

T CELL-SPECIFIC γ GENES IN C57BL/10 MICE

Sequence and Expression of New Constant and Variable Region Genes

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T cells recognize antigens with the use of cell surface receptors that are composed of an α and β chain heterodimer (1). The cloning of the β chain (2, 3), and subsequently the α chain (4, 5) of the T cell antigen receptors revealed that these genes are distantly related to immunoglobulin and MHC genes (6). In addition, these genes undergo somatic rearrangement during T cell maturation. The number of possible rearrangements and genes deduced from α and β chain transcripts appear to be large.

In the attempt to clone the α chain genes by construction of subtractive libraries and differential hybridization, Saito et al. (7) found and identified a third immunoglobulin-like mRNA that was capable of undergoing somatic gene rearrangements in T cells. This mRNA, designated a T cell-specific γ gene, appeared to be expressed mainly in CTL (8). Furthermore, it has been reported (8) that the repertoire for these T cell-specific γ genes is limited. The data (8) indicated that different T cell clones use identical V_γ (V10.8A), J_γ , and C_γ ($J_{C_\gamma 10.5}$) gene segments. Analysis of the γ gene genomic organization in BALB/c mice, however, revealed the existence of three cross hybridizing constant regions, each associated with its own J_γ gene segments (9). The DNA sequences of two of these constant regions, $C_\gamma 10.5$ and $C_\gamma 7.5$ have been reported. The sequence of the third constant region, designated $C_\gamma 13.4$ has not been reported yet. Sequences of the J_γ gene segments accompanying $C_\gamma 10.5$ and $C_\gamma 13.4$ have also been reported. In this study, we have analyzed cDNA sequences from cytotoxic T cell lines derived from the mouse strain C57BL/10 (B10). Our results indicate that a new set of J_γ and C_γ gene segments is used in one of the CTL clones. The genomic organization of T cell-specific γ genes in the B10 strain mice appears to be different from that reported for BALB/c mice.

Materials and Methods

cDNA Synthesis. AED (*N*-iodo-acetyl-*N*-(5-sulfonic-*l*-naphthyl)ethylene diamine)¹-specific, H-2K^b- or H-2D^b-restricted cytotoxic T cell lines of B10 origin were cultured as previously described (10). Total cellular RNA was extracted using the guanidinium thiocyanate-CsCl gradient method (11). cDNA was synthesized from total RNA according

¹ *Abbreviation used in this paper:* AED, *N*-iodo-acetyl-*N*-(5-sulfonic-*l*-naphthyl)ethylene diamine).

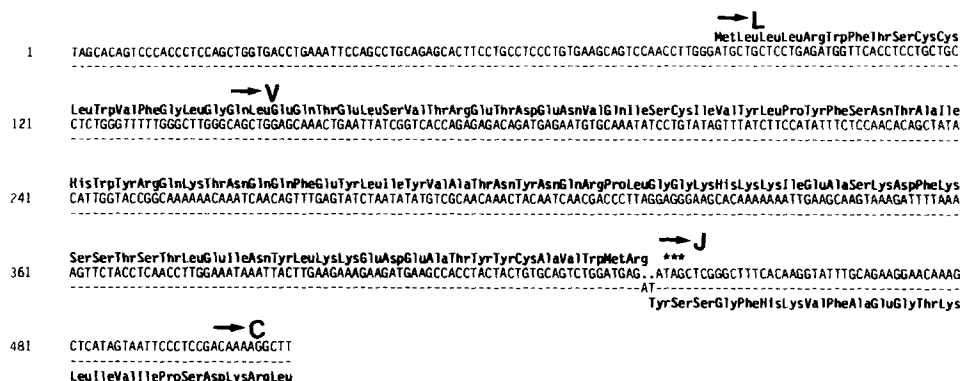


FIGURE 1. Nucleotide and deduced amino acid sequences of 8/10-2 γ 1.1 compared with those of pHDS4/203 (7). Nucleotides that are identical between the two sequences are indicated (-). The leader, variable, joining, and constant regions are designated as L, V, J, and C, respectively. The termination codon is indicated (***)

to a modified procedure of Gubler and Hoffman (12). After addition of the Eco RI linker, the cDNAs were cloned into λ gt10 and screened according to the published method (13). 32 P-radiolabeled γ probe was provided by Tim Skelton and Cox Terhorst.

