Brief Definitive Report

RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF THE GENE ENCODING THE α CHAIN OF THE HUMAN T CELL RECEPTOR

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The immune system uses B and T cells to identify and eliminate foreign antigens. For these cells to function, antigen receptors are required that can discriminate self from nonself. The T cell receptor is similar to the B cell receptor (Ig), in that it is clonally distributed and exhibits extensive diversity for antigen recognition. However, the two receptors differ functionally in that T cells only recognize antigen in conjunction with products of the major histocompatibility complex.

At present, it is known (1–3) that the T cell receptor for antigen is a 80–90 kilodalton (kD) molecule consisting of two glycosylated, disulfide-linked polypeptide chains, α and β. The chains have a similar organization to that of Ig, in that the functional chains are generated by recombinational events at the DNA level. The four gene segments required for a functional β chain are those for the variable (V), diversity (D), joining (J), and constant (C) regions (4–6). At least three similar gene segments (V, J, and C) are necessary for the α chain (7). The human β chain gene has been mapped to chromosome 7, whereas the α chain gene has been localized to chromosome 14, proximal to the Ig heavy chain locus (8–11).

Additional genetic information is required to establish linkage relationships, and to further analyze, at the molecular level, T cell function. A recent study (12) has shown a restriction fragment length polymorphism (RFLP) of one of the genes encoding the β chain of the human T cell antigen receptor. Segregation within families allowed the discrimination of allelic forms of the β chain gene. This report describes a similar polymorphism in the gene encoding the α chain of the human T cell antigen receptor, that also segregates within families, thus yielding at least two allelic forms of both the α and β chain genes of this crucial membrane molecule.

Materials and Methods

Preparation of Human DNA. Genomic DNA was isolated from HLA-D homozygous typing cell lines, using the procedure of Maniatis et al. (13). A similar protocol was used to isolate high molecular weight DNA from plasma-depleted peripheral blood obtained from normal individuals. Briefly, peripheral blood cells were washed three times with TE

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buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 12,000 *g* and lysed two times with cold lysis buffer (320 mM sucrose, 10 mM Tris HCl, pH 7.4, 1% Triton X-100, 5 mM $MgCl_2$) for 10 min, with centrifugation at 12,000 *g*. The cellular and nuclear membranes were treated with proteinase K (0.1 mg/ml) at 60°C for 3 h in 10 mM Tris HCl, 5 mM EDTA, 75 mM NaCl, 0.5% sodium dodecyl sulfate (SDS), pH 8.0. The samples were subsequently extracted with phenol, phenol/chloroform, and chloroform before transfer to dialysis tubing for DNase-free RNase (50 μg/ml) treatment. The samples were dialyzed overnight at 37°C against 20 mM Tris HCl (pH 7.5), 10 mM NaCl, 0.5 mM EDTA, pH 7.5, followed by phenol, phenol/chloroform, and chloroform extractions. The DNA was dialyzed against TE buffer for 4–5 d before ethanol precipitation.

Digestion, Electrophoresis, Transfer, and Hybridization. Genomic DNA was digested with Bgl II (2 U/μg DNA) (Boehringer Mannheim, Inc., Indianapolis, IN) in 100 mM Tris HCl (pH 7.5), 100 mM $MgCl_2$, 10 mM dithiothreitol for 18–20 h at 37°C. The samples were ethanol-precipitated, resuspended in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA) and electrophoresed in 0.6% agarose gel (15 μg/lane) for 18–20 h in TBE. The molecular mass markers used were Hind III–digested phage λ DNA, and Hae III–digested φX-174 (PL Biochemicals, Milwaukee, WS). Southern blot hybridization was performed using nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) (14). The filters were prehybridized at 42°C for 16–20 h in 50% deionized formamide, 5 × SSC (standard sodium citrate), 25 mM sodium phosphate buffer (pH 6.5), 5 × Denhardt's solution, 0.5% SDS, and 500 μg/ml herring sperm DNA. The probes used in these studies were either pGA5, a cDNA isolated from the human T cell leukemia line HPB-MLT, representing the leader (L), V, C, transmembrane, cytoplasmic, and 3′ untranslated (3′ UT) regions of the T cell α gene; or fragments thereof (Eco RI/Acc I L and V region probe; Acc I/Eco RI J, C, and 3′ UT region probe; Rsa I/Eco RI C and 3′ UT region probe; and Ava II/Eco RI 3′ UT probe. See Fig. 1) (15). The probe was nick-translated, and hybridization was carried out under the prehybridization conditions described above, with the following modifications: 20 mM sodium phosphate buffer, 1× Denhardt's solution, and 100 μg/ml herring sperm DNA. The filters were postwashed, with the final stringency being 1× SSC at 42°C for 1 h, and autoradiography was performed for 4 d. In some experiments, the filters were stripped twice with 0.1× SSC, 0.1% SDS (95°C) for 15 min before reprobing.

