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A new mouse TCR V_γ gene that shows remarkable evolutionary conservation

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We have identified a new mouse T-cell receptor V_γ gene segment, $V_{\gamma4.4}$, which frequently undergoes rearrangements in AKR thymomas, and at a lower frequency in fetal thymocytes. $V_{\gamma4.4}$ is the fourth and the most 5′ V gene segment in the γ4 cluster, being 7.3 kb from $V_{\gamma4.3}$. Surprisingly, $V_{\gamma4.4}$ is more homologous to eight human V_γ genes than to the other mouse V_γ genes. It has only a 38% nucleotide and 21% amino acid sequence homology to the most homologous mouse V_γ gene ($V_{\gamma4.1}$), whereas these homologies to the human $V_{\gamma8}$ gene are as high as 68% and 48% respectively.

Key words: T cell receptor/γ chain genes

Introduction

Recently a second type of T-cell receptor (TCRII) was described in man (Bank *et al.*, 1986; Brenner *et al.*, 1986) and mouse (Lew *et al.*, 1986). The function of the TCRII-bearing cells is still obscure although these cells can constitute up to 10% of human peripheral T lymphocytes (Lanier and Weiss, 1986). This receptor is a heterodimer, formed by association of γ chains with the products of still uncharacterized δ chain genes. It was at first suggested that γ chain diversity, expecially in mouse, is very limited due to the small number of V_γ genes available for rearrangement (Kranz *et al.*, 1985). However, three new V_γ gene segments have recently been identified (Garman *et al.*, 1986; Heiling and Tonegawa, 1986; Traunecker *et al.*, 1986), and we report here an additional V_γ gene which can undergo rearrangements in T lymphoid cells. Strikingly, this V_γ gene segment shows markedly higher nucleotide and amino acid sequence homology to eight human V_γ genes than to the murine ones.

Results and Discussion

Characterization of a new V_γ gene

We obtained a panel of thymic hybridomas (a gift from Dr Werner Haas) that were generated by fusing a thymoma cell line, BW 5147, to fetal day 15 thymocytes from (DBA/2 × C57BL/6)F_1 mice. *Eco*RI-digested hybridoma DNA samples were analysed for γ gene rearrangements by Southern blotting with a $C_\gamma1$ cDNA probe (probe c in Figure 1) which hybridizes efficiently to $C_\gamma1$, $C_\gamma3$ and $C_\gamma4$ gene segments [for the nomenclature see Figure 1 and Traunecker *et al.* (1986)]. In DNA from BW 5147, two rearrangements ($V_\gamma4.3$–$J_\gamma4$–$C_\gamma4$ and $V_\gamma1$–$J_\gamma1$–$C_\gamma1$) and an unrearranged $C_\gamma3$ gene can be detected, as 18.0-, 16.0- and 6.4-kb restriction fragments respectively. An additional 3.8-kb fragment also hybridized weakly to the $C_\gamma1$ probe. Surprisingly, six out of 62 hybridoma DNAs (e.g. HK 49I and HK 54I) had lost the 18.0-, 16.0- and 6.4-kb BW 5147-derived bands, but retained the faint 3.8-kb and fetal thymocyte derived bands (Figure 2A). A plausible explanation for this finding was that one chromosome 13 from BW 5147, containing the usual $_\gamma1$ and $_\gamma4$ rearrangements, was lost in these hybridomas, whereas the homologue from BW 5147 had a new type of rearrangement which deleted all but the one gene segment hybridizing to $C_\gamma1$ probe (3.8-kb band). The weakness of the signal produced from the 3.8-kb restriction fragment suggested that a rearrangement had occured in the $_\gamma2$ cluster because the $C_\gamma2$ gene segment has only a 77% nucleotide sequence homology to the $C_\gamma1$ probe. This was confirmed by the detection of the same 3.8-kb fragment with a $C_\gamma2$ specific probe (probe a in Figure 1).

