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Immunology

T-cell γ gene is allelically but not isotypically excluded and is not required in known functional T-cell subsets

(genomic sequencing/T-cell antigen receptor/gene rearrangement)

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ABSTRACT **The T-cell γ genes, structurally related to immunoglobulin genes and the T-cell antigen-receptor α- and β-chain genes, undergo somatic rearrangement in T-lineage cells. However, the role of the T-cell γ genes has not yet been determined. To determine the potential for γ gene expression in a set of well-characterized, cloned T-cell lines, we cloned all of the rearranged γ genes from each cell line. The genes were sequenced to determine if the junction of the variable and joining regions maintained the proper translational reading frame. We then attempted to correlate the presence of an in-frame γ gene with a T-cell subset. We were unable to establish such a correlation. We found evidence, however, that allelic exclusion influences the rearrangement of the γ gene. This is consistent with the idea that the γ gene product participates in establishing a clonally diverse population of T cells recognizing a polymorphic ligand. Isotypic exclusion does not apply to the γ gene, however, suggesting different roles for the different γ gene isotypes.**

Since the discovery of the T-cell γ gene in the murine system (1, 2), a wealth of information has accumulated about both the murine and the human T-cell γ genes, but it has been difficult to ascribe a function to the γ gene product. Because of structural similarities (1–6) to the genes encoding immunoglobulin and the α (7–12) and β (13–20) subunits of the T-cell antigen receptor, the γ gene has been presumed to encode a molecule involved in recognizing a polymorphic ligand important to T-cell activity. However, recognition of antigen associated with major histocompatibility complex (MHC)-encoded molecules appears to be the function of the T-cell αβ heterodimer (21, 22). Several results have directed attention towards the idea that the γ gene serves some function in the immature thymocyte. Studies of the levels of γ mRNA throughout development (23, 24) revealed that expression is greatest in the developing fetal thymus and in the $CD8^-$, $CD4^-$ subpopulation of adult thymocytes; both populations of cells are enriched in immature T cells (25). Mature peripheral T cells of adult mice, in contrast, contain the lowest level of γ mRNA (23, 24). Although the number of γ gene segments is not large, the greatest variety of γ gene segments has been found to be rearranged and expressed in fetal thymocytes (5, 6). Furthermore, analyses of γ gene rearrangement and transcription indicated that expression of the γ gene is unnecessary in at least some functional T cells (2, 26, 28). Recent experiments using antibodies directed against the receptor-associated T-cell surface antigen CD3 or against the γ polypeptide have identified γ gene products as one subunit of a heterodimer on the surfaces of immature thymocytes (29–31). A small fraction of adult human peripheral lymphocytes and cell lines derived from them also express the γ gene product (32–36). Thus, although the γ gene may be involved in a developmental event common to all T cells, it has been suggested (32, 36) that those cells expressing the γ gene product represent a distinct T-cell lineage. Resolution of this question has been impeded by the lack of data defining that population of cells in which the γ gene product functions.

All T-cell lines studied contain at least one rearranged γ gene (1, 2, 26–28, 34, 35, 37, 38). We have questioned why the γ gene should be found to be rearranged in all T cells representing the well-characterized T-cell subpopulations if it functions only in an as yet unrecognized subset of T cells. We have also sought to determine whether the γ gene is subject to allelic and isotypic exclusion—the mechanisms that prevent lymphocyte receptors of multiple specificities from being expressed in a single cell (39). We have addressed these questions by studying a collection of cloned T-cell lines with known function and specificity. From each cell line we have cloned all of the rearranged γ genes and determined the sequence at the junction of the variable (*V*) and joining (*J*) gene segments. From this study we hoped to determine whether the capacity to produce a functional γ gene product, as revealed by the presence of a rearranged γ gene in which the *V–J* junction maintains the proper translational reading frame, correlated with a recognized T-cell subset. No such correlation could be established. Evidence is presented that although the allelic-exclusion principle applies to the γ gene, isotypic exclusion does not. Although these data may also support the premise that the γ gene functions in an as yet unrecognized population of T cells, alternative interpretations will be discussed below.

MATERIALS AND METHODS

T-Cell Lines. The cell lines used in this study are described in Table 1. They were grown under the conditions indicated in their original reports (see Table 1).

