Identification of a T Cell Receptor \(\beta\) Chain Variable Region, V\(\beta\)20, That Is Differentially Expressed in Various Strains of Mice

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Summary

A cDNA library of TCR \(\beta\) chain transcripts from BALB/c thymocytes was constructed using anchored polymerase chain reaction (PCR). Screening of this library led to the identification of a \(V\beta\) gene segment, \(V\beta\)20, structurally related to \(V\beta\)3 and \(V\beta\)17. Genomic analysis of mice displaying deletions in their \(V\beta\) loci, together with mapping of cosmid clones, situated \(V\beta\)20 2.5 kb beside \(V\beta\)17. The expression of \(V\beta\)20 was estimated by PCR in mice of different H-2 and Mls types. Peripheral T cells from H-2\(^d\) and H-2\(^d\) mice did not express \(V\beta\)20, whereas in I-E-negative mice (C57B1/6 and SJL), \(V\beta\)20 transcripts were detected. The lack of \(V\beta\)20 transcripts in (C57B1/6 \times CBA/J)\(F_1\), (C57B1/6 \times BALB/c)\(F_1\), and in congenic B6.H-2\(^k\) mice suggests that the differential use of \(V\beta\)20 is due to an I-E-mediated clonal deletion process. The involvement of the Mls super antigens was excluded by analysis of all Mls type combinations. The nature of the \(V\beta\)20-deleting element(s) is discussed in the context of the I-E/superantigen systems controlling the expression of \(V\beta\)11 and \(V\beta\)17.

Study of the mouse TCR \(\beta\) chain repertoire led to the identification of 28 \(V\beta\) gene segments (1, 2). In BALB/c, 23 \(V\beta\)s are organized in 19 subfamilies, which are composed of a single member, except for \(V\beta\)5 and \(V\beta\)8, which both have three members, and five pseudogenes have not been yet attributed to any subfamily (3). The number of functional \(V\beta\) gene segments differs greatly among strains of mice. For example, \(V\beta\)17 and \(V\beta\)19 are found as pseudogenes in TCR \(\beta^0\) haplotype and functional in TCR \(\beta^1\) haplotype (3, 4), and several strains display genomic deletions that remove up to 60% of their \(V\beta\)s (5–7). Along with this variability of the germ-line repertoire, the usage of \(V\beta\)s by mature T lymphocytes depends on MHC products and on the expression of superantigens that eliminate T cells bearing particular \(V\beta\)s (8). The identification of the \(V\beta\)s was largely based on screening of thymus or T cell clone cDNA libraries, therefore greatly depending on the frequency of \(V\beta\) usage, or on probing of genomic clones with consensus \(V\beta\) oligonucleotides that may miss \(V\beta\)s differing in the region corresponding to the consensus primers. To overcome these problems, a cDNA library from BALB/c thymus was enriched in TCR \(\beta\) transcripts by anchored PCR (A-PCR), which precludes bias of consensus primers, generates a great number of TCR \(\beta\) clones, and therefore may detect rare \(\beta\) transcripts. We identified a yet unknown \(V\beta\) gene segment, tentatively named \(V\beta\)20, that maps near \(V\beta\)17. The analysis of the usage of \(V\beta\)20 by peripheral T cells in various strains of mice shows that \(V\beta\)20 expression is dependent on the MHC haplotype.

Materials and Methods

Animals. The inbred strains of DDO and WLA mice are maintained at the Institut Pasteur (Paris, France) (7).

cDNA Synthesis. RNAs were prepared using the hot-phenol method, and 10 \(\mu\)g of total RNA was converted to cDNA as described (9). For A-PCR, a homopolymeric C tail was added to cDNA by 15 U of terminal deoxynucleotidyl transferase (International Biotech, Inc., New Haven, CT) and 20 mM of dGTP in 50 \(\mu\)l of the supplier's buffer for 30 min at 37°C.