To characterize the rearrangement in detail we constructed a genomic DNA library from *Eco*RI-digested DNA of the hybridoma HK 49I and isolated nine clones which hybridized to the $C_\gamma2$ probe. Two clones were analysed extensively, one contained the rearranged 3.8-kb fragment (clone BW 3.8.1), and the other a 10.8-kb germ-line fragment (clone B6 10.8.1). The sequence comparison of rearranged and germ-line genes revealed a new V_γ gene which had undergone an unproductive rearrangement to $J_\gamma2$ (Figure 3). This new V gene has low nucleotide (32–38%) and amino acid (20–21%) sequence homology to other murine V_γ gene segments; however, it is much more homologous to human V_γ sequences (see below). The deduced amino acid sequence shows the residues His-Trp-Tyr (positions 38–40), the Tyr-Tyr-Cys stretch (positions 97–99) and the two Cys residues (positions 24 and 99), which have been conserved in all murine V_γ segments so far sequenced (Garman *et al.*, 1986; Hayday *et al.*, 1985; Heilig and Tonegawa, 1986; Traunecker *et al.*, 1986) (Figure 3B).

There are at least four V_γ genes in the $_\gamma4$ cluster

We performed Southern blot analysis for *Eco*RI-, *Hin*dIII- and *Pvu*II-digested DNA from BALB/c, AKR and (DBA/2 × C57BL/6)F1 liver cells, using this new V_γ gene segment as hybridization probe. In all DNA samples, only a single band was detected (data not shown). Thus there are no other V genes with sufficient sequence homology to hybridize with this V_γ probe. *Hin*dIII-digested DNA samples from eight AKR thymomas were also analysed to find out the normal rearrangement pattern of this V_γ gene. Four of these DNA samples had an identical rearrangement on a 5.0-kb restriction fragment (Figure 2B). The same 5.0-kb fragments also hybridized to the $J_\gamma1$ probe (Figure 2C), which detected rearrangements to $J_\gamma1$, $J_\gamma3$ and $J_\gamma4$ gene segments (Figure 2C, lane 1). Because of the size of this fragment and the disappearance of germline $J_\gamma4$ segments, we conclude that the 5.0-kb rearrangement was due to the joining of this new V_γ gene to $J_\gamma4$. As this new V_γ gene is the fourth V_γ gene to be described, which undergoes rearrangement to $J_\gamma4$, we have named it $V_\gamma4.4$. In the thymoma 439, $V_\gamma4.3$ [the most 5′ of previously known V_γ genes in $V_\gamma4$ cluster (Garman *et al.*, 1986)] has undergone rearrangements to $J_\gamma4$ in both chromosomes but $V_\gamma4.4$ is still retained in germ-line configuration (Figure 2B and C, line 8), suggesting that $V_\gamma4.4$ is the most 5′ of known V genes in the $_\gamma4$ cluster. This was confirmed by analysis of the cosmid clone BDFL 6.1 [isolated from BDFL

