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T cell receptor genes in an alloreactive CTL clone: implications for rearrangement and germline diversity of variable gene segments

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Both cDNA and genomic clones of the T cell receptor (TCR) α- and β-chain genes of the alloreactive cytotoxic T lymphocyte (CTL) clone F3 were examined. Two distinct rearrangement events, one functional and one non-functional, were found for both the α and β loci. Thus only a single functional TCR αβ heterodimer could be defined, consistent with allelic exclusion in the TCR genes. The V_α gene employed by F3 is part of a six-member V_α subfamily. Genomic clones containing each member of this subfamily were isolated and the V_α nucleotide sequences determined. Five of these six genes are functional; these genes differ from each other by 7-14% at the amino acid level. A single dominant hypervariable region was defined within this subfamily, in contrast to the pattern of variability seen between V_α genes in general.

Key words: allelic exclusion/germline diversity/hypervariability/polymorphism/T cell receptor genes

Introduction

The murine TCR is a disulfide-linked heterodimer composed of an acidic α- and a basic β-chain polypeptide, each containing a variable and a constant region (Kappler *et al.*, 1983; McIntyre and Allison, 1983). A functional α-chain variable region is encoded by two germline elements, a variable (V) and a joining (J) gene segment (Chien *et al.*, 1984b; Saito *et al.*, 1984), while a functional β-chain variable region is assembled from three germline elements, a V, a diversity (D), and a J gene segment (Chien *et al.*, 1984a; Siu *et al.*, 1984). During T cell ontogeny, V_α and V_β genes are assembled from these dispersed gene segments by a series of somatic DNA rearrangements analogous to those required to produce immunoglobulin heavy and light chain variable genes (Hood *et al.*, 1985; Tonegawa, 1983).

Somatic DNA rearrangement in B and T cell receptor genes is not necessarily complete or precise (Alt and Baltimore, 1982; Schnell *et al.*, 1980). Non-functional rearrangements have been observed and may be important because, in contrast to most other autosomal systems studied, the expression of immunoglobulin genes follows the rule of allelic exclusion (Coleclough *et al.*, 1981; Early and Hood, 1981). In any given B lymphocyte, only one of the two alleles of the heavy and light chain loci can encode a functional polypeptide; the other allele is phenotypically silent. Available evidence suggests that allelic exclusion may apply also to TCR genes, although its generality has not been established (Goverman *et al.*, 1985; Ikuta *et al.*, 1985; Dembic *et al.*, 1986).

Alterations of DNA sequences, such as N region diversity and somatic hypermutation, are known to accompany somatic DNA rearrangements among immunoglobulin genes (Alt and Baltimore, 1982; Crews *et al.*, 1981; Selsing and Storb, 1981). In the TCR, extensive N region sequences have been documented in β- but not in α-chain genes, and it has been suggested that the presence of N region is associated with the presence of D gene segments (Kronenberg *et al.*, 1986). Somatic hypermutation, however, is not thought to occur in TCR genes.

The murine V_β gene segments are largely organized into single element subfamilies which show only limited interstrain variability. In contrast, V_α subfamilies usually contain more than one member and exhibit extensive restriction fragment length polymorphism between mouse strains. Moreover, the murine V_α family has apparently undergone significant expansion and contraction, as evidenced by differences in the number of cross-hybridizing bands between inbred strains of mice on genomic Southern blot. To date, however, data are not available to assess the actual extent of germline diversity within each of the V_α subfamilies.

To examine the mechanisms responsible for generating the diversity of the TCR repertoire and to determine how the expression of TCR genes is related to the somatic DNA rearrangement events, we have characterized in detail the TCR genes of an alloreactive CTL clone, F3. We have cloned and sequenced both the α- and β-chain cDNAs and their genomic counterparts. We have also analyzed non-productive rearrangements at both α- and β-chain loci and compared all rearranged genes to their embryonic counterparts. Finally, using a V_α-specific probe from the α cDNA of this cell line, we have studied by direct sequence analysis the germline diversity present in gene segments of a V_α subfamily.

Results

TCR gene rearrangements in F3

F3 is an alloreactive CTL clone derived from a secondary C57BL/6-H-2^{bm1} anti-C57BL/6 (bm1 anti-B6) mixed lymphocyte culture. F3 shows strong lytic activity against cells from B6 mice as well as from bm5 and bm6 mutant mice. To characterize the structure of the antigen-specific TCR genes in the cell line F3, cDNA and genomic clones containing rearranged variable regions (V_α or V_β) were isolated and characterized. A cDNA library was constructed from F3 poly(A)$^+$ RNA and screened with C_α- and C_β-specific probes; nucleotide sequences of positive clones were determined. A representative full-length C_α-containing clone, cF3-20 (designated as V_αF3.2-J_αF3.1-C_α), is shown in Figure 1. The V_αF3.2 gene segment is homologous to the V_α8 of clone TA61; the J_αF3.1 is identical to the J_α of clone TA37, as reported by Arden *et al.* (1985). Similarly, a representative full-length C_β-containing clone, cF3-42, is shown in Figure 1. Clone cF3-42 contains the V_β11, D_β1.1, J_β2.7, and C_β2 gene segments [V_β nomenclature according to Barth *et al.* (1985) as described in Behlke *et al.* (1986)].

