Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1023–1026, February 1989
Immunology

T-cell receptor δ-chain diversity in peripheral lymphocytes

(antigen recognition/thymic development/N regions/polymerase chain reaction)

MICHAEL J. LACY, LESLIE KLIS MCNEIL, MATTHEW E. ROTH, AND DAVID M. KRANZ*

Department of Biochemistry, University of Illinois, 1209 West California Avenue, Urbana, IL 61801

Communicated by Herman N. Eisen, November 7, 1988

ABSTRACT **A small percentage (≈5%) of the cells in the adult thymus expresses a heterodimeric receptor, γδ, that exhibits extensive clonal diversity. The specificity and function of these cells are unclear. Furthermore, it is not known if their role in the immune system is primarily one that operates within the thymus during the selection of the T-cell repertoire or if they function primarily in an antigen-recognition capacity in the peripheral lymphoid system. To examine if $\gamma\delta^+$ T cells in the periphery are as diverse as those in the thymus, we used the polymerase chain reaction to amplify δ-chain transcripts from polyclonal populations of thymic and splenic lymphocytes (the latter were derived from allogeneic mixed lymphocyte cultures). The nucleotide sequences of δ chains from the spleen, like those from the thymus, were all different. Most of the diversity was present in the region between the variable (*V*) and joining (*J*) gene segments and was generated through the use of the two known diversity (*D*) elements, $D_{\delta 1}$ and $D_{\delta 2}$, and by the addition or deletion of bases at the $V_\delta D_{\delta 1}$, $D_{\delta 1} D_{\delta 2}$, and $D_{\delta 2} J_\delta$ junctions. The extensive γδ repertoire among peripheral cells suggests that they have the potential to recognize an array of ligands that could be as diverse as those recognized by $\alpha\beta^+$ cells. The amplification strategy described here can be used to analyze rapidly the diversity exhibited by any of the members of the immunoglobulin-like gene families that undergo rearrangement.**

Most helper and cytotoxic T lymphocytes express an αβ-heterodimeric receptor that recognizes antigen presented by a product of the major histocompatibility complex (MHC) (1–9). The function of these cells in the immune response has been appreciated for some time and thus the discovery several years ago of a gene, γ, that encoded one subunit of another clonally diverse receptor was quite unexpected (10–12). Despite the progress in identifying the T cells that express this surface structure as a heterodimer, γδ (13, 14), the ligand recognized by this receptor and the function of these cells remain unknown.

During the earliest stages of thymic ontogeny, most cells in the thymus express the γδ receptor, although by birth most cells express the αβ receptor (15–17). The adult thymus maintains a low proportion of $\gamma\delta^+$ cells, with limited diversity in the γ chain (18, 19) and more extensive diversity in the δ chain (20). Previous studies have shown that the γδ receptor is also expressed by a population of cells in the periphery (13, 21, 22) and that MHC-alloreactive $\gamma\delta^+$ cells can be generated from the spleens of athymic mice (23). Although δ-chain sequences have recently been obtained from several human cell lines derived from peripheral blood (24), it is not clear if the repertoire and diversity of $\gamma\delta^+$ cells in the periphery are similar to that of the thymus.

In this report, we use the polymerase chain reaction (PCR) (25) to amplify δ-chain transcripts from polyclonal thymocytes and polyclonal alloreactive spleen cells. Amplified transcripts were cloned and the partial nucleotide sequences of 25 δ chains derived from adult thymus and spleen were determined. All 25 of the transcripts differed in the region between the variable (*V*) and joining (*J*) gene segments. The diversity was generated through the use of the two known diversity elements, $D_{\delta 1}$ and $D_{\delta 2}$ (20), and by the addition or deletion of bases at the $V_\delta D_{\delta 1}$, $D_{\delta 1} D_{\delta 2}$, and $D_{\delta 2} J_\delta$ junctions. Twenty-one of the 25 nucleotide sequences are in-frame and thus each could encode a different δ-chain protein. The extensive γδ repertoire among peripheral cells suggests that the primary function of these cells requires the recognition of a diverse array of ligands.