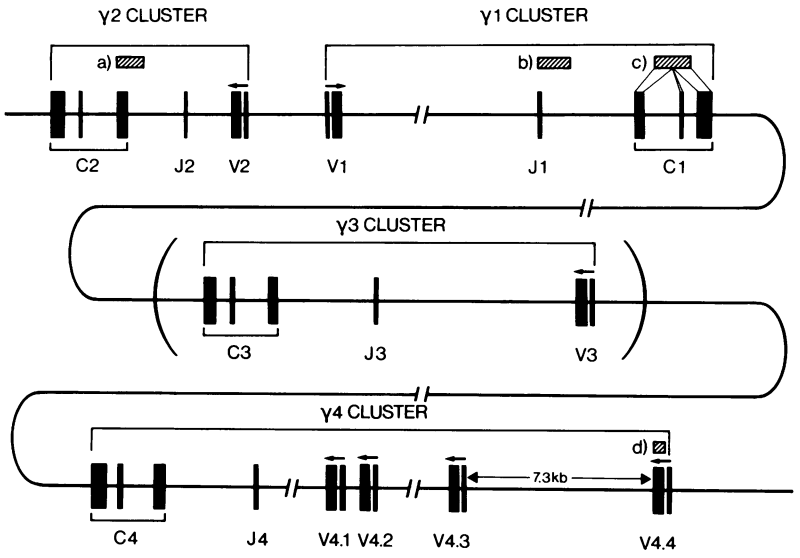


Fig. 1. The genomic organization and the nomenclature of the γ gene family. The nomenclature was developed emphasizing the cluster organization within which the rearrangements always occur. Our genetic maps are based on previously published maps (Hayday *et al.*, 1985; Garman *et al.*, 1986; Traunecker *et al.*, 1986). Gene segments connected with a solid line have been physically linked to each other. The linkage of $V_\gamma 4.4$ to $V_\gamma 4.3$ was done in this study and the distance is indicated in the figure. The transcriptional orientation of each cluster is indicated by arrows. This genomic organization is consistent with our findings in this study, except that the orientation of $_\gamma 3$ cluster is not known. The shadowed boxes indicate the hybridization probes used: (**a**) $C_\gamma 2$ probe, 1.0-kb *Eco*RI−*Hin*dIII genomic fragment; (**b**) $J_\gamma 1$ probe, 1.2-kb *Ava*I−*Hin*dIII genomic fragment; (**c**) $C_\gamma 1$ cDNA probe, 900-bp *Ava*I−*Eco*RI cDNA clone containing $C_\gamma 1$ coding region and its 3′ untranslated region as described before (Hayday *et al.*, 1985); (**d**) $V_\gamma 4.4$ probe, 200-bp *Hpa*II−*Hpa*II genomic fragment containing part of $V_\gamma 4.4$ coding region.

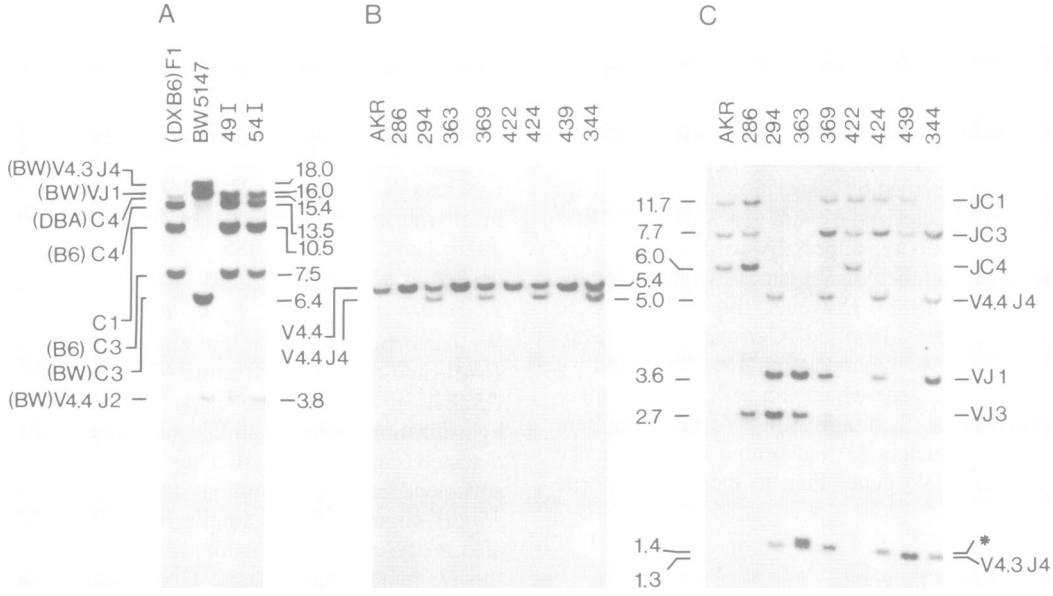
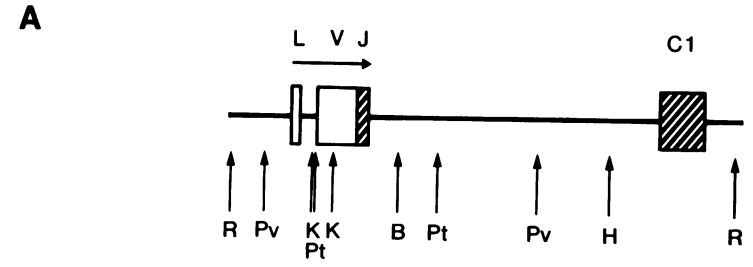