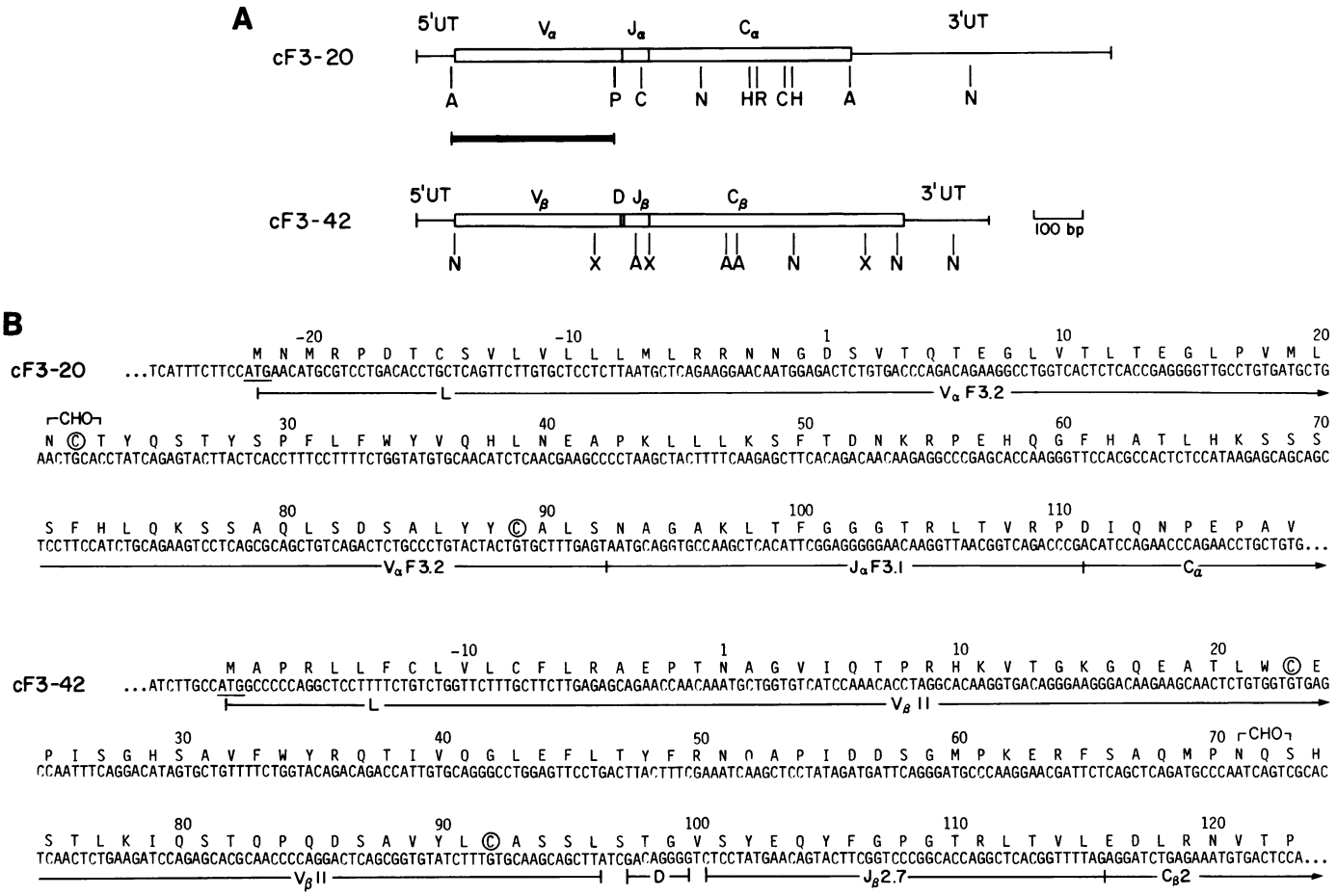

Fig. 1. Restriction maps and sequences of full-length α and β cDNA clones of the F3 CTL. (**A**) Partial restriction enzyme maps of α (cF3-20) and β (cF3-42) cDNA clones. Restriction enzyme abbreviations are as follows: A, *Ava*II; C, *Hinc*II; H, *Hin*dIII; N, *Nco*I; P, *Pvu*II; R, *Eco*RV; X, *Xho*II. The *Ava*II–*Pvu*II fragment of cF3-20 (dark bar) was subcloned into pUC 12 and used as a V_αF3-specific probe (see Figure 4). (**B**) Nucleotide and translated amino acid sequences of the two cDNA clones. Cysteines used in intra-chain disulfide bonds are circled and potential N-linked glycosylation sites are indicated. Codon numbering follows that of Patten *et al.* (1984) and Arden *et al.* (1985).

