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Haplotypic polymorphisms of the *TNFB* gene

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Abstract. The *TNFB* genes from two major histocompatibility complex (MHC) ancestral haplotypes have been compared. The genes carried by the ancestral haplotypes *8.1* (*A1,B8,BfS,C4AQ0, C4B1,DR3*) and *57.1* (*A1,B57, BfS,C4A6,C4B1,DR7*) were cloned and sequenced to determine the degree of polymorphism. In this report we show that the respective *TNF* genes are allelic and have unique nucleotide sequences. The data demonstrate the presence of three nucleotide differences between the *TNFB* alleles of *8.1* and *57.1*. Two of the differences occur in untranslated regions of the gene but the third nucleotide change results in an amino acid difference in the mature TNFB protein. These polymorphisms may have implications with respect to differential regulation in disease- and nondisease-associated haplotypes.

Introduction

The human major histocompatibility complex (MHC) is located on the short arm of chromosome 6 and spans the region from the class I *HLA* genes towards the telomere and the class II *HLA* genes towards the centromere. Between the class I and class II regions is a region of about 1 megabase which carries a multitude of non-*HLA* genes, including those encoding the complement components C4, C2, and Bf, the steroid 21 hydroxylase Cyp21, tumor necrosis factor (TNFA), and lymphotoxin (TNFB). In addition, there are a number of genes of which the transcripts are as yet poorly characterized, and whose presumptive products are of unknown function: these include *B144* (Tsuge et al. 1987) and the *BAT* genes (Spies et al. 1989a, b; Sargent et al. 1989; Banerji et al. 1990).

The cytokines TNFA and TNFB are polypeptide effector and signal molecules produced by activated macrophages (Nedwin et al. 1985) and stimulated lymphocytes (Aggarwal et al. 1984, 1985a), respectively. Both are potent cytotoxic agents when applied to tumor cells in vitro and in vivo (reviewed by Old 1985). In addition, both are capable of mediating a wide range of inflammatory reactions (Beutler and Cerami 1987). There are some indications that levels of TNF in various diseases may vary according to MHC haplotype (Bendtzen et al. 1988; Jacob et al. 1990).

The term "ancestral haplotype" has been used by us to denote haplotypes carrying particular combinations of MHC alleles [defined both serologically and by restriction fragment length polymorphism (RFLP) analysis] and spanning particular physical genomic distances as determined by pulsed-field gel electrophoresis (Tokunaga et al. 1988). In family studies of Western Australian Caucasoids, ancestral haplotypes and recombinations between limited pairs of ancestral haplotypes comprise almost 90% of all MHC haplotypes and consequently account for almost all of the observed linkage disequilibria between MHC alleles (Dawkins et al. 1989). A central tenet of the existence of ancestral haplotypes is that any locus within a particular ancestral haplotype should have the same genetic content as other similar ancestral haplotypes from unrelated individuals. In this paper we have isolated and sequenced the *TNFB* genes from the *57.1* and *8.1* ancestral haplotypes and show that the respective *TNF* genes are allelic and have unique nucleotide sequences.

Materials and methods

Construction of human haplotype-specific lambda genomic libraries. High relative mass genomic DNA was isolated from human Epstein-Barr virus-transformed, *HLA*-homozygous lymphoblastoid cell lines,

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number M55913.

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R6/12371 (*A1,B8,BfS,C4AQ0,C4B1,DR3*) and R6/12337 (*A1,B57, BfS,C4A6,C4B1,DR7*). DNA (1 mg) was partially digested with *Sau* 3A1 under conditions previously determined to yield an optimal proportion of fragments in the 16–22 kilobase (kb) size range. Partially digested DNA was size-fractionated over sucrose gradients and ligated to phosphatased *Bam* HI/*Eco* RI-digested EMBL3 bacteriophage arms (Promega Biotech, Madison, Wisconsin). The libraries were screened by standard methods using a TNFA cDNA probe (Benton and Davis 1977). Representative clones from both the *57.1* and *8.1* libraries were used to subclone the *TNFB* gene region prior to sequencing. The 2.4 kb *Eco* RI fragment corresponding to the 5′ portion of the *TNFB* gene contained in each was subcloned into the plasmid pGEM7. A Kpn I fragment containing the entire *TNFA* region from the 57.1 clone and a Kpn I/Sal I fragment containing the entire *TNFB–TNFA* region from the 8.1 lambda clone were subcloned into pGEM7 and pGEM3, respectively.