Fig. 2. Southern blot analyses of some hybridoma and AKR thymoma cell lines. **Panel A**, *Eco*RI-digested DNA samples, $C_\gamma 1$ cDNA probe (probes are described in legend to Figure 1). Restriction fragments hybridizing to this probe are indicated on the left side of the panel. **Panel B**, *Hin*dIII-digested DNA samples, $V_\gamma 4.4$ probe. **Panel C**, the same filter as in panel B was washed and re-hybridized with $J_\gamma 1$ probe. This probe detects rearrangements of $J_\gamma 1$, $J_\gamma 3$ and $J_\gamma 4$ as indicated on the right side of the panel, see also lane 1. The star indicates a 1.4-kb rearrangment that is likely to be $V_\gamma 4.1$ or $V_\gamma 4.2$ to $J_\gamma 4$ rearrangement in AKR background. (D × B6)F_1 and AKR indicate DNA samples from (DBA/2 × C57BL/6)F_1 and AKR liver cells. 49I (HK 49I) and 54I (HK 54I) are hybridoma cell lines originating from a fusion of (DBA/2 × C57BL/6)F_1 fetal day 15 thymocytes to BW 5147. Numbers indicate the following thymoma cell lines: 286 D/29 TC, 294 III/16 TC, 363 TC, 369 TC, 422 P1 TC, 424 TC, 439 TC and 344 TC.

cosmid library by Z.Dembić (Dembić *et al.*, 1986)]. This cosmid contains the $V_\gamma 4.3$ gene fused with $J_\gamma 4$ and 20 kb of the 5′ flanking sequence upstream of $V_\gamma 4.3$. Hybridization with the $V_\gamma 4.4$-specific probe showed that this gene is located 7.3 kb 5′ to $V_\gamma 4.3$ (Figure 1).

The rule that V_γ rearrangements take place most frequently within a V−J−C cluster also holds for $V_\gamma 4.4$, i.e. $V_\gamma 4.4$ practically always undergoes rearrangement to $J_\gamma 4$. The only exception we have seen, is the $V_\gamma 4.4$ to $J_\gamma 2$ rearrangement in BW 5147. This rearrangement causes a large deletion that suggests



B

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GACC ATG AAG AAC CCT GGC TCA CAA GCC CTG CTT CCT TTA TAT CTT CCC TGG GAA GCC A   Vγ4.3

GTTCTTATAGCACAAGGC ATG CTG TGG GCT CTG GCC CTA CTT CTA GCT TTC TTG CCG GCT G GT  Vγ4.4/Jγ2
AC CTG GCA GAT GAG -AT -CA CTC CTG AAA -TA G-C A-- T-C TTA -G- C-C TT- A-A T --  Vγ4.3

      ---CACTAAA --- TCA ACC T-- TG- CTT T-T --- --G T-- --G ACC TGT -T- T --  Vγ4.1
  AGTCCAAC-TT--G --- --- CTC CTG AGA TGG T-C ACC TCC -GC TG- C-C TG- -T- T --  Vγ1
               --- --- -T- --- --A --T --G --- --- --- --- C-- --T C-- - --  Vγ8(H)

AAGTACATGGCATAGATACCCATGACATTTCATT[                          ]TATCAGCAGGAAGCCATGCTG  Vγ4.4/Jγ2
G---TGCCACTG-CAGGT---TGTG--G[                                 ]CCGTC--CATT--TG-TTCT  Vγ4.3

----TA--TTTGCTTC-TTTTTCCCTT-CGTTA-TTTT[                     ]GTGTC-T-AGAT--CTGGGAA-A  Vγ4.1
----GG-CATTTCT-GGTG-AGGAGACAG-G-ACTCAT[                       ]CC---TTTCC-TTT-T-T-AA  Vγ1
---AGTGCT--C--C-G-GAGGCTCACAGGTT--ATTTTGTTTCGTTTTGTTTATTTTCT--TTTT-C---GGA-A-CA  Vγ8(H)

ATGAGTTGCTAATCTACCATTCTCTGTTGCTGGTACCACTGCAG GC AGA CAA ACA TCC TCC AAC TTG GAA  Vγ4.4/Jγ2
TCCCC-GC-AGTCA-T-ATGGG-G-T--T--C--TTGCT-----[        ]TT GG- CAT GGG --G --- --G  Vγ4.3
                                          T---[        ]-G GG- A-G AGT CT- AC- TC-  Vγ4.2
-AA-TGATTGGGAAA-T-TC-GATGTC-CT-CC-TG-TT-C---[        ]-T GG- GA- --- TGG A-A TCT  Vγ4.1
---CTG-TT-CT-TGCT---GAGAAC--TTCCC-TTGCT-----[        ]TT GGG CTT GGG C-G C-- --G  Vγ1
TACTAAGAAATGC--CATTACA-T-TG--T--T-C---T----- C- --T --G -A- --T --- --- --- ---  Vγ8(H)

