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Genetic Diversity of Tumour Necrosis Factor: Implications on Cardiovascular Complications of Polymorphisms at Position –308 In The Promoter Region

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Abstract: Recently, it has been reported that a genetic background may play a role in plasma cytokine levels induced by several conditions ranging from psoriasis, eclempsia, sepsis and post-operative complications. Among these, is the gene for tumor necrosis factor-alpha (TNF-"), a cytokine known to exert a range of inflammatory and immunomodulatory activities important in the host defence mechanisms. Indeed, TNF-" has been implicated in the pathogenesis of several conditions including cardiovascular, eclempsia, psoriasis, rheumatoid arthritis (RA), septic shock and myocardial dysfunction. Currently, the focus is on the mechanisms that modulate TNF-" production, which in turn impact on the disease-mediated inflammatory process. Various polymorphisms have been identified within and around the TNF-"-encoding gene located within the major histocompatibility complex (MHC). In the promoter region relative to the transcription start site, there are several single nucleotide polymorphisms (SNPs), at positions -1031 (T6C), -863 (C6A), -857 (C6A), -851 (C6T), -419 (G6C), -376 (G6A), -308 (G6A), -238 (G6A), -162 (G6A) and -49 (G6A). However, those at positions -419, -163, -49, are rare in Caucasians. This review has highlighted the conflicting results among various publications on the associations between –308 TNF SNPs and TNF production. In addition, we have specifically reviewed the association between genotype distribution and allele frequencies of TNF *NcoI* gene polymorphism at the –308 positions and the pathophysiologic changes induced by coronary heart disease.

Key words: Reactive oxidant species, cardiac surgery, single nucleotide polymorphism

INTRODUCTION

Tumour Necrosis Factor-alpha (TNF-"), a pleotropic cytokine produced mainly by macrophages and T-cells, is involved in cellular, inflammatory and immune reactions important in the host defence[1]. The over-expression of TNF-" has been implicated in the pathogenesis of several conditions including cardiovascular disease, rheumatoid arthritis (RA) and sepsis. The production of TNF-" at sites of inflammation has readily been demonstrated by immunohistochemistry (for the protein) and by *in situ* hybridisation (for mRNA). Circulating TNF-" levels are regulated at different stages, i.e., at gene transcription, post-transcription control of mRNA stability, cleavage of the membrane form to liberate the soluble form and the expression of receptors[2]. It mediates its functions by binding to TNF receptors (TNFRs) of which TNFR-2 has a higher affinity and seems to bind TNF-" better at lower concentrations. Signals through TNFRs influence T cell proliferation and proinflammatory responses[3] which are shed from the cell surface in a soluble form[4], thus adding another level to the regulation of TNF-" function. Soluble TNFRs neutralize TNF-" activity by competing with cell-bound receptors, but, at the same time, they stabilize the TNF-" molecule and prevent its degradation[5].

Recently, focus is on the mechanisms that modulate TNF-" production, during the disease process have gained attention. In general, an increased TNF-" level strongly correlates with the occurrence of the inflammatory responses. However, it is still unknown whether there is a relationship between TNF-" production and the severity of inflammatory conditions[2]. Several polymorphisms have been identified within the TNF-" encoding gene located within the major histocompatibility complex (MHC)[6]. Similarly, mutations in the TNFR-1 gene coding for the extracellular domain are known to be linked to several dominantly inherited auto-inflammatory syndromes. These mutations cause decreased shedding and increased cell surface expression of the TNFR-1[7].

As will be evident from the following review, there are many questions that need to be addressed regarding the frequency of the TNF polymorphism particularly at the positions –308 of the Tumour Necrosis Factor promoter region and whether this has any biological and disease implication.