DNA sequence analysis. DNA sequencing of supercoiled plasmid templates was performed using specific oligonucleotides by the dideoxy chain termination method (Chen and Seeburg 1985) using a Sequenase (US Biochemicals, Cleveland, Ohio) kit as recommended by the manufacturer. Unique-sequence oligonucleotides for use as sequencing primers were synthesized on an Applied Biosystems 38OB DNA synthesizer (Foster City, California). Sequence data were analyzed using the IBI Purstell computer programs.

Results

The *8.1* and *57.1* ancestral haplotype-specific libraries were screened with a TNFA cDNA clone (Pennica et al. 1984). Each library yielded at least six unique clones bearing the region encoding the *TNFA* gene. *Eco* RI and *Nco* I restriction mapping of the lambda 8.1 and lambda 57.1 clones revealed the organizations depicted in Figure 1. The *Eco* RI sites are in similar positions, relative to the *TNFA* gene, to those previously reported (Nedospasov et al. 1986).

However, a comparison of the positions of the equivalent *Nco* I sites in the 57.1 and 8.1 clones revealed that there was an additional *Nco* I site located in the 5′ portion of the *TNFB* gene of the *8.1* ancestral haplotype that was not present in the same position of the *57.1* ancestral haplotype. Originally, an *Nco* I polymorphism of the region was demonstrated using a TNFA probe (Choo et al. 1988; Dawkins et al. 1989; Fugger et al. 1989a, b). Previous Southern hybridization analysis of *57.1* revealed a 10.5 kb fragment whereas *8.1* gave a 5.5 kb fragment (Dawkins et al. 1989). Our current data indicate that the *TNFB* gene of *8.1* lies on two adjacent *Nco* I fragments of 5.5 kb and 5.0 kb, whereas the *57.1 TNFA* and *TNFB* genes both lie on a 10.5 kb *Nco* I fragment (Fig. 1). Consequently, our results indicate that the *Nco* I site located in the first intron of the *TNFB* gene of *8.1* is polymorphic.

Appropriate restriction fragments carrying the *TNFB* gene (see Materials and methods) were subcloned and sequenced to allow the *8.1* and *57.1* haplotype-specific *TNFB* genes to be characterized. A comparison of the nucleotide sequences of the *57.1* and *8.1* ancestral haplotype *TNFB* genes (Fig. 2) revealed that the two genes are very similar. However, three nucleotide differences were found (see Fig. 2 and Table 1). The first difference (at nucleotide position 87) was located in the 5′ untranslated exon 1. The second difference (at nucleotide position 329) occurred at the intronic polymorphic *Nco* I restriction site of *8.1*; the absence of the *Nco* I polymorphic site in *57.1* can therefore be explained as a single G to A nucleotide change. The third nucleotide difference occurred in the third exon of the gene (at nucleotide position 800) and translates into an amino acid difference at position 26 in the mature TNFB protein (Goeddel et al. 1986). This polymorphic amino acid is an asparagine in the *8.1* gene and a threonine in the case of *57.1*.

Comparison of the *8.1* and *57.1* genes with those of the *TNFB* genes from unspecified haplotypes published by Nedwin and co-workers (1985) and by Nedospasov and co-workers (1986) predicts identical organization of introns and exons. The *8.1* and *57.1* haplotype *TNFB* gene sequences, however, do exhibit several differences when compared with the published *TNFB* gene sequences.

