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Polymorphisms in the *TNF* gene cluster and *MHC* serotypes in the West of Scotland

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Abstract We examined the distribution of polymorphic elements within the tumor necrosis factor (*TNF*) gene cluster in 105 unrelated individuals and determined their relationship to class I and class II major histocompatibility complex (*MHC*) antigens, and to the highly polymorphic microsatellites *TNFA* and *TNFB*. The data demonstrate the contribution of elements within the *TNF* cluster to two extended haplotypes which are recognized to straddle the *MHC*. The *A1.B8.DR3* haplotype appears to contain the *TNF* alleles *TNFA2*, *TNFB3*, *LT.Nco-1.B*1*, and *TNF-308.2*, while the *A3.B7.DR2* haplotype is associated with the *TNF* alleles *TNFA11*, *TNFB6*, *TNFC1*, *LT.Nco-1.B*2*, *LT.AspH1.1*, *TNF-308.1*, and *TNFE1*. The presence of other extended associations which covered smaller parts of the *MHC* was also suggested. In most cases, the associations described here were in keeping with previously described extended haplotypes which dominate the structure of the *MHC*, but these did not always match completely. Taken together, these data suggest that the structure of the *TNF* locus is well integrated into the rest of the *MHC* but that important ethnic differences may exist.

Introduction

The gene coding for *TNF* is found within the *MHC* class III region in the *TNF* gene cluster (Carroll et al. 1987), which

also includes two lymphotoxin genes (*LTA* and *LTB*; Browning et al. 1993) and the human *B144* homologue, *Lst-1* (Holzinger et al. 1995). The *TNF* locus is 12 kilobases (kb) in length and contains several polymorphic areas (see Figure 1). Five polymorphic microsatellites (Jongeneel et al. 1991; Nedospasov et al. 1991; Udalova et al. 1993) and one restriction fragment length polymorphism (RFLP; Messer et al. 1991; in intron 1 of the *LTA* gene, defined by the enzyme *Nco* 1) have received particular attention although other polymorphic elements exist, notably at positions –308 (Wilson et al. 1992) and –238 (d'Alphonso and Momigliano-Richiardi 1994) of the promoter of the *TNF* gene; a frequent *AspH1* RFLP (Ferencik et al. 1992), a rare *Eco* R1 RFLP (Partanen and Koskimies 1988), and a rare *Pvu*-II RFLP (Abraham et al. 1993a; de Baey et al. 1995) have also been documented. Although the production of *TNF* in vitro has been linked with different DR antigens (Santamaria et al. 1989; Jacob et al. 1990), which are themselves capable of offering different degrees of signal transduction (Fleury et al. 1995), certain alleles within the *TNF* locus have been implicated in disease. Frequently, this is as part of a larger association containing other elements of the *MHC* (Wilson et al. 1994b; Monos et al. 1995), although this may not always be the case (McGuire et al. 1994; Mizuki et al. 1995). Some of the polymorphic areas associated with the *TNF* gene cluster also contain individual alleles which have been associated with an apparent genetic predisposition to high secretion of *TNF*. In particular, Pociot and co-workers (1993) have shown that the *TNFA2* and *TNFC2* microsatellite alleles were linked with high *TNF* secretion from LPS-activated monocytes. The *LT.Nco-1.B*2* allele has also been linked to high *TNF* secretion (e.g., Pociot et al. 1991) and the *TNF-308* site has been described as having some control over transcription rates of the *TNF* mRNA (Wilson et al. 1994a).

It has been demonstrated that extended haplotypes such as *A1.B8.DR3* exist in the human *MHC*. Recent work has suggested that this is one of a number of ancient extended haplotypes which form the basis of all haplotypic structures across the *MHC* (Degli-Esposti et al. 1992, 1995). This work has also sought to demonstrate that *TNF* alleles are an

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integral part of these haplotypes (Dawkins et al. 1989; Abraham et al. 1991, 1993b), suggesting that individual variations in *TNF* production are related to the whole haplotype rather than to the effect of individual *TNF* alleles (Abraham et al. 1993c).

Much of the work which positions *TNF* alleles within extended *MHC* haplotypes has been carried out on the tissue-typing panel of cell-lines gathered from all round the world (Abrahams et al. 1993b; Degli-Esposti et al. 1995). We wished to determine the extent to which this was the case in our local population in the West of Scotland. We therefore established the genotypes of up to 105 unrelated individuals at eight polymorphic sites within the *TNF* locus and examined the relationship of the various polymorphic elements to the two highly polymorphic microsatellites *TNFa* and *TNfb* as well as to the class I and class II serotypes.

