Abstract  Tumor necrosis factor (TNF)-α has been implicated in pathophysiological processes in coronary artery disease (CAD). TNF receptor 2 is of particular interest in mediating such effects. The gene for this receptor (TNFRSF1B) has, moreover, been implicated in hypertension, elevated cholesterol and insulin resistance. TNFRSF1B is thus a worthy candidate in studies of the genetic basis of CAD. We therefore conducted a case-control study of a microsatellite marker with five alleles (CA13-CA17) in intron 4 of TNFRSF1B in 1006 well-characterized white patients with angiographically confirmed CAD and a control group of 183 healthy subjects. We found a strong association of the TNFRSF1B marker with CAD (χ² = 40, P = 0.00000069). The frequency of the CA16 allele was 33% in CAD vs. 21% in control (odds ratio, OR, to have CAD for presence vs. absence of CA16 allele in CA16 homozygotes was 4.5, 95% CI 2.1–9.4, P < 0.0001; in CA16 heterozygotes OR was 1.3, 95% CI 0.94–1.89, P = 0.10). The frequency of the major allele (CA15) was 43% in CAD vs. 56% in controls (in CA15 homozygotes OR 0.33, 95% CI 0.20–0.52, P < 0.0001; in heterozygotes OR 0.41, 95% CI 0.26–0.63, P < 0.0001). In a stepwise logistic regression model the CA16 allele was significantly associated with overweight (OR 1.44, 95% CI 1.0–1.9, P = 0.027). Apolipoprotein A-I was elevated (P < 0.0001), as was high-density lipoprotein (P = 0.098), and severity of angina was decreased (P = 0.024) as a function of genotype. Plasma soluble (s) TNF-R2 was 5.1 ± 0.1 ng/ml in CAD vs. 3.2 ± 0.1 in control (P < 0.0001), 5.2 ± 0.1 in the presence vs. 4.6 ± 0.2 in the absence of vessel disease (P = 0.009), and rose with increasing severity of angina: 4.2 ± 0.2 (no angina), 5.0 ± 0.1 (stable angina), 5.4 ± 0.2 (unstable angina; P = 0.003). sTNF-R2 was correlated with age, cholesterol, creatinine, fibrinogen, transforming growth factor β and homocysteine and was influenced by TNFRSF1B genotype. Thus genetic variation in or near the TNFRSF1B locus may predispose to CAD.

Keywords  Tumor necrosis factor receptor · Tumor necrosis factor receptor superfamily member 1B gene · Coronary artery disease · Genetic polymorphism · Case-control study
Introduction

Tumor necrosis factor (TNF) is synthesized in the heart by myocardial macrophages and cardiac myocytes and is an autocrine contributor to cardiac dysfunction [1]. Elevated in TNF-α is seen in non-septic heart conditions such as coronary artery disease (CAD) [2], congestive heart failure [3], acute myocardial infarction, angina pectoris, ischaemia-reperfusion injury and cardiac hypertrophy [4, 5]. Left ventricular systolic and diastolic function are impaired [6], with the negative inotropic effect of TNF involving both nitric oxide dependent and independent (sphingosine kinase) mechanisms [3, 7]. The elevation in TNF-α in CAD could be from vascular damage or from infection by *Chlamydia pneumoniae* [8] or *Helicobacter pylori* [9].

The action of TNF involves two receptors – TNF-R1 and TNF-R2. TNF-R2 responds to TNF-α by undergoing a marked upregulation of its mRNA whereas there is little or no change in TNF-R1 mRNA [10, 11]. This is followed by rapid shedding of the N-terminal extracellular domain of 75 kDa, 415 residue, TNF-R2 by hydrolysis at amino acid 211 [12] to give 40-kDa plasma soluble (s) TNF-R2 [13]. The latter neutralizes TNF at high concentrations but, when low, preserves TNF activity, helps sequester TNF to its membrane receptors and therefore increases long-term effects [14]. TNF-R2, but not TNF-R1, is increased in ischaemic heart disease and peripheral vascular disease [15]. TNF and sTNF-R are both increased in congestive heart failure [3], and inhibition of TNF by recombinant sTNF-R2 has been used to advantage in treatment of such patients [16]. TNF-R2 has higher ligand affinity and faster dissociation, and may synergize with TNF-R1 to enhance the effects of the latter [17]. Enhancement by TNF-R2 of TNF-R1 promotes nuclear factor-κB activation and apoptosis [17]. TNF-R2 also has independent effects [18] that occur later, are of a long-term nature [10], and include cell proliferation [18]. In addition, TNF-R2 mediates the strong stimulation by the transmembrane (pro) form of TNF [19].

