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Detection and mapping of polymorphic KpnI alleles in the human T-cell receptor constant beta-2 locus

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SUMMARY

Southern blot analysis with human T-cell receptor (TcR) beta-chain specific cDNA probes revealed two novel allelic forms of the TcR beta-2 gene locus. Three different genotypes were noted based on the presence of polymorphic KpnI restriction fragments: I, 5·7 kb fragment only; II, 3·9 kb and 1·8 kb fragments only; III, all three polymorphic fragments. This hybridization pattern suggested that the presence or absence of a polymorphic KpnI site within the 5·7 kb fragment defines the two different allelic forms of the TcR beta chain locus. By Southern blot analysis of genomic DNA from T-cell lines with deleted C-beta-1 regions and computer-assisted restriction site mapping of germline and cDNA sequences of the C-beta-2 locus, the polymorphic KpnI site was localized at 24 bp 5′ to the third exon of the C-beta-2 gene. It was determined that the polymorphic KpnI site and the earlier described polymorphic BglII site located 5′ to the C-beta-2 gene are not co-inherited. No difference was noted in distribution of the KpnI genotypes and allelic frequencies between 26 normal individuals and 22 patients with systemic lupus erythematosus. However, this newly characterized polymorphism of the TcR locus should provide a useful tool to analyse the role of inherited genetic variations in the function of T lymphocytes under normal and pathological conditions.

Restriction fragment length polymorphisms (RFLP) of the TcR alpha, beta and gamma chain loci have recently been described (Hoover *et al.*, 1985; Robinson & Kindt, 1985; Berliner *et al.*, 1985; Robinson & Kindt, 1986; Concannon, Gatti & Hood, 1987; Posnett, Wang, Friedman, 1986; Li, Szabo & Posnett, 1988). These mutations in the recognition sites for restriction enzymes may be used to analyse the genetic variability in the immune response and the genetic background of autoimmune diseases. Recently, significant associations have been reported between the heterozygous genotype of the polymorphic BglII site (BglII+/BglII−) and the autoimmune diseases insulin-dependent diabetes mellitus (Hoover *et al.*, 1986), membranous nephropathy and Graves' disease (Demaine *et al.*, 1987). On the other hand, the polymorphic BglII site could not be associated with systemic lupus erythematosus (SLE) (Bentwich *et al.*, 1987).

RFLP of the TcR beta chain locus was investigated in 26 unrelated normal individuals and 22 unrelated patients with SLE diagnosed according to the ARA criteria (Tan *et al.*, 1982). High molecular weight genomic DNA was isolated from peripheral blood lymphocytes and granulocytes and analysed by Southern blot hybridization, as described earlier (Perl *et al.*, 1987). As source of DNA from T-cell lines, Jurkat and Molt-4 leukaemic T cells, as well as HTLV-I-transformed SLB-I cells and HTLV-II-transformed MO-T cells were used. DNA from lymphocytes and granulocytes of each donor was studied along with human placental DNA (P-DNA; Sigma, St Louis, MO) as a standard germline control to exclude the involvement of somatic rearrangement and clonal expansion in the detected polymorphic hybridization patterns. The status of the TcR beta chain gene locus was assessed using an 800 bp cDNA probe, Jur-beta-2. This cDNA probe contains diversity (D, bases 1–9), joining (J, 10–57) and constant regions (C, bases 58–800) of the human T-cell receptor beta-2 gene cluster (Yoshikai *et al.*, 1984).

While uniform hybridization patterns were noted with EcoRI, HindIII, BamHI, PvuII, PstI, SstI, and AvaI enzymes, a RFLP was found with the enzyme KpnI as shown in Fig. 1a. Hybridization of Jur-beta-2 with KpnI-digested DNA samples revealed three polymorphic (5·7 kb, 3·9 kb, and 1·8 kb) and three invariant fragments (7·5 kb, 3·5 kb, and 2·3 kb). Three different genotypes were noted based on the presence of the polymorphic fragments: I, 5·7 kb fragment only; II, 3·9 kb and 1·8 kb fragments only; and III, all three polymorphic fragments. This hybridization pattern suggested that the presence or absence of a polymorphic KpnI site within the 5·7 kb fragment defines the