DNA Sequencing. Nucleotide sequences were determined by the dideoxy chain-termination method of Sanger (14) after cloning specific restriction fragments into the phage vector M13mp8 or M13mp19.

Southern Blot Analysis. High molecular weight cellular DNA was extracted, digested with Eco RI and electrophoresed through 0.8% agarose gel (15). The DNA was transferred to a nitrocellulose filter and hybridized to nick-translated probes as described (16). Filters were washed at 65°C in 1 \times SSC and exposed to x-ray films at -70°C in the presence of intensifying screens.

Results

Sequences of γ cDNA Clones from Two Cytotoxic T Cell Lines of B10 Mice. Two functional CTL clones with well-defined MHC restriction and antigen specificities were chosen for analysis. These CTLs are specific for the hapten AED in association with either H-2K^b or H-2D^b (10). Northern blot analysis indicate that these CTLs express mRNAs of ~1.5 kb in length that crosshybridize with a murine γ chain probe (data not shown). cDNA libraries of two clones, designated 8/10-2 and 5/10-13, were constructed. 8/10-2 is K^b-AED-specific and 5/10-13 is D^b-AED-specific. The latter crossreacts with K^d-AED as well. cDNA libraries were screened with a γ chain probe, and the hybridizing cDNA clones were isolated. The nucleotide sequences of the cDNA clones with the longest inserts in each of the two libraries were determined. Fig. 1 shows the V γ , J γ , and partial C γ gene region sequences of the γ gene clone from 8/10-2 (8/10-2 γ 1.1) compared to the published γ chain cDNA sequence (pHDS4/203) (7). The two sequences are identical except for two extra nucleotides (AT) in PHDS4/203 at the V-J junction. The resulting translational frameshift in the 8/10-1 γ 1.1 sequence introduces a termination codon at position 441 that is not found in the pHDS4/203. The 8/10-2 γ 1.1 sequence presumably arose from a nonproductive gene rearrangement involving the same V γ 10.8A and J-C γ 10.5 gene segments which gave rise to the potentially functional pHDS4/203 message (7, 9).

The nucleotide sequence of the cDNA clone from CTL clone 5/10-13 (5/10-

13 γ 1.2) has an open reading frame from nucleotides 88–1,068 (Fig. 2). This may code for a functional message. Fig. 2 shows the 5/10-13 γ 1.2 sequence compared to the V γ , J γ , and C γ gene region sequences of pHDS4/203. All of the 5/10-13 γ 1.2 gene regions are different from those of pHDS4/203. The V γ gene region sequence of 5/10-13 γ 1.2 has more homology to V γ 10.8B (9). Only 2 nucleotides (at position 176 and 411) out of 351 are different between the V γ of 5/10-13 γ 1.2 and the V γ 10.8B exons (data not shown). Since 5/10-13 originates from B10, and published V γ 10.8B germline sequences are from BALB/c, the two-nucleotide difference might be due to strain polymorphism or somatic mutation. As the third V γ segment (V γ 5.7) seems to be deleted in B10 (discussed below), we conclude that 5/10-13 γ 1.2 uses V γ 10.8B. This is apparently the first message ever to use V γ 10.8B. With a deletion of 6 bp at the 3' end of the germline V γ 10.8B, 5/10-13 γ 1.2 is joined to a J γ gene segment that has not been previously described. Also, the C γ sequence of 5/10-13 γ 1.2 is considerably different from that of the published C γ 10.5 (9). The C γ region of 5/10-13 γ 1.2 is 69 bp longer than C γ 10.5 (shown by dots in Fig. 2). Excluding these 69 bp, the coding regions of the two constant regions are different in 117 bp out of 500 (23.4% difference). The comparison of the deduced amino acid sequences shows a difference of 57 amino acids out of 167 (34% difference). The constant region of 5/10-13 γ 1.2 is also considerably different (data not shown) from that of the germline C γ 7.5 gene, which is supposedly (9) nonfunctional. The C γ region of 5/10-13 γ 1.2 is 54 bp longer than that predicted by the exons of the pseudo-gene C γ 7.5. Excluding these 54 bp, the coding regions of the two constant regions are different in 130 base pairs out of 518 (25% difference). The sequence of the 3' untranslated region of the cDNA clone 5/10-13 γ 1.2 is completely different from those previously reported (7, 9) C γ genes. All of these differences are too great to be explained by strain polymorphism or somatic mutations. Located at nucleotide positions 889–897 is also a potential N-glycosylation site of Asn-Ala-Thr. In addition, a Lys residue is found in the transmembrane position of the deduced protein sequence.