A complete F3 genomic library was constructed in the EMBL3 vector and screened with a C_β-specific probe. Seven C_β-positive clones were obtained; five hybridized to a V_β11-specific probe, and two hybridized to a V_β8-specific probe (Figure 2). The nucleotide sequences of the V_β-containing regions of two representative clones, ϕA6-1 and ϕA6-2, were determined (Figure 3). The ϕA6-2 clone contains a functional rearrangement of a V_β11 gene segment to D_β1.1 and J_β2.7 and is the origin of the cDNA clone cF3-42. However, rearrangement of a V_β8.2 gene segment to D_β2.1 and J_β2.7 in ϕA6-1 is not functional due to a frameshift at the junction, creating a termination codon within the J_β2.7 gene segment.

The C_β-containing genomic clones were further analyzed by hybridization against a panel of V_β-specific probes. Unexpectedly, an additional region in ϕA6-1, located 5 kb 5′ to the rearranged V_β8.2 gene, also hybridized to the V_β8-specific probe. Sequence analysis indicates that a 0.85-kb *Eco*RI fragment contains a germline V_β8.3 gene segment (Figure 2). In addition, a V_β12-hybridizing region was localized 10 kb 5′ to the rearranged V_β11 gene in ϕA6-2 and was found to contain a germline V_β12 gene segment. Thus, V_β gene segments of the same or of different V_β subfamilies can be closely linked on the chromosome (H.Chou, in preparation).

Analysis of α-chain rearrangements is more difficult because the distance between the rearranged V_α–J_α and C_α can be very

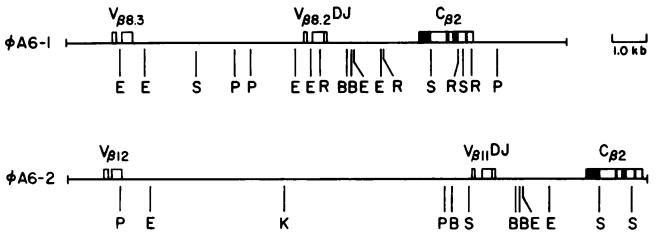

Fig. 2. Organization of genomic clones containing the rearranged β-gene loci in F3 CTL. Exons encoding V_β, D_β, J_β and C_β gene segments are indicated by vertical lines or boxes above the horizontal lines. Restriction enzyme sites are indicated and abbreviations are as follows: B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; P, *Pvu*II; R, *Eco*RV; S, *Sac*I.

large. To further analyze the TCR α-gene rearrangements in F3, a genomic Southern blot of B6 liver DNA was hybridized with a V_αF3-specific probe derived from cDNA clone cF3-20. Six bands were detected, with sizes of 2.2, 5.5, 6.5, 8.5, 9.0 and 14 kb, and are referred to as V_αF3.1, F3.2, F3.3, F3.4, F3.5 and F3.6, respectively (Figure 4). In contrast, a genomic Southern blot of F3 DNA shows that the V_αF3.1 and V_αF3.2 bands have been replaced by two new bands of 4.5 and 2.3 kb, referred to as V_αF3.2R and V_αF3.2R′, respectively. To establish the relationship between these V_αF3-hybridizing bands and the functional α cDNA clone cF3-20, genomic DNA clones containing