Chromosomal location of the TNF-alpha gene: The TNF-alpha gene is on the human chromosome 6p21.3 and is tandemly arranged with the TNF-" gene. Both these genes lie in the so-called class III region, between the genes encoding the MHC class II, human leukocyte

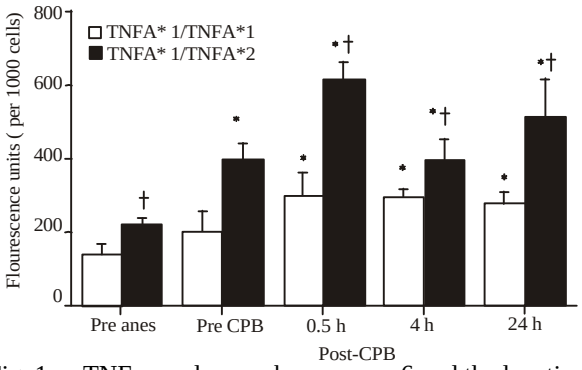

Fig. 1: TNF complex on chromosome 6 and the location of microsatellites and SNPs[2]

Table 1: Summary of the microsatellite markers close to the TNF-" gene

Microsatellite	TNFa	TNFb	TNFc	TNFd	TNFe
Repeat sequence	$(GT)_n$	$(GA)_n$	$(GA)_n$	$(GA)_n$	$(GA)_n$-like
Number of alleles	14	7	2	7	3
Number of repeats	99-125	125-131	159-161	124-136	98-102

antigen (HLA) class II cell surface molecules HLA-DP, DQ and DR and the MHC class I antigens, HLA-A, B and C (Fig. 1). The whole of the MHC occupies a mere 4,000 kilobase (kb) of DNA, while the distance between the HLA-DR and HLA-A genes is only 2,000 kb. These relatively short distances, perhaps accompanied by mechanisms that reduce genetic recombination, mean that all the genes on one strand of DNA in this interval tend to be inherited *en bloc* as a haplotype. This is referred to as genetic linkage disequilibrium implying that the genes in this region do not segregate independently or randomly assort. Genetic linkage disequilibrium leads to the appearance of extended haplotypes, such as HLA-A1, B8 and DR3 haplotypes associated with autoimmune disorders including Systemic Lupus Erythematosus (SLE) in the Caucasian population[6]. The inheritance of the TNF gene and indeed, certain genetic variants of the TNF gene occur in disequilibria with other genes in this relatively small region of the genome. Of particular relevance are the linkage disequilibria between the TNF microsatellite markers and the HLA genes.

TNF polymorphic microsatellite markers: So far, six TNF polymorphic microsatellites (a-f) have been described[8,9] with the original five polymorphic microsatellites, TNFa-e being studied extensively for disease associations in different populations. TNFa, b and d are multiallelic, highly polymorphic markers, while TNFc and TNFe are biallelic and triallelic, respectively (Table 1)[8].

TNF Single Nucleotide Polymorphisms (SNPs): There are many SNPs within the TNF gene including the promoter region relative to the transcription start site at positions: -1031 (T6C), -863 (C6A), -857 (C6A), -851 (C6T), -419 (G6C), -376 (G6A), -308 (G6A), -238 (G6A), -163 (G6A) and -49 (G6A), although those at positions -419, -162, -49 are rare in Caucasians[10]. This suggests that the 56 region of the TNF-" gene is highly polymorphic. In addition, there is an insertion of a cytosine at position +70 in the first exon 10, a G > A substitution at position +488 in the first intron 10 and a deletion of a guanine at position +691 in the first intron 11 of the TNF-" gene.

In contrast, the 36 region of the TNF-" gene appears to be highly conserved. Waldron-Lynch and co-workers[11] studied the TNF 3' Un-Translated Region (UTR) in Rheumatoid Arthritis (RA) patients and controls. The authors analyzed >800 bp of the 3' UTR of the TNF gene using secondary structure content prediction (SSCP) and by gene sequencing in 38 subjects with or without RA. In the study no polymorphisms were detected at the 3'-UTR, an observation that was in agreement with that of Becker *et al* [12] who investigated TNF-" gene polymorphisms in patients with connective tissue disease or type I diabetes. Interestingly, linkage disequilibria exist between SNPs at positions –1031 with –863 and–376 with –238. In addition, the polymorphism at position –308 in the TNF-" gene was linked to a polymorphism at codon 26 in the adjacent TNF-" (LT-") gene[13].

