BASIC RESEARCH

Tumour necrosis factor α gene polymorphism: a predisposing factor to non-ischaemic myocardial dysfunction?

C G Densem, I V Hutchinson, N Yonan, N H Brooks

Heart 2002;87:153–155

See end of article for authors' affiliations

Correspondence to:
Dr C G Densem,
Department of Cardiology,
Wythenshawe Hospital,
Southmoor Road,
Wythenshawe, Manchester
M23 9LT, UK;
cameron.d@virgin.net

Accepted
19 September 2001

Background: Tumour necrosis factor α (TNF α) is implicated in the pathophysiology of heart failure. Plasma TNF α is raised in patients with myocardial dysfunction in proportion to the symptoms.

Objective: To determine whether this genetic variant is over represented in heart transplant recipients.

Patients: 175 heart transplant recipients and a control group of 212 healthy volunteers were studied. The reason for transplantation was severe symptomatic myocardial dysfunction in all cases.

Methods: The TNF α genotype was determined by polymerase chain reaction and gel electrophoresis. The populations were compared for their fit to Hardy–Weinberg equilibrium by calculating the expected frequencies of each genotype and comparing them to the observed values. A χ^2 test was used to determine the significance of the difference between the observed and expected values.

Results: No differences were found in the frequency of the TNF2 allele between all heart transplant recipients taken together (54/175, 31%) and healthy volunteers (58/212, 27%). A higher proportion of TNF2 allele carriers was present in cardiac recipients with a pretransplant diagnosis of viral mediated or idiopathic heart failure than in those with ischaemic myocardial dysfunction (26/69 (37.7%) v 28/106 (26.4%), $p = 0.03$). Patients with a non-ischaemic aetiology had a higher prevalence of TNF2 than healthy volunteers (26/69 (37.7%) v 58/212 (27%), $p = 0.05$).

Conclusions: The TNF2 allele is overrepresented in patients with end stage non-ischaemic myocardial dysfunction. This may represent a genetic predisposition in a small subset of patients who could respond favourably to anti-TNF α treatment.

In 1985, patients with septic shock were found to have a circulating myocardial depressant substance.¹ This was later found to be tumour necrosis factor α (TNF α).² TNF α has since been strongly implicated in the pathogenesis of heart failure. Circulating concentrations of TNF α are raised in heart failure patients, and there is a correlation between increasing concentrations and severity, symptoms, cardiac cachexia, and survival.^{3–4} Animal studies have shown that overexpression^{5–6} or chronic infusion^{7–8} of TNF α leads to myocarditis, systolic dysfunction, ventricular dilatation and hypertrophy, myocardial fibrosis, myocyte apoptosis, and increased mortality, all of which can be reversed by TNF α binding proteins.⁹ Early human clinical trials of TNF α inhibition suggest that an improvement in symptoms and exercise capacity,¹⁰ as well as an increase in ejection fraction,^{10–11} can be achieved.

Levels of TNF α after lymphocyte¹² or monocyte¹³ stimulation vary between healthy individuals. This is partly the result of a guanine to adenosine substitution polymorphism located at position –308 in the promoter region of the gene.¹⁴ The “TNF2” allele is a more powerful transcriptional activator than the common allele, with a six- to sevenfold increase in the inducible level of TNF α gene transcription.¹⁵ Cell stimulation studies have shown that the TNF2 allele is associated with greater TNF α production in vitro.¹⁶ The allele is also in strong linkage disequilibrium with other genetic determinants of TNF α production, for example HLA-DR3.¹²

We hypothesised that the TNF2 genetic variant would be overrepresented in patients with end stage heart failure who required a heart transplant.

METHODS

Patients

We studied recipients of human cardiac allografts transplanted between 1987 and 1996. The control group consisted of 212 unrelated healthy volunteers.¹⁷

Sample collection

Patients gave informed consent to the collection and storage of blood, isolation of DNA, and determination of the TNF2 polymorphism. Ethical approval was obtained from the South Manchester medical research ethics committee. Blood samples were mixed with EDTA and stored at –80°C before further processing. DNA or stored leucocytes from deceased patients were acquired from the tissue typing laboratory, Manchester Royal Infirmary.

Primer sequences

Specific oligonucleotide primers were designed for the TNF α –308 polymorphism, based on the published sequence (fig 1). Lyophilised oligonucleotide probes were manufactured by Genosys, UK. The primers were prepared to a working concentration of 50 mM by the addition of measured quantities of PCR grade water.