Rearrangement of γ Genes in Cytotoxic T Cell Lines 8/10-2 and 5/10-13. Southern blot analyses were performed to determine whether the γ chain genes used in the CTL clones 8/10-2 and 5/10-13 exhibited different rearrangements than those of previously described CTL. Southern analysis of genomic DNA from CTL 5/10-13, CTL 8/10-2, and B10 liver cells were performed using the probes illustrated in Fig. 3. Results of these experiments are summarized in Fig. 4. After digestion of B10 liver DNA with restriction enzyme Eco RI, one distinct 10.8 kb band could be detected (Fig. 4A) with the 8/10-2 γ 1.1 V γ region probe (Fig. 3a). The 5.7 kb V γ hybridizing fragment (V γ 5.7) previously reported for BALB/c DNA (9) was missing. In the 5/10-13 cell line DNA both a rearranged band of 14 kb as well as the 10.8 kb germline band were detected. Only one rearranged band, of 14 kb, was detected in the 8/10-2 cell line DNA. When the J γ C γ region of 8/10-2 γ 1.1 (Fig. 3b) was used as a probe, two distinct bands, of 15 and 10.5 kb, could be detected in Eco RI-digested B10 liver DNA (Fig. 4B). In the 5/10-13 DNA digested with Eco RI, four bands, of 22, 16, 14, and 10.5 kb, were detected. While in similarly digested 8/10-2 DNA, four bands, of 22, 16, 14, and 7.5 kb, were detected.

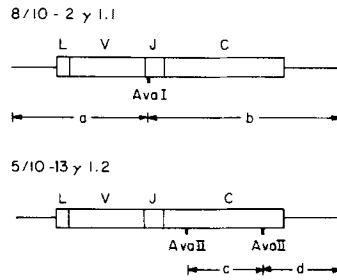


FIGURE 3. Probes used in this study. Each probe was purified twice by low melting-point agarose gel electrophoresis.

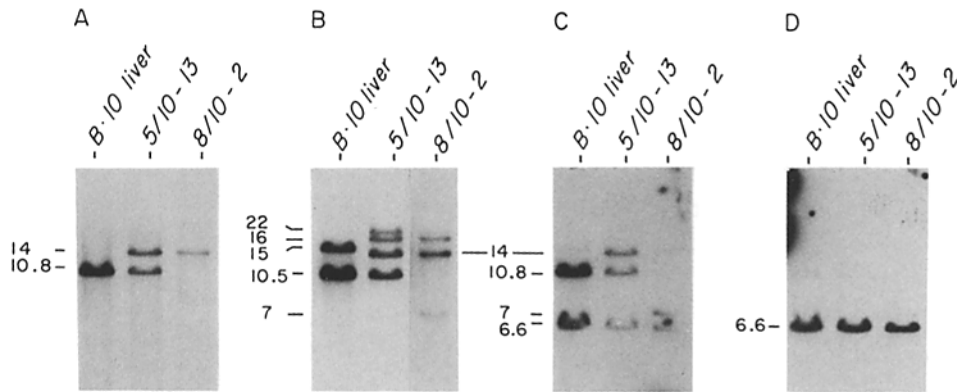


FIGURE 4. Southern blot analysis of Eco RI-digested genomic DNA samples from B10 liver and cytotoxic T cell lines. Probes are: A, probe a; B, probe b; C, probe c; D, probe d in Fig. 3.

Since the V_γ region of 5/10-13 γ 1.2 (V_γ 10.8B) and the V_γ region of 8/10-2 γ 1.1 (V_γ 10.8A) are >93% homologous (Fig. 2), they should crosshybridize. Therefore the expressed V_γ is probably contained in the 14 kb V_γ hybridizing fragment from both the 5/10-13 and 8/10-2 cell line DNA samples.

