Brief Definitive Report

GENETIC MARKERS OF THE ANTIGEN-SPECIFIC T CELL RECEPTOR LOCUS

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The antigen receptor of the T cell is a disulfide-linked heterodimer containing an acidic α chain and a basic β chain, both migrating at ~40,000 mol wt in sodium dodecyl sulfate–polyacrylamide gel electrophoresis. cDNA (complementary DNA) clones have been isolated that exhibit constant and variable regions, as do the subunits of the heterodimer (1–3). The loci encoding the α and β subunits of the heterodimer undergo gene rearrangements unique to T cells (4). The β chain gene locus, mapped to chromosome 6 (5, 6), consists of a set of variable region genes, presumably linked to two J-C (joining, constant region) clusters [referred to as Jβ-Cβ (Jβ1-Cβ1) and Jβ'-Cβ' (Jβ2-Cβ2)] contained within a 25 kilobase (kb) genomic clone (7).

A rat monoclonal antibody (mAb), KJ16-133, has been isolated that reacts with an allotypic determinant present on ~20% of the T cells of most mouse strains (including BALB/c), but fails to react with T cells from SJL, SJA, SWR, C57BR, and C57L mice (8, 9). KJ16-133 is believed to react with the antigen-specific and major histocompatibility complex (MHC)-restricted receptor on T cells because: (*a*) as soluble antibody, it inhibits synthesis of interleukin 2 (IL-2) induced when T cell hybridoma D0-11.10 interacts with its ligands, ovalbumin plus I-A^d; (*b*) as insolubilized antibody (bound to Sepharose beads), it mimics antigen plus class II–restricting element by inducing hybridomas to secrete IL-2; (*c*) it immunoprecipitates an 86 kilodalton heterodimer from some T cell hybridomas, and reacts with similar molecules present on the surface of some but not all T cells (8); and (*d*) the expression of the allotypic determinant is allelically excluded (9). Furthermore, the reaction of this antiallotype, KJ16-133, with T cells is independent of the antigen or MHC class I or II restriction specificity, and, hence, the effector function of the T cell (8).

We show herein that the Eco RI restriction endonuclease can be used to distinguish the BALB/c allele of the Jβ-Cβ region from that of SJL/J. Since KJ16-133 distinguishes an allotypic determinant present on the surface of T cells from BALB/c mice, and absent from those of SJL/J, the segregation of these two pairs of alleles was determined in a family of recombinant inbred (RI) strains made from BALB/c and SJL/J progenitors [(C × J)RI]. Complete concordance was found between the two markers, indicating that the Jβ-Cβ gene region is linked to the gene encoding the allotypically tagged subunit of the T cell heterodimer.

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Materials and Methods

DNA Preparation and Probes. 10 μg each of liver DNA from BALB/c, B10A, SJL/J, C57L, SWR, and a family of (C × J)RI mice were digested with Eco RI, (3 U/μg DNA), electrophoresed in horizontal 1% agarose gels in Tris-acetate buffer, blotted according to the method of Southern (10), and hybridized to DNA probes labeled with ^{32}P-labeled nucleotides by nick translation. The sequences included in clones specific for the β-chain are indicated in Fig. 1. The cDNA clone 86T5(2) consists of a diversity (D) region with its 5′ flanking sequence, Jβ3, and the coding region of Cβ. This probe hybridizes with these segments, and to Cβ′ because the nucleotide sequences of Cβ and Cβ′ are virtually identical (9–12). Probe 6, specific for the 5′(Jβ) cluster of J exons, is a 1.4 kb fragment extending from the Bam HI site 5′ to Jβ3, to the Pvu II site 3′ to Jβ6 (7). Probe 11, specific for the 3′ cluster of J exons (Jβ′) is a Pvu II–Cla I fragment spanning most of the Jβ′ region (4). A Cβ probe lacking J sequences was derived from 86T5 by subcloning in pUC9. The Sau 3A insert of this clone includes the first two domains of Cβ, and 43 nucleotides of the third domain. The Vκ21A probe, kindly provided by Robert Kleinfield and Roy Riblet (Fox Chase Institute for Cancer Research, Philadelphia, PA), is a 2.0 kb Bam HI fragment inserted into m13mp8. It includes 1.8 kb of flanking sequence 5′ to the coding region, and extends 180 nucleotides into the V region, to a Bam HI site.

