

Identification of a T Cell Receptor β Chain Variable Region, V β 20, 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 V β gene segment, V β 20, structurally related to V β 3 and V β 17. Genomic analysis of mice displaying deletions in their V β loci, together with mapping of cosmid clones, situated V β 20 2.5 kb beside V β 17. The expression of V β 20 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 V β 20, whereas in I-E-negative mice (C57Bl/6 and SJL), V β 20 transcripts were detected. The lack of V β 20 transcripts in (C57Bl/6 \times CBA/J)_{F1}, (C57Bl/6 \times BALB/c)_{F1}, and in congenic B6.H-2^k mice suggests that the differential use of V β 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 β 20-deleting element(s) is discussed in the context of the I-E/superantigen systems controlling the expression of V β 11 and V β 17.

Study of the mouse TCR β chain repertoire led to the identification of 28 V β gene segments (1, 2). In BALB/c, 23 V β s are organized in 19 subfamilies, which are composed of a single member, except for V β 5 and V β 8, which both have three members, and five pseudogenes have not been yet attributed to any subfamily (3). The number of functional V β gene segments differs greatly among strains of mice. For example, V β 17 and V β 19 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 V β s (5–7). Along with this variability of the germ-line repertoire, the usage of V β s by mature T lymphocytes depends on MHC products and on the expression of superantigens that eliminate T cells bearing particular V β s (8). The identification of the V β s was largely based on screening of thymus or T cell clone cDNA libraries, therefore greatly depending on the frequency of V β usage, or on probing of genomic clones with consensus V β oligonucleotides that may miss V β 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 β transcripts by anchored PCR (A-PCR), which precludes bias of consensus primers, generates a great number of TCR β clones, and therefore may detect rare β transcripts. We

identified a yet unknown V β gene segment, tentatively named V β 20, that maps near V β 17. The analysis of the usage of V β 20 by peripheral T cells in various strains of mice shows that V β 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 μ g of total RNA was converted in 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 μ 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 μ 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 β first exon) and XNSC10 (5' CACTC-GAGCGGCCGCGTCGACCCCCCCCC 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 V β 20, D18 was digested by KpnI, G tailed, and cut by BamHI. V β 20 was amplified with K9DO (complementary to positions 472–488 of V β 20) and XNSC10. V β 20-specific PCRs were done with K9UP (positions 241–256 of V β 20) and MTB primers, of TCR β transcripts with the MCTBUP (positions 3–22) and an equimolar mixture of the MTB1DO and MTB2DO primers (complementary to positions 474–493 of C β 1 and C β 2), of TCR α transcripts with MTCAUP (positions 1–20) and MTCADO2 primers (complementary to positions 263–279), and of V β 17 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 C β probe and with a panel of V β probes in either high (0.1 \times SSC, 0.05% SDS at 65°C) or low (0.5 \times 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 V β 20 probe was a 220-bp PstI fragment derived from K9 clone.

Results and Discussion

Identification of a New V β 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 V β s used. By screenings with C β and V β probes, we obtained a clone, K9, which hybridized with the V β 17 probe only at low stringency. The K9 nucleotide sequence revealed a stretch of 339 nucleotides upstream of D β region that does not correspond to the 5' flanking sequence of the D β 2.1 gene segment, and displays <75% of nucleotide identity with any of the known mouse V β s (Fig. 1). This suggests that the K9 clone contains a new V β gene segment, tentatively named V β 20. Three additional clones were ob-

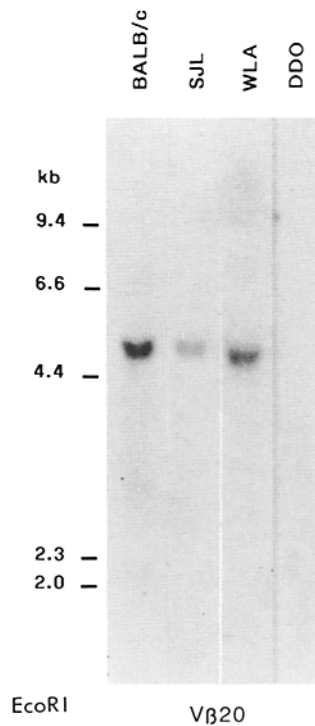


Figure 2. Southern blot analysis of genomic DNA from BALB/c, SJL, WLA, and DDO. DNA samples were digested by EcoRI restriction enzyme and hybridized with the V β 20 probe. The filter was later probed with C β probe to control that all lanes contained equivalent amounts of DNA. The size of λ HindIII fragments are given on the left.