Evidence of an association between HLA-DR Genes and TNF-" production: Several studies have shown an association between HLA DRB1 alleles and the *in vitro* production of TNF-" with HLA DR3, DR1, DR4 and DR7 associated with higher TNF-" production[14-17] whilst DR2 and DR5 were associated with lower TNF-" responses[14-18] Linkage disequilibrium between TNF-" polymorphisms and HLA-DR types probably explains this phenomenon, although other explanations cannot be ruled out. For example, a gene close to the DR locus and in linkage disequilibrium with DR alleles may control TNF-" gene transcription.

Associations between TNF-" Microsatellites and TNF-" production: A review of the literature has shown that there are conflicting views about the associations between TNF microsatellites and TNF production *in vitro*. Pocoit *et al*,[16]have reported that both TNFa2 and c2 were associated with high TNF-" production while TNFa6 and c1 were associated with low TNF-" production. In contrast, Derkx *et al*,[18] showed that TNFa2, a6, a10 were associated with lower TNF-" production whilst a4 and a11 were associated with higher TNF-" production. Although there did appear to be a relationship between TNF alleles and TNF-" production, the relationship was not a simple one. Despite the overall association between the TNFc1

allele and low TNF-" production, the extended haplotype found in the autoimmune conditions, HLA-DR3 (HLA DRB1* 0301), TNFa2, b1, c1, HLA-A1, B8 is usually associated with higher TNF-" production *in vitro*[18]. Furthermore, the microsatellite markers TNFa2 (associated with high and low TNF-" production in the studies by both Derkx *et al.*,[18]and Pociot *et al*[16], respectively) and TNFa6 (associated with low TNF-" production in the study by Derkx *et al*) are linked with HLA-DR4. Nevertheless, the HLA-DR4 extended haplotype DRB1*0401, TNFa2, b1, c1, HLA-B62 are associated with high TNF-" production while another HLA-DR4 extended haplotype, DRB1*0401, TNFa6, b5, c1, HLA-B44 is mostly associated with low TNF-" production. The findings from the two studies that used different methodologies indicate that another polymorphism within these extended haplotypes is likely to influence TNF-" production rather than the microsatellite sequences per se. Thus, as for the HLA-DR associations, the relationship between microsatellite markers and TNF-" production is probably due to linkage disequilibrium.

Other investigators such as Turner *et al*,[19] have suggested that TNFd3 was associated with higher TNF-" production when using a model of endotoxin stimulated whole blood. However, different stimuli might interact with different regions of the gene promoter. Moreover, certain regions of the promoter might be differentially regulated in different cell types, although, in a recent report, Yaqoob *et al*, [20] suggested that both whole blood cultures and Mononuclear Cell (MNC) cultures correlated very well in the same individual for the production of TNF-" *in vitro*. However, a differential count of the blood cells used in such studies may be helpful in identifying the odd sample with an unusual proportion of polymorphonuclear cells (PMNCs) to mononuclear cells (MNCs).

Association between SNPs and TNF-" production: Single nucleotide polymorphisms within the TNF-" gene itself are more likely to be of direct functional significance in terms of regulating TNF-" production as there are many SNPs within the TNF-" gene promoter. Many studies have concentrated on one SNP in isolation of others. In particular, there is interest in those polymorphic sites in the regulatory regions of the TNF-" gene that coincide with the DNA motifs to which transcription factors bind. Typical of these is the -308 G/A SNP that has been the most studied polymorphism. *In vitro* stimulation of TNF-" production by cells from 6308*G/G homozygous individuals and G/A heterozygote individuals has produced conflicting results. Two studies have reported higher TNF-" production by cells from G/A donors than by G/G cells[21,22], whilst other studies have reported no significant affect[23-25] However, it is interesting to note that these studies used different LPS concentrations and the number of individuals with the G/A genotype studied was in most cases small, affecting the power of the study to detect any significant difference between the genotypes.