Cytofluorimetric Analysis of T Cells. Preparation of a rat mAb KJ16-133, which recognizes the antigen–MHC receptor complex has been described (8). This was used as the primary antibody to stain nylon wool–purified lymph node T cells. Viable T cells stained with this antiallotype at 37°C, or a control antibody, anti-Thy-1 (T24/40.7, from Dr. Ian Trowbridge, The Salk Institute) at 4°C, were then incubated at 4°C with an appropriate dilution of an affinity-purified, fluorescein-conjugated, mouse anti–rat κ-chain mAb (R67/9.1 from Dr. T. A. Springer, Harvard Medical School, Boston, MA). The relative fluorescence intensities of individual cells were determined using the Cytofluorograf System 50-H (Ortho Diagnostic Instruments, Westwood, MA), and the percentage of stained cells was determined as previously described (13).

Results

Alleles of Jβ-Cβ Region Are Defined by Restriction Endonuclease Sites. Liver DNA from BALB/c and SJL/J mice can be distinguished by Eco RI restriction sites detectable with the cDNA probe 86T5. In BALB/c Eco RI cleavage of an 11 kb segment produces a 9.4 kb fragment containing Jβ and a 2.0 kb fragment carrying Cβ; both fragments are absent in SJL/J, which displays two other fragments, 7.8 and 3.1 kb, derived from the same 11 kb segment (Fig. 1). The

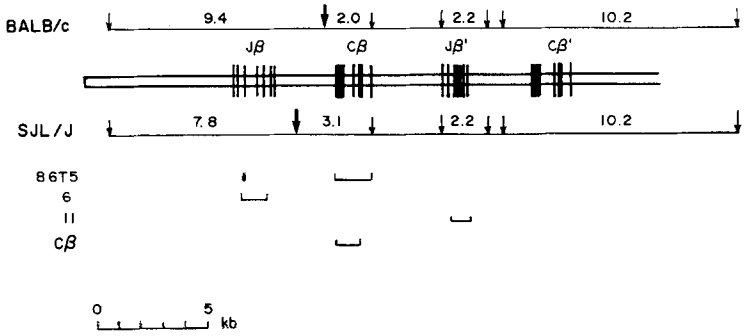

FIGURE 1. Organization of the Jβ-Cβ–Jβ′-Cβ′ region. Eco RI sites in this region are indicated as arrows. Those Eco RI sites that distinguish the alleles of BALB/c (*a*) and SJL/J (*b*) are designated by bold face arrows. Numbers indicate the size of the Eco RI fragments (in kb). The organization of this cluster is taken from Gascoigne et al. (7). Sequences included in the probes (described in Materials and Methods) are indicated.

allele defined by the loss of one Eco RI site and the gain of another is located, in SJL/J, in the intron of the Jβ-Cβ cluster. Hybridization of Eco RI-restricted BALB/c and SJL/J DNA to probes specific for either the 5′ Jβ (probe 6) or the 3′ Jβ′ (probe 11) cluster (Fig. 2), has shown the restriction site alleles to be in the Jβ, not Jβ′ region. Only probe 6 detected the alleles, i.e., a single 9.4 kb band in Eco RI-cut BALB/c DNA, and a single 7.8 kb band in similarly cut SJL/J DNA. Hybridization with a probe containing Cβ (but lacking the Jβ regions) detects fragments of 2.0 kb (Cβ) and 10.2 kb (Cβ′) in BALB/c, while in SJL, fragments of 3.1 kb (Cβ) and 10.2 kb (Cβ′) are found.

The 86T5 probe was used to follow the segregation of the restriction site alleles in a set of (C × J)RI strains (Fig. 2*D*). Only (C × J)1 and (C × J)11 display the restriction pattern observed in the BALB/c parent. Fragments typical of the SJL/J progenitor were observed in the seven other RI strains.

Gene Encoding Allotype Marker on β Chain of T Cell Receptor Is Linked to Eco RI Restriction Site Allele in Jβ-Cβ. We have mapped the gene encoding the allotype marker using the (C × J)RI strains. A representative experiment showing the frequency of lymph node T cells stained with KJ16-133 antiallotype is shown in Table I. While >95% of the purified T cell population from each strain was stained with anti-Thy-1, only BALB/c, (C × J)1, and (C × J)11 stained with KJ16-133. All other (C × J)RI strains and SJL/J failed to react with KJ16-133. Since no recombinants between the gene encoding the allotype marker and the Eco RI restriction site were found in the nine RI strains studied, these markers are separated by $<3 \pm 3$ centimorgans (at the 95% confidence level).

Discussion

The relationship between two genetic markers presumed to characterize the MHC-restricted, antigen-specific T cell receptor has been analyzed: (*a*) Eco RI restriction sites define a pair of alleles marking a gene locus, Jβ-Cβ, the attributes of which suggest that it encodes a subunit of the T cell receptor. (*b*) mAb KJ16-133 defines a pair of alleles that encode an allotypic determinant on the heterodimer which has attributes implying that it functions as the T cell receptor.

