Gene-antigen register

Polymorphic markers related to a single Tcrb-V6 gene segment

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Received September 12, 1990; revised version received October 22, 1990

The central role of the antigen-specific alpha/beta T-cell receptor (Tcr) in immune recognition has led to a search for Tcr gene polymorphism relevant to autoimmune diseases. Previous reports primarily emphasized associations with constant (C)-region (Millward et al. 1987; Demaine et al. 1989; Freimark et al. 1987) restriction fragment length polymorphisms (RFLPs) as opposed to polymorphisms of variable (V)-region genes, which determine the specificity of antigen-MHC recognition by the Tcr. Although Tcr-V- and C-region genes are linked, recent family studies have reported a lack of linkage disequilibrium between V- and C-region polymorphisms (Robinson and Kindt, 1987; Charmley et al. 1988; Charmley et al. 1990) indicating that C-region polymorphisms alone may be of limited value in studying Tcr disease association (Nivens et al. 1990; Charmley et al. 1990). Further study of disease associations has been hampered by the paucity of data regarding the extent of polymorphism of Tcr-V-region genes in normal Caucasian populations.

In the present study, we evaluated RFLPs related to Tcrb-V genes in 100 normal, unrelated, Caucasian individuals using five Tcrb-V gene specific cDNA probes, V4, V5, V6.1, V8.1 and V18 (Leiden and Strominger 1986; Yanagi et al. 1984). Southern blot analysis of genomic DNA digested with the restriction enzymes Bgl-II, Bam HI, Eco RI, and Taq I, was carried out as described (Southern 1975).

Polymorphic restriction enzyme sites were detected by two restriction enzymes, Taq I and Bgl II, with the Tcrb-V6.1 cDNA probe. Each probe/enzyme combination defines a bi-allelic polymorphism. Hybridization of the Tcrb-V6.1 probe to blots containing Bgl II digested DNA revealed a variant band of 5.7 kilobases (kb) whose intensity varied in a reciprocal fashion with the intensity of a ubiquitous 12.5 kb fragment (Fig. 1). Three hybridization patterns were observed. The ubiquitous presence of the 12.5 kb fragment suggests the existence of at least two cross-hybridizing 12.5 kb fragments per haplotype, only one of which contains a polymorphic Bgl II restriction site permitting the assignment of genotypes as indicated in Figure 1. The less intense staining of 12.5 kb fragment as compared to the 5.7 kb fragment observed in the 5.7 kb homozygotes indicates that the Tcrb-V gene segment within the ubiquitous cross-hybridizing 12.5 kb fragment displays a lesser degree of homology with the Tcrb-V6.1 cDNA probe than the Tcrb-V gene segment within the polymorphic Bgl II fragments. It was possible to assign one of these genotypes to each individual, consistent with the presence of a biallelic locus. Genotype assignments were verified by blinded evaluation of autoradiograms. In addition, double digest of 25 DNA samples with Eco RI and Bgl II were performed which permitted discrimination of 5.7/5.7 kb and 5.7/12.5 kb genotypes on a basis other than intensity of the 12.5 kb and 5.7 kb bands. A 4 kb band is consistently present in individuals assigned the 12.5/5.7 as well as the 12.5/12.5 genotypes and absent in those assigned the 5.7/5.7 genotype (Fig. 2).

The Tcrb-V6.1 Taq I probe/enzyme combination defines another biallelic polymorphism with variant bands at 6.5 kb and 5.3 kb permitting genotype assignments as indicated in Figure 3. The polymorphic 5.3 kb Taq I band constitutes the lower band of the doublet visible on Southern blots, whilst the upper band represents a weakly cross-hybridizing fragment. Verification of genotype assignments, particularly discrimination of the 5.3/6.5 genotype from the 6.5/6.5 genotype, was confirmed by performing double digests of 17 DNA samples with Taq I and Bgl II. The polymorphic 6.5 kb and 5.3 kb Taq I bands were unaltered by additional digestion with Bgl II whilst the upper band of the 5.3 kb doublet was no longer
visible (see below and Figure 4, lanes A, B, and E). To our knowledge, these particular polymorphic restriction sizes have not been described previously. Table 1 summarizes the distribution of Tcrb V6.1/Bgl II and Tcrb V6.1/Taq I RFLP genotypes in 174 normal, unrelated, Caucasian individuals. The bi-allelism of both markers was confirmed by the finding that genotypes met expectations based on Hardy-Weinberg equilibrium conditions (data not shown).

Calculations based on phenotypic frequencies of alleles of the two Tcrb V6 related loci also revealed evidence for strong linkage disequilibrium indicating close linkage between the two loci. In particular, the presence of Bgl II polymorphic restriction enzyme sites on both the Tcrb haplotypes of 87 individuals is absolutely associated with the presence of Taq I polymorphic restriction sites (genotype 5.3/5.3); conversely, all 43 individuals with an absent Taq I polymorphic restriction site (genotype 5.3/6.5 or 6.5/6.5) also have an absent Bgl II polymorphic restriction site on at least one Tcrb haplotype (genotype 5.7/12.5). Thus, the presence of a polymorphic 6.5 kb Taq I fragment is always associated with the presence of at least one polymorphic 12.5 kb Bgl II fragment.

The relation between the two polymorphic restriction sites was further analyzed by performing Bgl II/Taq I double digests on 17 DNA samples whose Tcrb V6.1 Bgl II and Taq I RFLP genotypes included the various combinations observed in the general population. The Taq I polymorphic bands were unaltered by additional digestion with Bgl II (Fig. 5) suggesting that, in view of the linkage disequilibrium between these RFLP alleles, these Taq I restriction sites are situated within the polymorphic Bgl II fragments. The finding of the original 5.3 kb Taq I band in double digests (Taq I/Bgl II) of DNA from individuals homozygous for the 5.3 kb Taq I and 5.7 kb Bgl II bands (Figure 4, lane C) is in agreement with this view.

This relationship of the Bgl II and Taq I sites to each other was also investigated further by the isolation of the 12.5 kb and 5.7 kb fragments from Southern gels of Bgl II digested DNA followed by DNA extraction and digestion with Taq I. The presence, still, of the original 5.3 kb and/or 6.5 kb Taq I bands confirms that the Taq I restriction sites are situated within the polymorphic Bgl II fragments (Fig. 5). Thus, isolation of the 5.7 kb Bgl II fragment from three double heterozygotes (genotype 5.7/12.5, 5.3/6.5) followed by Taq I digestion resulted in the appearance of 5.3 kb Taq I bands only (Fig. 5a).