Common primer	ATA GGT TTT GAG GGG CAT GG
G primer	TCT CGG TTT CTT CTC CAT CG
A primer	AAT AGG TTT TGA GGG GCA TGA

Figure 1 Primer sequences for the TNF α –308 polymorphism.

Abbreviations: PCR, polymerase chain reaction; TNF, tumour necrosis factor

Table 1 Frequency of the TNF α -308 polymorphisms in the transplant recipient, transplant donor, and healthy unrelated populations

TNF α polymorph	Transplant recipients (n=175)	Healthy volunteers (n=212)
1	121 (69%)	154 (73%)
2	54 (31%)	58 (27%)

No significant differences between groups.

Table 2 Frequency of the TNF2 polymorphism in transplant recipients and a healthy unrelated population

TNF α polymorph	Non-ischaemic dysfunction (n=69)	Ischaemic dysfunction (n=106)	Healthy volunteers (n=212)
2	26 (37.7%)	28 (26.4%)†	58 (27%)*

*p=0.05; †p=0.03.

Polymerase chain reaction

Each polymerase chain reaction (PCR) mixture comprised (in relative quantities) 10 $\times$ reaction buffer ($\times$ 0.22), 2 mM dNTP mix ($\times$ 0.22), 25 mM MgCl₂ ($\times$ 0.13), 60% (wt/vol) sucrose ($\times$ 0.42), thermoprime DNA polymerase ($\times$ 0.011). A 50 μ l aliquot of each of the common and specific primers (G or A) was mixed with 150 μ l of PCR grade water. Equal volumes of the reaction and primer mixtures were placed in separate thin walled PCR tubes. One tenth this volume of test DNA was added before PCR.

A Techne Genius (Techne Cambridge, Duxford, UK) controlled the thermal cycles of 95°C for one minute followed by 95°C for 15 seconds, 65°C for 50 seconds, and 72°C for 40 seconds (10 cycles), and 95°C for 20 seconds, 61°C for 50 seconds, and 72°C for 50 seconds (20 cycles). A final holding temperature of 4°C was used.

Electrophoresis of PCR products

The genotype was identified by running the PCR products through 2% agarose gel containing 5 mg/100 ml ethidium bromide. The PCR product size was 184 base pairs. A and G homozygotes (high and low producers, respectively) have one product band only in their respective lanes; GA heterozygotes (high producer) have a band in each lane.

Statistical analysis

The data were tested for their fit to the Hardy-Weinberg equilibrium by calculating the expected frequencies of each genotype and comparing them to the observed values. A χ^2 test was used to determine the significance of the difference between the observed and expected values.

RESULTS

No differences were found in the frequency of the TNF2 allele between all heart transplant recipients taken together—regardless of disease aetiology (54/175, 31%)—and healthy volunteers (58/212, 27%) (table 1). Carriers of the TNF2 allele were overrepresented in heart transplant recipients with a pretransplant diagnosis of viral mediated or idiopathic myocardial dysfunction (that is, non-ischaemic), in comparison with those with ischaemic myocardial dysfunction (26/69 (37.7%) *v* 28/106 (26.4%), *p* = 0.03) and healthy unrelated volunteers (26/69 (37.7%) *v* 58/212 (27%), *p* = 0.05) (table 2). The high producing TNF allele was therefore equally prevalent between patients with pretransplant ischaemic heart disease and the control cohort (26.4% *v* 27%, NS).

DISCUSSION

TNF α has been strongly implicated in the pathogenesis of heart failure.³⁻¹¹ In this study we showed that the TNF2 allele, which is associated with a six- to sevenfold increase in the inducible level of TNF α gene transcription,¹⁵ was more prevalent in transplant recipients with a preoperative diagnosis of non-ischaemic myocardial dysfunction. This finding suggests that a subset of individuals could have a genetic predisposition to end stage heart failure requiring transplantation. These patients may develop left ventricular dysfunction through a TNF α dependent pathway triggered, for example, by a viral infection. Our results are supported by a Japanese study of idiopathic dilated cardiomyopathy,¹⁸ in which the frequency of TNF2 in affected patients was significantly higher than in a healthy control group. In a non-transplant population, however, the polymorphism was not associated with the presence of heart failure,¹⁹ but this might reflect the select nature of our transplant cohort.