In the blots probed with the C_γ region of 5/10-13 γ 1.2 (Fig. 3C) the 14 kb band was detected only in 5/10-13 (Fig. 4C). In addition, this probe and the associated 3' untranslated region probe, which includes part of C_γ (Fig. 3d) detected a new germline band of 6.6 kb (Fig. 4C and D).

New Constant Region and J_γ Segment in B10 and BALB/c Mice. The nucleotide sequence and results of Southern blot analyses using B10 germline DNA suggest that a new C_γ region gene exists in the B10 mice. To further analyze the differences between B10 and BALB/c mice, Eco RI-digested germline DNA samples of B10 and BALB/c mice were examined by Southern blots using V_γ region probe a and C_γ region probes of b, c, and d (see Fig. 3). Two V_γ -

FIGURE 2. Nucleotide and deduced amino acid sequences of 5/10-13 γ 1.2 compared with these of pHDS4/203 composed of V_γ 10.8A and J_{C_γ} 10.8A (7). Nucleotides that are identical between the two sequences are indicated (-). Differences are explicitly indicated. The dots indicate sequence gaps introduced to maximize homology. The potential N-glycosylation site is indicated by a solid line. The leader, variable, joining, constant, transmembrane, and cytoplasmic regions are designated as L, V, C, J, TM, and CY, respectively.

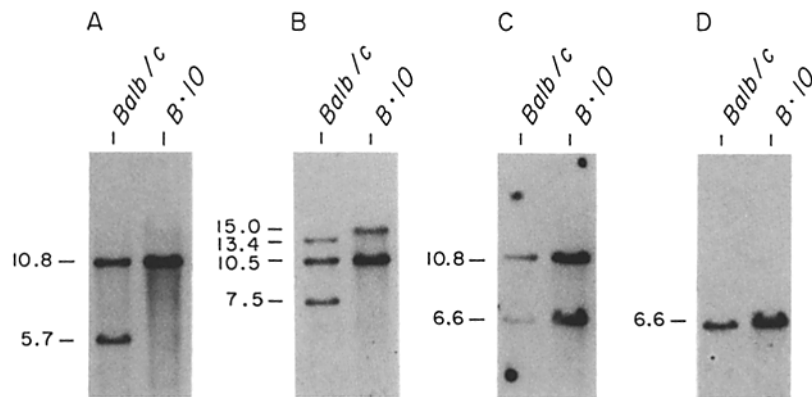


FIGURE 5. Southern blot analysis of Eco RI-digested genomic DNA samples from BALB/c and B10 liver. Probes are: A, probe a; B, probe b; C, probe c; D, probe d in Fig. 3.

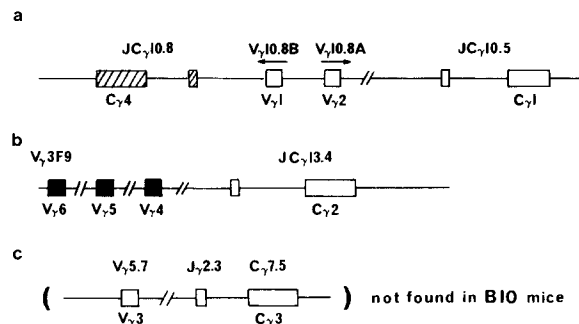


FIGURE 6. Schematic diagram of germline genomic organization of T cell γ genes. In the BALB/c mice genome, all of *a*, *b*, and *c* are present. Genomes of B10 mice contain only *a* and *b* as shown, but are missing *c*. Introns are not shown. Open boxes represent the regions whose presence has been reported (9). Hatched boxes indicate the new J_γ and the C_γ regions reported in this paper. Solid boxes indicate new variable regions according to the data from F. Rupp (unpublished data), Susumu Tonegawa (personal communication), and D. Raulet (personal communication). Nomenclature of the C_γ and V_γ regions are also proposed.