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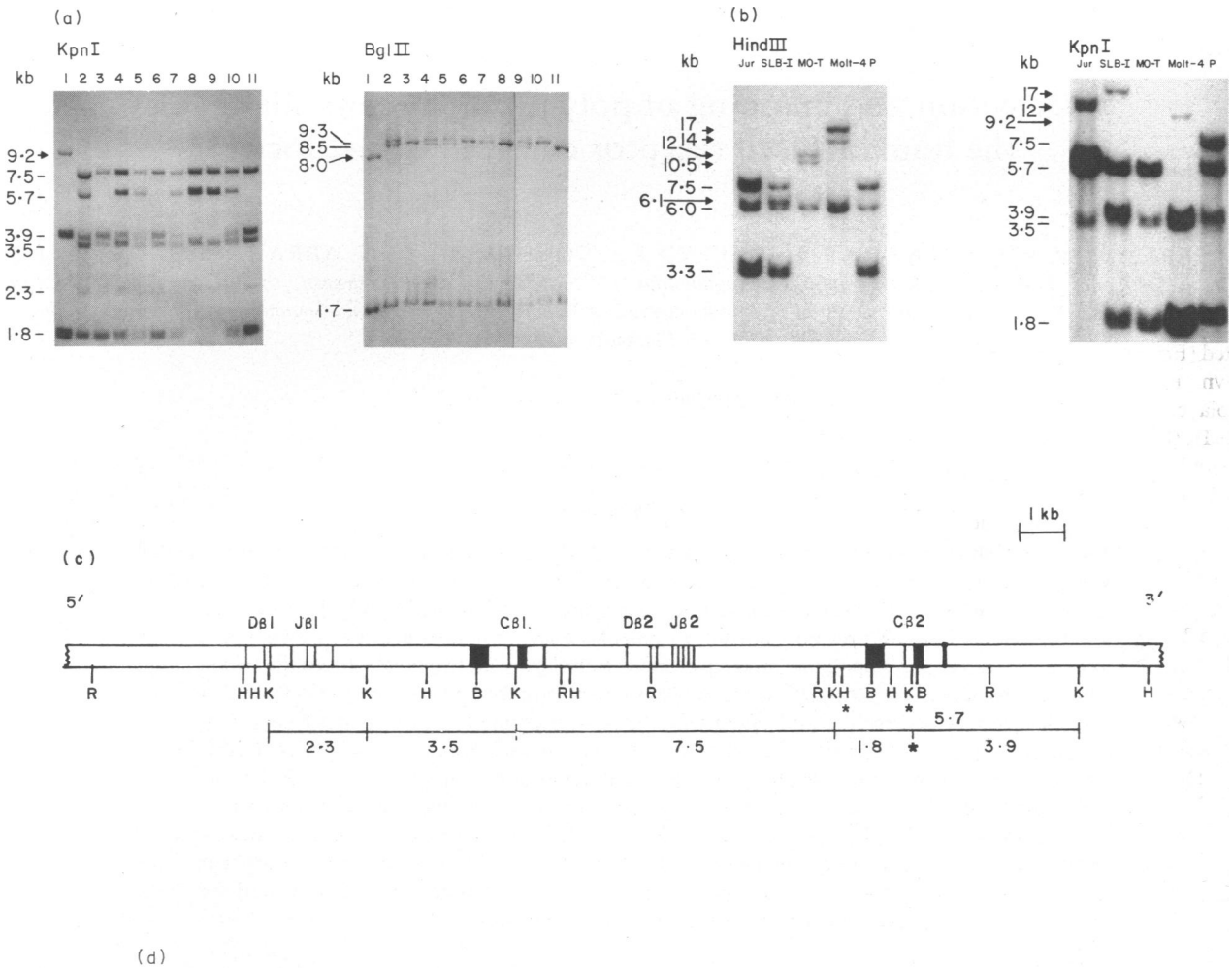

(d)