Gene reporter assays have been employed to investigate the 6 308 SNP and again, different results have been reported. Three studies suggested that the A allele has an influence on TNF-" gene transcription[26,27]while three other studies concluded that it does not[28,29]. There are many variables affecting the results of this type of experiment; including the length of the promoter sequence used, the presence or absence of the 3'-UTR, the cell type used for transfection and whether it is of human or non-human origin. Different studies have used different approaches, thus making it difficult to draw a general conclusion, although in general circulating TNF-" levels do not seem to correspond with the 6 308 TNF promoter polymorphisms. This may suggest that circulating TNF-" levels might be under a multi-factorial regulatory process and that specific polymorphisms might exert greater control and be of greater importance to local TNF-" concentration[30] In fact, linkage disequilibrium is strong in this area and it may be difficult to study the role of an SNP in isolation. In some populations the -376*A allele is in allelic association with the ~308*G and -238*A alleles[31]. Whilst this seems true in Caucasians, a study of Africans from the Gambia failed to show this allelic association.

Functional studies of the TNF polymorphism at position 6863 (C>A) revealed that this site binds NF-6B, both p65-p50 and p50-p50 dimers. The base substitution at this position inhibits p50-p50 binding and this may reduce the enhancer effect of NF-êB on TNF-" gene activation[32] Polymorphisms within the 36 UTR may be of importance in TNF-" gene regulation; a deletion of the 36 UTR of TNF-" in the mouse led to abnormally stable mRNA, with TNF-" being synthesized by cells that normally do not produce TNF-"[33]

An analysis of the extended MHC haplotypes that include the TNF region has been presented. Recently it was proposed that the modern complex TNF haplotypes were derived from three ancient extended MHC haplotypes through a process of mutation or crossover[34].

The –308*G, -238*G alleles were found to be associated with low TNF-" production while the –308* A and the –238*A alleles were associated with high TNF-" production. [34] Indeed, the evidence suggested that the regulations of TNF-" production may not be controlled by the upstream promoter sequences alone. Functional

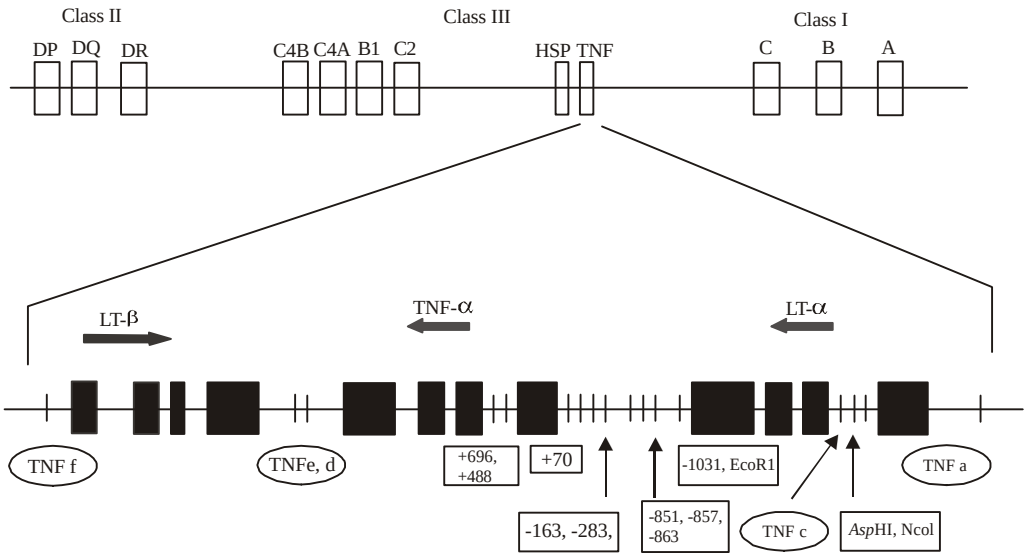

Fig. 1: TNF genotypes versus levels of oxidative stress at different time intervals (n=95). The data is represented as mean± SEM. *p<0.05 versus Pre-anaesthesia value; †P<0.05 versus corresponding TNFA*1/TNFA*1 genotypes

analysis data however, supports the hypothesis that TNF-" regulation is controlled by DNA sequences outwards of the gene, an idea that may explain the associations between TNF haplotypes with higher or lower TNF-" production in vitro. This implies that sequences controlling TNF-" production may not be within the MHC at all. In fact, a recent study on the loci running the opposite direction to the MHC genotype suggests that this appears to influence only the production of IFN-$[35] It has also been proposed that polymorphic *cis*-acting regulatory factors, occurring on chromosome 6p distinct from the TNF genes but in linkage disequilibrium with them plays a role in the regulation of the TNF-" gene. In this way, some of the polymorphisms in the TNF gene region could indirectly act as the genetic controls for TNF-" production.