Polymerase Chain Reactions. PCRs were performed with 10% of total single-strand cDNA, 20 pmol of primers, and 1 U of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) in 50 \(\mu\)l of the supplier's buffer and consisted in incubations at 94°C for 5 min, then 25 cycles of 10 s at 94°C, 1 min at 55°C, 15 s at 60°C, and 45 s at 72°C. A-PCR were done with MTB (complementary to positions 91–117 of C\(\beta\) first exon) and XNSC10 (5' CACTC-GAGCGCCGGCGTCGACCCCCCCCCC 3'). A second A-PCR was performed to yield larger amounts of products using XNSC10 and MTBSX (complementary to positions 17–37). For A-PCR of
germline VB20, D18 was digested by KpnI, G tailed, and cut by BamHI. VB20 was amplified with K9DO (complementary to positions 472-488 of V1520) and XNSC10. VB20-specific PCR products were done with K9UP (positions 241-256 of VB20) and MTB primers, of TCR β transcripts with the MCTBUP (positions 3-22) and an equimolar mixture of the MTBIDO and MTB2DO primers (complementary to positions 474-493 of Cβ1 and Cβ2), of TCR α transcripts with MTCAUP (positions 1-20) and MTCADO2 primers (complementary to positions 263-279), and of Vβ17 with MVB17S and MVB17FX, as previously described (10).

**Molecular Cloning and Nucleotide Sequence Analysis.** PCR products were cloned into M13 phages digested by SmaI restriction enzyme. The clones were screened with a Cβ probe and with a panel of Vβ probes in either high (0.1 x SSC, 0.05% SDS at 65°C) or low (0.5 x SSC, 0.5% SDS at 50°C) stringency conditions. The nucleotide sequences were determined by the dideoxynucleotide chain termination method (11) with a Sequenase kit (United States Biochemical Corp., Cleveland, OH).

**Southern Blots.** Conditions for Southern blots and probes were previously described (7). The VB20 probe was a 220-bp PstI fragment derived from K9 clone.

**Results and Discussion**

**Identification of a New Vβ Gene Segment.** A TCR β cDNA library was constructed from BALB/c thymocytes by the A-PCR method, which amplifies TCR β transcripts irrespectively of the VBs used. By screenings with Cβ and VB probes, we obtained a clone, K9, which hybridized with the Vβ17 probe only at low stringency. The K9 nucleotide sequence revealed a stretch of 339 nucleotides upstream of DIS region that does not correspond to the 5' flanking sequence of the Dβ3.1 gene segment, and displays <75% of nucleotide identity with any of the known mouse VtSs (Fig. 1). This suggests that the K9 clone contains a new Vβ gene segment, tentatively named Vβ20. Three additional clones were obtained after PCR with MTBSX and K9UP primers; their nucleotide sequences confirm the structure of Vβ20. The four cDNA clones result from recombination events with different Dβββ elements in the reading frame of Vβ20 (Fig. 1). To exclude that Vβ20 is a pseudogene such as Vβ19β, which has a shift of the reading-frame in the leader region (3), we cloned the germ-line Vβ20. The predicted translation begins with the initiation codon at position 71 and contains the six

![Figure 1. Nucleotide and amino acid structures of the Vβ20 gene segment. The structure of Vβ20 was derived from nucleotide sequences of genomic DNA amplification for the positions 1-448, of the cDNA clone K9 for positions 89 to Jβ, and finally of three independent cDNA clones (N, P, L) for positions 241 to Jβ. The predicted amino acid translation is presented above. For the cDNA clones N, K9, and L, only the recombination region is presented, and assignments of the V, D, and J gene segments are indicated on the top of the translation. Splicing signals are underlined and residues conserved in all V regions are indicated by a star. The Vβ20 nucleotide sequence is available from EMBL under accession number X59150.](image-url)
amino acids invariant among all Vβs, indicating that Vβ20 encodes a functional Vβ domain.

Localization of Vβ20 Gene Segment. Hybridization of BALB/c DNA with a Vβ20 probe showed a 4.8-kb EcoRI fragment (Fig. 2) and a 1.8-kb HindIII fragment (not shown), indicating that the probe detects a new Vβ subfamily composed of a single member. The same hybridizing fragment was observed for SJL and WLA, whereas DDO failed to hybridize with Vβ20 probe (Fig. 2). Comparison of the Vβ deletion extensions in SJL, WLA, and DDO strains (7), indicating that Vβ20 is located between Vβ19 and Vβ3. Restriction map analysis of the D6 and D18 cosmids spanning this region showed that Vβ20 is included in 8-kb BamHI and 11-kb KpnI fragments, which both bear Vβ17 (Fig. 3). PCRs were performed with Vβ17- and Vβ20-specific primers. Products were obtained only with primers corresponding to the coding strand of Vβ20 and to the complementary strand of Vβ17 (not shown). Therefore, Vβ20 is located <2.5 kb from Vβ17 in the same transcriptional polarity. Six Vβs, including Vβ20, clustered in 25 kb, are structurally more related to each other than to any other Vβ. Comparisons of the nucleotide sequence indicate that Vβ3, Vβ17, and Vβ20 display 74–75% of identity. Vβ17 presents 61%, VβN1 60%, and VβN2 69% of nucleotide identity with Vβ20 constituting more divergent individuals. These data strongly suggest that