hybridizing fragments, of 10.8 kb and 5.7 kb, were found in the BALB/c DNA, while only one was detected at 10.8 kb in the B10 genome (Fig. 5A). The C_γ region probe b detects the three germline bands of 13.4, 10.5, and 7.5 kb in the BALB/c DNA, while B10 DNA contains only two C_γ b-hybridizing fragments of 15 kb and 10.5 kb. These results for BALB/c are consistent with those reported by Hayday et al. (9). The fragments $C_\gamma 7.5$ and $V_\gamma 5.7$ are not present in B10 mice. A 2.3 kb $J_\gamma C_\gamma$ -hybridizing band was detected by a longer exposure of the filter shown in Fig. 5B in the BALB/c but not in the B10 lane (data not shown). Therefore, it appears that $J_\gamma 2.3$ is also missing in the genome of B10. When the C_γ region probe of 5/10-13 γ 1.2 (Fig. 3C) was used, fragments with sizes 10.8 and 6.6 kb in both B10 and BALB/c DNA were detected. The 6.6 kb band probably contains the 3' untranslated region, since probe d only hybridizes to a 6.6 kb band (Fig. 5D). Thus, since both B10 and BALB/c genomic DNA show these bands, the newly identified C_γ region of 5/10-13 γ 1.2 must be present in both strains of mice. Restriction enzyme mapping with Eco RI and Kpn I

indicates that this new constant region is located at the 5' end of the inverted V_γ gene segment, $V_\gamma 10.8B$ (9) on the same 10.8 kb Eco RI fragment (data not shown). This newly identified C_γ region is not that of $JC_\gamma 13.4$ reported (9) in BALB/c mice, since 5/10-13 γ 1.2 does not use the J_γ segment of $JC_\gamma 13.4$ reported (9) earlier. Furthermore, the constant region probe from 5/10-13 γ 1.2 does not crosshybridize to $JC_\gamma 13.4$ (Fig. 5C). Recently, a cDNA clone using a new V_γ gene that does not crosshybridize with the previously described V_γ genes and $JC_\gamma 13.4$ was found in BALB/c cytotoxic T cells (F. Rupp, unpublished data). Two additional V_γ are also known to be associated with $JC_\gamma 13.4$ (S. Tonegawa and D. Raulet, personal communication).

On the basis of these data, we have revised the germline genomic organization (Fig. 6) of BALB/c DNA reported previously (9). In addition, a germline genomic organization of B10 DNA is also proposed, with a new nomenclature of these C_γ and V_γ segments.

Discussion

Two cDNAs of the γ gene family have been isolated and characterized from two functional CTL clones of B10 mice. The nucleotide sequences of one of them (8/10-2 γ 1.1) indicates that the transcript is composed of the VJC ($V_\gamma 10.8A$, $JC_\gamma 10.5$) found in several CTLs (9). This cDNA, however, contains a stop codon and is therefore not functional. Southern blot analysis indicates that additional rearrangements have occurred in this CTL clone. It is not known at this time whether this rearrangement results in a functional transcript. The isolation of three nonfunctional messages from an alloreactive CTL clone (3F9) suggests that many of the γ chain gene rearrangements are nonfunctional (F. Rupp, unpublished data).

Sequence analysis of the γ gene of the other CTL clone (5/10-13 γ 1.2) indicates that previously unreported J_γ and C_γ gene segments are used. These new genes ($JC_\gamma 10.8$) do not correspond to the $JC_\gamma 13.4$ reported (9) in BALB/c mice for the following reasons. First, the C region of $JC_\gamma 10.8$ does not crosshybridize with $C_\gamma 13.4$ (Fig. 5c). Second, the cDNA clone 5/10-13 γ 1.2 uses a new J_γ segment not associated with $C_\gamma 13.4$. Finally, a cDNA clone using the new V_γ gene segment not crosshybridizing with the V_γ gene previously described (9) has been isolated. This newly identified V_γ gene segment is associated with the $JC_\gamma 13.4$ (F. Rupp, unpublished result). Therefore, we conclude that the $JC_\gamma 10.8$ in B10 mice does not correspond to $C_\gamma 13.4$ of BALB/c mice. $JC_\gamma 10.8$ rearranges with the inverted $V_\gamma 10.8B$ to form a potentially functional transcript. $JC_\gamma 10.8$ is located at the 5' end of 10.8 kb Eco RI fragment containing $V_\gamma 10.8A$ and the $V_\gamma 10.8B$. Since the entire new $J_\gamma C_\gamma$ sequences of CTL clone 5/10-13 γ 1.2 shows considerable variation from sequences of the $J_\gamma C_\gamma$ sequences reported previously, the isolation of this clone must have been due to crosshybridization of the V_γ region of the probe to the V_γ region of this cDNA clone. This new C_γ gene deduced protein sequence is only 66% homologous to the $C_\gamma 10.5$ reported before, and is thus potentially an isotype with a different function. One intriguing possibility is that the protein may combine with the $C_\gamma 10.5$ or $C_\gamma 13.4$ to produce a second heterodimer. The Lys residue in the transmembrane region is also characteristic of T cell receptor proteins. It has been suggested that this positive charge may