Materials and methods

Sample DNA

DNA was obtained from the panel of normal donors maintained by the Tissue-Typing Unit at Glasgow Royal Infirmary. Samples excluded any persons with known autoimmune or malignant disease or relatives of such persons; as far as it was possible to establish, the DNA was from healthy donors. These samples were *TNF*-typed by us anonymously and then the class I and class II serotypes were obtained for inclusion in the analysis. At this point, family members were identified within our test population and eliminated, leaving a population of 105 unrelated individuals on which the analysis was performed.

Typing of *TNF* alleles

The *TNF* alleles were typed by primer extension, with the inclusion of restriction endonuclease digestion where appropriate. Primer sequences and reaction conditions are described below. Following amplification, *TNFa*, *TNfb*, *TNfc*, and *TNfe* microsatellite alleles were resolved on denaturing polyacrylamide gels comprising 6% acrylamide (19:1 with bis-acrylamide, both from Gibco-BRL, Paisley, Scotland) and 7 M urea (7.65 M for *TNFa*; Appligene, Strasbourg, France), on BaceAce sequencing rigs (Stratagene, Cambridge, England). Products were internally labelled with α^{32} -P-dCTP in a reaction mix that contained 1 μ M each primer, 20 μ M dCTP, and 200 μ M dATP, dTTP, and dGTP (Pharmacia, Göttingen, Germany). 0.5 units of Primezyme thermostable DNA polymerase (Biometra, Milton Keynes, England) was used for the microsatellite reactions and MgCl₂ was kept at 1.5 mM using Primezyme "Optimal Buffer". The final reaction volume was 20 μ l. We found that accurate typing of the more polymorphic microsatellite elements within the *TNF* locus is absolutely dependent on the inclusion of several DNAs of known *TNF* genotype in each reaction run, and we used cell-lines (Udalova et al. 1993) for this purpose. The following cell lines were obtained from the Tissue-Typing Workshop panel as standards for the microsatellite reactions (Udalova et al. 1993): T7567 (*TNFa6*, *b5*, *c1*, *e4*), IBW9 (*TNFa4*, *b7*, *c2*, *e3*), LZL (*TNFa2*, *b1*, *c2*, *e1*), and OLL (*TNFa6*, *b5*, *c1*, *e3*). These cell lines were maintained in RPMI 1640 culture medium supplemented with 10% foetal calf serum, penicillin, and streptomycin. DNA was extracted from cell pellets by proteinase-K digestion (Stratagene) and phenol/chloroform treatment (Sigma, Poole, England). The cosmid clone M31A (Nedospasov et al. 1991) was also used (*TNFa8*, *b4*, *c1*, *e3*).

Oligonucleotide primers were synthesised (Cruachem Ltd, Glasgow, Scotland) according to published sequences (Udalova et al. 1993).
 For *TNFa* 5' GCC.TCT.AGA.TTT.CAT.CCA.GCC.ACA 3'
 5' CCT.CTC.TCC.CCT.GCA.ACA.CAC.A 3'
 For *TNfb* 5' GCA.CTC.CAG.CCT.AGG.CCA.CAG.A 3'
 5' GTG.TGT.GTT.GCA.GGG.GAG.AGA.G 3'
 For *TNfc* 5' GGG.AGG.TCT.GTC.TTC.CGC.CG 3'
 5' CGT.TCA.GGT.GGT.GTC.ATG.GG 3'
 For *TNfe* 5' GTG.CCT.GGT.TCT.GGA.GCC.TCT.C 3'
 5' TGA.GAC.AGA.GGA.TAG.GAG.AGA.CAG 3'

Single-step reactions were used throughout: for *TNFa* comprising an initial denaturation (94 °C, 5 min) followed by 40 cycles of 94 °C 25 s, 60 °C 1 min, 74 °C 1 min, then completed with a final extension at 72 °C for 5 min; for *TNfb* and *TNfc* comprising an initial denaturation (94 °C, 5 min) followed by 30 cycles of 94 °C 25 s, 60 °C 45 s, 74 °C 45 s, then completed with a final extension at 74 °C for 5 min; for *TNfe* comprising an initial denaturation (94 °C, 5 min) followed by 35 cycles of 94 °C 30 s, 62 °C 30 s, 74 °C 30 s, then completed with a final extension at 74 °C for 5 min.

