mass index (BMI) (GG, 28.1 ± 0.2; GA, 27.9 ± 0.3; AA, 27.3 ± 0.6; P = 0.560) or waist/hip ratio (GG, 0.95 ± 0.004; GA, 0.94 ± 0.01; AA, 0.96 ± 0.01; P = 0.482) among all patients. The findings suggest that the G−308 → A substitution at the TNFα locus may not be in linkage with obesity. However, it should be noted that more than 77% of patients in the present study were either obese (27.2% BMI > 30) or overweight (49.8% BMI 25–30). This highly skewed population could reduce the power for detecting the relationship. The results should be interpreted specifically with regard to the high-risk population.

We also explored whether the TNFα polymorphism was associated with the levels of extracellular superoxide dismutase (EC-SOD), an antioxidant existing in abundance in the extracellular space, and of total homocysteine, a potent oxidant. Patients with the EC-SOD Arg193 → Gly mutation, who have very high EC-SOD levels with unknown biological significance, were excluded from the analysis [14]. The levels of EC-SOD and homocysteine were higher in patients having the TNFα A allele both before and after the effects of cigarette smoking were taken into account (Table 1). The mechanism behind the positive association between the TNFα polymorphism and EC-SOD levels is not clear. It is established, however, that TNFα plays a significant role in oxidative stress. It can either directly or

Key words: atherosclerosis, homocysteine, obesity, polymorphism, superoxide dismutase.

Abbreviations: BMI, body mass index; EC-SOD, extracellular superoxide dismutase; TNFα, tumour necrosis factor α.

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support to the oxidative role of TNF polymorphism and homocysteine levels lend further

[17]. Findings of a positive association between the TNF polymorphism and homocysteine levels is significant (F = 5.573; P = 0.019; power = 0.654) after controlling for cigarette smoking.

Mn-dependent SOD by TNF-α protects from TNF-α-induced oxidation. This protective effect of EC-SOD would be particularly important, since EC-SOD is found in abundance in atherosclerotic lesions [17]. Findings of a positive association between the TNF polymorphism and homocysteine levels lend further support to the oxidative role of TNF-α and homocysteine, which was also suggested to exert oxidative stress [18]. The simultaneous elevation of TNF-α and homocysteine levels is a detrimental combination, since homocysteine can enhance TNF-α-mediated cytotoxicity [19].

By a simple χ² comparison, there was no association between TNF-α genotype and the number of significantly diseased vessels (χ² = 5.344; degrees of freedom = 6; P = 0.501). The A allele frequencies in patients with no, one, two and three significantly diseased vessels (≥ 50% luminal obstruction) were 0.168, 0.210, 0.169 and 0.145 respectively. The same non-significant association was also observed for patients with (A allele frequency 0.178) or without (0.161) angiographically demonstrable coronary disease. There was no association between the polymorphism and diabetes, hypertension, past history of myocardial infarction or unstable angina. These findings are consistent with those in a French population [20] which had a similar A allele frequency (0.157–0.242) to our population (0.174).

In summary, we report positive and significant associations between the TNF-α G−308→A polymorphism and levels of EC-SOD and homocysteine, which are consistent with TNF-α acting as an oxidative stress relevant to atherogenesis. However, the TNF-α polymorphism was not associated directly with the occurrence or severity of atherosclerosis documented angiographically. We have further demonstrated that the polymorphism is not associated with obesity, as measured by the BMI and the waist/hip ratio.

ACKNOWLEDGMENT

This project was funded by research grants from the National Health & Medical Research Council of Australia, Cardiac Services of the Prince of Wales Hospital and Eastern Heart Clinic, Sydney. We are most grateful to the cardiologists in the department for allowing us to study their patients.

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Received 25 October 1999