TNF polymorphisms on cardiovascular complications and operative morbidity: Features such as septic shock and myocardial dysfunction in patients undergoing cardiac operations frequently require inotropic support or intra-aortic balloon pump, all shown to be associated with an increased TNF-" production[36]. Its administration reproduces all the deleterious effects of endotoxin and bacteria, including hypotension, activation of the coagulation cascade and organ dysfunction[37]. Despite this strong evidence for a causal relationship between TNF-" and septic shock, the mechanism by which TNF-" may contribute to the pathophysiology of other disease conditions such as heart failure remains unknown. It has recently been reported that a genetic background may play a role in influencing the cytokine plasma levels induced by surgery[38]. Indeed, previous work demonstrated that TNF-" plasma levels significantly increased after the first 24-hour perioperative period in patients undergoing Cardiopulmonary bypass Operations (CPB)[39]. The release of reactive oxygen species by blood leukocytes in the extracorporeal circuits stimulates the expression of a variety of genes including inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-"), interleukin-8 and 6. Although, the extracorporeal circuits during CPB are standard, the overall effect in terms of the induction of the inflammatory reaction in patients is diverse, despite the fact that a genetic association has yet to be demonstrated. Previous studies have shown that the variation in the gene promoter for TNF-" is associated with increased TNF-" production in various pathological conditions although the mechanisms are not known. In view of these observations, we evaluated the genotype distribution and allele frequency of the TNF *NcoI* gene polymorphism at –308 positions with regards to levels of the oxidative stress and incidence of complications in patients undergoing on-pump coronary artery bypass graft surgery. The study was approved by our local ethics committee and 95 patients elective for cardiac operations gave informed written consent.

Blood samples were collected before the induction of general anaesthesia, before the start of cardiopulmonary bypass (CPB) or extracorporeal circulation, 30 min, 4 h and 24 h after the initiation of CPB. Cellular oxidative burst

activity was determined using a whole blood assay as described previously[40,41] Briefly, heparinized whole blood (100 µL) was incubated in a 37°C shaking water bath incubator for 30 min with 10 µL of 750 ìmol LG¹ 2,7 dihydrorhodamine. 2,7-dihydrorhodamine passively enters the blood leukocytes and converts to fluorescent rhodamine when exposed to Reactive Oxygen Species (ROS). Leukocytes were separated from whole blood by Ficoll-Hypaque density centrifugation and subsequent lysing of residual red blood cells. The leukocytes were fixed in Coulter Immunoprep Epics Leukocyte preparation system (Multi-Q-Prep; Coulter Miami, FL). Fluorescence measurements were performed by an optical flow cytometer (Becton-Dickinson, UK) (22) using a 15-mW argon ion laser with an excitation filter of 488-nm and emission band filter of 525 nm. Forward versus side scatter plots were used to gate for monocytes electronically. Fluorescence intensity was determined on a log scale from 1 to 10000 by 5000 acquired events using Becton Dickinson software, version 1.0. Results were expressed as mean ± SEM fluorescence[42].The oxidative stress levels before and after CPB surgery was comparable between the patients. However, in assigning patients to different TNF gene polymorphisms significantly higher oxidative stress was observed in patients heterozygous for the TNFA*2 allele compared with patients homozygous for TNFA*1 was observed (Fig. 2).