The RFLP sites in intron 1 of *LTA* marked by *Asp* H1 (Ferenick et al. 1992) and *Nco* 1 (Messer et al. 1991) were analyzed using primers and conditions described by the original authors, except that *Bsi*HKA1 (an isoschizomer of *Asp* H1; New England Biolabs, Beverly, MA) was used. Similarly, the *TNF*-308 polymorphism was investigated using the original method of digesting the primer extension product with *Nco* 1 (after the method of Wilson et al. 1992).

The polymorphism at position -238 of the *TNF* promoter was defined using a novel PCR/RFLP technique developed in our laboratory (H-H Oh). Briefly, primers were designed to give a 192 bp product:

5' TTC.CTG.CAT.CCT.GTC.TGG.AAG.TAA.GAA 3'
 5' AGG.ATA.CCC.CTC.ACA.CTC.CCC.ATC.CTC.CCg.GaT.C 3'

These complemented areas (-396 to -370) and (-237 to -204) of the *TNF* promoter, respectively. Two nucleotide substitutions (at -235 and -233; lower case) were included in the downstream primer to generate a *Bam* H1 restriction endonuclease site in the presence of the common allele (nucleotide G at position -238). Target DNA was amplified in a 50 μ l reaction comprising an initial denaturation of 94 °C 5 min followed by 35 cycles of 94 °C 1 min, 55 °C 1 min, and 72 °C 1 min with a concluding extension step of 72 °C 5 min. Following overnight digestion with *Bam* H1 (Stratagene), alleles were visualized as for *TNF*-308. The presence of the common allele (and hence the *Bam* H1 site) was indicated by digestion to a 164 bp product.

In the RFLP-type reactions, primer extensions were carried out using 0.5 units of Applied Biotechnologies thermostable DNA polymerase in 1.5 mM MgCl₂ using Applied Biotechnologies Buffer IV in 50 μ l reactions containing 1 μ M each primer and 200 μ M of each deoxyribonucleotide. Biometra "Uno" thermoblocks were used in all cases.

In order to provide a consistent nomenclature for these RFLP sites, we adopted the following convention: where the enzyme cut the reaction product, this was designated "allele 1" and where cutting failed to occur, this was designated "allele 2". Thus, the *G/A* alleles at *TNF*-238 (d'Alphonso et al. 1994) were designated alleles 1 and 2, respectively. Similarly, the *G/A* alleles at *TNF*-308 (Wilson et al. 1992) were designated 1/2 and the *C/G* alleles at the *Asp* H1 site in intron 1 of *LTA* (Ferenick et al. 1992) were designated 1/2. We retained the established *B*1/B*2* nomenclature for the alleles in intron 1 of *LTA* defined by *Nco* 1, where *B*1* represents the cut allele.

Statistical analysis

The proportion of each allele present was calculated assuming no null alleles, as previously described for the *TNF* microsatellites (Jongeneel et al. 1991). Associations between loci were estimated by constructing a series of 2 \times 2 tables, which were then analyzed by the Chi-square test (using Minitab software). The probability (p) obtained was corrected (Bonferroni) for multiple comparisons (pc) according to the number of alleles observed. Associations between alleles ($P < 0.05$) were considered "strong" where $PC < 0.05$.

Table 1 Distribution of alleles at polymorphic loci within the human *TNF* cluster

<i>TNF</i> locus	Allele	Number observed	Percentage distribution
<i>TNFB</i>	<i>TNFB1</i>	18	15.5%
	<i>TNFB2</i>	1	0.9%
	<i>TNFB3</i>	14	12.1%
	<i>TNFB4</i>	43	37.1%
	<i>TNFB5</i>	30	25.9%
	<i>TNFB6</i>	3	2.3%
	<i>TNFB7</i>	7	6.0%
<i>TNFA</i>	<i>TNFA1</i>	2	1.1%
	<i>TNFA2</i>	37	20.3%
	<i>TNFA3</i>	3	1.7%
	<i>TNFA4</i>	17	9.3%
	<i>TNFA5</i>	12	6.6%
	<i>TNFA6</i>	33	18.1%
	<i>TNFA7</i>	12	6.6%
	<i>TNFA8</i>	1	0.6%
	<i>TNFA9</i>	3	1.7%
	<i>TNFA10</i>	16	8.8%
	<i>TNFA11</i>	38	20.9%
	<i>TNFA12</i>	1	0.6%
	<i>TNFA13</i>	7	3.9%
<i>TNFC</i>	<i>TNFC1</i>	140	78.7%
	<i>TNFC2</i>	38	21.3%
<i>LT.AspH1</i>	<i>LT.AspH1.1</i>	32	41.0%
	<i>LT.AspH1.2</i>	46	59.0%
<i>LT.Nco-1</i>	<i>LT.Nco-1.B*1</i>	66	36.6%
	<i>LT.Nco-1.B*2</i>	116	63.7%
<i>TNF-308</i>	<i>TNF-308.1</i>	130	79.3%
	<i>TNF-308.2</i>	34	20.7%
<i>TNFE</i>	<i>TNFE1</i>	26	21.3%
	<i>TNFE2</i>	4	3.3%
	<i>TNFE3</i>	92	75.4%
	<i>TNFE4</i>	0	0.0%