tained after PCR with MTBSX and K9UP primers; their nucleotide sequences confirm the structure of V β 20. The four cDNA clones result from recombination events with different D β J β elements in the reading frame of V β 20 (Fig. 1). To exclude that V β 20 is a pseudogene such as V β 19^b, which has a shift of the reading-frame in the leader region (3), we cloned the germ-line V β 20. The predicted translation begins with the initiation codon at position 71 and contains the six

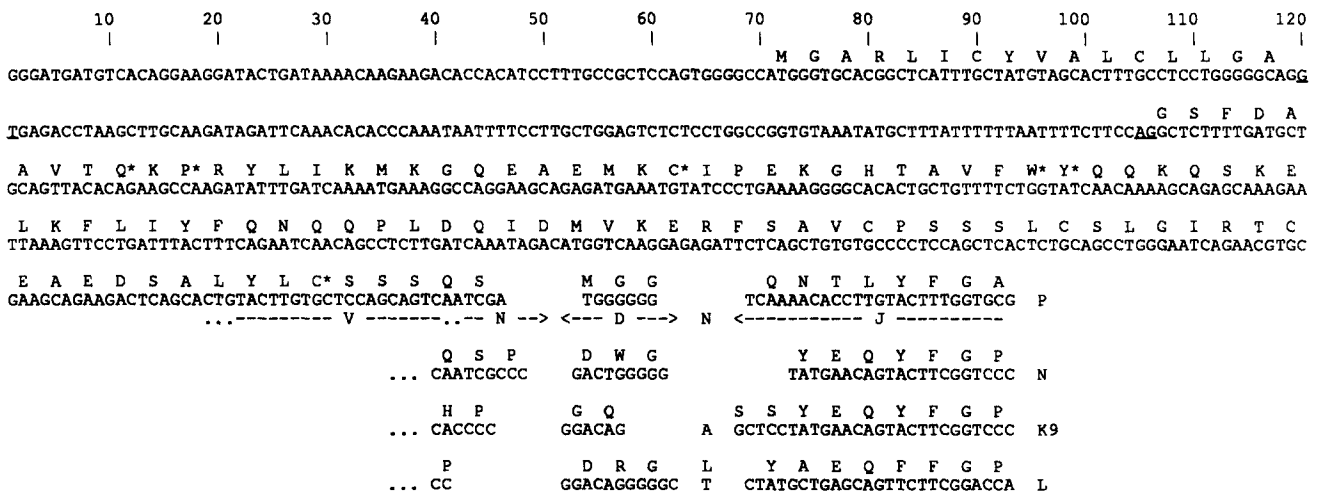


Figure 1. Nucleotide and amino acid structures of the V β 20 gene segment. The structure of V β 20 was derived from nucleotide sequences of genomic DNA amplification for the positions 1–448, of the cDNA clone K9 for positions 89 to J β , and finally of three independent cDNA clones (N, P, L) for positions 241 to J β . 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 V β 20 nucleotide sequence is available from EMBL under accession number X59150.

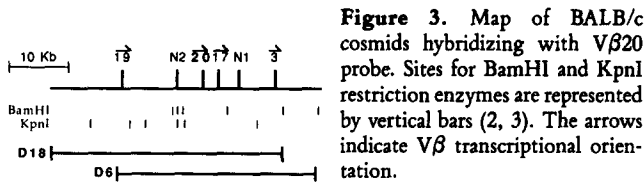


Figure 3. Map of BALB/c cosmids hybridizing with V β 20 probe. Sites for BamHI and KpnI restriction enzymes are represented by vertical bars (2, 3). The arrows indicate V β transcriptional orientation.

amino acids invariant among all V β s, indicating that V β 20 encodes a functional V β domain.

Localization of V β 20 Gene Segment. Hybridization of BALB/c DNA with a V β 20 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 V β subfamily composed of a single member. The same hybridizing fragment was observed for SJL and WLA, whereas DDO failed to hybridize with V β 20 probe (Fig. 2). Comparison of the V β deletion extensions in SJL, WLA, and DDO strains (7), indicating that V β 20 is located between V β 19 and V β 3. Restriction map analysis of the D6 and D18 cosmids spanning this region showed that V β 20 is included in 8-kb BamHI and 11-kb KpnI fragments, which both bear V β 17 (Fig. 3). PCRs were performed with V β 17- and V β 20-specific primers. Products were obtained only with primers corresponding to the coding strand of V β 20 and to the complementary strand of V β 17 (not shown). Therefore, V β 20 is located <2.5 kb from V β 17 in the same transcriptional polarity. Six V β s, including V β 20, clustered in 25 kb, are structurally more related to each other than to any other V β . Comparisons of the nucleotide sequence indicate that V β 3, V β 17, and V β 20 display 74–75% of identity. V β 19 presents 61%, V β N1 60%, and V β N2 69% of nucleotide identity with V β 20 constituting more divergent individuals. These data strongly suggest that

this genetic region underwent complex and sequential duplications leading to gene expansion.