Results

T Cell α Chain Polymorphism Revealed by RFLP Analysis. An initial screening of six different restriction enzymes yielded only one enzyme, Bgl II, that could discern RFLP of the T cell antigen receptor α chain gene. Analysis of Bgl II–digested genomic DNA revealed that seven fragments hybridized with the pGA5 probe (Fig. 2, *A*). Five of these fragments (1–4, and 7) were invariant among different individuals and cell lines tested, while two fragments (5 and 6) varied. Bands 2 and 4 in Fig. 2 appear to be broad, and were initially thought to

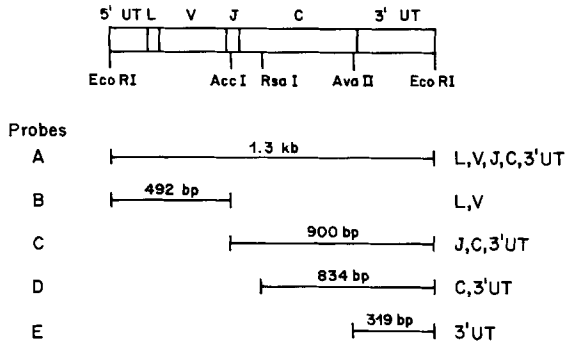

FIGURE 1. Schematic of the pGA5 insert and fragments thereof used as specific probes.