MATERIALS AND METHODS

Polyclonal Cell Lines. Polyclonal T cells from the thymus and the spleen were prepared as follows. Immature thymocyte "double negatives" (DN) were isolated by treatment of thymocytes from 6- to 12-week-old BALB/c or BALB.B mice with anti-CD4 (GK1.5) and anti-CD8 (3.1688 and AD4.5) monoclonal antibodies (26, 27); this was followed by treatment with complement (GIBCO; Cedarlane Laboratories, Hornby, ON, Canada) and removal of dead cells by centrifugation through Ficoll-Paque (Pharmacia). DN cells were further enriched by panning on plates containing affinity-purified anti-rat immunoglobulin. Nonadherent cells were characterized by fluorescence-activated cell sorting to be >80% $CD4^-CD8^-$. Splenic T cells were isolated from alloreactive BALB/c anti-BALB.B mixed lymphocyte cultures (MLCs) using spleens from BALB/c mice that had not received *in vivo* injections (I°) or spleens from BALB/c mice that had received two *in vivo* injections (III°) of the $H\text{-}2^b$ tumor cell EL4. Such alloreactive cells have been shown to express γ-chain transcripts (28). After 5–10 days in culture, nonviable cells were removed by centrifugation through Ficoll-Paque and cells at the interface were washed two times with cold phosphate-buffered saline (pH 7.2) prior to the preparation of RNA.

Oligonucleotides. Synthetic oligonucleotides (Fig. 1) were obtained from the Genetic Engineering Facility at the University of Illinois.

RNA Preparation and cDNA Synthesis. RNA from cell preparations was isolated by the guanidinium isothiocyanate/CsCl method (29). Ten micrograms of total RNA was used for the synthesis of cDNA using the constant region, C_δ, primer (7.5 μM), 1 mM deoxynucleotides, 4 mM sodium pyrophosphate, 50 mM Tris·HCl (pH 8.3), 10 mM $MgCl_2$, 12 units of RNasin, and 50 units of reverse transcriptase for 30 min at 43°C.

PCR. One-half of the cDNA sample was heated to 95°C for 5 min and the PCR was performed in the presence of 2 μM $V_{\delta 4}'$ primer, 67 mM Tris·HCl (pH 8.8), 6.7 mM $MgCl_2$, 16.6

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: C, constant; D, diversity; DN, double-negative $CD4^-CD8^-$; J, joining; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PCR, polymerase chain reaction; V, variable.

*To whom reprint requests should be addressed.

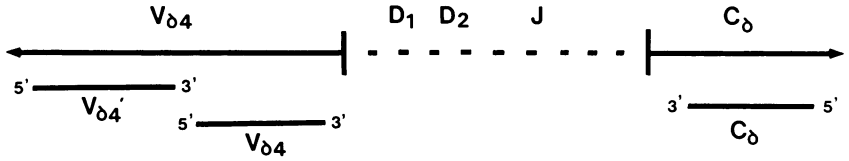

FIG. 1. Oligonucleotides used in the amplification of δ-chain transcripts. A *C* region oligomer (C_δ, 3'-CGGAGGCCGGTTTGGTAGACGT-CAG-5') containing a *Pst* I site (GACGTC) for cloning was used as a primer for cDNA synthesis and subsequent PCR. A *V* region oligomer ($V_{\delta 4}'$, 5'-GCTGGGGGATCCTGCGACG-3') containing a *Bam*HI site (GGATCC) for cloning was used as a primer in the PCR. An oligomer ($V_{\delta 4}$, 5'-TATTTCTGTGCTCTCATG-3') internal to C_δ and $V_{\delta 4}'$ was used for detection of amplified transcripts that were ≈130 bases in length.

mM ammonium sulfate, 10 mM mercaptoethanol, 6.7 μM EDTA, 150 μM deoxynucleotides, 10% dimethyl sulfoxide, and *Thermus aquaticus* polymerase (30) at 92°C for 1 min, 50°C for 1 min, and 65°C for 2 min for each cycle.

DNA Blot Analysis. Five microliters of the amplified material was denatured in 0.25 M NaOH, diluted 10^{-3} to 10^{-1} into 0.375 M NaCl/0.125 M NaOH/0.0375 M sodium citrate (0.15× SSC), and the solution was added to GeneScreen*Plus* (DuPont). Filters were prehybridized in 1% bovine serum albumin/7% $NaDodSO_4$/1.5 mM EDTA/250 mM NaCl/0.52 M sodium phosphate, pH 7.2, at 52–54°C and were hybridized overnight with the ^{32}P-labeled $V_{\delta 4}$ oligomer. Blots were washed in 2× SSC and 0.5% $NaDodSO_4$ at 52–54°C and exposed to film for 1–2 days.