In recent studies involving a microsatellite marker in intron 4 of the TNF-R2 gene (TNFRSF1B) we have found an association with essential hypertension and elevation in cholesterol [20]. Others have since reported an association of this marker with familial combined hyperlipidaemia [21]. In addition, another TNFRSF1B marker has been associated with obesity, leptin and insulin resistance in type 2 diabetes [22]. The possibility that TNFRSF1B has a role in the genetic basis of CAD led us to conduct an association study involving well-characterized CAD patients.

Materials and methods

Study population

The study included 1006 white patients referred to the Eastern Heart Clinic at Prince of Wales Hospital, Sydney, for coronary angiogram, with a provisional diagnosis of CAD. Recruitment was confined to patients aged under 65 years. Each patient's medical history was obtained using a questionnaire with standardized choices of answers to be ticked during the interview, and DNA samples were collected for each patient as described previously [23]. The severity of CAD was determined by the number of significantly stenosed coronary arteries. Each angiogram was classified as revealing either normal coronary arteries or having no coronary lesion with at least 50% luminal stenosis or as having one, two, or three major epicardial coronary arteries with more than 50% luminal obstructions. The control group consisted of 183 healthy white subjects recruited from the Sydney Red Cross Blood Bank, who exclude subjects with any cardiovascular disease. Control group characteristics were: age, 48±10 years, 58% male, body mass index (BMI) 26±4, systolic/diastolic blood pressure 120±11/73±8 mmHg, total cholesterol 5.2±0.1 mmol/l, triglycerides 1.5±0.08 mmol/l, high-density lipoprotein (HDL) cholesterol 1.3±0.04 mmol/l, and low-density lipoprotein (LDL) cholesterol 3.2±0.08 mmol/l. All subjects were of British/northern European descent.

Genotyping

Leucocyte DNA was used for genotyping by PCR. Primers were: forward 5′-GTG ATC TGC AAG ATG AAC TCA C-3′ (HEX-labelled); reverse 5′-ACA CCA CGT CTG A TTG TTT CA-3′, synthesized by GeneWorks (Adelaide, South Australia). Each 25 µl PCR mix contained 100 ng genomic DNA, 5.5 mmol each primer, 0.25 mmol/l each dNTP, 1 U AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, CT, USA), 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.3, 1.7 mmol/l MgCl₂. After an initial step at 95°C for 12 min, PCR involved 10 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 12 min, followed by 15 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, then 20 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, finishing with a step at 72°C for 30 min. PCR products were electrophoresed on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA), and genotypes were assigned using ABI Genotyper software. Alleles were seen as PCR products of 263, 267, 269, 271, 273 and 275 bp. Since the 263-bp allele was rare (n=5, 0.2%), data for this were combined with those for the 267-bp allele, which it was most closely related to. The five alleles were termed CA13, CA14, CA15, CA16 and CA17 to indicate the number of CA repeats each possessed.

Plasma assays

Concentration of sTNF-R2 in plasma was measured using a MEDGENIX enzyme amplified sensitivity immunoassay (BioSource Europe, Fleurus, Belgium). Plasma concentrations of total cholesterol, triglyceride and HDL were measured by the Clinical Chemistry Department, Prince of Wales Hospital, with standard colorimetric methods. Concentrations of plasma apolipoprotein (apo) A-1, B and lipoprotein A [Lp(a)] were measured by in-house enzyme-linked immunosorbent assay as described previously [24, 25, 26]. An IMX autoanalyser was used for the determination of plasma homocysteine [27]. Concentration of total and active transforming growth factor (TGF) β1, and superoxide dismutase were measured by immunoassay as previously described [28, 29].

Abbreviations

ANOVA: Analysis of variance · apo: Apolipoprotein · BMI: Body mass index · CAD: Coronary artery disease · HDL: High density lipoprotein · LDL: Low-density lipoprotein · Lp(a): Lipoprotein A · OR: Odds ratios · sTNF-R: Plasma soluble TNF-R fragment · TGF: Transforming growth factor · TNF: Tumor necrosis factor · TNF-R: Tumor necrosis factor receptor