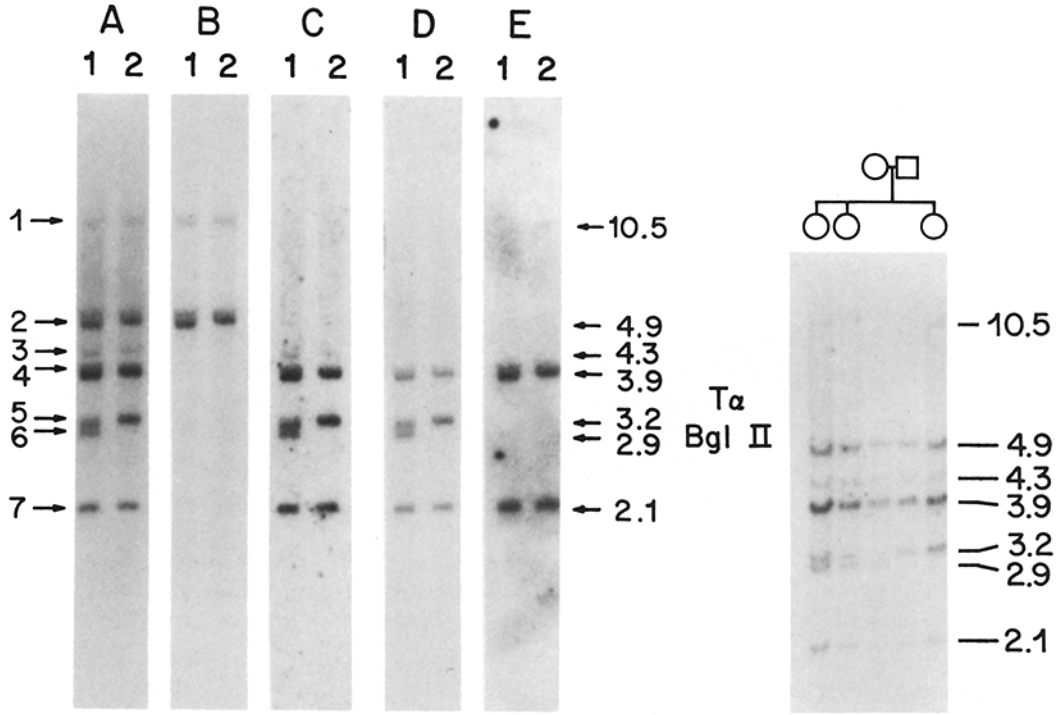

FIGURE 2. Bgl II–digested genomic DNA from a heterozygous (3.2/2.9 kb) and a homozygous (3.2/3.2 kb) individual for the T cell antigen receptor α chain gene hybridized with probe A. *A*, the Eco RI pGA5 insert; *B*, an Eco RI/Acc I L, V probe; *C*, Acc I/Eco RI J, C, 3′ UT probe; *D*, Rsa I/Eco RI C, 3′ UT probe; *E*, Ava II/Eco RI 3′ UT probe.

FIGURE 3. Bgl II–digested genomic DNA of five members of a family was electrophoresed, blotted and probed with pGA5.

TABLE I

Summary of Hybridization of Various T Cell Receptor α Chain Probes on Bgl II–digested Human DNA

Fragment	Size	Probe					Gene region
		A	B	C	D	E	
	kb						
1	10.5	+	+	−	−	−	V
2	4.9	+	+	−	−	−	V
3	4.3	+	−	+	−	−	J
4	3.9	+	−	+	+	+	3′ UT
5	3.2	+	−	+	+	−	C
6	2.9	+	−	+	+	−	C
7	2.1	+	−	+	+	+	3′ UT

Probe letters refer to the left side of Fig. 1. Fragment number refers to the left side of Fig. 2.

represent two separate restriction fragments. However, in other experiments (for example, see Fig. 3) both appear to be single restriction fragments.

Additional analyses of these Bgl II fragments using a single filter reprobed several times with different probes corresponding to the V, J, C, and/or 3′ UT region of the T cell α cDNA revealed that the 10.5 and 4.9 kilobase (kb) fragments (bands 1 and 2) represent portions of the V region of the T cell antigen receptor α chain gene (Fig. 2, *B*, and Table I). The 4.3 kb fragment

TABLE II

Distribution of T Cell Receptor α Chain Gene RFLP In B Cell Lines and Normal Individuals

Restriction fragment pattern	B cell lines ($N = 19$)	Normal individuals ($N = 15$)	Total ($N = 34$)
3.2/3.2	14 (73.7%)	12 (80%)	26 (76.5%)
3.2/2.9	4 (21.1%)	3 (20%)	7 (20.6%)
2.9/2.9	1 (5.3%)	0	1 (2.9%)

(band 3) hybridizes to the J, C, 3′ UT probe, but not to the C, 3′ UT probe (Fig. 2, *C* and *D*, and Table I), therefore, band 3 must encode the J segment of the T cell receptor α chain gene. The polymorphic 3.2 and 2.9 kb fragments (bands 5 and 6) hybridize to the C, 3′ UT probe, but not to the 3′ UT probe (Fig. 2, *D* and *E*, and Table I). Thus, bands 5 and 6 must represent the C region of the T cell receptor α chain gene. Furthermore, since the 3′ UT probe hybridizes to the 3.9 and 2.1 kb fragments (bands 4 and 7), these must encode 3′ UT gene segments. (Fig. 2, *E*, and Table I).

Distribution of the T Cell Antigen Receptor α Chain RFLP in the Population. Screening 19 B lymphoblastoid cell lines and 15 normal individuals revealed three patterns: the two homozygous patterns (2.9/2.9 and 3.2/3.2) and the heterozygous pattern, 2.9/3.2. The frequency of the homozygous 3.2/3.2 situation is 76.5%, whereas the homozygous 2.9/2.9 is only 2.9% (Table II). The heterozygous state comprises 20.6% of the population. The gene frequencies are 0.87 for the 3.2 kb fragment, and 0.13 for the 2.9 kb band.

When DNA from several families was examined, segregation of the T cell receptor α chain gene RFLP was demonstrated (Fig. 3). In this example, the father was homozygous for the 3.2 kb fragment, as was sibling 3 (far right), whereas the mother and siblings 1 and 2 were heterozygous. Since each child would inherit a 3.2 kb paternal fragment, the 2.9 kb fragment seen in siblings 1 and 2 is of maternal origin. Sibling 3 inherited a 3.2 kb fragment from both parents.

