Brief Definitive Report

Identification of a T Cell Receptor β Chain Variable Region, Vβ20, That Is Differentially Expressed in Various Strains of Mice

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Summary

A cDNA library of TCR β chain transcripts from BALB/c thymocytes was constructed using anchored polymerase chain reaction (PCR). Screening of this library led to the identification of a Vβ gene segment, Vβ20, structurally related to Vβ3 and Vβ17. Genomic analysis of mice displaying deletions in their Vβ loci, together with mapping of cosmid clones, situated Vβ20 2.5 kb beside Vβ17. The expression of Vβ20 was estimated by PCR in mice of different H-2 and Mls types. Peripheral T cells from H-2^k and H-2^d mice did not express Vβ20, whereas in I-E-negative mice (C57Bl/6 and SJL), Vβ20 transcripts were detected. The lack of Vβ20 transcripts in (C57Bl/6 x CBA/J)F1, (C57Bl/6 x BALB/c)F1, and in congenic B6-H-2^b mice suggests that the differential use of Vβ20 is due to an I-E-mediated clonal deletion process. The involvement of the Mls superantigens was excluded by analysis of all Mls type combinations. The nature of the Vβ20-deleting element(s) is discussed in the context of the I-E/superantigen systems controlling the expression of Vβ11 and Vβ17.

Study of the mouse TCR β chain repertoire led to the identification of 28 Vβ gene segments (1, 2). In BALB/c, 23 Vβs are organized in 19 subfamilies, which are composed of a single member, except for Vβ5 and Vβ8, which both have three members, and five pseudogenes have not been yet attributed to any subfamily (3). The number of functional Vβ gene segments differs greatly among strains of mice. For example, Vβ17 and Vβ19 are found as pseudogenes in TCR β^b haplotype and functional in TCR β^a haplotype (3, 4), and several strains display genomic deletions that remove up to 60% of their Vβs (5-7). Along with this variability of the germ-line repertoire, the usage of Vβs by mature T lymphocytes depends on MHC products and on the expression of superantigens that eliminate T cells bearing particular Vβs (8). The identification of the Vβs was largely based on screening of thymus or T cell clone cDNA libraries, therefore greatly depending on the frequency of Vβ usage, or on probing of genomic clones with consensus Vβ oligonucleotides that may miss Vβs differing in the region corresponding to the consensus primers. To overcome these problems, a cDNA library from BALB/c thymus was enriched in TCR β transcripts by anchored PCR (A-PCR), which generates a great number of TCR β clones, and therefore may detect rare β transcripts. We identified a yet unknown Vβ gene segment, tentatively named Vβ20, that maps near Vβ17. The analysis of the usage of Vβ20 by peripheral T cells in various strains of mice shows that Vβ20 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 μg of total RNA was converted to cDNA as described (9). For A-PCR, a homopolymeric G 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 μl 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 μl 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 CB first exon) and XNSC10 (5'CACCTGACGCGCGGCGTCGACCCCCCCCC 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