GAA AGA ATA ATG TCA ATC ACC AAG CTA GAG GGG TCC TCT GCT ATA ATG ACT TGT GAT ACT  Vγ4.4/Jγ2
C-- CCT GA- --A --- --T T-- -GA -C- AGA -AT GAG A-- --A CA- --A T-C --- A-A GT-  Vγ4.3
CCT CTG GGG TCA -AT G-- -T- --- AGG A-A --A AAT A-G --- T-T C-C -AA --- C-A -TA  Vγ4.2
C-G GAT CAG C-C --C T-T --- CGA AG- CCA AAC AAG A-G -TG CAC --A -G- --C A-G CTC  Vγ4.1
C-- -CT GA- T-A --G G-- --- -GA GAG ACA -AT GAG AA- -TG CA- --A T-C --- ATA GT-  Vγ1
-GG --- -C- -A- --- G-- --- -G- -C- ACT --- --A --A --- G-- --C --- --- --- CT-  Vγ8(H)

CAC AGA ACA[                ]GGC ACT TAC ATC CAC TGG TAC CGA TTC CAG AAA GGG AGG  Vγ4.4/Jγ2
TT- -TC GA- AGC TTT AGG[    ]A-T GTA AC- --A --- --- --- --G CAG A-A CC- AAC CAA  Vγ4.3
A-A -C- -GT GTT CAG AAG CCC -AT G-A --- --A --- --- --- -A- GAG A-- CC- --C CA-  Vγ4.2
TCT G-G GTT CCC CTT CAT[    ]AA- --C ATT G-G --- --- --- -A- C-G A-A G-- --- GA-  Vγ4.1
T-T CTT C-- TAT TTC TCC[    ]AA- --A GCT --A --T --- --- --G CAA A-A -C- AAT CAA  Vγ1
-CT GT- GA- AAT[            ]-C- GTC --- -C- --- --- --- -T- CA- --- G-G --- -A-  Vγ8(H)

GCC CCA GAG CAC CTT CTC TAC TAT AAC TTC GTC AGT TCC ACA ACT GTG GTG GAT TCC AGA  Vγ4.4/Jγ2
-GT TT- --- TTT --A T-A --T GTC CTT GCA AC- CC- A-- CAT -T-[    ]T-C TTA GAT -AG  Vγ4.3
CGT -TC C-A AGA A-G --G -GT AG- TCT -CA AAA GAA A-A -TT GTC TAT -A- A-A GAT TTT  Vγ4.2
C-- -TG AGA -GA A-C T-- --T GGC TCA G-- AAA -C- -A- -A- CAA[            ]GA- -AG  Vγ4.1
CAG TTT --- T-T --A A-A --T GTC GCA ACA AA- TAC AAT CA- CGA[    ]CCC TTA GGA G-G  Vγ1
--- --- C-- -GT --- --G --- --- G-- -C- TA- -AC --- -GG GT- --- T-- --A --A G--  Vγ8(H)

TTC AAT TCA GAG AAA TAT CAT GTT TAT GAA GGC CCG GAC AAG AGG TAT AAA TTT GTG CTT  Vγ4.4/Jγ2
GAG T-C AAG A-A -TG G-G GCA AG- A-A A-T CCT AGT -CT TCT -CA -CG -T- --G ACA A-A  Vγ4.3
AGT G-C GA- AGA T-T G-G GCA AGG ACA TGG CAG AGT --T TT- TCT -CA GTC C-C ACC A-A  Vγ4.2
-C- C-C --C CGC TTG G-A AT- -A- G-G A-G[    ]GAT --T GGT -CC -T- T-C C-G A-A A-C  Vγ4.1
AAG C-C AA- A-A -TT G-A GCA AG- A-A --T TTT AAA AGT TCT -CC -CA -CC --G -AA A-A  Vγ1
A-- -G- CG- --A --G --- --- AC- --- -C- A-- A-A -GG --- --C CT- --- --- A-A --G  Vγ8(H)