Expression of V β 20. Studies of V β usage by stainings with anti-V β antibodies and by RNA hybridizations with V β probes demonstrated the clonal elimination of mature T cells bearing certain V β domains in mice that carry appropriate self superantigen and H-2 combinations (reviewed in reference 12). As V β 20 displays all features of a functional V β , we analyzed its expression in peripheral T cells by PCR. V β 20 PCR products were obtained with C57Bl/6 and SJL, whereas they are barely detected in the other strains (Fig. 4). The different V β 20 expressions are not due to variations in the gene copy number nor to differences in frequency of rearrangements, since all strains possess a single V β 20 copy and yielded equivalent V β 20 levels in unselected thymocytes. The level of V β 20 in the (C57Bl/6 \times CBA/J)F₁ and (C57Bl/6 \times BALB/c)F₁ hybrids is as low as in the negative parents, showing that this phenotype is dominant and supporting a V β 20 clonal deletion process. Mls systems, known to regulate several V β s usage, are not involved, since none of the H-2^k strains express V β 20 irrespective of Mls combination. Strikingly, the usage of V β 20 by peripheral T cells correlates with the lack of I-E molecule, as C57Bl/6- and SJL-positive mice carry nonfunctional I-E α genes. The role of H-2^k product(s) was confirmed by the lack of V β 20 expression in the congenic B6.H-2^k.

The strain distribution of V β 20 expression follows that observed for V β 17a1 and V β 11, 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 V β 17a1-bearing T cells (13, 14), and integrated sequences related to mouse mammary tumor virus prevent V β 11 expression (15–17). Critical residues determining

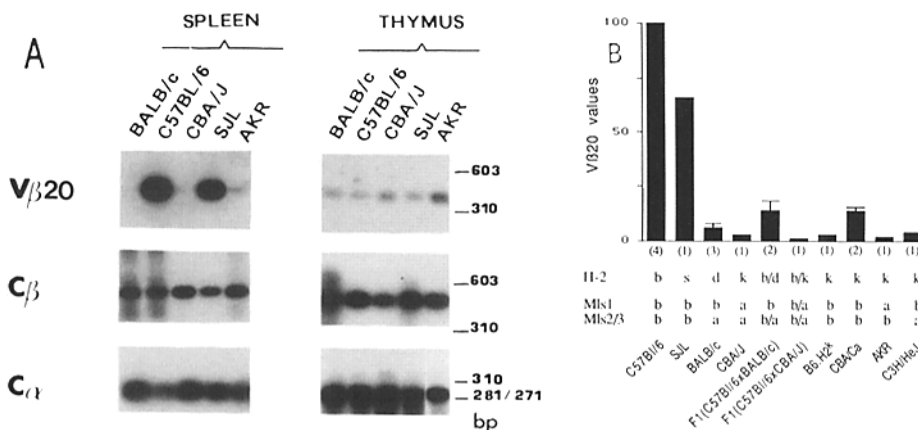


Figure 4. Analysis of V β 20 expression by PCR. First-strand cDNA were synthesized from splenocyte and thymocyte RNA preparations and amplified by PCR with K9UP and MTBSX primers for V β 20, with MTBUP, MTCB1D0, and MTCB2D0 for C β , and with MTCAUP and MTCADO2 for C α . (A) Products were analyzed on 1% agarose gel and hybridized with V β 20, C β , and C α probes. The size of ϕ X174 HaeIII fragments is given on the right. (B) Normalized PCR values of V β 20 expression in the spleen. Relative amounts of V β 20 and C β were determined by scanning the autoradiograms. V β 20 values were normalized by dividing by respective C β values. For each experiment, the V β 20 levels are expressed relatively to values obtained with C57Bl/6: $100 \times (V\beta20/C\beta) / (V\beta20_{C57Bl/6}/C\beta_{C57Bl/6})$. 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 V β 17a1 drastically alter its reactivity toward Mls2/3 (10). In this region, V β 20 exhibits no significant structural similarities with V β 11 and V β 17a alleles, giving no indications about the nature of V β 20 deleting element(s). None of the V β 20-related V β s is actually used by the peripheral T cells of BALB/c:

V β 17b, V β 19b, V β N1, and V β N2 possess defects in their coding regions (3, 4), and the functional V β 3 and V β 20 are deleted from mature T cells (19, our data). However, SJL uses V β 3, V β 17a, V β 19a, and V β 20. Thus, the deletion of 10 V β s in SJL β locus may be compensated for by the use of the V β s 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 V β 20, which is identical to the BALB/c V β 20 presented here, was recently published by Smith et al. (20).