Cloning and Sequencing of Amplified δ-Chain Transcripts. Approximately one-third of the PCR-amplified cDNA was digested with *Pst* I and *Bam*HI and the digested material was ligated to pUC19 (*Pst* I and *Bam*HI digested). The ligation mixture was used to transform RR1 *Escherichia coli* cells, and ampicillin-resistant colonies were hybridized with ^{32}P-labeled $V_{\delta 4}$. Both strands of the plasmid insert from $V_{\delta 4}$-positive colonies were sequenced by the dideoxy method using Sequenase (United States Biochemical). Secondary transformations were performed with some plasmid preparations in which ambiguous sequences were initially obtained. We believe that after amplification and prior to pUC19 cloning, complementary strands that have base pair mismatches may anneal.

RESULTS

Analysis of Polyclonal Populations by the PCR. Four different T-cell preparations were examined in this study. Two were derived from adult thymus and two were derived from adult spleen. The thymus preparations were DN cells isolated from either BALB/c or BALB.B mice. The spleen preparations were BALB/c anti-BALB.B MLC in which the responding BALB/c mice either did not receive an *in vivo* priming (I° MLC) or received two *in vivo* primings (III° MLC). Previous studies have suggested that $\gamma\delta^+$ cells can be elicited in such alloreactive MLC (23, 28).

To analyze δ-chain diversity among these preparations, we took advantage of the PCR technique to amplify δ-chain cDNAs that utilize the $V_{\delta 4}$ gene segment in combination with the δ-chain *C* region (C_δ) (Fig. 1). The $V_{\delta 4}$ gene was first isolated from DN thymocytes (31) and was subsequently shown to be used relatively frequently among adult DN thymocytes (20). $V_{\delta 4}C_\delta$-amplified cDNA from BALB/c DN thymocytes was compared with unamplified cDNA in a dot blot using an internal ^{32}P-labeled $V_{\delta 4}$ probe to determine the efficiency of the PCR under these conditions (Fig. 2*A*). Thirty cycles of amplification yielded detectable $V_{\delta 4}$ transcripts. $V_{\delta 4}$ transcript was also detected in blots of amplified material from the two thymus and the two MLC preparations (Fig. 2*B*). The signal from DN thymocytes was severalfold higher than that from MLC, probably because of the enrichment of $\gamma\delta^+$ cells in DN cells relative to the MLC. An $\alpha\beta^+$ T-cell line and other $\gamma\delta^-$ preparations did not yield ^{32}P-$V_{\delta 4}$ signal after 30 cycles of PCR under the same conditions (data not shown).

We have also used a V_α gene primer (pHDS58, ref. 6) to successfully amplify $V_{\alpha 58}C_\delta$ transcripts from DN thymocytes and III° MLC preparations (data not shown). Peripheral cells can therefore generate diversity in δ chains by using either V_δ or V_α gene segments, as shown for another V_α gene ($V_\alpha TA1$) in DN cells from adult thymus (20). $V_{\alpha 58}C_\delta$ transcripts were not examined further and studies described below were performed with $V_{\delta 4}C_\delta$-amplified material.

δ-Chain Nucleotide Sequences. $V_{\delta 4}C_\delta$ transcripts were examined by cloning amplified material into pUC19 (see Fig. 1 legend), detecting positive colonies (≈1% of the recombinants) with the ^{32}P-labeled $V_{\delta 4}$ probe, and sequencing the inserts. The nucleotide sequences of 10 $V_{\delta 4}C_\delta$ transcripts from BALB.B thymus, 6 $V_{\delta 4}C_\delta$ transcripts from BALB/c thymus, and 9 $V_{\delta 4}C_\delta$ transcripts from III° MLC are shown in Fig. 3. All of the δ chains use the $V_{\delta 4}$ gene segment, 23 use the $J_{\delta 1}$ gene segment, and 2 use the $J_{\delta 2}$ gene segment. Hence, J_δ gene segment use (i.e., $J_{\delta 1} > J_{\delta 2}$) appears to be consistent with that described in a previous report (20). The nucleotide sequences located between V_δ and J_δ vary extensively among these clones. All of the transcripts from the thymus and the spleen differed in this region. As shown for δ-chain cDNAs from adult thymus (20, 32), this diversity is generated by the use of two *D* gene segments ($D_{\delta 1}$ and $D_{\delta 2}$) and by the addition or deletion of bases at the $VD_{\delta 1}$ (N_1 position), $D_{\delta 1}D_{\delta 2}$ (N_2 position), and $D_{\delta 2}J_\delta$ (N_3 position) junctions.

