Limited diversity of the rearranged T-cell γ gene

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The immunoglobulin-related, T-cell specific γ gene is rearranged in a wide variety of murine T lymphocytes. We detected γ-gene transcripts in all cloned cytotoxic T lymphocytes examined but in only 1 of 11 T-helper cell lines or hybridomas. Although in cytotoxic T cells, the rearranged γ gene seems to have been assembled from the same germ-line variable and joining gene segments, the transcribed gene exhibited distinct sequence diversity near the junction between these segments.

THE populations of B and T lymphocytes that collectively constitute the immune system share many properties. Both populations are made up of many different clones, each distinguished by its ability to react specifically with only one or a few out of a practically limitless number of foreign antigens. The specificity of B cells for particular antigens results from clonally distinctive amino-acid sequences in the variable (V) domains of the heavy and light chains of the surface immunoglobulin molecules that serve as antigen-specific receptors for these cells. Thus, each clone of B cells normally produces two distinctive *V* domain sequences, one for its immunoglobulin heavy chains and the other for its light chains. T cells, like B cells, have surface antigen-specific receptor molecules also made up of two polypeptide chains[1-3]. These T-cell receptor chains, α and β, are also clonally diversified by virtue of variable amino acid sequences in the N-terminal domain of each chain[4-13]. However, analysing a clone of cytotoxic T lymphocytes (CTLs) in which the genes for the α- and β-subunits are expressed, we found that there is also expressed a similar third gene, represented by cDNA clone pHDS4/203 (refs 10 and 11), hereafter called the γ gene.

This gene has many properties in common with α and β genes which include: (1) assembly from gene segments resembling the gene segments for immunoglobulin *V*, joining (*J*) and constant (*C*) regions; (2) rearrangement and expression of these gene segments in T cells and not in B cells; (3) low but distinct sequence homology to immunoglobulin V, J and C regions; (4) other sequences reminiscent of the transmembrane and intracytoplasmic regions of integral membrane proteins; (5) a cysteine residue at the position expected for a disulphide bond linking two subunits of a dimeric membrane protein molecule. The function of the γ gene is unknown, but because of its shared properties with the α and β genes and the intriguing finding of three, rather than two, expressed *V*-region gene segments in a single clone of T cells, we seek here to learn more about its properties. We show that the gene is assembled in diverse T cells from predominantly the same *V* and *J* gene segments but that it is nonetheless diversified due to sequence variations in the region of the junction of its rearranged *V* and *J* gene segments; although the rearranged γ gene is transcribed in all the cloned CTLs examined, it seems to be transcribed only in infrequent T-helper cell lines and hybridomas. This suggests that the γ-gene product participates in determining the specificity of CTLs.

Rearrangements in T-cell lines

The germ-line configuration of the γ gene is rearranged in CTL clone 2C but not in myeloma cell lines[10]. To determine if rearrangement occurs in other types of T cells, we examined DNA from various T-cell lines using separate probes for the *V* and *C* regions of the γ gene. We also used a full-length (*V*+*C*) β-gene probe (pHDS11) to compare the variability of the rearrangements of the β gene with that of the γ gene. Consistent with previous findings[14-16], a unique rearrangement pattern for the β gene was observed in almost all of the T-cell clones examined (Fig. 1*a*). The exceptions were CTL clones 2.1.1 and 1.5.2 which exhibited indistinguishable patterns; these clones are of BALB.B origin and are specific for the same antigen (L^d), but each arose in a different mouse.