Results

Alleles in the *TNF* gene cluster

The distribution of the various alleles is tabulated in Table 1. The G at position -238 of the *TNF* promoter (allele *TNF-238.1*) was found to be non-polymorphic in our normal population (although we observed the rare allele (*TNF-238.2*) in certain disease populations, data not shown), as was the *Eco* R1 site in the 3'UTR of *LTA*; these loci are therefore not referred to in Table 1. Little or no differences were observed from the previously reported distribution for the *LT.AspH1*, *LT.Nco-1*, and *TNF-308* loci (Ferenick et al. 1992; Messer et al. 1991; Wilson et al. 1992). The observed distributions for the *TNFA*, *TNFB*, and *TNFC* microsatellites were also in keeping with those previously reported in other European populations (Jongeneel et al. 1991; Nedospasov et al. 1991; Crouau-Roy et al. 1993); but please see also Table 2. The distribution at the *TNFE* microsatellite shown here represents the first reported population study at this locus.

Table 2 Percentages of *TNFA* microsatellite alleles in nine groups of healthy individuals

<i>TNF</i> allele	Study group								
	1	2	3	4	5	6	7	8	9
<i>TNF a1</i>	0.00	1.10	3.10	0.00	2.00	11.60	0.00	0.70	0.70
<i>a2</i>	11.00	20.33	20.80	21.00	21.50	25.00	30.00	30.70	32.00
<i>a3</i>	0.00	1.65	3.10	1.20	3.40	3.50	0.00	3.30	3.30
<i>a4</i>	9.00	9.34	4.20	3.70	4.00	4.50	9.00	11.30	10.00
<i>a5</i>	4.00	6.59	3.10	11.00	4.00	3.60	5.00	6.70	6.70
<i>a6</i>	22.00	18.13	9.40	13.40	10.10	7.10	19.00	14.00	14.00
<i>a7</i>	15.00	6.95	12.50	7.30	13.40	17.00	9.00	3.30	4.00
<i>a8</i>	0.00	0.55	5.20	0.00	4.00	0.00	1.00	0.00	0.00
<i>a9</i>	7.00	1.65	0.00	8.50	0.00	2.70	1.00	3.30	3.30
<i>a10</i>	15.00	8.79	10.40	13.40	15.40	10.70	10.00	8.70	9.30
<i>a11</i>	13.00	20.88	18.70	9.80	12.80	8.90	15.00	16.00	14.70
<i>a12</i>	2.00	0.55	7.30	1.20	7.40	0.00	0.00	1.30	1.33
<i>a13</i>	2.00	3.85	2.10	8.50	1.30	5.40	1.00	0.70	0.70

Distribution of *TNFA* alleles in nine different studies of normal individuals, arranged in order of increasing frequency of allele *TNFA2*. The normal population used in this study (Scottish) is study group 2. Other groups are: Group 1, Russian (Nedospasov et al. 1991); group 3, French (Crouau-Roy et al. 1993); group 4, Greek (Crouau-Roy et al. 1993); group 5, French (Jongeneel et al. 1991); group 6, Basque (Crouau-Roy et al. 1993); group 7, American (Honchel et al. 1996); group 8, Danish (Pociot et al. 1993); group 9, Danish (Crouau-Roy et al. 1993)