The development of virus induced autoimmune myocarditis is associated with local production and secretion of TNF α .²⁰ Administration of TNF α can promote coxsackievirus B3 myocarditis in genetically resistant B10A mouse models.²¹ In a mouse model of encephalomyocarditis virus infection, exogenous recombinant TNF α increased the virus concentration in the myocardium, produced myocardial necrosis and cellular infiltration, and exacerbated the myocarditis. TNF α antibodies improved survival and the myocardial lesions.²² Murine models of cardiac myosin induced myocarditis closely resemble the chronic stages of virus induced myocarditis in humans. Antibodies against TNF α also had a beneficial effect in this condition.²³ Raised serum TNF α was found in 46% of patients presenting with viral myocarditis, compared with 35% with dilated cardiomyopathy.²⁴

Possible mechanisms of TNF α mediated heart failure include reduction in intracellular calcium,²⁵ desensitisation of cardiac myocytes to calcium, interference with nitric oxide synthase function, activation of metalloproteinases, direct negative inotropic effects,²⁶ cardiomyocyte apoptosis,²⁷ or increased production of superoxide anions.²⁸ Cardiac myocytes appear to be the main source of TNF α .⁵ Alternatively, raised TNF α may be secondary to heart failure and not a causative factor. TNF α concentrations may reflect a common pathophysiological background resulting from peripheral hypoperfusion and ischaemia²⁹ or increased right atrial pressure resulting in splanchnic congestion. This may lead to increased bowel permeability with consequent bacterial translocation, endotoxin release, and increased systemic TNF α production.²⁹ Also, increasing myocardial wall stress could elicit TNF α expression.

The findings of this study could have practical implications. If a subgroup of patients do have TNF α mediated heart failure then they may respond to anti-TNF α therapy. Treatment with TNF α binding protein prevents and reverses the negative inotropic effects in vitro.¹⁵ An adenovirus encoding the 55 kDa TNF α receptor-IgG fusion protein improved left ventricular size and myocardial inflammation in TNF α overexpressor mice.³⁰ Clinical trials of TNF α inhibition in humans using pentoxifylline¹¹ or TNF α receptor (p75) fusion protein¹⁰ suggest an improvement of symptoms and exercise capacity, an increase in ejection fraction, and a reduction in ventricular size and mass.¹⁰ TNF α , however, is an auto/para/juxtacrine agent with biological activity determined by the target cell type as well as by the intracellular milieu. Results obtained with systemic administration of a cytokine inhibitor must be interpreted with this in mind.

Conclusions

We have found that the TNF2 allele, which confers increased TNF α production, is over represented in patients with end stage non-ischaemic myocardial dysfunction. This may represent a genetic predisposition in a small subset of patients who could respond favourably to anti-TNF α treatment.

ACKNOWLEDGEMENT

CGD was supported by a grant from the National Heart Research Fund. The study was funded by the Wythenshawe Cardiology Research Fund and the New Heart, New Start appeal. Many thanks to Dr Steve Sheldon, St Mary's Tissue Typing Laboratory, for supply of some DNA and leucocyte samples.

Authors' affiliations

C G Densem, N Yonan, N H Brooks, Cardiothoracic Transplant Unit, Wythenshawe Hospital, Manchester, UK
I V Hutchinson, School of Biological Sciences, Manchester University, UK