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1    ATGGCGTAGT CCCCAAAGAA CGAGGACCTA GTAACATAAT TGTGCTTCAT
51   TATGGTCCTT TCCCGGCCTT CTCTCTCACA CATACACAGA GCCCCTACCA
101  GGACCAGACA GCTCTCAGAG CAACCCTAGC CCCATTACCT CTTCCCTTTC
151  CAGAGGACCT GAAAAACGTG TTCCCACCCG AGGTCGCTGT GTTTGAGCCA
201  TCAGAAGCAG AGATCTCCCA CACCCAAAAG GCCACACTGG TGTGCCTGGC
251  CACAGGCTTC TACCCCGACC ACGTGGAGCT GAGCTGGTGG GTGAATGGGA
301  AGGAGGTGCA CAGTGGGGTC AGCACAGACC CGCAGCCCCT CAAGGAGCAG
351  CCCGCCCTCA ATGACTCCAG ATACTGCCTG AGCAGCCGCC TGAGGGTCTC
401  GGCCACCTTC TGGCAGAACC CCCGCAACCA CTTCCGCTGT CAAGTCCAGT
451  TCTACGGGCT CTCGGAGAAT GACGAGTGGA CCCAGGATAG GGCCAAACCT
501  GTCACCCAGA TCGTCAGCGC CGAGGCCTGG GGTAGAGCAG GTGAGTGGGG
551  CCTGGGGAGA TGCCTGGAGG AGATTAGGTG AGACCAGCTA CCAGGGAAAA
601  TGGAAAGATC CAGGTAGCGG ACAAGACTAG ATCCAGAAGA AAGCCAGAGT
651  GGACAAGGTG GGATGATCAA GGTTCACAGG GTCAGCAAAG CACGGTGTGC
701  ACTTCCCCCA CCAAGAAGCA TAGAGGCTGA ATGGAGCACC TCAAGCTCAT
751  TCTTCCTTCA GATCCTGACA CCTTAGAGCT AAGCTTTCAA GTCTCCCTGA
801  GGACCAGCCA TACAGCTCAG CATCTGAGTG GTGTGCATCC CATTCTCTTC
851  TGGGGTCCTG GTTTCCTAAG ATCATAGTGA CCACTTCGCT GGCACTGGAG
901  CAGCATGAGG GAGACAGAAC CAGGGCTATC AAAGGAGGCT GACTTTGTAC
951  TATCTGATAT GCATGTGTTT GIGGCCTGTG AGTCTGTGAT GTAAGGCTCA

1001 ATGTCCTTAC AAAGCAGCAT TCTCTCATCC ATTTTTCTTC CCCTGTTTTC
1051 TTTCAGACTG TGGCTTCACC TCCGGTAAGT CAGTCTCTCC TTTTTCTCTC
1101 TATCTTTCGC CGTCTCTGCT CTCGAACCAG GGCATGGAGA ATCCACGGAC
1151 ACAGGGGCGT GAGGGAGGCC AGAGCCACCY GTGCACAGGT ACCTACATGC
1201 TCTGTTCTTG TCAACAGAGT CTTACCAGCA AGGGGTCCTG TCTGCCACCA
1251 TCCTCTCTGA GATCTTGCTA GGGAAGGCCA CCTTGTATGC CCTGCTGGTC
1301 AGTGCCCTCG TGCTGATGGG CATGGTAAGG AGGAGGGTCG GATAGGGCAG
1351 ATGATGGGGG CAGGCGATGG AACATCACAC ATGGGCATAA AGGAATCTCA
1401 GAGCCAGAGC ACAGCCTAAT ATATCCTATC ACCTCAATGA AACCATAATG
1451 AAGCCAGACT GGGGAGAAAA TGCAGGGAAT ATCACAGAAT GCATCATGGG
1501 AGGATGGAGA CAACCAGCGA GCCCTACTCA AATTAGGCCT CAGAGCCCGC
1551 CTCCCCTGCC CTACTCCTGC TGTGCCATAG CCCCTGAAAC CCTGAAAATG
1601 TTCTCTCTTC CACAGGTCAA GAGAAAGGAT TCCAGAGGCT AGCTCCAAAA
1651 CCATCCCAGG TCATTCTTCA TCCTCACCCA GGATTCTCCT GTACCTGCTC
1701 CCAATCTGTG TTCCTAAAAG TGATTCTCAC TCTGCTTCTC ATCTCCTACT
1751 TACATGAATA CTTCTCTCTT TTTTCTGTTT CCCTGAAGAT TGAGCTCCCA
1801 ACCCCCAAGT ACGAAATAGG CTAAACCAAT AAAAAATTGT GTGTTGGGCC
1851 TGGTTGCATT TCAGGAGTGT CTGTGGAGTT CTGCTCATCA CTGACCTATC
1901 TTCTGATTTA GGGAAAGCAG CATTCGCTTG GACATCTGAA GTGACAGCCC
1951 TCTTTCTCTC CACCCAATGC TGCTTTCTCC TGTTCATCCT GATGGAAGTC
2001 TCAACACA
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Figure 1. (a) Southern blot analysis of KpnI- and BglII-digested genomic DNA samples using the Jur-beta-2 cDNA probe. Lanes shown are: (1) DNA from Molt-4 T-cell line; (2) human placenta DNA; (3–11) lymphocyte DNA from nine unrelated normal individuals. Dashes indicate germline bands, while arrows show rearranged fragments. (b) Southern blot analysis of HindIII- and KpnI-digested DNAs from Jurkat (Jur), SLB-I, MO-T and Molt-4 T-cell lines, and from human placenta (P). The blot was hybridized to the Jur-C-beta-2 probe. (c) Schematic map of the TcR constant region loci. The restriction sites for EcoRI (R), HindIII (H), and BglII (B) and the localization for coding exons (shaded areas) were obtained from Tunacliffe *et al.* (1985) and Toyonaga *et al.* (1985). The KpnI sites (K) and the arrangement of invariant and polymorphic KpnI fragments were derived from the results described in this paper. The polymorphic KpnI and BglII sites are indicated by asterisks. (d) Organization of the four exons and location of the polymorphic KpnI site in the nucleotide sequence of a germline C-beta-2 gene, as adapted from Tunacliffe *et al.* (1985).