Here, we show that these two markers are linked by analyzing a set of (C × J)RI strains. The marker, herein defined by the Eco RI restriction sites, is within the intron between Jβ and Cβ (Fig. 1). It is likely that the β chain exon encoding the allotype is of a J region, rather than a V or C region. Only 20% of peripheral T cells (Table I) or randomly chosen T cell hybridomas (from a mouse strain positive for the allotype) express the marker. Furthermore, the limited expression of the allotypic marker on the heterodimer of T cells is independent of effector function, stage of ontogeny, class of MHC (I or II) recognized, and antigenic specificity of the cell (8). We conclude, therefore, that the frequency of expression of this marker reflects the frequency of utilization of the exon encoding it. If the allotypic marker were encoded by the C region, it would have to be expressed by both Cβ and Cβ′, because the amino acid sequences of the domains of Cβ and Cβ′ external to the membrane encoded by the 5′ exons, are identical (7, 12). This leaves unexplained why only 20% of T cells express the allotype. The probability that a V region gene encodes the allotypic marker seems low, since this would require that the V region be expressed 20% of the time. Furthermore,

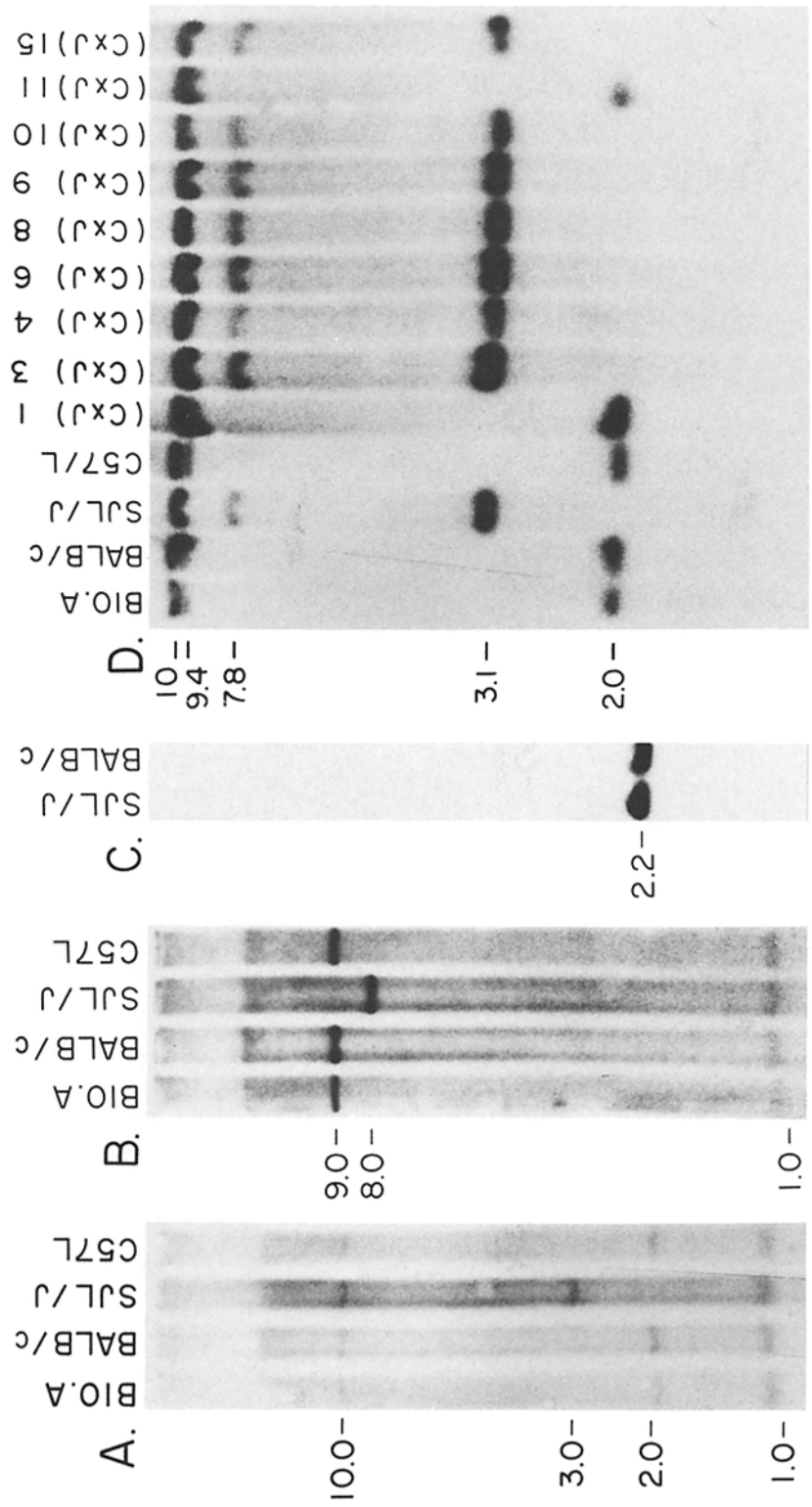

FIGURE 2. The localization of the allelic Eco RI sites to Jβ-Cβ by hybridization to probes specific for Jβ, Jβ′, and Cβ′. (*A*) Hybridization of Eco RI-cut liver DNA to a Cβ probe. A 2.0 kb Cβ fragment is found in BALB/c, B10.A, and C57L, while this fragment in SJL is 3.1 kb. All strains possess the 10.2 kb Cβ′ fragment. (*B*) Hybridization of probe 6 to a 9.4 kb Jβ-containing fragment in BALB/c, B10.A, and C57L, and to a 7.8 kb fragment in SJL. In (*C*), probe 11 is used. An identical 2.2 kb Jβ′ fragment is detected in SJL/J and BALB/c. (*D*) Segregation of the alleles defined by the endonuclease Eco RI are shown in the (C × J)RI strains. Only (C × J)1 and 11 possess the BALB/c alleles. The other RI strains resemble SJL/J.

TABLE I

Segregation of Alleles Defined by Allotype and Eco RI Restriction Sites in (C × J)RI Mice

Mouse strain	T cells detected by antiallotype mAb KJ16-133*	Designation of haplotype		
		Allotype	Jβ-Cβ‡	V_x21A§
	%			
BALB/c	17.7	C‖	C	C
SJL/J	<0.1	J	J	J
$(C \times J)_1$	19.8	C	C	C
$(C \times J)_3$	<0.1	J	J	C
$(C \times J)_4$	<0.1	J	J	J
$(C \times J)_6$	<0.1	J	J	J
$(C \times J)_8$	<0.1	J	J	J
$(C \times J)_9$	<0.1	J	J	J
$(C \times J)_{10}$	<0.1	J	J	J
$(C \times J)_{11}$	19.7	C	C	J
$(C \times J)_{15}$	<0.1	J	J	C

* Shows staining of purified peripheral T cells, using KJ16-133 as primary antibody. Flow cytometry analysis is as indicated in text.