Expression of Vβ20. Studies of Vβ usage by stainings with anti-Vβ antibodies and by RNA hybridizations with Vβ probes demonstrated the clonal elimination of mature T cells bearing certain Vβ domains in mice that carry appropriate self superantigen and H-2 combinations (reviewed in reference 12). As Vβ20 displays all features of a functional Vβ, we analyzed its expression in peripheral T cells by PCR. Vβ20 PCR products were obtained with C57Bl/6 and SJL, whereas they are barely detected in the other strains (Fig. 4). The different Vβ20 expressions are not due to variations in the gene copy number nor to differences in frequency of rearrangements, since all strains possess a single Vβ20 copy and yielded equivalent Vβ20 levels in unselected thymocytes. The level of Vβ20 in the (C57Bl/6 × CBA/J)F1 and (C57Bl/6 × BALB/c)F1 hybrids is as low as in the negative parents, showing that this phenotype is dominant and supporting a Vβ20 clonal deletion process. Mls systems, known to regulate several Vβs usage, are not involved, since none of the H-2k strains express Vβ20 irrespective of Mls combination. Strikingly, the usage of Vβ20 by peripheral T cells correlates with the lack of I-E molecule, as C57Bl/6- and SJL-positive mice carry nonfunctional I-Eα genes. The role of H-2k product(s) was confirmed by the lack of Vβ20 expression in the congenic B6.H-2^k.

The strain distribution of Vβ20 expression follows that observed for Vβ17a1 and Vβ11, which are controlled in I-E-positive mice by two kinds of self superantigens: a nonpolymorphic B cell–specific product of an unknown nature mediates the deletion of Vβ17a1-bearing T cells (13, 14), and integrated sequences related to mouse mammary tumor virus prevent Vβ11 expression (15–17). Critical residues determining

Figure 3. Map of BALB/c cosmids hybridizing with Vβ20 probe. Sites for BamHI and KpnI restriction enzymes are represented by vertical bars (2, 3). The arrows indicate Vβ transcriptional orientation.

Figure 4. Analysis of Vβ20 expression by PCR. First-strand cDNA were synthesized from splenocyte and thymocyte RNA preparations and amplified by PCR with KguP and MTBSX primers for Vβ20, with MTBUP, MTCBIDO, and MTCB2DO for CB, and with MTCAUP and MTCADO2 for Cα. (A) Products were analyzed on 1% agarose gel and hybridized with Vβ20, CB, and Cα probes. The size of 6×174 HaeII fragments is given on the right. (B) Normalized PCR values of Vβ20 expression in the spleen. Relative amounts of Vβ20 and CB were determined by scanning the autoradiograms. Vβ20 values were normalized by dividing by respective CB values. For each experiment, the Vβ20 levels are expressed relatively to values obtained with C57Bl/6:100 × (Vβ20/Cβ)/(Vβ20/Cβ). The number of mice independently tested is indicated in parenthesis. Mls genotypes are designated according to Abe and Hodes (8).
specificity toward deleting elements are located in a loop distant from the site of interaction with MHC/antigenic peptide complex (18). Minor alterations in this region of Vβ17a1 drastically alter its reactivity toward Mls2/3 (10). In this region, Vβ20 exhibits no significant structural similarities with VB11 and Vβ17a alleles, giving no indications about the nature of Vβ20 deleting element(s). None of the Vβ20-related Vβs is actually used by the peripheral T cells of BALB/c: Vβ17b, Vβ19b, VβN1, and VβN2 possess defects in their coding regions (3, 4), and the functional Vβ3 and Vβ20 are deleted from mature T cells (19, our data). However, SJL uses Vβ3, Vβ17a, Vβ19a, and Vβ20. Thus, the deletion of 10 Vβs in SJL β locus may be compensated for by the use of the Vβs absent from mature repertoires of other strains such as BALB/c.

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Note added in proof: The partial nucleotide sequence of C57Bl/6 Vβ20, which is identical to the BALB/c Vβ20 presented here, was recently published by Smith et al. (20).

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