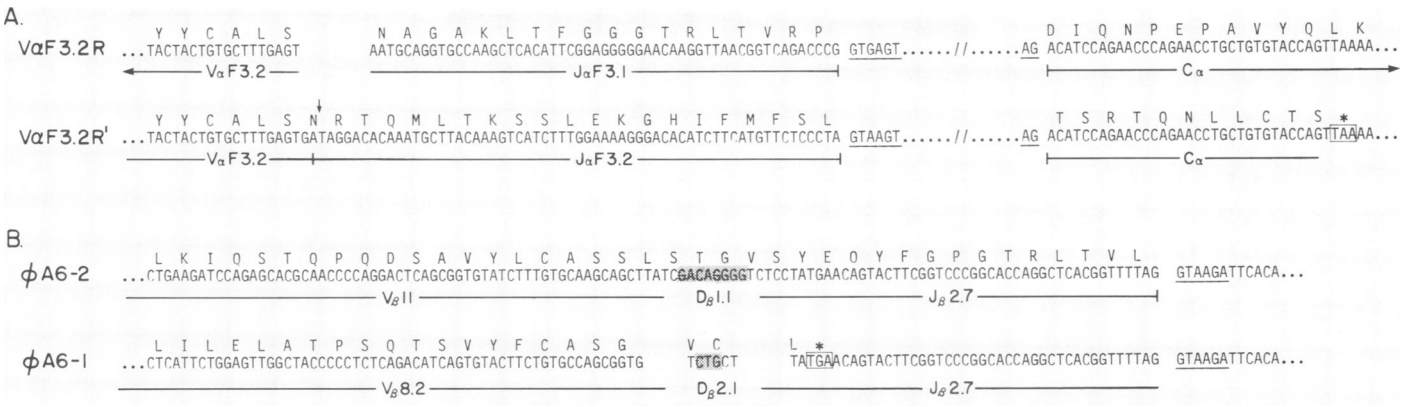

Fig. 3. Allelic exclusion in F3 CTL. (**A**) Comparison between the productively (**top**) and non-productively (**bottom**) rearranged V_αF3.2 gene segments. The introns between J_α and C_α are indicated by a dotted line; the consensus RNA splice signals are underlined. The vertical arrow indicates the $V_\alpha - J_\alpha$ junction where the frameshift in J_αF3.2 begins. The termination codon within frameshifted C_α is boxed and indicated by an asterisk. (**B**) Comparison between the productive $V_\beta 11 - D_\beta 1.1 - J_\beta 2.7$ (**top**) and the non-productive $V_\beta 8.2 - D_\beta 2.1 - J_\beta 2.7$ (**bottom**) rearrangements at the β-gene loci. The nucleotide sequences contributed by the D gene segments are stippled.

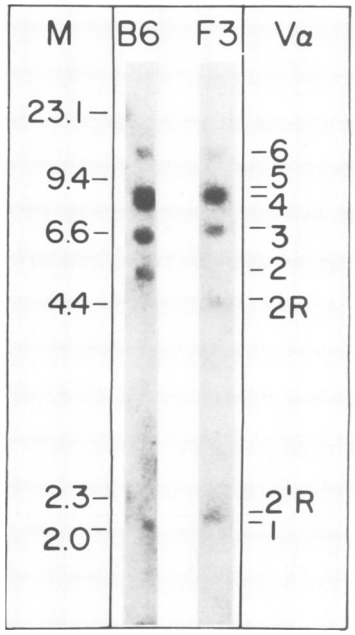

Fig. 4. Southern blot analysis of V_α rearrangement in the F3 CTL. Approximately 10 μg of genomic DNA prepared from C57BL/6 liver (B6) and from the F3 CTL clone were digested with *Eco*RI, fractionated through a 0.8% agarose gel, transferred to a nitrocellulose filter, and hybridized to the V_αF3-specific probe described in Figure 1. The hybridizing bands are designated on the right. Size markers (M) are indicated in kilobases.

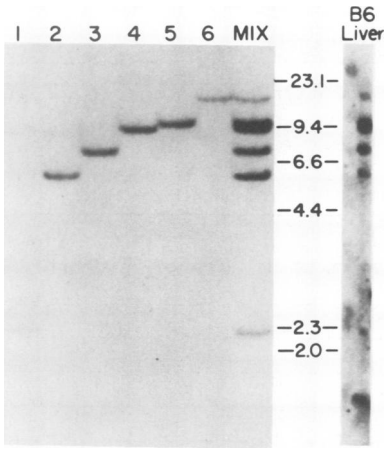

Fig. 5. Reconstruction of genomic Southern blot from representative phages containing gene segments of the V_αF3 subfamily. Approximately the same amount of each phage DNA was digested with *Eco*RI and separated on a 0.9% agarose gel. The DNA was transferred to a nitrocellulose filter and hybridized to a nick-translated V_αF3-specific probe as indicated in Figure 1. **Lanes 1–6** are DNA of representative phages containing V_αF3.1 to V_αF3.6, respectively.

V_αF3.1, V_αF3.2, V_αF3.2R, and V_αF3.2R′ were isolated and their nucleotide sequences determined.

Sequence analysis reveals that V_αF3.2R contains a functional rearrangement of the V_αF3.2 gene segment to the J_αF3.1 gene segment and is the origin of the clone cF3-20 (Figure 3). The rearrangement of a second V_αF3.2 gene segment to the J_αF3.2 gene segment in V_αF3.2R′ creates frameshifts in the J_αF3.2 and in the C_α, resulting in a termination codon at the beginning of the C_α gene segment. Thus, V_αF3.2R′ represents an abortively rearranged gene. V_αF3.1 is presumably the most J_α-proximal gene segment of this subfamily and was deleted on both chromosomes as a result of the rearrangements of the more distal V_αF3.2 gene segments.