REFERENCES

- Parrillo JE, Burch C, Shelhamer JH, *et al*. A circulating myocardial depressant substance in humans with septic shock. *J Clin Invest* 1985;**76**:1539–53.
- Michie HR, Manogue KR, Spriggs DR, *et al*. Detection of circulating tumour necrosis factor after endotoxin administration. *N Engl J Med* 1988;**318**:1481–6.
- Levine B, Kalman J, Mayer L, *et al*. Elevated circulating levels of tumour necrosis factor in severe chronic heart failure. *N Engl J Med* 1990;**323**:236–41.
- McMurray J, Abdullah I, Dargie HJ, *et al*. Increased concentrations of tumour necrosis factor in "cachectic" patients with severe chronic heart failure. *Br Heart J* 1991;**66**:356–8.
- Bryant D, Becker L, Richardson J, *et al*. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor- α . *Circulation* 1998;**97**:1375–81.
- Kubota T, McTiernan CF, Frye CS, *et al*. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- α . *Circ Res* 1997;**81**:627–35.
- Bozkurt B, Kribbs SB, Clubb FJ, *et al*. Pathophysiologically relevant concentrations of tumor necrosis factor- α promote progressive left ventricular dysfunction and remodeling in rats. *Circulation* 1998;**97**:1382–91.
- Murray DR, Freeman GL. Tumor necrosis factor- α induces a biphasic effect on myocardial contractility in conscious dogs. *Circ Res* 1996;**78**:154–160.
- Kapadia S, Torre-Amione G, Yokoyama T, *et al*. Soluble TNF binding proteins modulate the negative inotropic properties of TNF- α in vitro. *Am J Physiol* 1995;**268**:H517–25.
- Bozkurt B, Torre-Amione G, Warren MS, *et al*. Results of targeted anti-tumor necrosis factor therapy with etanercept (ENBREL) in patients with advanced heart failure. *Circulation* 2001;**103**:1044–7.
- Sliwa K, Kudicky D, Candy G, *et al*. Randomised investigation of effects of pentoxifylline on left-ventricular performance in idiopathic dilated cardiomyopathy. *Lancet* 1998;**351**:1091–3.
- Jacob CE, Fronek Z, Lewis GD, *et al*. Heritable major histocompatibility complex class II associated differences in production of tumor necrosis factor α : relevance to genetic predisposition to systemic lupus erythematosus. *Proc Natl Acad Sci USA* 1990;**87**:1233–7.
- Molvig J, Baek L, Christensen P, *et al*. Endotoxin-stimulated human monocyte secretion of interleukin 1, tumour necrosis factor alpha, and prostaglandin E₂ shows interindividual differences. *Scand J Immunol* 1988;**27**:705–16.
- Wilson AG, di Giovine FS, Blakemore AIF, *et al*. Single base polymorphism in the human tumour necrosis factor alpha (TNF α) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1992;**1**:353.
- Wilson AG, Symons JA, McDowell TL, *et al*. Effects of a polymorphism in the human tumour necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997;**94**:3195–9.
- Louis E, Franchimont D, Piron A, *et al*. Tumour necrosis factor (TNF) gene polymorphism influences TNF- α production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998;**113**:401–6.
- Perry C, Pravica V, Sinnott PJ, *et al*. Genotyping for polymorphisms in interferon- γ , interleukin-10, transforming growth factor- β 1 and tumour necrosis factor- α genes: a technical report. *Transplant Immunol* 1998;**6**:193–7.
- Ito M, Takahashi H, Fuse K, *et al*. Polymorphisms of tumor necrosis factor- α and interleukin-10 genes in Japanese patients with idiopathic dilated cardiomyopathy. *Jpn Heart J* 2000;**41**:183–91.
- Kubota T, McNamara DM, McTiernan CF, *et al*. Effects of tumour necrosis factor gene polymorphisms on patients with congestive heart failure. *Circulation* 1998;**97**:2499–501.
- Lane JR, Neumann DA, Lafond Walker A, *et al*. Role of IL1 and tumor necrosis factor in coxsackie virus-induced autoimmune myocarditis. *J Immunol* 1993;**151**:1682–90.
- Lane JR, Neumann DA, Lafond Walker A, *et al*. Interleukin 1 or tumour necrosis factor can promote coxsackie B3-induced myocarditis in resistant B10.A mice. *J Exp Med* 1992;**175**:1123–9.
- Yamada T, Matsumori A, Sasayama S. Therapeutic effect of anti-tumor necrosis factor alpha antibody on the murine model of viral myocarditis induced by encephalomyocarditis virus. *Circulation* 1994;**89**:846–51.
- Smith SC, Allen PM. Neutralisation of endogenous tumor necrosis factor ameliorates the severity of myosin induced myocarditis. *Circ Res* 1992;**70**:856–63.
- Matsumori A, Yamada T, Suzuki H, *et al*. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. *Br Heart J* 1994;**72**:561–6.
- Yokoyama T, Vaca L, Rossen RD, *et al*. Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. *J Clin Invest* 1993;**92**:2302–12.
- Finkel MS, Oddis CV, Jacob TD, *et al*. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992;**257**:387–9.
- Krown KA, Page MT, Nguyen C, *et al*. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes: involvement of the sphingolipid signalling cascade in cardiac cell death. *J Clin Invest* 1996;**98**:2854–65.
- Matsubara T, Tiff M. Increased superoxide anion release from human endothelial cells in response to cytokines. *J Immunol* 1986;**137**:3295–8.
- Anker SD, Egerer KR, Volk H, *et al*. Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. *Am J Cardiol* 1997;**79**:1426–30.
- Kubota T, Bounoutas GS, Miyagishima M, *et al*. Soluble tumor necrosis factor receptor abrogates myocardial inflammation but not hypertrophy in cytokine-induced cardiomyopathy. *Circulation* 2000;**101**:2518–25.