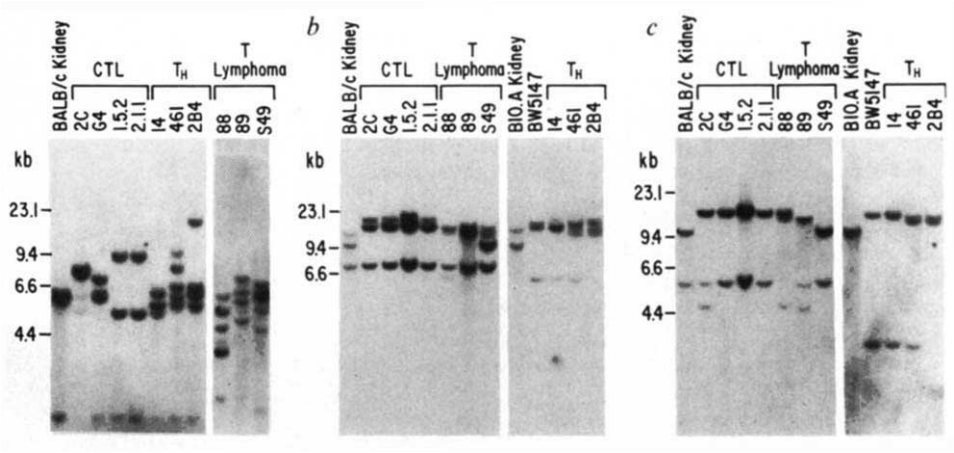

Fig. 1 Southern blot analyses[26] of DNA from various T-cell lines. DNA was isolated from four alloreactive BALB.B CTL clones[27] (2C, 2.1.1 and 1.5.2, anti-L^d; G4, anti-D^d), three T-helper (T_H) hybridomas derived from a BW5147 × B10.A spleen cell fusion[28] (14; anti-GAT; 461 anti-dinitrophenyl ovalbumin; 2B4 anti-pig cytochrome *c*) and three BALB/c T-cell lymphomas[29] (88,89 and S49). Blots were hybridized with the following ^{32}P-labelled, nick-translated probes (ref. 10): *a*, total insert of cDNA clone pHDS11 (cDNA clones were derived from the cDNA library of CTL clone 2C), corresponding to *V*+*C* regions of the β-chain gene; *b*, an *Ava*I fragment containing 840 bp at the 3′ end of the pHDS4 insert, corresponding to the *C* region of the γ gene; *c*, an *Ava*I fragment containing 450 bp at the 5′ end of the pHDS203 insert, corresponding to the *V* region of the γ gene.
Methods. DNA was digested with *Pvu*II (*a*) or *Eco*RI (*b*, *c*), electrophoresed through 0.8% agarose gels and blotted onto nitrocellulose. Blots were hybridized at 42 °C in 50% formamide and 5 × SSC and filters were washed at 65 °C in 0.2 × SSC.

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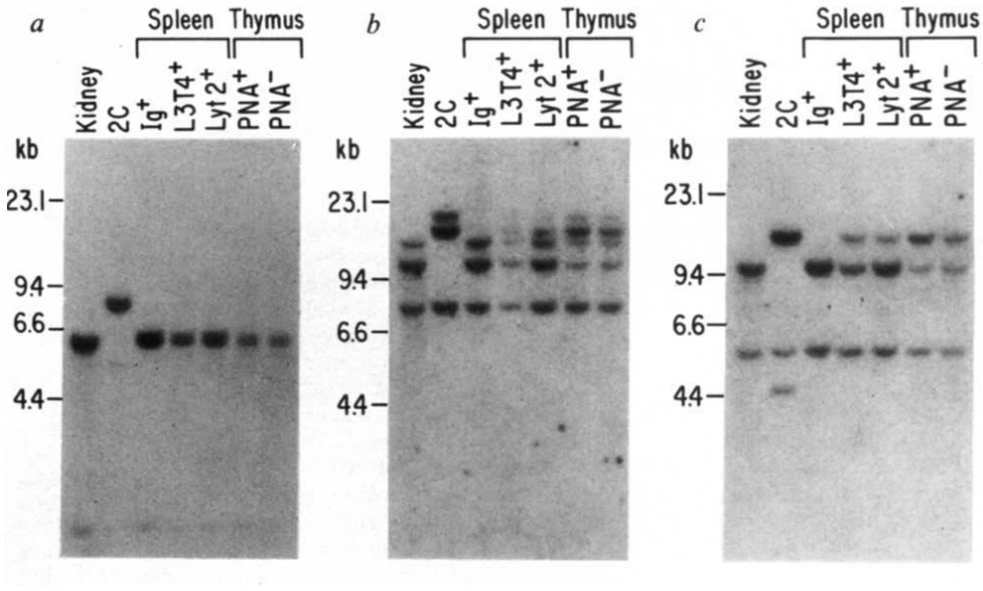