Discussion

This report uses RFLP analysis to show that there are at least two allelic forms of the T cell antigen receptor α chain gene. The majority of the individuals and cell lines tested were homozygous for the 3.2 kb Bgl II fragment that hybridized with a cDNA for the T cell α chain gene. In contrast, only 3% of the genomic DNA screened were homozygous for the other allele (2.9 kb fragment). The appearance of the 2.9 kb fragment is not the result of gene rearrangement, as it is observed at the same frequency in DNA from peripheral blood and clonal B cell lines. Both of these sources should yield germline banding patterns. Furthermore, the ability to demonstrate that this 2.9 kb fragment segregates in families further supports the existence of two allelic forms of the human T cell receptor α chain gene.

The location of the Bgl II site that gives rise to the RFLP described herein is either 5′ or 3′ of the constant region exon(s) of the T cell receptor α chain gene. Inspection of the published nucleotide sequences of the two cDNA clones of the T cell antigen receptor α chain reveals a single Bgl II site three nucleotides into the 3′ UT of one clone (15), but not the other (9). Therefore, this sequence

analysis, together with the data presented in Fig. 2 of two 3′ UT Bgl II fragments and the polymorphic C region gene segments, suggests that the 3′ UT region of the gene must be encoded by three separate exons. If, however, the sequence differences reported are technical in nature, two 3′ UT exons could explain the data. Thus, the precise location of the Bgl II site explaining the RFLP, either 5′ or 3′ of the C region exon, or within the first 3′ UT, must await further study. The location of the 3′ Bgl II site, either within the intron or within the 3′ UT exon may reflect sequence differences that may be important in processing, and therefore ultimately in gene expression. In addition, the two allelic forms may also differ within their coding segments, and these differences were not detected, either because the changes do not alter restriction enzyme sites, or because not enough enzymes were used. Hopefully, the allelic variation revealed by this RFLP is reflective of the entire chromosomal segment, and is associated with other (possibly V region) variations. Nonetheless, within a family, it serves as a marker for paternal/maternal chromosomes and allows an analysis of other characteristics of that haplotype.

The recent demonstration (12) of at least two allelic forms of T cell antigen receptor β chain gene, in conjunction with the data presented here of two allelic forms of the T cell antigen receptor α chain gene, suggests that *trans* association of allelic α and β chains could occur on normal T cells. This offers one opportunity of identifying paternal and maternal genes expressed in individual T cell clones of heterozygous individuals, as well as the possibility of testing for correlations between T cell antigen receptor alleles and receptor function. Since helper T cells recognize antigen in association with HLA class II molecules, and the α and β chains of certain class II molecules (HLA-DQ) also *trans* associate (16), a significant degree of altered reactivity could occur in the heterozygous offspring of parents homozygous at each of these loci. Further studies examining polymorphism of the T cell gene family will enhance our knowledge of recognition by the T cell antigen receptor, as well as facilitate genetic linkage studies concerning T cell function.

Summary

Two allelic forms of the T cell antigen receptor α chain gene were discerned by restiction fragment length polymorphism (RFLP) employing the T cell antigen receptor α chain probe pGA5, and the restriction enzyme Bgl II. Analysis revealed that the polymorphic fragments are detected by a probe specific for the constant region exon of the T cell antigen receptor α chain gene. Furthermore, the polymorphic fragments were shown to segregate within families. The two allelic forms yield two homozygous states, 3.2/3.2 and 2.9/2.9, at a frequency of 76.5 and 2.9%, respectively, within the normal population. The heterozygous state was observed in 20.6% of the population. The discovery of allelic forms of both the α and β chains of the T cell antigen receptor genes may provide a unique opportunity to study heritable markers of T cell function in several human diseases.

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Note added in proof: A second cDNA clone from the Jurkat T cell line has been sequenced (Yanagi, Y., A. Chan, B. Chin, M. Minden, and T. Mak. 1985. Analysis of cDNA clones specific for human T cells and the α and β chains of the T-cell receptor heterodimer from a human T-cell line. Proc. Natl. Acad. Sci. USA. 82:3430. This sequence differs from the previous isolate in that it possesses a Bgl II site three nucleotides into the 3′ UT, as does the isolate from the HBP-MLT T cell line. Therefore, since previously discussed sequence differences appear to be of a technical nature, the 3′ UT region likely consists of two exons, and the Bgl II site in the 3′ UT probably does not explain the RFLP described herein.

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