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germline \( V_{\beta}20 \), D18 was digested by KpnI, G-tailed, and cut by BamHI. \( V_{\beta}20 \) was amplified with KgDO (complementary to positions 472–488 of V1520) and XNSC10. \( V_{\beta}20 \)-specific PCR was done with KguP (positions 241–256 of VB20) and MTB primers, of TCR \( \beta \) transcripts with the MCTBUP (positions 3–22) and an equimolar mixture of the MTBIDO and MTB2DO primers (complementary to positions 474–493 of C131 and C132), of TCR \( \alpha \) transcripts with the MTCAUP (positions 1–20) and MTCADO2 primers (complementary to positions 263–279), and of \( V_{\beta}17 \) with MVBI7S and MVBI7FX, 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 \( C_{\beta} \) probe and with a panel of \( V_{\beta} \) probes in either high (0.1 x SSC, 0.05% SDS at 65°C) or low (0.5x 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 \( V_{\beta} \) Gene Segment.** A TCR \( \beta \) cDNA library was constructed from BALB/c thymocytes by the A-PCR method, which amplifies TCR \( \beta \) transcripts irrespectively of the \( V_{\beta} \)s used. By screenings with \( C_{\beta} \) and \( V_{\beta} \) probes, we obtained a clone, K9, which hybridized with the \( V_{\beta}17 \) 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 D/32.1 gene segment, and displays <75% of nucleotide identity with any of the known mouse \( V_{\beta} \)s (Fig. 1). This suggests that the K9 clone contains a new \( V_{\beta} \) gene segment, tentatively named \( V_{\beta}20 \). Three additional clones were obtained after PCR with MTBSX and K9UP primers; their nucleotide sequences confirm the structure of \( V_{\beta}20 \). The four cDNA clones result from recombination events with different D\( \beta\)\( \beta \) elements in the reading frame of \( V_{\beta}20 \) (Fig. 1). To exclude that \( V_{\beta}20 \) is a pseudogene such as \( V_{\beta}19b \), which has a shift of the reading-frame in the leader region (3), we cloned the germ-line \( V_{\beta}20 \). The predicted translation begins with the initiation codon at position 71 and contains the six
amino acids invariant among all VBs, indicating that VB20 encodes a functional VB domain.

Localization of VB20 Gene Segment. Hybridization of BALB/c DNA with a VB20 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 VB subfamily composed of a single member. The same hybridizing fragment was observed for SJL and WLA, whereas DDO failed to hybridize with VB20 probe (Fig. 2). Comparison of the VB deletion extensions in SJL, WLA, and DDO strains, indicating that VB20 is located between VB19 and VB3. Restriction map analysis of the D6 and D18 cosmids spanning this region showed that VB20 is included in 8-kb BamHI and 11-kb KpnI fragments, which both bear VB17 (Fig. 3). PCRs were performed with VB17- and VB20-specific primers. Products were obtained only with primers corresponding to the coding strand of VB20 and to the complementary strand of VB17 (not shown). Therefore, VB20 is located <2.5 kb from VB17 in the same transcriptional polarity. Six VBs, including VB20, clustered in 25 kb, are structurally more related to each other than to any other VB. Comparisons of the nucleotide sequence indicate that VB3, VB17, and VB20 display 74–75% of identity. VB19 presents 61%, VB1N1 60%, and VB1N2 69% of nucleotide identity with VB20 constituting more divergent individuals. These data strongly suggest that this genetic region underwent complex and sequential duplications leading to gene expansion.

Expression of VB20. Studies of VB usage by stainings with anti-VB antibodies and by RNA hybridizations with VB probes demonstrated the clonal elimination of mature T cells bearing certain VB domains in mice that carry appropriate self superantigen and H-2 combinations (reviewed in reference 12). As VB20 displays all features of a functional VB, we analyzed its expression in peripheral T cells by PCR. VB20 PCR products were obtained with C57Bl/6 and SJL, whereas they are barely detected in the other strains (Fig. 4). The different VB20 expressions are not due to variations in the gene copy number nor to differences in frequency of rearrangements, since all strains possess a single VB20 copy and yielded equivalent VB20 levels in unselected thymocytes. The level of VB20 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 VB20 clonal deletion process. Mls systems, known to regulate several VBs usage, are not involved, since none of the H-2k strains express VB20 irrespective of Mls combination. Strikingly, the usage of VB20 by peripheral T cells correlates with the lack of I-E molecule, as C57Bl/6- and SJL-positive mice carry nonfunctional I-Eex genes. The role of H-2k product(s) was confirmed by the lack of VB20 expression in the congenic B6.H-2k.

The strain distribution of VB20 expression follows that observed for VB1a and VB11, 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 VB1a-bearing T cells (13, 14), and integrated sequences related to mouse mammary tumor virus prevent VB11 expression (15–17). Critical residues determining
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 Vβ11 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 C57B1/6 Vβ20, which is identical to the BALB/c Vβ20 presented here, was recently published by Smith et al. (20).

References