TNFA and *TNFB* microsatellite alleles and MHC class I and class II loci

The associations observed at the *TNFA* microsatellite and their significance levels are summarized in Table 3. Strong associations were observed between the *TNFA* microsatellite allele *TNFA11* and the *MHC* serotypes *HLA-A3* ($X^2 = 39.066$, $PC = 0.00071$), *HLA-B7* ($X^2 = 39.845$, $PC = 0.00088$) and *HLA-DR2* ($X^2 = 22.368$, $PC = 0.00059$). We believe that this positions the *TNFA11* allele firmly as part of the *A3.B7.DR2* extended haplotype in our population. In addition, multiple relationships were observed for two other *TNFA* microsatellite alleles. The *TNFA7* allele was strongly associated with *HLA-A29* ($X^2 = 24.033$, $P = 0.00071$), *HLA-DR7* ($X^2 = 17.814$, $PC = 0.00059$) and more weakly associated with *HLA-B12* ($X^2 = 4.974$, $P = 0.0257$), thereby placing *TNFA7* into *A29.B12.DR7*. Finally, *TNFA2* was in strong association with *HLA-DR3* ($X^2 = 11.854$, $PC = 0.0354$) and with *HLA-B8* ($X^2 = 9.337$, $P = 0.0022$) and *HLA-A1* ($X^2 = 6.858$, $P = 0.0088$). This probably positions the *TNFA2* allele within the *A1.B8.DR3* haplotype; a secondary, lesser association between *TNFA2* and *HLA-B17* ($X^2 = 6.549$, $P = 0.0105$) was also noted.

Other strong ($PC < 0.05$), but isolated, associations existed between *TNFA5/HLA-B35* ($X^2 = 17.543$, $PC = 0.00088$) and *TNFA13/HLA-B16* ($X^2 = 13.133$, $PC = 0.0264$). Weaker ($PC > 0.05$) associations were observed between *TNFA4/HLA-DR6* ($X^2 = 6.015$, $P = 0.0142$), *TNFA6/HLA-B15* ($X^2 = 8.238$, $P = 0.0041$), and *TNFA10* with both *HLA-B21* ($X^2 = 7.204$, $P = 0.0073$) and *HLA-A11* ($X^2 = 7.103$, $P = 0.0077$).

Table 3 Associations between *TNFA* and *TNFB* microsatellite alleles and *MHC* class I or class II serotypes in a population of unrelated individuals from the West of Scotland

<i>TNF</i> allele	<i>HLA-A</i>	<i>HLA-B</i>	<i>HLA-DR</i>
<i>TNFA2</i>	A1*	B8*, B17*	DR3‡
<i>TNFA4</i>	#	#	DR6*
<i>TNFA5</i>	#	B35‡	#
<i>TNFA6</i>	#	B15*	#
<i>TNFA7</i>	A29§	B12*	DR7↓
<i>TNFA10</i>	A11*	B21*	#
<i>TNFA11</i>	A3§	B7§	DR2↓
<i>TNFA13</i>	#	B16‡	#
<i>TNFB1</i>	A29*	#	#
<i>TNFB3</i>	A1‡	B8‡	DR3‡
<i>TNFB6</i>	#	B7‡	DR2↓
<i>TNFB7</i>	#	#	DR3*

* $P < 0.05$, $PC > 0.1$ † $0.05 < PC < 0.1$ ‡ $0.005 < PC < 0.05$ § $0.0005 < PC < 0.005$ ↓ $PC < 0.0005$

No association detected

Fewer relationships were apparent which involved the *TNFB* microsatellite alleles. *TNFB3* was strongly ($PC < 0.05$) associated with *HLA-A1* ($X^2 = 13.717$, $PC = 0.0078$), *HLA-B8* ($X^2 = 15.406$, $PC = 0.005$) and *DR3* ($X^2 = 11.753$, $PC = 0.0156$), while *TNFB6* was strongly associated with *HLA-B7* ($X^2 = 11.328$, $PC = 0.04$) and *HLA-DR2* ($X^2 = 22.683$, $PC = 0.00039$). Weaker associations ($PC > 0.05$) were observed between *TNFB1/HLA-A29* ($X^2 = 5.495$, $P = 0.0191$) and *TNFB7/HLA-DR3* ($X^2 = 5.617$, $P = 0.0178$). Taken together, these data suggest that *TNFA/b* alleles are a firm component of two extended *MHC* structures within our population: A3.B7.*TNFA11b6.DR2* and A1.B8.*TNFA2b3.DR3*, with a probable third comprising A29.B12.*TNFA7.DR7*.