In summary, we have isolated two types of rearrangements for each of the V_α and V_β loci, but found only one pair of functional α- and β-chain genes in the F3 CTL clone.

Genes of the V_α subfamily

To assess the extent of germline diversity present within the V_αF3 subfamily, we screened B6 and F3 genomic libraries with a V_αF3-specific probe (Figure 1) and isolated 22 V_αF3-positive clones. The sizes of *Eco*RI fragments containing V_αF3-related genes in these clones were compared to those of *Eco*RI fragments seen in a genomic Southern blot of B6 DNA. We isolated at least one recombinant phage clone for each of the six V_αF3-related genes. An *Eco*RI digest of a mixture of these six unique phages reconstitutes the genomic Southern-blot banding pattern (Figure 5). We conclude that V_αF3-related genes contained in these phages represent the complete V_αF3 subfamily.

The nucleotide sequences of the V_α portions of these six clones were determined and are shown in Figure 6. Five of the six genes contain open reading frames and are presumed to be functional V_α gene segments. The V_αF3.2 gene segment, employed in both the functional and the abortive rearrangements in F3, is used as

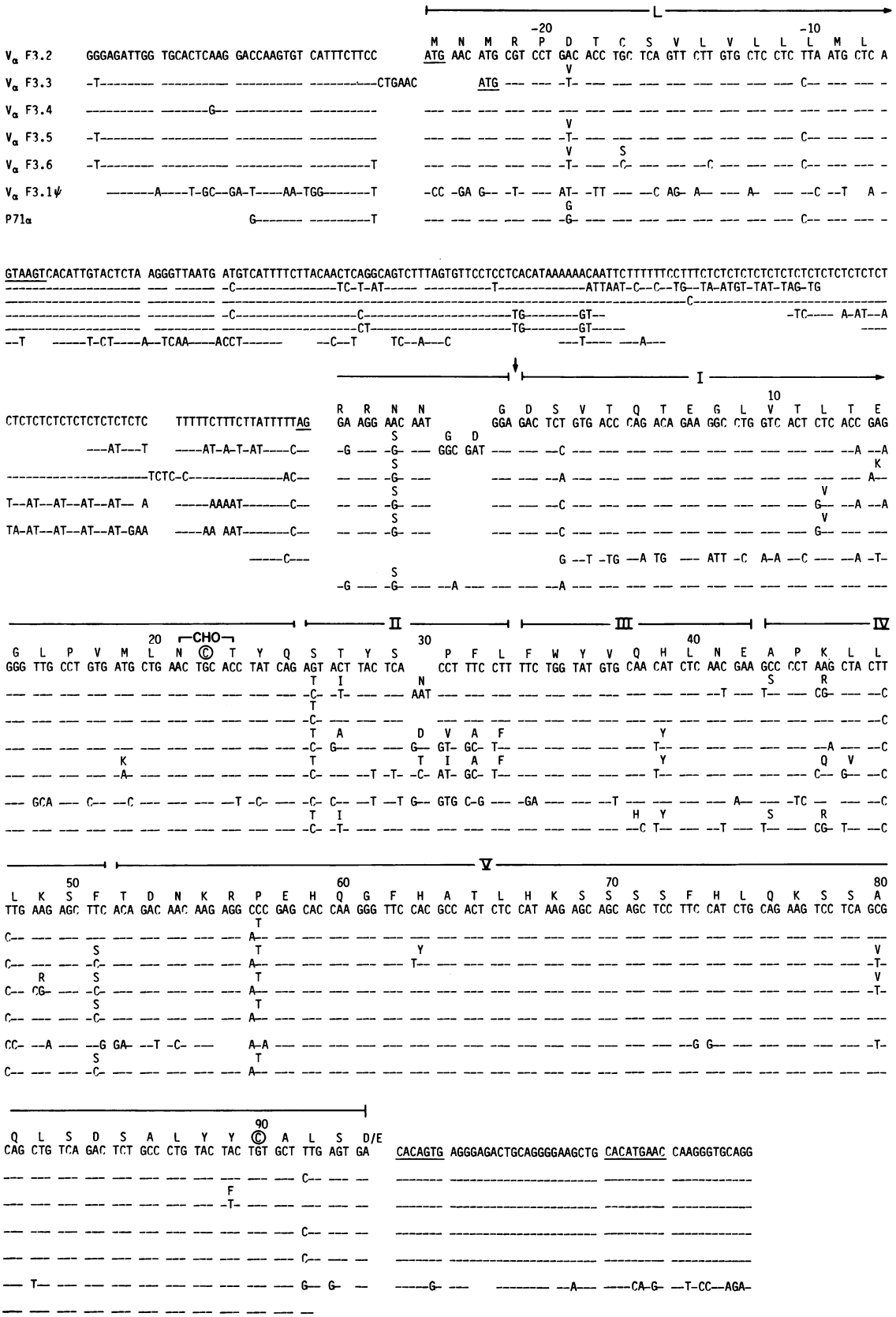

Fig. 6. Nucleotide and translated amino acid sequences of the germline V gene segments of the $V_\alpha F3$ subfamily. The complete nucleotide and amino acid sequences of the $V_\alpha F3.2$ gene segment are presented in the top line. The sequences of the remaining five genes of this subfamily are listed below. Gaps are introduced to maximize the homology. Dashes are used to indicate sequence identity to the top line, and amino acid residues different from that of the top line are indicated. Potential N-linked glycosylation sites are marked; the invariant cysteine residues are circled. Putative leader peptide cleavage sites are deduced according to Von Heijne (1983) and are indicated by an arrow. For ease of sequence comparison, the V coding regions are arbitrarily divided into five subregions, as indicated. P71α, from Becker *et al.* (1985), is the TCR α chain of a BALB/c anti-D^b CTL clone. Consensus RNA splice sites, nonamer and heptamer sequences, and putative initiation codons are underlined.

a reference for sequence comparison. The V_αF3.1 gene segment is a pseudogene which lacks an initiation codon at the expected position and contains multiple deletions which lead to premature termination within the V_α coding region. Each V_α gene is preceded by a leader of 66 bp which is separated from the V_α coding sequence by an intron of 140–170 bp in length. The V_α coding regions are 281 bp long and are followed by the heptamer sequence CACAGTG, a 22-bp spacer, and the nonamer sequence CACATGAAC. The heptamer and nonamer sequences are the consensus signals for somatic DNA recombination commonly observed at the 3′ end of immunoglobulin V genes and TCR V_α and V_β genes.

Discussion

F3 contains only one pair of productively rearranged TCR genes