The complete sequence of the human TNF- gene promoter from –511 to +11 was carried out as described previously[10]. The amplification of the 5'-untranslated region, a 107-base pair fragment (position –327 to –220) of the TNF-" promoter was carried out by PCR using primer sets A1 (sense) and A2 (antisense) described previously[27] The A1 primer has been designed to incorporate the TNFA polymorphic site –308 into an *N*coI restriction sequence. Genomic DNA was obtained from 10 mL of EDTA-anticoagulated blood using the TRIzol method (Life Technologies Ltd, Paisley, UK). Amplification of the 5'-UTR of the TNF-" gene by PCR using A1 primer (5'-AGGCAATAGGTTTTGAGGGCCAT-3') and A2 primer (5'-TCCTCCCTGCTCCGATTCCG-3') was carried out in 0.2 mL tubes containing 1-5 µL digest, 60 mmol LG¹ Tris HCl, 15 mmol LG¹ ammonium sulphate (Invitrogen Corporation, Carlsbad, CA), 0.2 mmol LG¹ of each deoxynucleoside triphosphate, 1 µL of each primer and 1 unit Taq polymerase. Cycling was as follows: 95^0C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 60^0C for 30 seconds and 72°C for 30 seconds. The PCR products were digested overnight at 37°C with *Nco*I at 4 U/20 µL reaction. Restriction patterns were visualised under UV light after electrophoresis (150 V for 2.5 h) through 2% agarose gel. *Nco*I digestion of PCR-amplified genomic DNA produced 20-base pair and 87-base pair fragments corresponding to the TNF*1 allele and a single 107-base pair fragment of TNF*2 allele.

Table 2: Tumour Necrosis Factor Genotypes and Allelic Frequencies within the study group (n = 95)

Genotypes		Allele Frequencies	
TNFA*1/TNFA*1	TNFA*1/TNFA*2	TNFA*1	TNFA*2
60 (63.2%)	35 (36.8%)	155 (81.6%)	35 (18.4%)

Table 3: Binary logistic regression analysis of known surgical risk factors with postoperative complications

Variable	OR	95% CI for OR	P-value
Hypercholesteremia	0.90	0.86-1.00	0.010
Preoperative MI	1.02	1.01-1.03	0.003
Hypertension	1.01	0.99-1.02	0.621
Diabetes Mellitus	1.03	1.00- 1.06	0.027
Gender	0.43	0.19- 0.93	0.032
Angina Status	1.02	1.01- 1.03	0.031
TNFA*1/TNF*2	2.72	1.30-5.70	0.0007

OR=Odds ratio; CI= confidence intervals; MI=myocardial infarctions

Nucleotide substitution at positions –308 (from guanine to adenine) of TNF gene promoter results in the loss of the *Nco*I restriction site in the TNF*2 allele, this gives a 107-base pair fragment. *Nco-I* digestion of the amplified polymerase chain reaction product revealed homozygosity for the allele TNFA*1 in 63.2% of the patients (60 of 95) versus 36.8% heterozygous patients (35 of 95) for the allele TNFA*2. Allelic frequencies for homozygous TNFA*1 and heterozygous TNFA*2 were 81.6% and 18.4%, respectively with no homozygous TNF*2 alleles detected (Table 2).

Logistic regression analysis was used to determine the relationship between the TNFA*2 genotype and the occurrence of the postoperative complications as dependent variables with binary outcome (Table 3). The results showed that there was an association between TNFA*2 genotype with morbidity as well as the occurrence of increased oxidative stress at 30 minutes after the initiation of CPB that remained elevated for up to 24 h later.

Biological regulation of TNF-" production and function: There are many biological steps, apart from the influence of the gene polymorphisms on TNF-" production, for which the control, production and release of TNF-" and its activity are regulated. These include the stability of mRNA and direct feedback inhibition of TNF-" production.

Stability of messenger RNA: The translation of the TNF-" transcript depends on the stability of the mRNA. In mice, the 3' end of the mRNA molecule plays an important role in the stability of the TNF-" transcript[43] with deletion of this region of the TNF-" gene leading to abnormally stable transcripts.