Southern Blot Analysis. Analysis was performed as described (6).

Genomic Libraries. DNA was prepared from each cell line and digested to completion with *Eco*RI. Between 50 and 100 μg of digested DNA was loaded onto a 0.75% low-melting-point agarose gel prepared in TBE (0.05 M Tris borate/1 mM EDTA, pH 8.2). Electrophoresis was carried out at 4°C and 35 V for ≈60 hr, after which the region of the gel containing DNA fragments between 12 and 20 kilobases long was cut out. The agarose slices containing DNA were melted at 65°C, cooled to 42°C, and extracted with TE-equilibrated phenol also at 42°C. After centrifugation, the aqueous phase was extracted once more with phenol, then twice with phenol/

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Abbreviations: *V*, variable-region gene; *J*, joining-region gene; *C*, constant-region gene; T_h cell, helper T lymphocyte; T_c cell, cytolytic T lymphocyte; T_s cell, suppressor T lymphocyte; MHC, major histocompatibility complex.

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Table 1. Description of cell lines and summary of analysis of γ gene rearrangement

Cell line	Description*				γ gene rearrangement†									
	Type	Specificity	Phenotype	Ref.	$V_2J_2C_2$		$V_4J_1C_1$		$V_1J_4C_4$		V_5		V_6	
3D10	T_s	KLH	$CD5^-8^-$	40	++	−	++	X	d	−	D	D	D	D
A15.1.17	T_c	Allo/class II	$CD4^+8^-$	41	++	+	+	−	−	−	D	−	D	−
20.2	T_h	Allo/class II	$CD4^+8^-$	2	−	−	+	−	−	−	D	−	D	−
OI6	T_h	HY/class II	$CD4^+8^-$	‡	+	+	−	−	−	−	−	−	−	−
OH2	T_c	HY/class I	$CD4^-8^+$	‡	++	+	+	−	−	−	D	−	D	−
OH8	T_c	HY/class I	$CD4^-8^+$	‡	+	+	+	X	−	−	D	D	D	D
4K3	T_c	Allo/class I	$CD4^-8^+$	27	+	+	+	+	−	−	D	D	D	D
2C	T_c	Allo/class I	$CD4^-8^+$	1	++	+	+	+	−	−	D	D	D	D

*From the indicated reference or source. The one T-suppressor (T_s) cell line studied (3D10) is specific for keyhole limpet hemocyanin (KLH). The specificities of the cytolytic T (T_c) and T-helper (T_h) cell lines are given as antigen/MHC product.

†Determined by Southern blot analysis (Fig. 2 and ref. 6). Minus sign (−) indicates unrearranged V gene allele; +, rearranged; ++, functionally rearranged; D, deletion of gene from chromosome; X, absence of rearranged gene, presumably by chromosomal deletion (see text); d, presumed local deletion not resulting in V_1J_4 juxtaposition (see text).

‡Characterized by, and gifts of, O. Kanagawa (Eli Lilly Research Laboratories, La Jolla, CA).

$CHCl_3$ (1:1, vol/vol) and twice with $CHCl_3$/isoamyl alcohol (24:1). The DNA was precipitated with ethanol, treated with calf intestinal alkaline phosphatase, and ligated with *Eco*RI-digested λ phage EMBL4 arms prepared and used as described (42).

DNA Sequence Determination. DNA was prepared from 200-ml lysates of purified phage clones containing γ gene sequences as described (43). After complete digestion with *Rsa* I (with one exception, see legend to Fig. 3) ≈15 μg of DNA was subjected to the standard base-specific chemical cleavage reactions of Maxam and Gilbert (44). Standard 8% acrylamide sequencing gels were run and the DNA was transferred to GeneScreen membrane (New England Nuclear) and hybridized as described (45, 46). The probe, indicated in the legend to Fig. 3, was radiolabeled as described by Reilly *et al.* (47). The filters were washed three times in 0.3 M NaCl/0.03 M sodium citrate, pH 7/0.1% $NaDodSO_4$ warmed to 65°C and three times in 0.15 M NaCl/0.015 M sodium citrate, pH 7/0.1% $NaDodSO_4$ warmed to 65°C. Sequences were read from films exposed overnight to the filters. The $V_1J_4C_4$ clone was sequenced by conventional Maxam and Gilbert methods (44).