References

1. Lai, E., R.K. Barth, and L. Hood. 1987. Genomic organization of the mouse T-cell receptor β -chain gene family. *Proc. Natl. Acad. Sci. USA.* 84:3846.
2. Chou, H.S., C.A. Nelson, S.A. Godambe, D.D. Chaplin, and D.Y. Loh. 1987. Germline organization of the murine T cell receptor β -chain genes. *Science (Wash. DC).* 238:545.
3. Louie, M.C., C.A. Nelson, and D.Y. Loh. 1989. Identification and characterization of new murine T cell receptor β chain variable region (V β) genes. *J. Exp. Med.* 170:1987.
4. Wade, T., J. Bill, P.C. Marrack, E. Palmer, and J.W. Kappler. 1988. Molecular basis for the nonexpression of V β 17 in some strains of mice. *J. Immunol.* 141:2165.
5. Behlke, M., H.S. Chou, K. Huppi, and D.Y. Loh. 1986. Murine T cell receptor mutants with deletions of beta-chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 83:767.
6. Haqqi, T.M., S. Banerjee, G.D. Anderson, and C.S. David. 1989. RIII S/J (H-2r). An inbred mouse strain with a massive deletion of T cell receptor V β genes. *J. Exp. Med.* 169:1903.
7. Jouvin-Marche, E., N.S. Trede, A. Bandeira, A. Tomas, D.Y. Loh, and P.-A. Cazenave. 1989. Different large deletions of T cell receptor V β genes in natural populations of mice. *Eur. J. Immunol.* 19:1921.
8. Abe, R., and R.J. Hodes. 1989. Properties of the Mls system: a revised formulation of Mls genetics and an analysis of T-cell recognition of Mls determinants. *Immunol. Rev.* 107:5.
9. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
10. Cazenave, P.-A., P.N. Marche, E. Jouvin-Marche, D. Voegtlé, F. Bonhomme, A. Bandeira, and A. Coutinho. 1990. V β 17 gene polymorphism in wild-derived mouse strains: two amino acid substitutions in the V β 17 region greatly alter T cell receptor specificity. *Cell.* 63:717.
11. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.
12. Herman, A., J.W. Kappler, P. Marrack, and A.M. Pullen. 1991. Superantigens: Mechanism of T-cell stimulation and role in immune response. *Annu. Rev. Immunol.* 9:745.
13. Kappler, J.W., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987. A T cell receptor V β segment that imparts reactivity to a class II major histocompatibility complex product. *Cell.* 49:263.
14. Marrack, P.C., and J.W. Kappler. 1988. T cells distinguish between allogeneic major histocompatibility complex products on different cell types. *Nature (Lond.).* 332:840.
15. Vacchio, M.S., and R.J. Hodes. 1989. Selective decreases in T cell receptor V β expression. *J. Exp. Med.* 170:1335.
16. Woodland, D.L., M.P. Happ, K.J. Gollob, and E. Palmer. 1991. An endogenous retrovirus mediating deletion of $\alpha\beta$ T cells? *Nature (Lond.).* 349:529.
17. Dyson, P.J., A.M. Knight, S. Fairchild, E. Simpson, and K. Tomonari. 1991. Genes encoding ligands for deletion of V β 11 T cells cosegregate with mammary tumour virus genomes. *Nature (Lond.).* 349:531.
18. Pullen, A.M., T. Wade, P.C. Marrack, and J.W. Kappler. 1990. Identification of the region of T cell receptor β chain that interacts with self-superantigen Mls-1a. *Cell.* 61:1365.
19. Pullen, A.M., P. Marrack, and J.W. Kappler. 1988. The T-cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. *Nature (Lond.).* 335:796.
20. Smith, L.R., D.H. Kono, and A.N. Theofilopoulos. 1991. Complexity and sequence identification of 24 rat V β genes. *J. Immunol.* 147:375.