Table 4: Evidence of strong correlations between TNF-" Polymorphism and disease outcomes

Study	Study Design	Population	Results	Conclusion
Mira JP *et al*., 1999[49]	Case control	89 septic patients and 87 healthy controls	Increased TNF2 allele in patients, P =0.002, reduced survival with increased TNF2 production (P=0.008)	TNF2 is allele associated with susceptibility to septic shock and death.
McGuire W *et al.*, 1994[50]	Case control	Gambian children with malaria	TNF2/TNF2, R/R 7 for death and neurological	Disease association is independent of HLA class sequeleI and II. TNF2/TNF2 at frequency of 0.16 in the Gambia implies an increased risk of malaria.
Yee LJ *et al*., 2000[51]	Case control	30 chronic hepatitis C (HCV) Caucasians with cirrhosis, 114 HCV - infected but free of cirrhosis	TNF2 at variant -308A conferred a 5.1-fold risk of cirrhosis (P = 0.03)	Polymorphisms in the TNF-" promoter is associated with variability in the histological severity of chronic HCV
Rood MJ *et al*., 2000[52]	Case control	99 Caucasian systemic lupus erythamatosis (SLE) patients177 Caucasian controls	Increased TNF-308A/A &-308G/A in SLE (odds ratio 5.0)	TNF-308A alleles are independent susceptibility factors for SLE
Hajeer AH *et al*., 2000[53]	Case control	179 Rheumatoid Arthritis (RA) patients; 145 controls	Increased risk of RA in rheumatoid arthritis Shared Epitope (SE) negative and SE-heterozygous with carriage TNF1 allele and were not associated with erosive or seropositive disease. Increased TNFa2 with erosive disease was independent of SE.	TNF1 polymorphism associated with reduced TNF-" production and increased risk factor for RA
Rudwaleit M *et al*., 2001[54]	Case control	PBMC from 25 HLA -B27 positive patients with active ankylosing HLA-B27 positive controls and 22 healthy HLA -B27 positive controls	Increased TNF-" was related to the genotype of the TNF- " promoter at the -308 polymorphisms (p=0.005) spondylitis 18 healthy	HLA-B27 positive subjects TNF2 at-308 or a linked gene results in higher TNF-" production
Monos DS *et al*., 1995[55]	Case control	48 Insulin Dependent Diabetes Mellitus (IDDM) Caucasian and 97 controls	Increased TNFa1b5 in IDDM (P<0.0005) DR3-B8 and DR7	TNFa2b3 and TNFa7b4 were in linkage disequilibrium with
Azzawi M *et al*., 2001[56]	Clinical study	119 heart transplant	Acute cellular rejecters were positive (heterozygous) for increased TNF-" producer allele (6308*A)(P < 0.0001)	Determination of TNF-" genotype in heart transplant recipients may be useful to select the optimal immunosuppression
Poli F *et al*., 2000[57]	clinical study	169 kidney recipients	TNF1/TNF1 gives a low TNF- ", TNF1/TNF 2 and TNF2/TNF2 gives a high TNF-"	TNFA polymorphism, is related to the clinical outcome of kidney transplantation
Sahoo S *et al*, 2000[58]	clinical study	45 renal transplant	26 recipients were *N*coI-positive produced low TNF- " whilst 19 *NcoI*- negative recipients had high TNF-". *N*coI- positive recipients producing low TNF-" had increased infection	Lower doses of immunosuppressant may benefit from inhibitors of TNF- " transcription

Feedback Inhibition of TNF-" Production: TNF-" protein itself can suppress the production of more TNF-". This effect is mediated through the TNFR-1 and TNFR-2 cell surface receptors for TNF-". Cells from mice deficient for TNFR-1 or TNFR-2 produce substantially more TNF-" upon stimulation[44]. Although TNF-" may be produced by many cell types macrophages are the main source of this cytokine[1]. The enzyme involved in the enzymatic cleavage of TNF-" molecule is a metalloproteinase disintegrin called TNF-" Converting Enzyme (TACE). Remarkably, TACE also acts on membrane anchored TNFR-2 protein thus controlling the amount of soluble circulating TNFR-2. This adds another level to the regulation of TNF-" function because soluble TNF receptor affects the activity of TNF-".