Fig. 2 Southern blot analyses of DNA from spleen and thymus subpopulations. DNA from subpopulations of BALB/c splenic lymphocytes and thymocytes were examined by hybridization with the following probes: *a*, $V_\beta + C_\beta$; *b*, C_γ; *c*, V_γ.

Methods: Surface immunoglobulin positive cells (sIg^+) were obtained by panning of BALB/c splenic lymphocytes (refs 30, 31) on plates precoated with rabbit anti-mouse immunoglobulin. Non-adherent cells were incubated with rat anti-Lyt-2 monoclonal antibodies (a mixture of monoclonal antibodies 2.43.5, 3.155.2, 3.239.2, 3.168.8, all provided by Dr Frank Fitch; ref. 32) or a rat anti-L3T4 monoclonal antibody (GK1.5, provided by Dr Katherine Wall, ref. 33) for 30 min. After washing, cells were incubated on plates precoated with rabbit anti-rat immunoglobulin and the adherent cells (Lyt 2^+ or $L3T4^+$) were recovered. BALB/c thymocytes were separated into mature and immature subpopulations by agglutination with peanut agglutinin (PNA^- mature; PNA^+ immature)[34]. DNA was extracted from the enriched subpopulations, digested with *Pvu*II (*a*) or *Eco*RI (*b*, *c*), and electrophoresed at approximately equivalent concentrations through 0.8% agarose gels. DNA was blotted onto nitrocellulose and hybridized as described in Fig. 1 legend.

In contrast to the highly diverse rearrangement patterns obtained with the β-gene probe, all CTL clones exhibited the same rearrangement pattern with the γ-gene probe, well illustrated by clone 2C. When *Eco*RI-digested clone 2C DNA was hybridized with the probe for the γ-gene C region, two rearranged bands of 16 and 22 kilobases (kb) and one 7.5-kb germ-line band were observed (Fig. 1*b*). We conclude that the 16-kb band contained the rearranged and expressed γ gene because it was the only C_γ-positive band in clone 2C that also hybridized with the probe for the V region of the γ gene (Fig. 1*c*). The rearranged 16-kb fragment arose from the joining of a J-C segment in the 10.5-kb germ-line fragment with a V segment contained in the 10.8-kb germ-line fragment[17]. As neither the C_γ-positive 10.5 nor the V_γ-positive 10.8-kb germ-line *Eco*RI fragments remain in clone 2C DNA, the V and C (or J-C) copies on the homologous chromosome must also have undergone rearrangement in this CTL line. The 10.5- and 10.8-kb gene segments are also joined non-productively in CTL clone 2C (see cDNA clone pHDS34, below).

The nature of the rearrangement(s) responsible for the disappearance of the C_γ-positive 13.4-kb germ-line band and the appearanace of the 22-kb C_γ-positive V_γ-negative band is unknown, but both copies of the 13.4-kb fragment must also have undergone rearrangement. The V_γ gene probe also detected an additional band (5 kb) in clone 2C DNA absent in kidney DNA and possibly representing yet another rearrangement (Fig. 1*c*).

As shown in Fig. 1*b*, *c*, the rearrangement pattern obtained with the γ-gene probes in the other CTL clones was the same as in clone 2C, suggesting strongly that the same germ-line V and C (or J-C) γ-gene segments are rearranged in CTLs having different specificities. The rearrangement patterns of the T-helper hybridomas varied; T-helper 14 was similar to the parental line BW5147 although T helpers 461 and 2B4 each had a rearranged fragment distinct from that of BW5147. Each of the three BALB/c-derived T lymphomas (88, 89 and S49) also displayed a slightly different rearrangement pattern. The diversity observed with the γ-gene probe was much less than with the β-gene probe (Fig. 1*a*), consistent with the suggestion that only a very limited number of germ-line gene segments are used for γ-gene rearrangements.