‡ Allele defined by Eco RI site in J_β present in strain.

§ Allele of V_x defined by restriction site polymorphisms detected by cutting with Bam HI and probing with V_x21 (Huppi, personal communication).

‖ C indicates that the observed behavior or pattern is BALB/c-like, while J indicates that it is SJL/J-like.

it has been shown that KJ16-133 binds to a determinant distant from the clone-specific determinant recognized by "idiotype-specific" reagents (8).

We have shown that BALB/c and SJL/J differ by the loss of one Eco RI site and the gain of another (Fig. 1), and that this change falls within the intron between Jβ and Cβ. Since several other restriction site alleles exist in the Jβ-Cβ/Jβ′-Cβ′ region as well (e.g., Msp I, Hind III, detectable with the 86T5 probe), it is reasonable to assume that multiple events have occurred to distinguish the genomic region encoding the β chain of SJL/J from that of BALB/c. Of the many murine strains that have been examined by ourselves and others (6), only SJL/J and SWR (Epstein and Kanagawa, unpublished data) exhibit restriction patterns different from that displayed by the major class typified by BALB/c. Although C57L, like SJL/J and SWR, lacks the allotypic marker, its restriction pattern (Fig. 2) is indistinguishable from that of BALB/c, supporting the view that the events resulting in the loss of the allotypic marker (e.g., amino acid replacement or inactivation of the entire Jβ-Cβ region) occurred independently of those generating the allelic DNA sites defined by restriction enzyme cleavage.

Summary

The restriction enzyme Eco RI reveals DNA cleavage sites that serve to distinguish the gene locus believed to encode the β subunit of the major histocompatibility complex (MHC)-restricted, antigen-specific receptor of the T cell in BALB/c mice from that of SJL/J mice. A monoclonal antibody, KJ16-133, also distinguishes BALB/c and SJL/J, because it recognizes an allotypic marker present on a cell-surface heterodimer believed to function as the MHC-restricted, antigen-specific T cell receptor. This study has shown that these two markers cosegregate in a set of BALB/c × SJL/J recombinant inbred (RI) mouse strains, permitting the conclusion that they are linked to within 3 centimorgans

of each other, and to the κ locus on chromosome 6. The tight linkage between these independently derived, totally different T cell markers substantially strengthens the argument that they characterize the MHC-restricted antigen-specific receptor of the effector T cell.

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