RESULTS

Fig. 1 depicts the various γ gene segments in their presumed germ-line configuration. Only certain rearrangements seem to occur. As indicated by arrows these are $V_1J_4C_4$, $V_2J_2C_2$, $V_4J_1C_1$, $V_5J_1C_1$, and $V_6J_1C_1$ (1, 3–6). The rearrangement of V_3 to J_3C_3 can occur, but as C_3 is defective the rearranged gene is nonfunctional (3). In order to determine the pattern of γ gene rearrangement in the cell lines described in Table 1, Southern blot analyses were performed. Fig. 2 *a* and *b* display the rearrangement patterns seen using the V_6 and C_4 probes, respectively; the results with probes for the other genes have been reported (6). The results of the Southern blot analyses are tabulated in Table 1. With this information we were able to isolate, from genomic DNA libraries, clones representing each of the rearranged γ genes of each cell line. We then determined the nucleotide sequence spanning the junction of the *V* and *J* regions of a number of clones from each library until unique sequences were obtained for each rearranged gene (Fig. 3). These sequences were then evaluated for the maintenance of the reading frame through the *V–J* region that would permit translation of the complete γ gene. Those genes

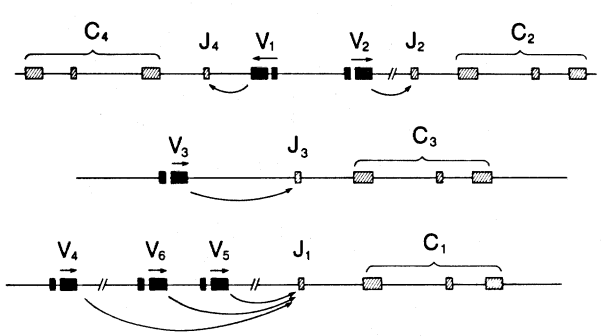

FIG. 1. Organization of the γ genes. The position of the three gene clusters with respect to one another has not been determined. The straight arrows above the *V* regions indicate direction of transcription. The curved arrows below the lines indicate the possible *V–J* rearrangements. C_1–C_4 are constant-region genes.

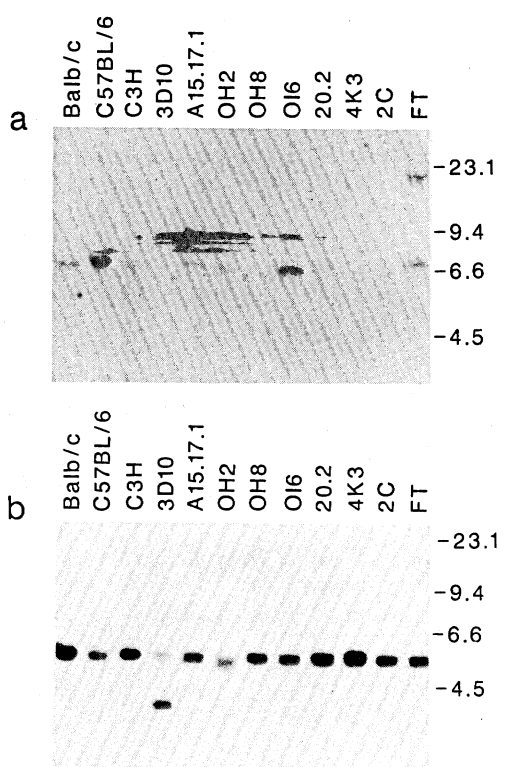

FIG. 2. Southern blot analyses of T-cell lines included in this study. (Line A15.1.17 is mistakenly labeled as A15.17.1.) (*a*) *Eco*RI-digested DNA hybridized with a probe detecting V_6. (*b*) *Kpn* I-digested DNA hybridized with a V_4 probe. Results of these blots and those described in ref. 6 are tabulated in Table 1. Markers at right indicate the positions and sizes (kilobase pairs) of the *Hin*dIII fragments of phage λ DNA used as molecular size markers.

demonstrating this capacity are referred to as functionally rearranged and are noted in Fig. 3.