associate with the Asp residue in the transmembrane portion of the T3 molecules (17). Unlike the previous $C_\gamma 10.5$, which contains no potential *N*-glycosylation site, this new C_γ has one potential *N*-glycosylation site.

Also of interest is the description of the differences between the γ chain genes of B10 mice and those of the BALB/c mice reported previously (9). Although we can confirm the existence of the potential pseudogenes $V_\gamma 5.7$, $J_\gamma 2.3$, and $C_\gamma 7.5$ in BALB/c mice, they are lacking in the genome of the B10 mouse. On the basis of these data, the new germline genomic organization of BALB/c mice has been revised (Fig. 6). A germline organization of B10 DNA is also proposed (Fig. 6).

Although the pseudogenes of BALB/c mice are not present in the B10 mouse strain, all of the known potentially functional V_γ , J_γ , and C_γ genes are conserved between the two strains of mice. These data strongly argue for the importance of these genes.

While it is possible that the γ chain genes are important in the development of T cells, the role it may play is far from clear. Although it has been postulated (8, 19) that γ genes may be involved in the recognition of class I MHC gene products on the target cells, proof of such a hypothesis requires further testing, as, (a) no protein coded for by γ chain messages have been identified to date, (b) γ transcripts are also found in helper and autoreactive T cell clones (18), and (c) a high frequency of nonfunctional γ chain messages are found in mature T cells (Yoshikai, Y., and T. W. Mak, unpublished data). It is possible that these genes may play a role in very early T cell ontogeny. Nonetheless, the isolation of a γ chain message using $V_\gamma 10.8B$ and a new $J_\gamma C_\gamma 10.8$ in this report indicates that the repertoire of the murine γ sequences may be more diverse than postulated previously (7-9, 19). We hope that the further elucidation of their genomic structures, together with the description of new and potentially functional γ messages may help develop experiments to discover the function of these γ chain genes. Perhaps the use of gene transfer technology described recently by Ohashi et al. (20) in the reconstitution of a functional T cell antigen receptor may uncover the role of these interesting genes.

Summary

The T cell-specific γ genes in C57BL/10 (B10) mice have been analyzed. Based on the cDNA sequences of these genes from antigen-specific MHC-restricted cytotoxic T cells, we found that the repertoire of these genes is not as limited as previously postulated (8). T cells from the B10 mice express an identical copy of $V_\gamma J_\gamma C_\gamma$ ($V_\gamma 10.8A$ - $J_\gamma C_\gamma 10.5$) transcript previously found in T cells of BALB/c mice. In addition, a potentially functional mRNA using $V_\gamma 10.8B$ and newly identified J_γ and C_γ gene segments were found. The new $J_\gamma C_\gamma$ ($J_\gamma C_\gamma 10.8$) is located 5' to the inverted $V_\gamma 10.8B$ in the germline DNA of both B10 and BALB/c mice. This new C_γ is only 77 and 66% homologous to the $C_\gamma 10.5$ at the nucleotide and deduced protein sequences, respectively, thus making it a potential isotype of the C_γ genes reported previously. The $V_\gamma 5.7$, $J_\gamma 2.3$ gene segments and pseudogene $C_\gamma 7.5$ found in the germline DNA of BALB/c mice are absent in B10 mice. The loss of this γ chain pseudogene in the B10 mouse strain, and

the retention of all potentially functional V_γ , J_γ , and C_γ genes with highly conserved coding sequences supports the importance of these genes.

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