Although all of the transcripts contain nucleotides contributed by the $D_{\delta 2}$ gene segment, there are several that apparently do not use the $D_{\delta 1}$ gene segment. It is possible that other D_δ gene segments may be used in some of these cDNAs since a number of the N-region sequences share three or more bases of a possible sequence (e.g., TCTCCCCT). Interest-

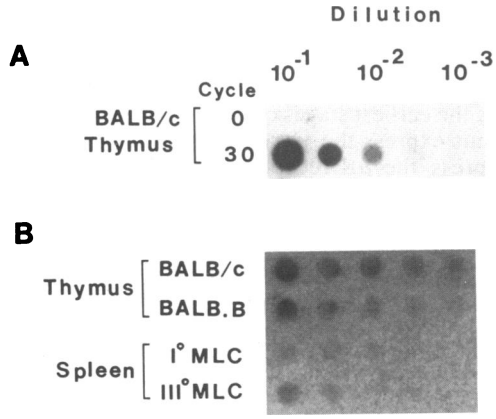

FIG. 2. Dot-blot analysis of amplified δ-chain transcripts. (*A*) RNA from BALB/c DN thymocytes was amplified by the PCR ($V_{\delta 4}' \rightarrow C_\delta$) and dilutions (by a factor of 3.2) of cycle 0 (prior to polymerase addition) and cycle 30 material were blotted and probed with ^{32}P-labeled $V_{\delta 4}$. (*B*) RNAs from BALB/c and BALB.B DN thymocytes and from I° and III° BALB/c anti-BALB.B MLCs were amplified by the PCR ($V_{\delta 4} \rightarrow C_\delta$) and dilutions (by a factor of 3.2) of cycle 30 material were blotted and probed with ^{32}P-$V_{\delta 4}$.