Because the goal of our study was to determine if a correlation exists between T-cell subsets and the potential for γ gene expression, we chose cells representing a cross-section of the T-cell subsets. Two T_h cell lines were studied, OI6 and 20-2, and were found to contain no functionally rearranged γ genes. These cells both express CD4, but not CD8, and both are specific for class II MHC molecules. Line 20-2 is an alloreactive cell line, whereas OI6 is specific for the male-specific antigen HY. Another of the cell lines we studied, A15.1.17, shares many of these characteristics—i.e., allospecificity, class II specificity, and CD4 expression—but is a T_c cell line. It contains a single, functionally rearranged $V_2J_2C_2$ gene. In A15.1.17 the other $V_2J_2C_2$ gene is not functionally rearranged, and the one $V_4J_1C_1$ rearranged gene is also nonfunctional.

Four additional T_c cell lines were studied, all class I-specific and $CD8^+$, $CD4^-$. Two of these (2C and 4K3) are allospecific and two (OH2 and OH8) recognize the HY antigen. Cell lines 2C and OH2 each contain a single functional $V_2J_2C_2$ rearrangement. As in A15.1.17, all other rearranged genes in these two cell lines are nonfunctional. In cell lines 4K3 and OH8, however, no functionally rearranged γ genes are present. In 4K3 four unique nonfunctional sequences were found. In OH8 all $V_4J_1C_1$ clones contained the same sequence, which is nonfunctional; possible explanations of this will be discussed below. A single T_s cell line, 3D10, was available for our study and it was found to contain two functionally rearranged genes: one is a $V_2J_2C_2$ rearrangement, the other a $V_4J_1C_1$ rearrangement. In this cell an aberrant rearrangement appears to have occurred at one of the $V_1J_4C_4$ loci. We presume that a deletion has resulted in the shortening of the distance between V_1 and C_4, although this has not resulted in union of V_1 and J_4. Whatever the mechanism of this deletion, it did not involve the hepatamer/nonamer nucleotide sequences recognized as being involved in lymphocyte receptor gene rearrangement (48–51). As was the case with OH8, only one $V_4J_1C_1$ sequence was obtained from 3D10. The Southern blot analyses of OH8 and 3D10 (see Table 1 and ref. 6) indicated that no V_4 or C_1 genes remain in the germ-line configuration. Because we sequenced five $V_4J_1C_1$ clones from 3D10 and ten from OH8, we believe that only one $V_4J_1C_1$ gene remains in these cell lines. As all γ gene segments are located on chromosome 13 (52) and each cell contains two V_2 and two C_2 gene segments, we conclude that at least the $V_4J_1C_1$-containing region of one chromosome 13 has been deleted in both 3D10 and OH8.

DISCUSSION

Functional γ Gene Is Unnecessary in Mature T Cells. It is apparent from our study that none of the recognized T-cell subsets can be shown to consistently contain cells in which the γ gene is functionally rearranged. While this may lend credence to the hypothesis that γ gene product is necessary

germline V_4 --- TCC TAC GGC T . . . .

germline V_2 --- GTC TGG ATG AG . . . .

Cell				In Frame ?
3D10	V_4 --- TCC TAC GGC T	**T**	T AGC TCA --- J_1	yes
	V_2 --- GTC TGG ATG A		AT AGC TCA --- J_2	yes
A15-17-1	V_4 --- TCC TAC GGC T	CC AT	AT AGC TCA --- J_1	no
	V_2 --- GTC TGG ATG AG	**C GAA**	AGC TCG --- J2	yes
	V_2 --- GTC TCG AT		AGC TCG --- J_2	no
OH2	V_4 --- TCC TAC GGC T	AA TAT G	GC TCA --- J_1	no
	V_2 --- GTC TGG	TGA	AGC TCG --- J_2	no
	V_2 --- GTC TG	**C CAA T**	AT AGC TCG --- J_2	yes
OH8	V_4 --- TCC TAC GGC T	AA T	AT AGC TCA --- J_1	no
	V_2 --- GTC TGG A	GG GT	AT AGC TCG --- J_2	no
	V_2 --- GTC TGG ATG	TC	AGC TCG --- J_2	no
OI6	V_2 --- GTC TGG AT	GGT TCA T	AT AGC TCG --- J_2	no
	V_2 --- GTC TGG	TAA TT	T AGC TCG --- J_2	no
20.2	V_4 --- TCC TAC GGC T	CC AT	AT AGC TCG --- J_1	no
4K3	V_4 --- TCC TAC GGC T	AA CGT AC	AGC TCA --- J_1	no
	V_4 --- TCC TAC GGC T		AGC TCA --- J_1	no
	V_2 --- GTC TGG ATG AG	GGT	AT AGC TCG --- J_2	no
	V_2 --- GTC TGG ATG AG		AGC TCG --- J_2	no
2C	V_4 --- TCC TA	T TAG AT	AT AGC TCA --- J_1	no
	V_4 --- TCC TAC GGC	GC	AT AGC TCA --- J_1	no
	V_2 --- GTC TGG ATG AG	AT	AT AGC TCG --- J_2	yes
	V_2 --- GTC TGG	CCC AT	AT AGC TCG --- J_2	no