Rearrangements in T-cell subpopulations

To determine whether the rearrangements observed are peculiar to cultured T-cells, we analysed DNA from several spleen and thymus subpopulations. Southern blot analysis with the β-gene probe revealed a decrease in the intensity of the unrearranged 6.0-kb fragment in *Pvu*II-digested DNA from the splenic T-cell subpopulations and thymus subpopulations when compared with similarly digested DNA from kidney or B-cell preparations (Fig. 2*a*). As expected from the marked clonal variability in β-gene rearrangements (Fig. 1*a*), however, well-defined rearranged β-gene bands were not detected in these highly polyclonal T-cell populations.

In contrast, hybridization with the C_γ and V_γ probes displayed distinct and consistently rearranged fragments in all T-cell populations (Fig. 2*b*, *c*). In fact, the rearranged fragments in these populations and in all of the CTL lines examined seemed indistinguishable, containing both the rearranged fragment (16 kb) with $V_\gamma + C_\gamma$ sequences and the C_γ-positive/V_γ-negative rearranged 22-kb fragment. Thus, even in the highly polyclonal T-cell populations, the γ-gene rearrangements are highly restricted and primarily involve the joining of a single V_γ to a single C_γ gene segment.

Like the β-chain gene, the γ-gene rearrangement must occur early in T-cell development as it was seen in both mature (PNA^-) and immature (PNA^+) thymocytes. In addition, CTLs and T-helper cells seem to exhibit γ-gene rearrangement as both the Lyt-2^+ and $L3T4^+$ subpopulations contained rearranged bands. It is not clear whether the persistent germ-line bands (C_γ region at 10.5 kb and V_γ region at 10.8 kb) in the heterogeneous T-cell preparations are due to unrearranged γ-gene segments in some T cells, or to the presence of some contaminating B cells, or other non-T cells that do not rearrange this gene.

Transcription

The γ gene is transcribed and gives rise to a 1.5-kb poly$(A)^+$ RNA component in CTL clone 2C (ref. 10). Although this gene is rearranged in the other three cytolytic T-cell clones and presumably in some helper T cells (as T-helper hybridomas and the $L3T4^+$ spleen cell subpopulation seem to exhibit rearrangements), it remained to be determined whether the γ gene is transcribed in all of these cells. Northern blot analyses[18] of poly$(A)^+$ RNA from two B-cell lymphomas, four CTL clones and two T-helper hybridomas were therefore performed with the C_γ probe (Fig. 3*b*) and, for comparison, with the β-gene probe (Fig. 3*a*). The cloned CTL and T-helper hybridomas contained approximately equal levels of transcript (1.1 and 1.3 kb) hybridizing to the β probe. In contrast, γ-hybridizing transcript was detected in four out of four CTLs, but in only one

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of the two T-helper hybridomas and in none of five additional T-helper hybridomas and four L3T4$^+$, Lyt-2$^-$ T-cell lines (data not shown). Thus, transcription of the γ gene was seen consistently in CTLs but only rarely in T-helper cells or hybridomas. As reported previously[10], no transcripts of either the β or γ gene were observed in the two B-cell lymphomas.

V–J junctional variability

To confirm that different CTL clones express the same germ-line *V* and *C* gene segments and also to search for clonal diversity, we isolated and sequenced three different cDNA clones from two different CTL clones. Two of the cDNA clones (pHDS4/203 and pHDS34) were isolated from CTL clone 2C (alloreactive, BALB.B anti-L^d)[10] and the third (DFL12) from a cDNA library of CTL clone DFL12, which arose in a mouse of the DBA/2 strain and is specific for the fluorescein group in the context of the D^d class I molecule[19].