two different allelic forms of the TCR beta chain locus. Thus, patterns I and II represent homozygous constellations, while pattern III shows the heterozygous pattern.

In order to determine if the presence or absence of the KpnI site is coinherited with the polymorphic BglII site of the TcR beta locus, hybridization patterns of the Jur-beta-2 probe to KpnI- and BglII-digested genomic DNA samples were compared in individuals homozygous for at least one of the two polymorphic loci. By hybridization of the Jur-beta-2 probe to BglII-digested genomic DNA samples, two homozygous patterns (I, 9·3 kb fragment; II, 8·5 kb fragment) and one heterozygous pattern (III, 9·3 kb and 8·5 kb fragments) were noted (Fig. 1a). As shown in Fig. 1a, donors 8 and 9 lacking the polymorphic KpnI site (homozygous type I KpnI genotype) displayed either a type II (homozygous) BglII genotype, or a type III (heterozygous) BglII pattern. On the other hand, donors 3, 6 and 11 displayed the type II (homozygous) KpnI genotype, carrying the polymorphic KpnI site on both alleles. While donors 3 and 11 showed the type II BglII genotype, donor 6 displayed the type I BglII pattern. These data and comparative analysis of 12 additional unrelated donors (not shown) revealed no exclusive association between the KpnI and BglII genotypes of the TCR beta locus.

To localize the polymorphic KpnI site within the TcR beta locus, T-cell lines with clonal TcR beta chain gene rearrangements were analysed. The status of the TcR beta locus was assessed in Jurkat, Molt-4, SLB-I and MO-T cells by digestion with HindIII- and EcoRI-restriction enzymes. Using the Jur-beta-2 probe, digestion with HindIII results in three germline fragments: a 3·3 kb band that represents C-beta-1, a 6 kb fragment that harbors the 3′ portion of C-beta-2, and a 7·5 kb fragment that contains D-beta-2, J-beta-2 and the 5′ portion of C-beta-2 (Fig. 1b and 1c). Rearrangements involving the C-beta-2 region result in the deletion of the fragment harboring the C-beta-1 gene and an alteration in size of the fragment carrying the D-beta-2, J-beta-2 and C-beta-2 genes. The absence of 3·3 kb and 7·5 kb HindIII fragments, as well as the detection of two rearranged bands in MO-T and Molt-4 DNAs, suggests that in these cell lines rearrangement of both C-beta-2 alleles and deletion of both C-beta-1 regions occurred. Hybridization of EcoRI-digested DNA samples to the Jur-beta-2 probe results in two germline fragments: an 11 kb fragment harboring the beta-1 complex, and a 4 kb fragment containing the beta-2 complex (Fig. 1c). While not shown, annealing of the Jur-beta-2 probe to EcoRI digested DNAs showed the rearrangement of one of the C-beta-1 alleles in Jurkat and SLB-I cells (one rearranged fragment and the 4 kb germline fragment) and confirmed the deletion of the beta-1 complex of both TcR beta loci in MO-T and Molt-4 cells (detection of a solitary 4 kb germline fragment).