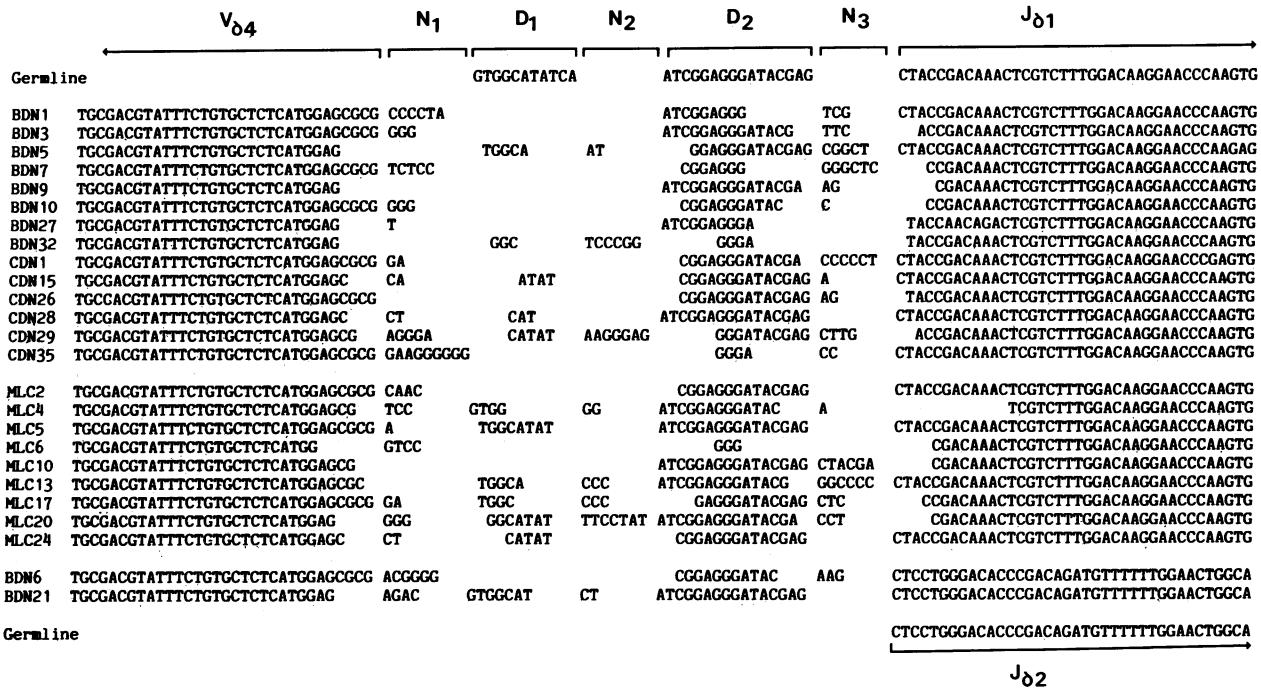

FIG. 3. Nucleotide sequences of $V_{\delta 4}$ transcripts from thymus and MLC. DNA sequences are aligned with published germ-line sequences (32) for $D_{\delta 1}$, $D_{\delta 2}$, $J_{\delta 1}$ (top), and $J_{\delta 2}$ (bottom). N regions (N_1, N_2, and N_3) correspond to nucleotide additions at the *VD*, *DD*, and *DJ* junctions, respectively. Sequences obtained from DN ($CD4^-CD8^-$) thymocytes of BALB.B mice (BDN) and BALB/c mice (CDN) are grouped separately from those obtained from III° MLC of BALB/c anti-BALB.B spleen cells (MLC). Two transcripts (BDN6 and BDN21) that used the $J_{\delta 2}$ gene segment are shown at the bottom.

ingly, the N_1 and N_2 positions of the CDN29 transcript exhibit a 5-base sequence (AGGGA) that is also within the $D_{\delta 2}$ gene segment. Mechanisms that contribute to the diversity at these positions remain to be examined.

δ-Chain Amino Acid Sequences. Twenty-one of the $V_{\delta 4}$ transcripts encode in-frame amino acid sequences, each of which could be expressed in combination with a γ chain as a cell-surface receptor. As expected from the nucleotide sequence data, the amino acid sequences that are contained between the *V* and *J* encoded regions vary extensively among these transcripts (Fig. 4). The diversity consists not only of amino acid residue variability but also of widely varying

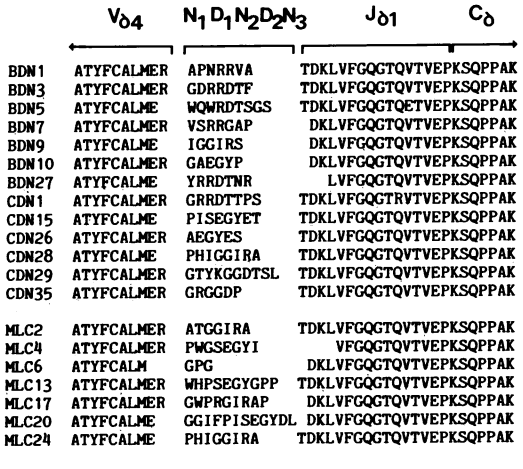

FIG. 4. Partial amino acid sequences of in-frame δ transcripts from thymus and MLC. Amino acid sequences were determined from nucleotide sequences shown in Fig. 3. Only transcripts that have in-frame *V* to *C* coding sequences are shown. BDN21, which has an in-frame sequence with the $J_{\delta 2}$ gene segment, was omitted. Residues encoded by N regions and *D* elements are within brackets ($N_1D_1N_2D_2N_3$) to emphasize the diversity in this region.

peptide lengths in this region. For example, the MLC6 chain is 10 amino acid residues shorter than either the MLC20, MLC13, or CDN29 chain. Since this region of the protein is likely to reside within the T-cell receptor binding site, by analogy with immunoglobulins, the potential diversity in residues that could contact the ligand would be enormous (see ref. 20 for discussion). It is clear from a study with λ light chains (33) that even a single amino acid change at the *V–J* junction can dramatically affect antigen binding by the intact immunoglobulin.

DISCUSSION

It is not known if $\gamma\delta^+$ T cells recognize foreign antigens and if they, like $\alpha\beta^+$ T cells, are restricted by a product of the MHC. Regardless, it is clear that the extensive diversity in the δ chain (this report and ref. 20) may make it difficult to define structural correlates between the use of particular γδ receptors and their recognition of specific antigens or MHC products, if such correlations exist. For example, two strains of mice that differ at the MHC (BALB/c, *H-2*^{d}; BALB.B, *H-2*^{b}) both express a heterogeneous repertoire of $V_{\delta 4}{}^+$ DN thymocytes (Figs. 3 and 4).