We have shown previously that cDNA clone pHDS4/203 is composed of three regions (*V*, *J* and *C*) homologous with the corresponding regions of genes for immunoglobulins and α- and β-chains[10,11]. As shown in Fig. 4, the nucleotide sequences of the three γ-gene cDNA clones are strikingly similar; indeed, the 5′-untranslated and *V*-region sequences of these clones are identical. The base-pair (bp) differences between 2C and DFL12 in the *C* and 3′-untranslated regions could be due to strain polymorphism (BALB.B versus DBA/2), the use of different *C*-region genes or somatic mutation. The latter possibility seems unlikely because no base-pair differences were found in the *V* region, where somatic mutation should be more common[20]. Clone pHDS34 also contains a deletion of 30 bp (883–912), encoding the short peptide sequence that links the *C* domain with the transmembrane peptide. As this sequence is encoded by its own exon (ref. 17) and the extent of the deletion corresponds exactly to the ends of this exon, the poly(A)$^+$ RNA corresponding to the pHDS34 cDNA clone probably arose either from a deletion of the exon at the DNA level or by skipping the exonic sequence during RNA splicing.

Apart from this deletion, the most striking differences between the γ-gene clones pHDS4/203, pHDS34 and DFL12 are at the boundary between the *V*- and *J*-encoded regions. Here, DFL12 has a deletion of the 9 bp coding for the tripeptide Trp-Met-Arg in pHDS 4/203 and pHDS34 has three consecutive cytidines (corresponding to a codon for Pro) in place of a pentanucleotide ATGAG, which corresponds to the codon for Met and the first two bases of an Arg codon in pHDS4/203. Thus, the polypeptide chain encoded by DFL12 differs substantially from that encoded by pHDS4/203 in the region of the *V–J* junction, whereas pHDS34 is in a wrong reading frame after the *V–J* junction and can specify only a *V*-encoded protein fragment. The junctional differences observed between pHDS4/203 and DFL12 could be attributed to polymorphism between the BALB.B-derived CTL clone 2C and the DBA/2-derived CTL clone DFL12; however, the finding that two cDNA clones (pDHS4/203 and pHDS34) from the same cell line (clone 2C) also differ at the *V–J* junction suggests strongly that such variability is a consequence of either somatic joining events, or the use of different diversity (*D*) or *J* gene segments, or terminal transferase activity[21].

Discussion

The existence on T cells of antigen-specific receptors with immunoglobulin-like α- and β-subunits leads to the expectation that a T cell, like a B cell, should express only two V domains, one for each receptor subunit. However, an extensively studied clone of CTLs, 2C, expresses an additional gene, γ, (identified previously as cDNA clone pHDS4/203; refs 10, 11) that is also immunoglobulin-like; for example, it has ~20% sequence homology with immunoglobulin V and C domains and its 5′ (amino-terminal) region codes for all of the invariant residues found in every heavy-chain V, light-chain V, V_α and V_β domain sequenced to date (J. Novotny, in preparation). To learn about possible functions of the γ gene, we have here evaluated the extent of its variability using *V* and *C*-region hybridization probes and analysing DNA from diverse sources. We find γ-gene rearrangements in a wide variety of T cells (CTLs, T-helper hybridomas and polyclonal T-cell populations from spleen and thymus). The rearrangement patterns were identical in all CTLs, as though primarily the same V_γ gene segment was joined to the same J_γ gene segment.