germline J_2^1 --- AT AGC TC$_G^A$ GG$_T^C$ TTT . . . .

FIG. 3. Sequences of the *V–J* junctions of rearranged γ genes. The sequence of each rearranged γ gene of each cell line is indicated. (Line A15.1.17 is mistakenly labeled as A15-17-1.) Only the sequence flanking the junction is given. The germ-line sequences of V_2 and V_4 are at the top; those of J_1 and J_2 are at the bottom. V_2 sequence is from ref. 3; the precise 3′ end of the V_4 coding region is not known. Functional junctions are indicated in bold type. The upper V_4 sequence from cell line 4K3 was sequenced from a *Cla* I site in the V_4 region (see ref. 6) because of the *Rsa* I site created at the *V–J* junction. The probe used to determine these sequences was a synthetic tetracontamer corresponding to the 34 nucleotides at the 3′ end of the *J* region and 6 noncoding nucleotides (see ref. 3). Its sequence, 5′ to 3′, is GCA GAA GGA ACT AAG CTC ATA GTA ATT CCC TCC G GTAAGT.

only to members of a novel subset of T cells (32, 36), at least two other explanations can be offered. Additional members of the γ gene family may exist that have not yet been discovered and hence are not included in our survey. Initially, only a single combination of γ gene segments, $V_2J_2C_2$, was shown to be expressed (1, 2), although two other V gene segments, V_1 and V_3, and two C segments, C_1 and C_3, were recognized and cloned by virtue of their cross-hybridization to the $V_2J_2C_2$ probe (3). Subsequently, the γ gene repertoire was found to be larger, when the V_4, V_5, and V_6 gene segments were found by virtue of their rearrangement to C_1 (5, 6, 28, 37). An additional C region, C_4, was detected (4) because of its rearrangement to V_1. Although an extensive search has not been made, preliminary results of Southern analyses performed at low stringency suggest that other γ gene segments may in fact exist (unpublished data). An alternative explanation for the absence of functional γ genes in some mature-T-cell lines is that secondary rearrangements have occurred. If the γ gene is not required beyond a certain developmental stage, a functionally rearranged γ gene could be displaced by subsequent rearrangement of another variable gene segment, resulting in a nonfunctional γ gene. Although evidence for such events has not been found for the γ gene, neither has it been sought. Approximately 20% of the rearranged γ gene clones obtained from a genomic library made from day-17 fetal thymocytes contained $V_5J_1C_1$ or $V_6J_1C_1$ sequences, and all of those sequenced were functional (ref. 5; K. Sizer and D. Raulet, personal communication). In the functional T-cell clones examined here, and in peripheral T cells (5), however, the V_5 and V_6 gene segments are either deleted or in the germ-line (unrearranged) configuration. Therefore, genes having primary functional rearrangements to V_5 or V_6 could undergo secondary rearrangements to V_4, yielding nonfunctional genes. If such events occur in cells between the time the γ gene functions in its hypothetical role in the thymus and the mature stage represented by the T-cell lines studied here, the dearth of functional $V_4J_1C_1$ rearrangements observed in this study could be explained. There is evidence that secondary rearrangements occur in the immunoglobulin system (53, 54), and the heptamer sequence thought to be required for such rearrangement is present in the V_5 and V_6 gene segments (5).