The extent of this heterogeneity is perhaps more apparent by our examination of the $V_{\delta 4}$ chains from BALB/c anti-BALB.B alloreactive cells (Figs. 3 and 4). Even these chains, which we initially thought may be more restricted than the entire repertoire of $V_{\delta 4}$ chains from either the spleen or the thymus, exhibit extensive diversity. From inspection of our sequence data alone, it is therefore impossible to determine whether the $\gamma\delta^+$ cells in the periphery may have been previously selected in the thymus. Such selection would presumably yield a peripheral γδ repertoire that is a subset of the thymic γδ repertoire.

In the study presented here, the phenotype of the cells within the MLCs that expressed the δ chains is unknown. Previous studies of cytotoxic (34) and helper (35) T lympho-

cytes showed that in most cases γ transcripts expressed by these cells are out-of-frame. Although in-frame γ transcripts have been obtained from polyclonal MLC preparations (28) and some $\alpha\beta^+$ cell lines (36), a cell that expresses both $\alpha\beta$ and $\gamma\delta$ on the cell surface has not been observed and thus it has been assumed that $\alpha\beta^+$ cells do not express a $\gamma\delta$ receptor. The results presented here suggest that the high proportion of out-of-frame γ transcripts observed in $\alpha\beta^+$ T-cell lines is not likely to hold true for δ-chain transcripts, probably because the δ-chain gene is deleted in most $\alpha\beta^+$ T cells. Since most $\gamma\delta^+$ cells in the periphery are $CD4^-$ and $CD8^-$ (21), we feel that it is likely the δ chains described in this report were derived from DN cells in the MLC.

In summary, the diversity of δ chains from peripheral T cells is extensive. A previous study has shown that peripheral $\gamma\delta^+$ cells that are specific for an allogeneic MHC product can be elicited in athymic BALB/c mice (23). However, it is not known if $\gamma\delta^+$ cells that are reactive with nominal antigen in the context of a MHC product can be elicited. The large repertoire of different $\gamma\delta^+$ cells in the periphery of normal BALB/c mice suggests that the ligands recognized by these cells, like $\alpha\beta^+$ cells, may include a wide range of antigenic structures. The function(s) of these $\gamma\delta^+$ cells compared to the more well-known functions of $\alpha\beta^+$ cells remains an issue of considerable interest.

Finally, the amplification strategy that was used to examine δ-chain diversity in this report has two major advantages over conventional cloning methods. First, the specificity of the primers can be designed to selectively examine any of the known *V–C* combinations of immunoglobulin or T-cell receptor genes, even if the cells to be examined represent a relatively minor subpopulation. Second, the extent of amplification, which we have estimated to be ≈100,000-fold (37) under these conditions, allows the analysis of low-abundance transcripts and small quantities of RNA (e.g., 1 μg of total RNA). Thus, a relatively small number of cells (10^6) can be used for cloning and sequencing analyses. This feature should allow the analysis of some cell types, such as $\gamma\delta^+$ T cells, that have been difficult to clone and expand in culture.

Note Added in Proof. We now routinely use whole thymus, spleen, and lymph node preparations to amplify and sequence δ transcripts. We also note that it has recently been shown that some populations of $\gamma\delta$ cells, such as intestinal epithelial lymphocytes (38) and dendritic epidermal cells (39), may express very restricted δ-chain repertoires.

We thank Julie Auger of the Cell Science Center at the University of Illinois for the flow cytometry analysis and we are grateful to Katy Thorpe for preparation of the manuscript. This work was supported by a grant from the National Institutes of Health (AI24635) and the Searle Scholars Program.