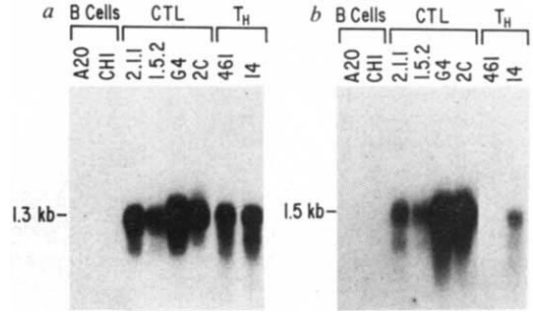

Fig. 3 RNA blot analyses (ref. 18) of poly(A)$^+$ RNA from various B- and T-cell lines. RNA from B-cell lymphomas A20-2J and CH1, CTLs 2C, G4, 1.5.2 and 2.1.1 and T-helper hybridomas 14 and 461 were hybridized with the following probes: *a*, $V_\beta + C_\beta$; *b*, C_γ. **Methods.** Approximately 1.5 μg poly(A)$^+$ RNA was denatured with glyoxal and electrophoresed through 1% agarose gel in 10 mM sodium phosphate buffer, *p*H 6.5. RNA was blotted onto nitrocellulose and hybridized as described in Fig. 1 legend with ^{32}P-labelled nick-translated probe for $V_\beta + C_\beta$ (*a*). The same filter was washed in boiling water at 100 °C for 5 min and hybridized with the probe for C_γ (*b*).

In agreement with this suggestion, three cDNA clones (from two CTLs of different specificities and different mouse strains) had identical 5′ *V*-region sequences and identical *J*-region sequences (Fig. 4), but these clones differed in sequence from each other near the *V*- and *J*-region junction. These clonal differences probably reflect junctional variability due to *V–J* joining events, the use of different *D*-gene segments, or terminal transferase activity.

These γ-gene properties demonstrate its marked resemblance to the genes encoding immunoglobulin λ chains in inbred mice[22]. The V region of each type of λ chain is encoded essentially by a single *V* and a single *J* gene segment and these chains similarly exhibit diversity at the V-J junction. Moreover, amino acid replacements at this junction can have a pronounced effect on ligand binding activity of λ-containing immunoglobulins[23], suggesting similar possibilities for the products of rearranged γ genes. Junction region variations are actually greater among the three rearranged γ-cDNA clones than has so far been observed in λ-chain genes and may in part be due to the involvement of *D* segments.

In speculating about the possible functions of the γ-gene product, we assume that: (1) the diversity at the *V–J* junction regions is clonal in nature and (2) the rearranged γ gene is expressed in CTLs but not ordinarily in T-helper cells. As cytotoxic and helper T cells are restricted usually in their recognition of antigen by different major histocompatibility complex (MHC) elements (CTLs by class I and T helpers by class II molecules, reviewed in ref. 24) it is possible that the product of the γ gene is (or is part of) a second receptor, concerned in CTL clones with restriction by MHC class I molecules. In suggesting this possibility, however, we do not wish to imply that the $\alpha\beta$ heterodimer has no relevance for recognition of MHC-restricting elements.

The hypothesis that T cells have two receptors, one for antigen and the other for MHC molecules, together with the contrasting one-receptor hypothesis, has been debated extensively. Although some experimental data seem to eliminate some versions of the two-receptor hypothesis[25], various other versions have not been eliminated. The properties of the γ gene force us to consider seriously two-receptor models where we visualize