Early studies indicated that the antigen-specific receptors on T cells are monospecific and clonally distributed, implying that one of the two alleles encoding the receptor is phenotypically silent (Bevan, 1975). Based on the KJ16-133 staining of F_1 T cells, Roehm *et al.* (1985) suggested that the expression of this TCR V_β gene marker follows the principle of allelic exclusion. Molecular genetics studies have shown that germline or incomplete D–J rearrangements can co-exist with a productively rearranged V_β gene (Goverman *et al.*, 1985; Kronenberg *et al.*, 1985; Malissen *et al.*, 1986). Furthermore, Dembic *et al.* (1986) recently reported evidence for allelic exclusion at both α- and β-gene loci in a CTL clone.

To determine the nature of the TCR in F3, we have isolated and characterized all of the rearranged α- and β-chain genes. Isolation of F3 genomic clones containing V_α and V_β rearrangements distinct from those corresponding to cF3-20 and cF3-42 provides further evidence that allelic exclusion holds for TCR α and β gene loci. Sequence analysis reveals that F3 contains only a single productive rearrangement for each of the α- and β-chain genes and therefore can express only one complete α and one complete β polypeptide chain to form a single TCR αβ heterodimer despite the presence of two distinct V_α and V_β rearrangements. Thus, available data support the conclusion that allelic exclusion is a common, if not a universal, phenomenon in T cells.

Pattern of TCR rearrangement

TCR β-chain genes appear to rearrange before α-chain genes in the thymus (Raulet *et al.*, 1985). Moreover, rearranged β-chain genes show significant N region diversity. Analysis of α-chain genes reveals the absence of both N-region insertions and identifiable D_α gene segments (Hayday *et al.*, 1985). Comparison of cF3-20 to the unrearranged V_αF3.2 gene segment and to another J_αF3.1-containing cDNA, TA37 (Arden *et al.*, 1985), shows that nucleotide sequences at the V_α–J_α junction can be fully accounted for by the germline sequences. Similarly, Hayday *et al.* (1985) concluded that the germline V_α2C and J_α2C sequences fully account for the sequence of pHDS 58 at the V_α–J_α junction. It is unclear at this point whether the N region diversity is causally related to the presence of D gene segments or to the developmental stages at which rearrangement occurs. Interestingly, N region sequences have been documented in the V_γ–J_γ junction (Heilig *et al.*, 1985), and rearrangement of γ-chain genes precedes that of α- and β-chain genes (Raulet *et al.*, 1985; Snodgrass *et al.*, 1985). There is no evidence, however, that D_γ gene segments exist. Based on available data, N region diversity seems to correlate better with rearrangement early in ontogeny than with the presence of D gene segments. Template-independent insertion of nucleotides by terminal transferase has been suggested as a mechanism for N region diversity (Desiderio *et al.*, 1984; Kunkel *et al.*, 1986). Whether stage-specific insertion of N region sequences correlates with the availability of terminal transferase is not known.