                                  GAGCTCCTGTTTTTGTAGAAGCGCTGAACAATGTG T+CA GGC   Jγ2(GL)
CGG AAT GTG GAG GAG TCC GAT TCT GCT CTG TAC TAC TGT GCC TCC TGG GCT GGA CA GGC  Vγ4.4/Jγ2
TAT TCC T-- --- --A GAA --C GAA --- A-C --- --- --- T-- -A- G-C        +       Vγ4.3
-AC C-A --- ACA --A GAG --C A-G -GA ACT --T --- --- --A -G- ---                Vγ4.2
AAC --- --T -TC ACA --G --- GAA --C AC- --- --- --- --- -G- ---                Vγ4.1
AAT T-C T-- A-- A-A GAA --- GAA --C ACC --- --- --- --A GT- --- ATC A-         Vγ1
GAA --- C-A ATT --A CGT --C --- -GG G-C --T --- --- --- A-- --- -A- A-G         Vγ8(H)

ACA TCA TGG GTC AAG ATA TTT GCC AAA GGG ACA AAG CTC GTA GTA ATT CCC CCA G GTAAG  Jγ2(GL)
ACA TCA TGG GTC AAG ATA TTT GCC AAA GGG ACA AAG CTC GTA GTA ATT CCC CCA G GTAAG  Vγ4.4/Jγ2

TAAGCTAAGCTGTTGTGTTGTTATCTTAGAGGGAATAACCATTGAGATTTTTTTCTTGGTTTTAGAAAAA         Jγ2(GL)
TAAGCTAAGCTGTTGTGTTGTTATCTTAGAGGGAATAACCATTGAGATTTTTTTCTTGGTTTTAGAAAAA         Vγ4.4/Jγ2
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Fig. 3. (**A**) Restriction map of the clone 3.8.1 that contains $V_\gamma 4.4$ to $J_\gamma 2$ rearrangement. Restriction enzyme sites are indicated by arrows and the following letters: R, *EcoRI;* Pv, *Pvu*II; Pt, *Pst*I; K, *Kpn*I; B, *Bgl*II, H, *Hin*dIII. C1 indicates the first exon of $C_\gamma 2$ genes. (**B**) The comparison of $V_\gamma 4.4$ nucleotide sequence to other V_γ sequences. The sequences are identified on the right with the nomenclature used in Figure 1. To optimize the homologies we have introduced gaps. Nucleotides which are identical with $V_\gamma 4.4$ sequence are indicated by −. The sequences for $V_\gamma 4.3$ (Garman *et al.*, 1986; Heilig and Tonegawa, 1986; Traunecker *et al.*, 1986), for $V_\gamma 4.2$ and $V_\gamma 4.1$ (Garman *et al.*, 1986), for $V_\gamma 1$ (Hayday *et al.*, 1985) and for human $V_\gamma 8$ (LeFranc *et al.*, 1986) are previously published. The sequence of $V_\gamma 4.4$ to $J_\gamma 2$ rearrangement and $J_\gamma 2$ germ-line were determined in this study. The germ-line sequence of $J_\gamma 2$ is placed on top of $V_\gamma 4.4-J_\gamma 4$ sequence. Arrows indicate the most probable site of the $V_\gamma 4.4-J_\gamma 2$ junction.

that $_{\gamma}2$ and $_{\gamma}4$ clusters are located at the opposite ends of the chromosomal region containing γ clusters and that they are in the same transcriptional orientation (Figure 1).