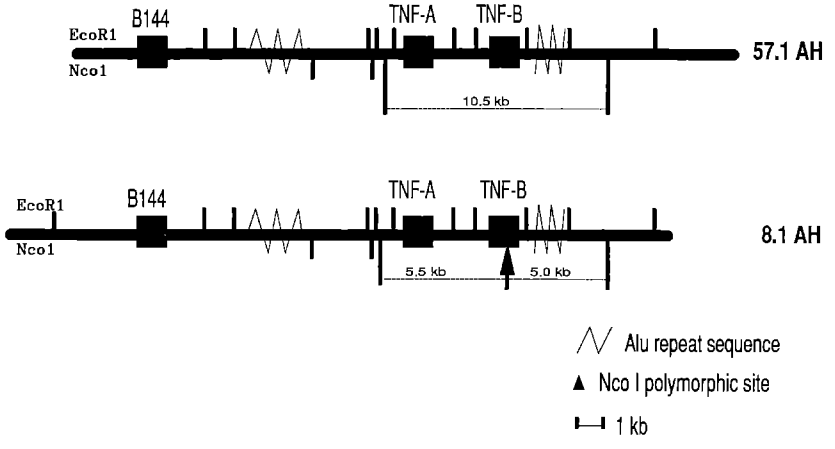
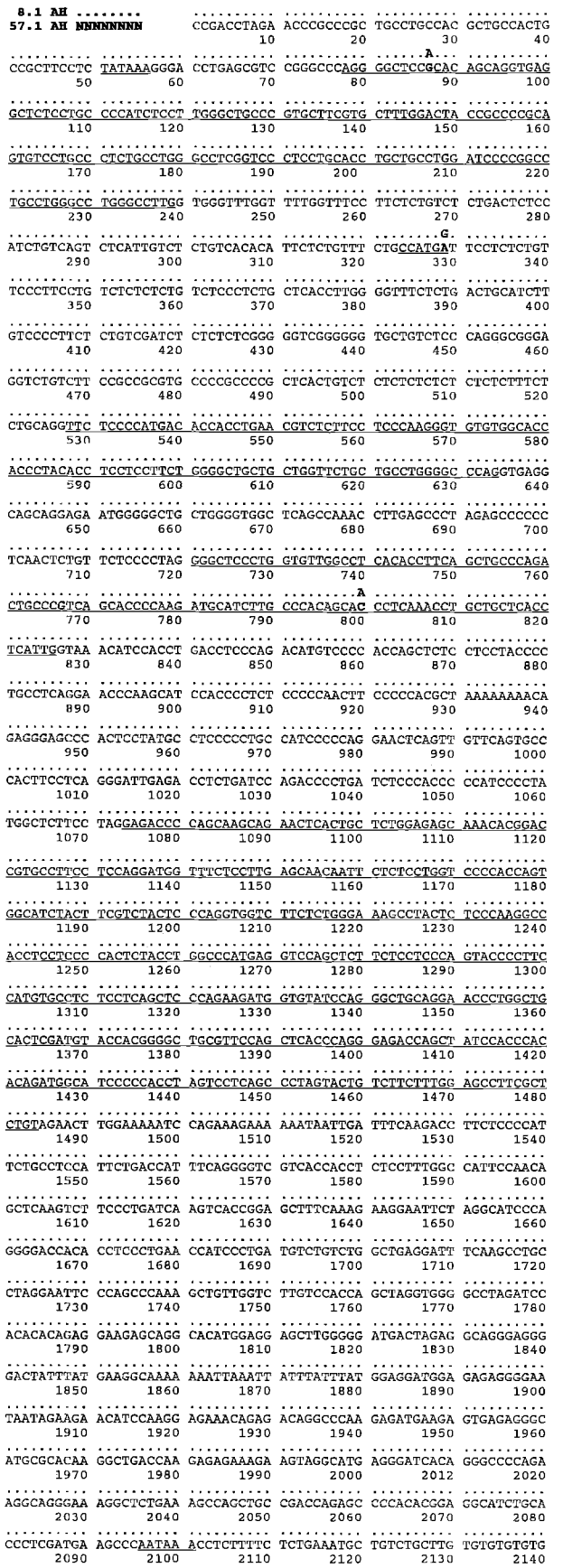