1. Haskins, K., Kubo, R., White, J., Pigeon, M., Kappler, J. & Marrack, P. (1983) *J. Exp. Med.* **157,** 1149–1169.
2. Meuer, S. C., Fitzgerald, K. A., Hussey, R. E., Hodgdon, J. C., Schlossman, S. F. & Reinherz, E. (1983) *J. Exp. Med.* **157,** 705–719.
3. Allison, J. P., McIntyre, B. W. & Bloch, D. (1982) *J. Immunol.* **129,** 2293–2300.
4. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **308,** 153–158.
5. Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S. P., Aleksander, I. & Mak, T. W. (1984) *Nature (London)* **308,** 145–149.
6. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **312,** 36–40.
7. Chien, Y., Becker, D. M., Lindsten, T., Okamura, M., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **312,** 31–35.
8. Dembic, Z., Haas, W., Weiss, S., McCubrey, J., Kiefer, H., von Boehmer, H. & Steinmetz, M. (1986) *Nature (London)* **320,** 232–238.
9. Saito, T., Weiss, A., Miller, J., Norcross, M. A. & Germain, R. N. (1987) *Nature (London)* **325,** 125–130.
10. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **309,** 757–762.
11. Kranz, D. M., Saito, H., Heller, M., Takagaki, Y., Haas, W., Eisen, H. N. & Tonegawa, S. (1985) *Nature (London)* **313,** 752–755.
12. Hayday, A. C., Saito, H., Gillies, S. D., Kranz, D. M., Tanigawa, G., Eisen, H. N. & Tonegawa, S. (1985) *Cell* **40,** 259–269.
13. 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. (1986) *Nature (London)* **322,** 145–149.
14. Bank, I., DePinho, R. A., Brenner, M. B., Cassimeris, J., Alt, F. W. & Chess, L. (1986) *Nature (London)* **322,** 179–181.
15. Raulet, D. H., Garman, R. D., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **314,** 103–107.
16. Pardoll, D. M., Fowlkes, B. J., Bluestone, J. A., Kruisbeek, A., Maloy, W. L., Coligan, J. E. & Swartz, F. H. (1987) *Nature (London)* **326,** 79–81.
17. Bluestone, J. A., Pardoll, D., Sharrow, S. O. & Fowlkes, B. J. (1987) *Nature (London)* **326,** 82–84.
18. Garman, R. D., Doherty, P. J. & Raulet, D. H. (1986) *Cell* **45,** 733–742.
19. Heilig, J. S. & Tonegawa, S. (1986) *Nature (London)* **322,** 836–840.
20. Elliott, J. F., Rock, E. P., Patten, P. A., Davis, M. M. & Chien, Y.-h. (1988) *Nature (London)* **331,** 627–631.
21. Lanier, L. L., Federspiel, N. A., Ruitenberg, J. J., Philips, J. H., Allison, J. P., Littman, D. & Weiss, A. (1987) *J. Exp. Med.* **165,** 1076–1094.
22. 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.
23. Matis, L. A., Cron, R. & Bluestone, J. A. (1987) *Nature (London)* **330,** 262–264.
24. Hata, S., Satyanarayana, K., Devlin, P., Band, H., McLean, J., Strominger, J. L., Brenner, M. B. & Krangel, M. S. (1988) *Science* **240,** 1541–1544.
25. Saiki, R., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230,** 1350–1354.
26. Dialynas, D. P., Wilde, D. B., Marrack, P., Pierres, A., Wall, K. A., Havran, W., Otten, G., Loken, M. R., Pierres, M., Kappler, J. & Fitch, F. W. (1983) *Immunol. Rev.* **75,** 29–56.
27. Raulet, D. H., Gottlieb, P. D. & Bevan, M. J. (1980) *J. Immunol.* **125,** 1136–1143.
28. Jones, B., Mjolsness, S., Janeway, C. & Hayday, A. C. (1986) *Nature (London)* **323,** 635–638.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
30. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
31. Chien, Y.-h., Iwashima, M., Kaplan, K. B., Elliott, J. F. & Davis, M. M. (1987) *Nature (London)* **327,** 677–682.
32. Chien, Y.-h., Iwashima, M., Wettstein, D. A., Kaplan, K. B., Elliott, J. F., Born, W. & Davis, M. M. (1987) *Nature (London)* **330,** 722–727.
33. Azuma, T., Igras, V., Reilly, E. B. & Eisen, H. N. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6139–6143.
34. Reilly, E. B., Kranz, D. M., Tonegawa, S. & Eisen, H. N. (1986) *Nature (London)* **321,** 878–880.
35. 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.
36. Heilig, J. S. & Tonegawa, S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8070–8074.
37. Roth, M. E., Lacy, M. J., McNeil, L. K. & Kranz, D. M. (1988) *Science* **241,** 1354–1358.
38. Bonneville, M., Janeway, C. A., Jr., Ito, K., Haser, W., Ishida, I., Nakanishi, N. & Tonegawa, S. (1988) *Nature (London)* **336,** 479–481.
39. Asarnow, D. M., Kuziel, W. A., Bonyhadi, M., Tigelaar, R. E., Tucker, P. W. & Allison, J. P. (1988) *Cell* **55,** 837–847.