Allelic Exclusion of the γ Gene. Our study has revealed that although mature functional T cells always include at least one rearranged γ gene, they may exhibit a variety of rearrangement patterns for the γ gene segments, including many segments that remain in the unrearranged, germ-line configuration (e.g., line 20.2). We have found that mature T cells may contain no functional rearrangements, but we have never found a cell exhibiting more than one functional rearrangement at a particular γ gene locus. At least three other groups have reported similar results (27, 28, 38). These results suggest that γ gene rearrangement is subject to pressure to avoid multiple functional rearrangements. This is consistent with the idea that the γ gene is subject to allelic exclusion (39). For both immunoglobulin and T-cell receptors, allelic exclusion restricts functional rearrangement of the appropriate receptor gene to a single allele. This ensures that only receptor of a single specificity will be assembled in any one cell. Allelic exclusion is a rare phenomenon; both alleles of most autosomal genes are generally expressed. Allelic exclusion seems to have evolved under the pressure to ensure monospecificity of lymphocytes and seems to be tightly coupled with clonal diversity of these cells. Hence, the demonstration of allelic exclusion of the γ gene strongly suggests that the putative γδ heterodimeric receptor, wherever expressed, displays clonal diversity. This supports the prediction that the ligand for the γδ receptor is polymorphic.

The γ Gene Is Not Subject to Isotypic Exclusion. By virtue of its multiple C-region genes, the γ gene family can be considered to most closely resemble the λ light chain gene family of murine immunoglobulin (see ref. 55 for review). As in the λ gene family, there are multiple C_γ gene segments each associated with a single J gene segment. In the case of the immunoglobulin light chain, there are four functional loci at which a light chain gene may be productively rearranged: three λ loci and the single κ locus. Presumably to prevent a single B cell from producing receptors of multiple specificity, isotypic exclusion ensures that only one isotype is productive; allelic exclusion ensures that only one allele of the isotype is productive. This follows from the fact that all four light chain loci provide the equivalent function to the B cell. In cell line 3D10 we find functional rearrangements at both the $V_1J_4C_4$ locus and the $V_2J_2C_2$ locus. This is in apparent violation of isotypic exclusion. This may mean that the C_1 and C_2 γ gene segments are not isotypic in the strict sense and that they are involved in different T-cell functions. What these functions are remains unclear.

The absence of isotype exclusion in the γ genes is curious in light of information available about the γ gene product. By use of antibodies directed against either CD3 or the γ gene product, a protein has been detected on the surfaces of immature T cells in both the human (29, 32–36) and the mouse (30, 31). There is also evidence that human peripheral T cells express a product of the γ gene (34–36). The human γ gene family, although organized differently than in mouse, contains two C-region gene segments (56–61). Two distinct forms of human γ polypeptide have been reported (32, 34, 35) and are distinguished by whether or not they are members of a disulfide-linked heterodimer. The nucleotide sequences of the C gene segments (56–61) clearly account for this difference and indicate that the two forms of γ polypeptide are the products of different genes. Differential expression of these genes in the development of the different human T-cell subsets has not been studied. Studies with the mouse indicate that the expression of C_1 and C_2 differ in that the C_1 gene segment (as detected by V_4 probe) is not expressed in peripheral T cells or cloned T-cell lines (5, 6, 23, 24), whereas the C_2 gene segment often is expressed in T-cell lines (1, 2, 6). Using an antibody directed against the murine γ polypeptide, Lew *et al.* (30) determined that only C_1 is expressed by immature thymocytes. The differential expression of the C_1 and C_2 gene segments in mouse and the identification of significantly different forms of the γ protein in humans suggest that, rather than being isotypic, the different γ genes provide different functions.

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1. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **309**, 757–762.
2. Kranz, D. M., Saito, H., Heller, M., Takagaki, Y., Haas, W., Eisen, H. N. & Tonegawa, S. (1985) *Nature (London)* **313**, 752–755.
3. Hayday, A. C., Saito, H., Gillies, S. D., Kranz, D. M., Tanigawa, G., Eisen, H. N. & Tonegawa, S. (1985) *Cell* **40**, 259–269.
4. Iwamoto, A., Rupp, F., Ohashi, P., Walker, C., Pircher, H., Joho, R., Hengartner, H. & Mak, T. W. (1986) *J. Exp. Med.* **163**, 1203–1212.
5. Garman, R. D., Doherty, P. J. & Raulet, D. H. (1986) *Cell* **45**, 733–742.