Fig. 1. Genomic organization of the *TNF* region of the *57.1* and *8.1* ancestral haplotypes. The *Eco* RI and *Nco* I restriction maps deduced after characterization of overlapping lambda clones. The position of the polymorphic *Nco* I site in the *8.1* genome is indicated by an *arrow*. Also shown are the positions of two Alu repeat sequences and the human homologue of the mouse *B144* gene.



Discussion

The present report shows that the human *TNFB* gene is relatively conserved: little difference was observed between the two different ancestral haplotypes that we have chosen to study. This is clearly in contrast to other well-characterized genes in the MHC, such as the *HLA* class I and class II and the complement genes, that are extremely polymorphic. However, three nucleotide differences were found in the *TNFB* genes. One of these changes was found to be located in the 5′ untranslated region of the gene. Another of the nucleotide differences was found in the first intron and was found to be coincident with an *Nco* I site in the *8.1* ancestral haplotype. Our data demonstrate that the polymorphic *Nco* I site, indicated by RFLP analysis using a *TNFA* gene-specific probe, is in fact located in the *TNFB* gene of *8.1*. Although these two single nucleotide changes may result in differential regulation of the *TNF* genes, this is not likely. However, the third nucleotide difference results in a polymorphism in the mature TNFB protein. The properties of TNF are such that both quantitative and qualitative differences may be responsible for the association between certain ancestral haplotypes and particular autoimmune diseases (Zhang et al. 1990; French and Dawkins 1990; Jacob et al. 1990). For instance, the threonine to asparagine change seen in the *8.1 TNF* allele may affect the way in which the TNF molecule interacts with other components of the cytokine cascade, such as the TNF receptor. Indeed, the amino acid difference seen in the two haplotypes occurs close to a conserved region between TNFA and TNFB (Goeddel et al. 1986) and so may have functional significance, as both cytokines bind to the same receptor (Aggarwal et al. 1985a, b). Considering the association of the *8.1* ancestral haplotype with many autoimmune diseases such as systemic lupus erythematosus, generalized myasthenia gravis, and especially complications of rheumatoid arthritis, it is possible that the polymorphism detected may be relevant to disease susceptibility. We are currently examining many different ancestral haplotypes from various racial groups to determine whether the *TNFB* gene polymorphism we have detected correlates with other disease susceptibility haplotypes. Differences in regulation are also possible (Zhang et al. 1990; Jacob et al. 1990). The full extent of the polymorphism of the *TNF* genes remain to be determined. We have shown three nucleotide differences in the *TNFB* genes carried by two

Fig. 2. Nucleotide sequences of the *TNFB* genes derived from the *57.1* and *8.1* ancestral haplotypes. The complete sequence of *57.1* is shown. Identity between the *57.1* and *8.1* sequences is indicated by *dots*. Nucleotide differences in *8.1* are as indicated *above* the *57.1* sequences. The exonic sequences are *underlined*. Also *underlined* are the TATAA box (51–56), the *Nco* I site (324–329), and the polyadenylation site (2096–2101).

Table 1. *TNFB* gene nucleotide sequence differences between *57.1* and *8.1*.

Sequence number	Nucleotide*		Position
	8.1	*57.1*	
87	A	G	Exon 1
329	G (*Nco* I)	A	Intron 1
800	A Asn	C Thr	Exon 3

* The nucleotide and the associated amino acid differences are indicated. Also shown is the nucleotide responsible for the polymorphic *Nco* I restriction site.

haplotypes and sequences obtained by others indicate that other polymorphisms exist (Nedwin et al. 1985; Nedospasov et al. 1986).

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