TNFA and *TNFB* microsatellite alleles and other alleles within the *TNF* locus

The relationships observed at the *TNFA* microsatellite locus and their significance levels are summarized in Table 4a. The most notable were again those involving the *TNFA* microsatellite allele *TNFA11*, which was strongly associated with *TNFB6* ($X^2 = 12.594$, $PC = 0.0188$) and *LT.AspH1.1* ($X^2 = 10.611$, $PC = 0.0231$), and more weakly associated with *TNFC1* ($X^2 = 4.921$, $P = 0.0265$), *LT.Nco1.B*2* ($X^2 = 8.336$, $P = 0.0039$) and *TNF-308.1* ($X^2 = 5.414$, $P = 0.02$), but not at all with *TNFE*; a weak association with *TNFB4* was also observed ($X^2 = 8.090$, $P = 0.004$). Similarly, *TNFA2* was strongly associated with *TNF-308.2* ($X^2 = 13.846$, $PC = 0.0046$) and to a lesser extent with *TNFB3* ($X^2 = 7.680$, $P = 0.0051$) and *LT.AspH1.2* ($X^2 = 8.686$, $P = 0.0032$); a weak association with *TNFB1* was also noted ($X^2 = 6.894$, $P = 0.0086$). The *TNFA5* microsatellite allele was strongly associated with *TNFB7* ($X^2 = 25.515$, $PC = 0.00047$) and also associated with *TNFE2* ($X^2 = 7.259$, $P = 0.0071$). Also, *TNFA6* was strongly

Table 4 Associations between the *TNFA* and microsatellite alleles and other alleles in the *TNF* locus in a population of unrelated individuals from the West of Scotland. Where two associations are shown *TNFB* the first is stronger

A						
<i>TNFA</i> alleles	<i>TNFB</i>	<i>TNFC</i>	<i>LT.AspH1</i>	<i>LT.Nco1</i>	<i>TNF-308</i>	<i>TNFE</i>
<i>TNFA2</i>	b3*, b1*	#	AspH1.2*	#	-308.2§	#
<i>TNFA3</i>	b1↓	#	#	#	#	#
<i>TNFA4</i>	#	#	#	B*1*	#	#
<i>TNFA5</i>	b7↓	#	#	#	#	e2*
<i>TNFA6</i>	b5↓	#	AspH1.2*	B*1↓	#	#
<i>TNFA7</i>	b4*	#	#	#	#	#
<i>TNFA10</i>	#	#	AspH1.1*	B*2†	#	#
<i>TNFA11</i>	b6‡, b4*	c1*	AspH1.1‡	B*2*	-308.1*	#
B						
<i>TNFB</i> allele	<i>TNFA</i>	<i>TNFC</i>	<i>LT.AspH1</i>	<i>LT.Nco1</i>	<i>TNF-308</i>	<i>TNFE</i>
<i>TNFB1</i>	a3↓, a2*	c2↓	#	B*2‡	-308.1*	e1↓
<i>TNFB3</i>	a2*	#	#	#	-308.2↓	#
<i>TNFB4</i>	a11*, a7*	#	#	#	#	#
<i>TNFB5</i>	a6↓	#	#	B*1↓	#	#
<i>TNFB6</i>	a11‡	#	AspH1.1‡	#	#	e1*
<i>TNFB7</i>	a5↓	#	#	#	#	#

* $P < 0.05$, $PC > 0.1$ † $0.05 < PC < 0.1$ ‡ $0.005 < PC < 0.05$ § $0.0005 < PC < 0.005$ ↓ $PC < 0.0005$

No association detected

associated with *TNFB5* ($X^2 = 21.713$, $PC = 0.00047$), *LT.Nco1.B*1* ($X^2 = 30.524$, $PC = 0.00026$) and *LT.AspH1.2* ($X^2 = 5.510$, $P = 0.0189$). *TNFA10* was associated with *LT.Nco1.B*2* ($X^2 = 8.385$, $P = 0.0038$) and *LT.AspH1.1* ($X^2 = 6.503$, $P = 0.0108$). *TNFA3* was only associated with a single other allele, *TNFB1* ($X^2 = 28.431$, $PC = 0.00047$), while similar but weaker single associations were observed between *TNFA4/LT.Nco1.B*1* ($X^2 = 8.644$, $P = 0.0011$) and *TNFA7/TNFB4* ($X^2 = 6.943$, $P = 0.0084$). It should be noted that despite the close physical proximity of these polymorphic elements (the whole *TNF* locus contains ten polymorphic sites in only 12 kb), some of these relationships are quite weak.