Table I. Homology between the V_αF3 subfamily genes[a]

	V_αF3.1	V_αF3.2	V_αF3.3	V_αF3.4	V_αF3.5	V_αF3.6	P71α[b]
V_αF3.1	–	76	76	79	77	75	76
V_αF3.2	–	–	95	96	93	93	95
V_αF3.3	–	91	–	94	94	93	97
V_αF3.4	–	92	93	–	93	94	95
V_αF3.5	–	87	88	90	–	95	93
V_αF3.6	–	86	87	88	92	–	93
P71α	–	91	95	93	89	87	–

[a]Numbers above the diagonal indicate the percent homology at the nucleotide sequence level; numbers below the diagonal indicate the percent homology at the amino acid level. V_α coding sequences, including the leaders, are compared.
[b]From Becker *et al.* (1985).

In addition to N region insertions, somatic hypermutation is known to increase the diversity in immunoglobulin genes (Bothwell *et al.*, 1981; Crews *et al.*, 1981). We compared sequences of the rearranged V genes in F3 to their germline counterparts to determine if somatic hypermutation has occurred in a T cell clone that has been repeatedly stimulated to proliferate *in vitro*. We found sequence identity in all V_α and V_β genes compared (Figures 3 and 6; germline V_β sequences not shown). Our data extend earlier observations that somatic hypermutation does not occur in TCR genes (Chien *et al.*, 1984a; Goverman *et al.*, 1985).

Germline diversity in the V_αF3 subfamily

While the germline repertoire of murine V_β gene segments appears to be limited, the V_α gene segments are organized into at least ten subfamilies, each containing one to ten members as defined by the number of cross-hybridizing bands on genomic Southern blots. Therefore, the extent of functional V_α diversity available to an organism is significantly influenced by the size of individual V_α subfamilies and by the variability present within each subfamily. The detailed sequence analysis of the V_αF3 subfamily provides the first insight into the extent of germline diversity present in a V_α subfamily.

Of the six gene segments present in the V_αF3 subfamily in the B6 mouse, V_αF3.1 is a pseudogene which cannot encode the V region of a functional TCR α-chain polypeptide. The remaining five gene segments all contain open reading frames. These genes possess the conserved amino acids that are invariant in both immunoglobulin and TCR V gene families, including the cysteine residues involved in the intra-chain disulfide bond. These five functional V_αF3 gene segments are highly homologous to each other; pairwise comparisons show 93–96% homology at the nucleotide level, which translates into 86–93% homology at the amino acid level (Table I). The extent of diversity seen in the V_αF3 subfamily is similar to that previously observed in immunoglobulin NP, NP-equivalent, and PC V_H subfamilies (Bothwell *et al.*, 1981; Loh *et al.*, 1983; Perlmutter *et al.*, 1985). Although the five functional V_αF3 gene segments are highly homologous, the 7–14% divergence seen at the protein level implies that five clearly distinct V regions for functional α-chain polypeptides can be encoded in this subfamily. We conclude that the number of cross-hybridizing bands on genomic Southern blots

Table II. Comparison of nucleotide differences in the V_αF3 subfamily

Subregion	R	S	R/S ratio	% Base change per base pair
L	4	3	1.33	2.1 (7/330)
I	3	4	0.75	1.9 (7/375)
II	12	1	12.0	10.8 (13/120)
III	1	1	1.0	1.5 (2/135)
IV	6	3	2.0	6.7 (9/135)
V	4	1	4.0	0.8 (5/645)
All	30	13	2.31	2.5 (43/1740)

The V_α coding region is divided into subregions as indicated in Figure 6. R = replacement differences (those that change an amino acid), S = silent differences (those that result in a synonomous codon), L = leader sequence.

realistically estimates the size of the V_α germline repertoire, since only one of the six V_αF3 gene segments is non-functional.

Nucleotide substitutions among members of the V_αF3 subfamily are not uniformly distributed within the coding region, but tend to cluster in certain areas (Figure 6). To facilitate analysis, we arbitrarily divided the V_α coding region into five subregions. Extensive nucleotide variability is clustered in subregion II (codons 26 to 33). This subregion contains unusual 3-nucleotide insertions/deletions between subfamily members which do not disrupt the proper reading frame. Dividing the total number of nucleotide substitutions by the number of base pairs yields a 10.8% substitution rate; in contrast, an overall substitution rate of 2.5% is seen in the coding region (Table II). The high ratio of replacement to silent changes seen in this subregion suggests that there is no selection against these replacement changes. By analogy to immunoglobulins, we speculate that this area of increased diversity may be important in antigen/MHC recognition. Subregion II in the V_αF3 subfamily corresponds to the first hypervariable region of immunoglobulins, a 5- to 10-amino acid stretch upstream from the highly conserved Trp-Tyr-X-Gln sequence (Kabat *et al.*, 1983; Wu and Kabat, 1970). Arden *et al.* (1985) similarly noted this area of increased variability in the V regions of α-chain cDNAs.