6. Heilig, J. S. & Tonegawa, S. (1986) *Nature (London)* **322,** 836–840.
7. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **312,** 36–39.
8. Chien, Y.-h., Becker, D. M., Lindsten, T., Okamura, M., Cohen, D. J. & Davis, M. M. (1984) *Nature (London)* **312,** 31–35.
9. Arden, B., Klotz, J. L., Siu, G. & Hood, L. E. (1985) *Nature (London)* **316,** 783–787.
10. Hayday, A. C., Diamond, D. J., Tanigawa, G., Heilig, J. S., Folsom, V., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **316,** 828–832.
11. Winoto, A., Mjolsness, S. & Hood, L. (1985) *Nature (London)* **316,** 832–836.
12. Yoshikai, Y., Clark, S. P., Taylor, S., Sohn, U., Wilson, B. I., Minder, M. D. & Mak, T. W. (1985) *Nature (London)* **316,** 837–840.
13. Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S. P., Aleksander, I. & Mak, T. W. (1984) *Nature (London)* **308,** 145–149.
14. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. (1984) *Nature (London)* **308,** 149–153.
15. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **308,** 153–158.
16. Chien, Y.-h., Gascoigne, N., Kavaler, J., Lee, N. E. & Davis, M. M. (1984) *Nature (London)* **309,** 322–326.
17. Siu, G., Clark, S. P., Yoshikai, Y., Malissen, M., Yanagi, Y., Strauss, E., Mak, T. W. & Hood, L. (1984) *Cell* **37,** 393–401.
18. Mallissen, M., Minard, K., Mjolsness, S., Kronenberg, M., Goveriman, J., Hunkapiller, T., Prystowsky, M. B., Yoshikai, Y., Fitch, F., Mak, T. W. & Hood, L. (1984) *Cell* **37,** 1101–1110.
19. Gascoigne, N., Chien, Y.-h., Becker, D. M., Kavaler, J. & Davis, M. M. (1984) *Nature (London)* **310,** 387–391.
20. Siu, G., Kronenberg, M., Strauss, E., Haars, R., Mak, T. W. & Hood, L. (1984) *Nature (London)* **311,** 344–350.
21. Yagüe, J., White, J., Coleclough, C., Kappler, J., Palmer, E. & Marrack, P. (1985) *Cell* **42,** 81–87.
22. Dembić, Z., Haas, W., Weiss, S., McCubrey, J., Kiefer, H., von Boehmer, H. & Steinmetz, M. (1986) *Nature (London)* **320,** 232–238.
23. Raulet, D. H., Garman, R. D., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **314,** 103–107.
24. Snodgrass, H. R., Dembić, Z., Steinmetz, M. & von Boehmer, H. (1985) *Nature (London)* **315,** 232–233.
25. Fowlkes, B. J., Edison, L., Mathieson, B. J. & Chused, T. M. (1985) *J. Exp. Med.* **162,** 802–822.
26. Heilig, J. S., Glimcher, L. H., Kranz, D. M., Clayton, L. K., Greenstein, J. L., Saito, H., Maxam, A. M., Burakoff, S. J., Eisen, H. N. & Tonegawa, S. (1985) *Nature (London)* **317,** 68–70.
27. Reilly, E. B., Kranz, D. M., Tonegawa, S. & Eisen, H. N. (1986) *Nature (London)* **321,** 878–880.
28. Rupp, F., Frech, G., Hengartner, H., Zinkernagel, R. M. & Joho, R. (1986) *Nature (London)* **321,** 876–878.
29. Bank, I., DePinho, R. A., Brenner, M. B., Cassimeris, J., Alt, F. W. & Chess, L. (1986) *Nature (London)* **322,** 179–181.
30. Lew, A. M., Pardoll, D. M., Maloy, W. L., Fowlkes, B. J., Kruisbeek, A., Cheng, S.-F., Germain, R. N., Bluestone, J. A., Schwartz, R. H. & Coligan, J. E. (1986) *Science* **234,** 1401–1405.
31. Nakanishi, N., Maeda, K., Ito, K., Heller, M. & Tonegawa, S. (1987) *Nature (London)* **325,** 720–723.
32. Brenner, M. B., McLean, J., Dialynas, D. P., Strominger, J. L., Smith, J. A., Owen, F. L., Seidman, J. G., Ip, S., Rosen, F. & Krangel, M. S. (1986) *Nature (London)* **322,** 145–149.