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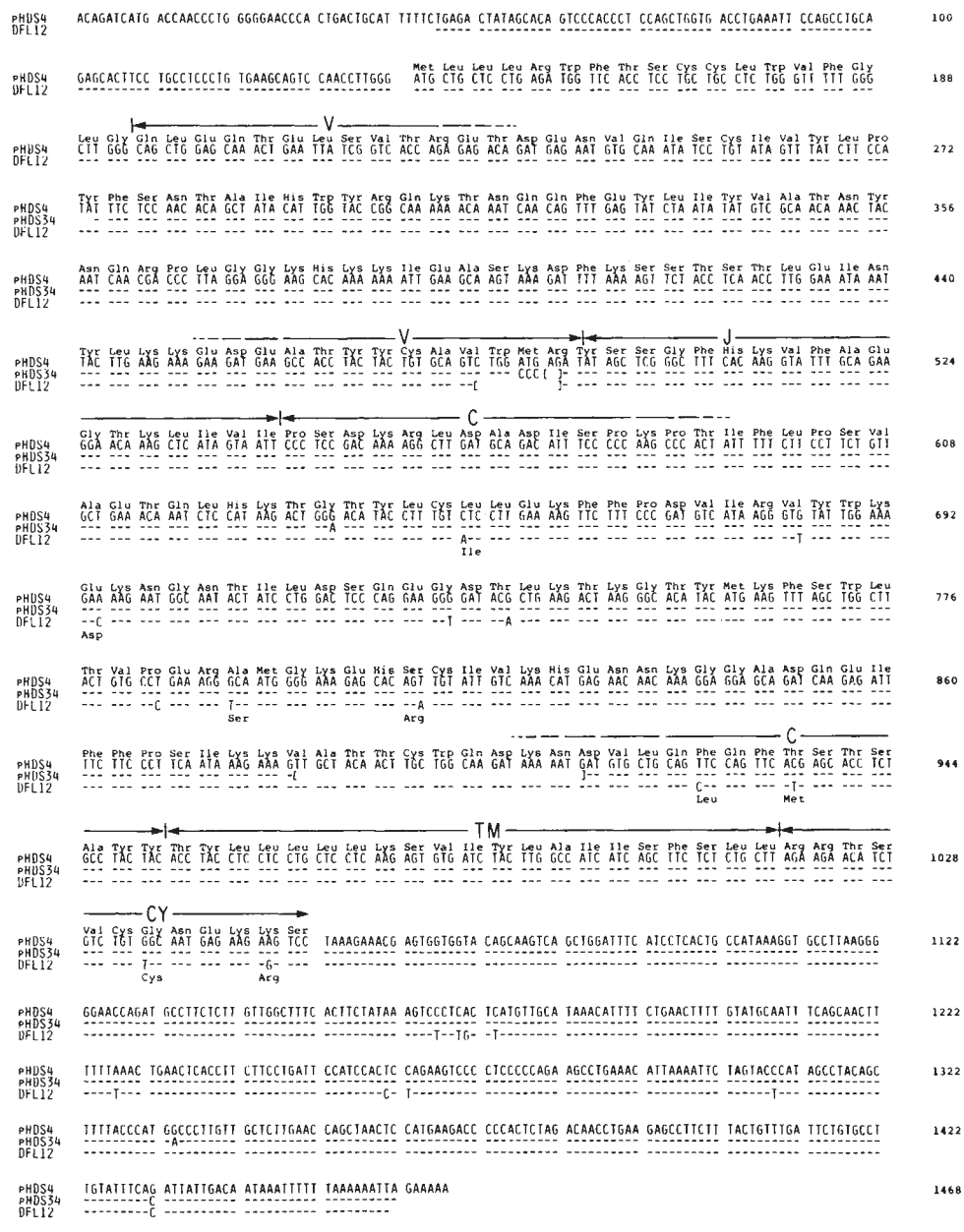

Fig. 4 Comparison of the nucleotide and deduced amino acid sequences of three γ-gene cDNA clones. The two cDNA clones pHDS4 (described previously as pHDS 4/203, ref. 10) and pHDS34 were isolated from the BALB.B CTL clone 2C. The cDNA clone DFL12 was isolated from the DBA/2 CTL clone DFL12 (ref. 19). The predicted amino-acid sequence of cDNA clone pHDS4 is shown above the nucleotide sequence. DFL12 amino-acid residue positions that differ from pHDS4 are shown below the nucleotide sequence. Numbers on the right indicate nucleotide positions. The *V*, *J*, *C*, transmembrane (TM) and cytoplasmic (CY) regions are indicated by the horizontal arrows. Nucleotides in pHDS34 or DFL12 that are identical with those in pHDS4 are indicated by —. Missing nucleotides are indicated by [ ].