A Wu–Kabat analysis of all known V_α genes reveals multiple areas of hypervariability (Arden *et al.*, 1985; Becker *et al.*, 1985). Because V_αF3 subfamily contains only one significant area of hypervariability, we predict that other V_α subfamilies will have areas of increased sequence variability distinct from the hypervariable area in the V_αF3 subfamily.

Interstrain polymorphism

Comparison of the six V_αF3 gene segments to other available α cDNA sequences reveals that the V_α of P71α, the α-chain cDNA of an alloreactive CTL reported by Becker *et al.* (1985), also belongs to the V_αF3 subfamily. P71α is likely to be the BALB/c allelic counterpart of V_αF3.3 in B6, because these two genes share the highest degree of homology among all the pairwise comparisons shown in Table I and the substitutions and insertions unique to V_αF3.3 are also present in the V region of P71α.

Comparison of these two V_α allelic genes yields an overall divergence rate of 3%. In contrast, the C_α coding regions show no sequence divergence between BALB and B6 mice [pHDS 58 of Saito *et al.* (1984) and cF3-20 respectively]. Given that the BALB/c and B6 mouse strains diverged only recently, this high rate of sequence divergence may indicate that selection for V_α variability favors the accumulation of nucleotide substitutions. Gene conversion-type events may also be operating to exchange sequences between non-allelic genes in the V_αF3 subfamily by mechanisms similar to those suggested for immunoglobulin genes (Bentley and Rabbitts, 1983; Ollo and Rougeon, 1983). If most V_α alleles are found to be polymorphic, the V_α repertoire available to the mouse species will be significantly larger than that of V_β, since V_β genes are fewer in number and are generally non-polymorphic between strains.

Materials and methods

Cell culture

The F3 CTL clone was derived by the limiting dilution method from a secondary bm1 anti-B6 mixed lymphocyte culture. Cells were maintained in RPMI 1640 supplemented with 5% fetal calf serum (FCS) and were propagated by repetitive stimulation with irradiated C57BL/6 spleen cells at weekly intervals, as described previously (Howe and Russell, 1983).

Construction and screening of F3 cDNA library

Approximately 2×10^8 F3 cells were harvested 3 days after the last re-stimulation and were separated from the irradiated filler cells by centrifugation through a Ficoll gradient. Total cellular RNA was prepared by the guanidinium isothiocyanate method as described by Chirgwin *et al.* (1979) and poly(A)$^+$ RNA was selected on a 1-ml oligo(dT)–cellulose column (Maniatis *et al.*, 1982). A cDNA library was constructed in the λgt10 cloning vector by the method of Huynh *et al.* (1984), with modifications as outlined by Gubler and Hoffman (1983). Recombinant phages were screened with C_α- and C_β-specific probes; positive cDNA clones were subcloned into pUC12 and sequences were determined by the method of Maxam and Gilbert (1980).

Construction and screening of genomic libraries

Total genomic libraries were constructed from F3 and B6 liver DNA partially digested with *Mbo*I in the λ replacement vector EMBL3 (Frischauf *et al.*, 1983) according to Maniatis *et al.* (1978). Approximately 10^6 recombinant phages were screened with C_β- and V_αF3-specific probes. For isolation of V_αF3.1, V_αF3.2 and V_αF3.6 gene segments, size-selected genomic libraries were constructed. Briefly, high mol. wt DNA from B6 liver was digested to completion with *Eco*RI and fractionated through a 0.8% agarose gel; DNA fragments with sizes corresponding to 2.2, 5.5 and 14.0 kb were isolated. The 2.2 and 5.5 kb fractions were individually ligated into the λgt10 vector; the 14 kb fraction was cloned into the EMBL3 vector at the *Eco*RI site. Approximately 10^5 recombinant phages of each size-selected library were screened with a V_αF3-specific probe by the method of Benton and Davis (1977).

Genomic Southern blot analysis

High mol. wt DNA from B6 liver or F3 was digested to completion with *Eco*RI, separated on 0.8% agarose gels and transferred to nitrocellulose filters (Southern, 1975). Filters were hybridized to a nick-translated V_α probe (sp. act. of $1-3 \times 10^8$ c.p.m./μg) at 68°C for 12–16 h under standard conditions (Maniatis *et al.*, 1982), and washed in 2 × SSC at 68°C.

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