33. Weiss, A., Newton, M. & Crommie, D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6998–7002.
34. Borst, J., Van de Griend, R. J., van Oostveen, J. W., Ang, S.-L., Melief, C. J., Seidman, J. G. & Bolhuis, R. L. H. (1987) *Nature (London)* **325,** 683–688.
35. Brenner, M. B., McLean, J., Scheft, H., Riberdy, J., Ang, S.-L., Seidman, J. G., Devlin, P. & Krangel, M. S. (1987) *Nature (London)* **325,** 689–694.
36. Moingeon, P., Jitsukawa, S., Faure, F., Troalen, F., Triebel, F., Graziani, M., Forestier, F., Bellet, D., Bohuon, C. & Hercend, T. (1987) *Nature (London)* **325,** 723–726.
37. Quetermous, T., Murre, C., Dialynas, D., Duby, A. D., Strominger, J. L., Waldman, T. A. & Seidman, J. G. (1986) *Science* **231,** 252–255.
38. Traunecker, A., Oliveri, F., Allen, N. & Karjalainen, K. (1986) *EMBO J.* **5,** 1589–1593.
39. Pernis, B. G., Chiappino, G., Kelus, A. S. & Gell, P. G. H. (1965) *J. Exp. Med.* **122,** 853–876.
40. Nakauchi, H., Ohno, I., Kim, M., Okumura, K. & Tada, T. (1984) *J. Immunol.* **132,** 88–94.
41. Pierres, A., Schmitt-Verhulst, A. M., Bufesne, M., Golstein, P. & Pierres, M. (1982) *Scand. J. Immunol.* **15,** 619–625.
42. Frischauf, A.-M., Lehrach, H., Poustha, A. & Murray, N. (1983) *J. Exp. Med.* **170,** 827–842.
43. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
44. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
45. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
46. Ephrussi, A., Church, G. M., Tonegawa, S. & Gilbert, W. (1985) *Science* **227,** 134–140.
47. Reilly, E. B., Reilly, R. M., Breyer, R. M., Sauer, R. T. & Eisen, H. N. (1984) *J. Immunol.* **133,** 471–475.
48. Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. (1979) *Nature (London)* **280,** 288–294.
49. Sakano, H., Maki, R., Kurasawa, Y., Roeder, W. & Tonegawa, S. (1980) *Nature (London)* **286,** 676–683.
50. Max, E. E., Seidman, J. G. & Leder, P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3450–3454.
51. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. (1980) *Cell* **19,** 981–992.
52. Kranz, D. M., Saito, H., Disteche, C. M., Swisshelm, K., Pravtcheva, D., Ruddle, F. H., Eisen, H. N. & Tonegawa, S. (1985) *Science* **227,** 941–944.
53. Reth, M., Gehrmann, P., Petrac, E. & Wiese, P. (1986) *Nature (London)* **322,** 840–842.
54. Kleinfeld, R., Hardy, R. R., Tarlinton, D., Dang, J., Herzenberg, L. A. & Weigert, M. (1986) *Nature (London)* **322,** 843–846.
55. Eisen, H. N. & Reilly, E. (1985) *Annu. Rev. Immunol.* **3,** 337–366.
56. Murre, C., Waldmann, R. A., Morton, C. C., Bongiovanni, K. F., Waldmann, T. A., Shows, T. B. & Seidman, J. G. (1985) *Nature (London)* **316,** 549–552.
57. LeFranc, M. P., Forster, A., Baer, R., Stinson, M. A. & Rabbits, T. H. (1987) *Cell* **45,** 237–246.
58. LeFranc, M. P. & Rabbits, T. H. (1986) *Nature (London)* **316,** 464–466.
59. Quertermous, T., Murre, C., Dialynas, D., Duby, A. D., Strominger, J. L., Waldman, T. A. & Seidman, J. G. (1986) *Science* **231,** 252–255.
60. Quertermous, T., Strauss, W., Murre, C., Dialynas, D. P., Strominger, J. L. & Seidman, J. G. (1986) *Nature (London)* **322,** 184–187.
61. Dialynas, D. P., Murre, C., Quertermous, T., Boss, J. M., Leiden, J. M., Seidman, J. G. & Strominger, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2619–2623.