Methods. A subtraction library of cDNA clones from 2C mRNA was constructed in the vector pBR322 (ref. 10). A library of cDNA clones from DFL12 mRNA was constructed in the pcD vector system by the method of Okayama and Berg[35]. Nucleotide sequences were determined by the method of Maxam and Gilbert[36] following the restriction map and strategy described previously[10].

that one receptor is the αβ heterodimer recognized already at the protein level and the second receptor includes the γ-gene product. Whether the latter is a homodimer, a heterodimer or a multimer composed of the product of the γ gene and the product(s) of another gene(s), which may or may not be rearranged in T cells, remains unknown.

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1. Allison, J. P., McIntyre, B. W. & Bloch, D. *J. Immun.* **129,** 2293-2300 (1982).
2. Meuer, S. C. *et al. J. exp. Med.* **157,** 705-719 (1983).
3. Haskins, K. *et al. J. exp. Med.* **157,** 1149-1169 (1983).
4. Acuto, O. *et al. Cell* **34,** 717-726 (1983).
5. Kappler, J. *et al. Cell* **34,** 727-737 (1983).
6. McIntyre, B. W. & Allison, J. P. *Cell* **34,** 739-746 (1983).
7. Yanagi, Y. *et al. Nature* **308,** 145-149 (1984).
8. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308,** 153-158 (1984).
9. Acuto, O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 3851-3855 (1984).
10. Saito, H. *et al. Nature* **309,** 757-762 (1984).
11. Saito, H. *et al. Nature* **312,** 36-40 (1984).
12. Chien, Y-H. *et al. Nature* **312,** 31-35 (1984).
13. Hannum, T. H., Kappler, J. W., Trowbridge, I. S., Marrack, P. & Freed, J. H. *Nature* **312,** 65-67 (1984).
14. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308,** 149-153 (1984).
15. Toyonaga, B., Yanagi, Y., Suciu-Foca, N., Minden, M. & Mak, T. W. *Nature* **311,** 385-287 (1984).
16. Hedrick, S. M. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Hayday, A. C. *et al. Cell* (in the press).
18. Thomas, P. S. *Meth. Enzym.* **100,** 255-266 (1983).
19. von Boehmer, H. & Haas, W. *Immun. Rev.* **54,** 27-56 (1981).
20. Tonegawa, S. *Nature* **302,** 575-581 (1983).
21. Alt, F. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4118-4122 (1982).
22. Reilly, E. B., Blomberg, B., Imanishi-Kari, T., Tonegawa, S. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81,** 2484-2488 (1984).
23. Azuma, T., Igras, V., Reilly, E. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81,** 6139-6143 (1984).
24. Zinkernagel, R. M. & Doherty, P. C. *Adv. Immun.* **27,** 52-177 (1980).
25. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. *J. exp. Med.* **153,** 1198-1214 (1981).
26. Southern, E. *J. molec. Biol.* **98,** 503-517 (1975).
27. Kranz, D. M., Sherman, D. H., Sitkovsky, M. V., Pasternack, M. S. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81,** 573-577 (1984).
28. Samuelson, L. E., Germain, R. N. & Schwartz, R. H. *Proc. natn. Acad. Sci. U.S.A.* **80,** 6872-6976 (1983).
29. Harris, A. W. *Expl Cell Res.* **60,** 341-353 (1970).
30. Wysocki, L. J. & Sato, V. L. *Proc. natn. Acad. Sci. U.S.A.* **75,** 2844-2848 (1978).
31. Mage, M. G., McHugh, L. L. & Rothstein, T. L. *J. immun. Meth.* **15,** 47-56 (1977).
32. Sarmineto, M., Glasebrook, A. L. & Fitch, F. W. *J. Immun.* **125,** 2665-2672 (1980).
33. Dialynas, D. P. *et al. J. Immun.* **131,** 2445-2451 (1983).
34. Reisener, Y., Linker-Issracli, M. & Sharon, N. *Cell. Immun.* **25,** 129-134 (1976).
35. Okayama, H. & Berg, P. *Molec. cell Biol.* **2,** 161-170 (1982).
36. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499-560 (1980).

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