The relationships involving *TNFB* microsatellite alleles were determined (Table 4b). In addition to those involving *TNFA* alleles (above), other strong associations were noted. *TNFB6* was moderately associated with *LT.AspH1.1* ($X^2 = 9.283$, $PC = 0.0299$) and *TNFE1* ($X^2 = 6.148$, $P = 0.0132$). *TNFB3* was closely associated with *TNF-308.2* ($X^2 = 20.747$, $PC = 0.00047$). *TNFB5* was closely associated with *LT.Nco1.B*1* ($X^2 = 19.886$, $PC = 0.00014$). However, *TNFB1* was strongly associated with many of the polymorphic sites across the whole locus: with *TNFC2* ($X^2 = 25.388$, $PC = 0.00014$), *LT.Nco1.B*2* ($X^2 = 8.832$, $PC = 0.039$), *TNF-308.1* ($X^2 = 5.396$, $P = 0.0202$) and *TNFE1* ($X^2 = 17.522$, $PC = 0.00021$). This suggests that these alleles may well form an extended relationship in the absence of a demonstrable link to any of the *MHC* class I or class II alleles in this study population and that they may behave as a haplotype.

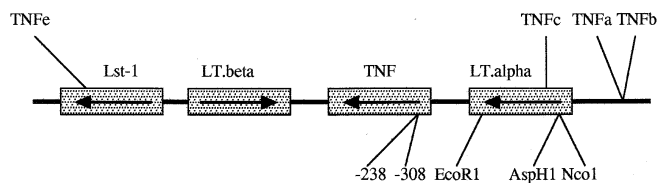


Fig. 1 A schematic representation of the human *TNF* gene cluster and the polymorphic elements investigated in this study. The polymorphic elements investigated here were distributed across the *TNF* gene clusters as shown. The relevant references are given in the text. Note that to date no polymorphic elements have been described within the *LTB* gene

Discussion

We have described the genotypes of 105 unrelated individuals at the *TNF* gene cluster. This is the first extensive report of a UK population across the *TNF* gene cluster; in total, nine polymorphic elements were examined. Also, we present here the first reported population study for the *TNFe* microsatellite (although its distribution in a panel of cell lines has been described (Udalova et al. 1993)). These data were used to identify potential relationships between the highly polymorphic *TNFa* and *TNFb* microsatellite loci and other elements, both within the *TNF* locus and outside, to the class I and class II *MHC*.

Using the associations with *TNFa* and *TNFb* alleles, we suggest the presence of two extended associations in our population which integrate alleles across the *TNF* locus with the *MHC* class I and class II elements on either side: *A3;B7;TNFb6;TNFa11;TNFc1;LT.AspHI.1;LT.Nco1.B*2;TNF-308.1;TNFe1;DR2A1;B8;TNFb3;TNFa2;LT.AspHI.2;TNF-308.2;DR3*

In addition, a probable extended association exists which is contained within the *TNF* locus:

*TNFb1; TNFa3; TNFc2; LT.Nco1.B*2; TNF-308.1; TNFe1*

Although this (*b1/a3/c2*, etc.) may be weakly associated with *HLA-A29* (see Table 4), the absence of an association into *HLA-B* (closer than *HLA-A*) or of associations between *TNFa3* and any *HLA* allele suggests that this may be spurious. This opinion is reinforced by the strength of the association of *HLA-A29* with *TNFa7*, in the extended association:

A29; B12; TNFb4; TNFa7; DR7,

which has also been observed in a Swiss population (Jongeneel et al. 1991).

Our data also suggest other possible associations between *TNF* locus alleles and the *MHC*: extended studies over larger sample populations will be needed resolve this completely. Such studies would also clarify the situation regarding the less complete or weaker apparent extended associations which can be inferred as being within our population, such as:

*A11; B21; TNFa10; LT.AspHI.1; LT.Nco1.B*2*

B35; TNFb7; TNFa5; TNFe2; DR3

*B15; TNFb5; TNFa6; LT.AspHI.2; LT.Nco1.B*1, and TNFb4; TNFa6; LT.Nco1.B*1*

Much work has been carried out seeking to describe ancestral haplotypes which cover the whole *MHC* locus (Degli-Esposti et al. 1992, 1995) and recently these have incorporated elements of the *TNF* locus (Dawkins et al. 1989; Abraham et al. 1991, 1993b). In some cases, our data are in keeping with this analysis. For example, *TNFa2/b3* has been linked to the ancestral haplotype 8.1 and this fits our observations, but we failed to show a direct association between *TNFa2* or *TNFb3* and the *LT.Nco1.B*1* allele, which is an integral part of the 8.1 extended haplotype (although our data do associate it with *B8* and *DR3* directly; $P = 0.0132$, $P = 0.001$, respectively). Similarly, *TNFa11* is associated with *A3.B7.DR2* in our population as it is in the ancestral haplotype 7.1 of Dawkins' group (Degli-Esposti et al. 1995), but in our population, *TNFa11* is firmly linked with *TNFb6* (which is itself independently associated with *B7.DR2*) and not *TNFb4*. Here again, there are relationships between *DR2/LT.Nco1.B*2* ($P = 0.0067$), *DR2/TNF-308.1* ($PC = 0.0016$), *DR2/AspHI.1* ($PC = 0.0024$), and *B7/LT.AspHI.1* ($P = 0.004$), which reinforce the hypothesis that this is a strong extended structure. The strong link between *HLA-B35*, *TNFa5*, and *TNFb5* (Degli-Esposti et al. 1995) is different in our population, where *TNFb5* is replaced by *TNFb7*. Such differences may have their basis in the number of subjects studied or they may represent true differences between populations; we carried out our analysis on a large number of unrelated individuals from a defined local area rather than the extensive international cell-line panel used by others (Abrahams et al. 1993b; Degli-Esposti et al. 1995) and so believe the data are truly representative of our local population.

Differences in allelic distribution appear to exist between populations at both the *TNFa* and *TNFb* loci. This appears particularly true with regard to the *TNFb6* allele, which was absent from three of four ethnic groups in the recent study of Crouau-Roy and co-workers (1993). When our study group was compared with the four reported groups in this latter study, some differences and similarities were apparent. It should be emphasized that this is a general comparison made within the limitations of examining only published material, but for example, it may be the case that the *a11.b4.c1* haplotype of Crouau-Roy and co-workers (their Table 3) is replaced in our population by *a11.b6.c1* and their *a2.b1.c2* by our *a3.b1.c2*. With regard to such differences between our study population and those of others (Crouau-Roy et al. 1993; Degli-Esposti et al. 1995), it should be remembered that the association between *TNFa11* and *TNFb4* is significant in our population, but weaker than that between *TNFa11* and *TNFb6* (see Table 4), which is why we chose to place *TNFb6* in the context of the extended association and not *TNFb4*. Similarly, the association between *TNFa2* and *TNFb1* is also significant, but weaker than that observed between *TNFa3* and *TNFb1* (Table 4). Crouau-Roy and co-workers (1993) also report relationships which are present in our population; for example *TNFa6/b5* and *TNFa7/b4*, as do Degli-Esposti and co-workers (1995); for example, *TNFa2/b3*, *TNFa2/b1*, *TNFa6/b5*, and *TNFa7/b4*. It is interesting to note that the distribution of *TNFa* alleles can vary widely

between test populations. For example, collating the published data at this locus reveals that the *TNFA2* allele varies from 11% to 32% (see Table 2 legend for references). Such variation may account for the different associations between *TNF* alleles observed in different studies.

Taken together, our data suggest that the alleles at the *TNF* locus are in some cases integral parts of the overall *MHC* structure, but that alleles at certain loci may vary between individual ethnic populations. It was of interest that the *Eco* R1 RFLP reported to be present in the 3'UTR of *LTA* (Partenen and Koskimies, 1988) in a Finnish population and the point mutation at position -238 of the *TNF* promoter (d'Alphonso and Momigliano-Richiardi, 1994) were both wholly absent from our study groups. This would mitigate against the assumption that the structure of the DNA between *HLA-B* and *HLA-DR* is immutable when classified into the ancestral haplotypes. Many of our other data (Table 3) would also argue for some flexibility within ancient haplotypes, as do recent studies from other laboratories (de Baey et al. 1995).

In conclusion, we present an analysis of the *TNF* locus in a large cohort of unrelated individuals from the West of Scotland and demonstrate that strong associations exist with other elements of the *MHC*. These associations are similar, but not identical, to those reported in populations studied elsewhere, suggesting that variations exist within the *TNF* locus even when the *MHC* haplotype is the same. If the *TNF* locus is to be shown to have an independent causal relationship with disease, it can probably only be identified in groups of patients which are carefully control matched at other *MHC* loci, preferably at both class I and class II.

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