To further localize the polymorphic KpnI site, the first 80 bases of the Jur-beta-2 cDNA clone containing D-beta-2 and J-beta-2 elements were removed after cleaving with AvaI. The AvaI restriction site was selected in order to generate a C-beta-2-specific probe after sequence analysis of the Jur-beta-2 clone using the University of Wisconsin Genetics Computer Group software. The 720-base long C-beta-2-specific probe (Jur-C-beta-2) was then hybridized to KpnI-digested DNA samples. The only difference in the hybridization patterns of KpnI-digested DNA samples was the detection of the weak invariant 2·3 kb fragment by the entire Jur-beta-2 probe (Fig. 1a) but not the Jur-C-beta-2 probe (Fig. 1b). Southern blot analysis of KpnI-digested DNA samples from the T-cell lines demonstrated a heterozygous pattern in SLB-I and MO-T cells, a type I homozygous pattern in Jurkat, and a type II homozygous pattern in Molt-4 cells. Since both MO-T and Molt-4 cells have deleted beta-1 regions, the polymorphic KpnI site is within the C-beta-2 locus. The 7·5 kb, 3·5 kb and 2·3 kb invariant germline bands, clearly visualized in Fig. 1a, were not present in Molt-4 cells, suggesting that they represent the beta-1 complex deleted in this T-cell line. The 2·3 kb invariant fragment was not detected in placenta cells by the Jura-C-beta-2 probe (Fig. 1b), suggesting that it contains the D-beta-1 and J-beta-1 regions. A schematic map of the TcR beta loci with arrangement of the invariant and polymorphic KpnI fragments is presented in Fig. 1c. The polymorphic KpnI site was further localized within the C-beta-2 region, 24 bp 5′ to exon III of the C-beta-2 gene, by comparative restriction-site analysis of germline and cDNA sequences of the TcR beta-2 locus available in Genbank (Yoshikai *et al.*, 1984; Yanagi *et al.*, 1985; Tunacliffe *et al.*, 1985; Toyonaga *et al.*, 1985). The polymorphic KpnI site corresponds to base positions 1188–1193 in a germline C-beta-2 gene sequence, as shown in Fig. 1d (Tunacliffe *et al.*, 1985). The polymorphic BglII site is located 675 bp to the 5′ side of the C-beta-2 gene (Toyonaga *et al.*, 1985) which is 523 bp 5′ to the startpoint of the sequence shown in Fig. 1d. Thus, the polymorphic BglII site is 1711 bp upstream from the polymorphic KpnI site of the C-beta-2 locus.

The TcR C-beta-2 KpnI genotypes and the frequencies of alleles with or without the polymorphic KpnI site were evaluated in 26 healthy donors and 22 patients with SLE. KpnI-digested DNAs from lymphocytes and granulocytes within any particular donor showed identical hybridization pattern confirming the germline origin of the individual genotypes. As shown in Table 1, no significant differences were noted either in the distribution of genotypes or in the allelic frequencies between normals and SLE patients. In accordance with Bentwich *et al.* (1987), we detected no difference in the distribution of BglII genotypes of the TcR beta-2 locus between patients with SLE and normals (data not shown). While the data suggest that these RFLP alone are not responsible for the development of SLE, they may influence disease susceptibility in association with other polymorphic gene loci encoding the TcR. Complex evaluation of all molecular genotypes of the TcR alpha/beta and gamma/delta loci is needed to characterize their involvement in T-cell function under normal and pathological conditions.

Table 1. Distribution of TcR C-beta-2 KpnI RFLP and allelic frequencies in normal individuals and patients with SLE

KpnI genotypes	Normals ($n=26$)	SLE patients ($n=22$)
I (KpnI−/KpnI−)	4 (15·4%)	1 (4·5%)*
II (KpnI+/KpnI+)	12 (46·2%)	9 (40·9%)
III (KpnI+/KpnI−)	10 (38·4%)	12 (54·6%)
Allelic frequencies		
KpnI+	0·65	0·68
KpnI−	0·35	0·32

* The slight difference in the frequency of genotype I between normals and SLE patients is not significant ($\chi^2=1·62$, $P>0·2$).

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