The rearrangement frequency of $V_{\gamma}4.4$ in different cell populations

The frequency of $V_{\gamma}4.4$ rearrangements was also estimated in fetal thymocytes by analysing DNA samples from 62 different day 15 fetal thymic hybridomas (mentioned above) whose TCR gene rearrangements are representative of the day 15 fetal thymocyte population as a whole (J.Pelkonen *et al.*, unpublished data). In contrast to the 50% rearrangement frequency in thymomas, $V_{\gamma}4.4$ undergoes rearrangements rarely in fetal thymocytes: only one out of 62 hybridomas had a $V_{\gamma}4.4$. rearrangement, which is comparable to the frequency of $V_{\gamma}4.2$ rearrangement (J.Pelkonen *et al.*, unpublished data; see also Garman *et al.*, 1986). Thus, thymomas may represent a population of thymocytes in which $V_{\gamma}4.4$ is actively used. However, the high number of other V_{γ}, as well as TCR β gene, rearrangements (β rearrangements not shown) in these thymomas rather suggests that they originate from aged thymocytes which have undergone multiple rearrangements; also $V_{\gamma}4.4$ rearrangements simply reflect the high general rearrangement frequency in thymomas. The high number of $V_{\gamma}4.4$ rearrangements relative to other $V_{\gamma}4$ rearrangements (the ratio of $V_{\gamma}4.4$ rearrangements to other $V_{\gamma}4$ rearrangements is 1/18 in fetal thymocytes whereas it is 1/2 in these thymomas) is consistent with the idea that $V_{\gamma}4.4$ can replace already rearranged V genes in the $_{\gamma}4$ cluster by the same mechanism as described for IgH chain genes (Kleinfeld *et al.*, 1986; Reth *et al.*, 1986). The heptamer sequence TACTGTG that is shown to function as a rearrangement acceptor site can be found in three out of four V_{γ} gene sequences (Figure 3; Garman *et al.*, 1986).

V_{γ} 4.4 has high homology to human V_{γ} sequences

Although $V_{\gamma}4.4$ has low homology to murine V_{γ} genes, it has high nucleotide (60–68%) and amino acid (42–48%) sequence homology to all eight human subgroup I V_{γ} genes (four of them are functional; $V_{\gamma}2$, $V_{\gamma}3$, $V_{\gamma}4$ and $V_{\gamma}8$), highest to $V_{\gamma}8$ [for human V_{γ} genes see LeFranc *et al.* (1986)]. $V_{\gamma}4.4$ as well as all other murine V_{γ} genes have very low homology to the fifth functional human V_{γ} gene ($V_{\gamma}9$, subgroup II) which shares <30% amino acid sequence homology with other human V_{γ} genes. The comparison of $V_{\gamma}4.4$. to human V_{γ} genes reveals conserved but also highly variable regions. The differences have a tendency to accumulate into the hypervariable regions, which were first pointed out for human V_{γ} chains by LeFranc *et al.* (1986). The most extreme example is human $V_{\gamma}3$ which has only 12% amino acid sequence homology to $V_{\gamma}4.4$ in hypervariable regions but as high as 60% outside of these regions. The result of this evolutionary comparison is consistent with the idea that the more conserved areas form the framework regions where the negative selection is strong, and the hypervariable regions, which are free to mutate, form part of the combining site analogous to the Ig variable regions. It is, of course, possible that $V_{\gamma}4.4$ is not the only V_{γ} gene conserved in mammalian evolution. It may well be that there are unidentified human V_{γ} genes that are homologous to other murine V_{γ} genes. It is perhaps not a coincidence to find that the mouse V_{γ} gene with the highest degree of similarity to a human counterpart exists within the $_{\gamma}4$ cluster because this cluster resembles most the single human γ cluster. Both clusters contain multiple V_{γ} genes which can differ markedly from each other (Garman *et al.*, 1986; LeFranc *et al.*, 1986) (the other functional murine γ clusters contain only a single V_{γ} gene each). In addition, murine $_{\gamma}4$ and human γ constant region genes code for glycosylated polypeptide chains, unlike the murine $_{\gamma}1$ constant region gene (Hayday *et al.*, 1985; Garman *et al.*, 1986; LeFranc *et al.*, 1986). Interestingly, as recently reported (Lew *et al.*, 1986) most, if not all, mouse TCRII-positive thymocytes use glycosylated $_{\gamma}4$ chains.

Materials and methods

Cells

Hybridoma cell lines were obtained from Dr Werner Haas (Roche Central Research Unit, Basel Switzerland). These hybridomas were generated by fusing a thymoma cell line, BW 5147, to fetal day 15 thymocytes from (DBA/2 × C57BL/6)F1 mice as previously described (Haas *et al.*, 1985). The thymoma cell lines originate from spontaneous AKR thymomas adapted *in vitro* culture by Drs Max Schreier and Peter Krammer.

Recombinant techniques

All the procedures are from published protocols (Maniatis *et al.*, 1982). The genomic phage library from the hybridoma HK49I was constructed into λgt wes vector. The DNA sequencing was carried out using the dideoxy chain termination method (Sanger *et al.*, 1977) by subcloning of fragments into M13mp18 and M13mp19 vectors.

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