Table-5: No correlation was reported between TNF-" Polymorphism and disease outcomes

Study	Study Design	Population	Results	Conclusion
Weitkamp JH *et al.*, 2000 [59]	Pilot study	23 preterm and term neonates, with bacterial sepsis	7 TNFB2/TNFB2, 12 TNFB1/TNFB2, 4 TNFB1/ TNFB1 (P=0.31)	Biallelic *Ncol* within the TNF locus not a prognostic marker for disease progression
Hohler T *et al*., 1998[60]	Case control	71 Hepatitis B (HBV) patients including 32 of the subjects that recovered from the acute HBV illness. This was compared with 99 healthy controls	Prevalence of the variant at position-308 was similar. P=NS	No association found

Evidence of how the variation in TNF-" Production influences the disease processes: The biological functions of the TNF-" are complex and are related to the concentration and the duration of exposure to TNF-". In the acute situation, local production of TNF-" is clearly beneficial. It increases the expression of adhesion molecules on the vascular endothelium to allow immune cells, in particular neutrophils and macrophages, to traffic to sites of tissue damage and infection[45]. Furthermore, TNF-" activates phagocytes to engulf and clear infectious agents and cellular debris. However, systemic or protracted exposure to TNF-" may be harmful. High levels of circulating TNF-" is associated with toxic shock (similar to that induced by bacterial endotoxins)[46] and the derangements of metabolism in surgery or trauma patients that may be related to the cachetic properties of this cytokine. TNF-" induces IL-1 and IL-6 production that leads to an elevated temperature, sleepiness and the release of glucocorticoids[47] that in the short-term may be valuable in combating certain infections, but in the longer-term is likely to be detrimental. These observations suggest that polymorphism in the human TNF-" gene encoding high TNF-" levels may enhance disease susceptibility or severity (Table 4) whilst other reports contradict these findings (Table 5).

Biological significance of TNF-" gene polymorphism: TNF-" is believed to be a pivotal pro-inflammatory mediator important in the pathogenesis of the systemic inflammatory response syndrome. In addition, excessive production of TNF- " may lead to organ dysfunction and death[43]. Recently it has been suggested that a genomic restriction fragment length polymorphism within TNF locus at –308 positions correlated with increased TNF- " plasma concentrations and poor prognosis[48]

Compared with other cytokines the TNF-" gene and the regions of DNA flanking it are highly polymorphic. The first possibility is that the TNF-" gene is situated in the middle of a region of the genome that is inherently variable in a way that ensures diversity of the immune responses. A closer look at the HLA-DRB1 gene reveals that there are over 200 alleles[2]. The HLA-DR molecules are very important in binding of peptide antigens and presenting them for recognition by the receptors of T helper (CD4-positive) lymphocytes. It may be then that the mechanisms that act to create and maintain polymorphism of the HLA molecules also does the same for other molecules in the HLA complex.

The second possibility, which is not mutually exclusive, is that environmental pressures have positively selected and maintained mutations that have arisen at a normal rate because they happen to beneficially influence the expression of a very important gene. For example, TNF-" has been shown to be an important pro-inflammatory cytokine in infection and autoimmunity. In cerebral malaria, the high responder genotypes, -308*A and –376*A alleles are associated with higher incidences of cerebral malaria[46]. Similar to the reported findings, we observed significantly higher levels of oxidative stress over different time intervals and incidences of complications in patients heterozygous for the TNFA*2 allele after the initiation and termination of CPB compared with patients homozygous for TNFA*1 allele. We demonstrated that 36.8% of the CPB patients were carriers of the TNFA*2 allele an allele shown in our study to be associated with higher intraoperative oxidative stress.

CONCLUSIONS

TNF polymorphisms are found in a region of great polymorphic variation and they are in linkage disequilibrium with the HLA genes and with each other. Because of differences in the distribution of HLA alleles one might expect variation in associations between TNF polymorphisms and various conditions in different geographical areas. Nonetheless, the results observed from published work have greatly elucidated the role of human inflammatory cytokine TNF-" gene promoter diversity in the disease process. It is in our opinion that determining a patient's TNF genotype before treatment may permit the selection of a homogeneous group of high-risk patients who could benefit from treatment with anti-TNF. Such a possibility deserves further study; since an effective therapy for systemic inflammatory syndrome would have important clinical and economic consequences.

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