T CELL RECEPTOR GENE USAGE IN THE RESPONSE TO
λ REPRESSOR cI PROTEIN

An Apparent Bias in the Usage of a Vα Gene Element

By MING-ZONG LAI,* SHU-YING HUANG,* THOMAS J. BRINER,*
JEAN-GERARD GUILLET,* JOHN A. SMITH,‡ AND MALCOLM L. GEFTER*

*From the *Department of Biology, Massachusetts Institute of Technology, Cambridge,
Massachusetts 02139; the ‡Departments of Molecular Biology and Pathology, Massachusetts General
Hospital; and the Department of Pathology, Harvard Medical School,
Boston, Massachusetts 02114*

T lymphocytes recognize foreign antigen in the context of cell surface molecules encoded by the MHC. The TCR that mediates this recognition is a cell surface heterodimer consisting of an α and a β chain (1–4). The genes encoding these two chains have been isolated and sequenced (5–8). Each chain is composed of regions encoded by separate gene segments: variable (V), junction (J), and constant (C) elements; in addition, the β chain contains a diversity (D) segment. These gene elements are organized and rearranged in a fashion similar to Ig genes (9–15). Gene transfection experiments demonstrate that the α/β TCR dimer is sufficient to endow the T cell with both antigen and MHC specificity (16–18). However, the precise molecular interactions between the TCR, peptide antigen, and MHC molecules remains unclear. Studies of Unanue, Grey, and their colleagues have demonstrated a specific interaction between peptide antigen and Ia protein (19, 20). We have shown that each peptide antigen interacts with the single peptide binding site of Ia protein, possibly by displacing an "internal ligand" (21, 22). In the present study, we have undertaken structural analysis on the TCR that recognizes a particular Ia-peptide complex.

T cell response to λ repressor cI has been demonstrated to be focused to a particular peptide segment of the protein, encompassing residues 12 to 26 (P12–26) in both BALB/c and A/J mice (23). This enabled us to analyze the TCRs that recognize the same peptide presented by different Ia molecules ($I-A^d$ and $I-E^k$). In addition, the fine-specificity of these P12–26-specific T cell hybridomas have been well characterized (23). The primary structure of TCR can thus be studied in the context of differential T cell responses to various P12–26 analogs.

Materials and Methods

T Cell Hybridomas.   T cell hybridomas were produced by fusion of T cell BW5147 with lymph node cells obtained from mice immunized with antigen and stimulated in vitro with

the same antigen (23). For P12-26-specific hybrids: 7B7.3, 8F8.4, 8F8.10, 9C127, and 15C9.6 are hybridomas made from BALB/c mice; 3I, 4I, 7II, 8I, 10I, 14I, 14II, 22I, 24I, 26IV, 31II, 39I, and 147IV are hybridomas produced from A/J mice.

Construction of cDNA Libraries. Total cellular RNA was prepared from each T cell hybridoma by the guanidinium isothiocyanate method (24). Poly (A)$^+$ RNA was purified from oligo-dT)-cellulose column (25). cDNA libraries were prepared by the method of Gubler and Hoffman (26) and cloned into λgt10 (27) after Eco RI linker addition. Recombinant phages were screened with ^{32}P-labeled nick-translated (28) probes of the constant or variable region of TCR. At least two independent cDNA inserts were subcloned into pGEM4 (Promega Biotec, Madison, WI) and sequenced by dideoxy chain-terminating method (29) or Maxam-Gilbert method (30).

Northern Analysis. 16 µg of total RNA was glyoxalated and subjected to electrophoresis on a 1.2% agarose gel accordingly to McMaster and Carmichael (31). RNA was transferred to Gene Screen Plus nylon membrane (New England Nuclear, Boston, MA) and hybridized with ^{32}P-labeled probe in 5X SSC, 1% SDS, 5% Denhart's (32), 100 µg/ml salmon sperm DNA, 10% dextran sulfate, and 50% formamide. The washed blots were autoradiographed by exposure at −70°C with intensifying screens. Blots that were probed several times were stripped by boiling in 0.1% SDS, 0.1X SSC for 20 min.

Southern Analysis. T cell hybridomas were lysed with 0.5% NP-40, the nuclei sedimented and treated with proteinase K (50 µg/ml) at 37°C overnight. DNA was extracted with phenol, chloroform, and was dialyzed against TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). After RNase (100 µg/ml) treatment, proteinase A and phenol-chloroform steps were repeated. The dialyzed DNA was digested with restriction enzyme and subjected to electrophoresis on 0.8% agarose at 25 V. The DNA was then transferred to Gene Screen Plus nylon membrane by the method of Southern (33) and was hybridized with a ^{32}P-labeled probe as in Northern analysis.

DNA Probes. The constant region of TCR β chain (p86T5.91) and the subclones of V$_α$FN1-18, V$_α$2B4, V$_α$MDA, V$_α$P71, V$_α$C5, and V$_α$E1 were a gift of Dr. M. Davis (Stanford University, Stanford, CA). The α chain constant region gene (pHDS-58) was provided by Dr. S. Tonegawa (MIT, Cambridge, MA). The probes of V$_β$1, V$_β$2, V$_β$3, V$_β$4, V$_β$5.1, V$_β$8.1, V$_β$8.3, V$_β$10, and V$_β$14 were a gift of Dr. L. Hood (Caltech, Pasadena, CA). The probes of V$_β$7, V$_β$11, V$_β$12, V$_β$12, V$_β$13, V$_β$15, and V$_β$16 were obtained from Dr. D. Loh (Washington University, St. Louis, MO).

Oligonucleotide Probes. The oligonucleotide probes were synthesized with a DNA synthesizer (model 380A; Applied Biosystems, Inc., Foster City, CA) by using the silica-based solid-phase method (34) and the proton-activated nucleotide phosphoramidite method (35). The oligonucleotides were purified by PAGE and desalted on a C$_{18}$ column. The probe was labeled to a specific activity of 4–10 × 10^8 cpm/µg by using T4 polynucleotide kinase and [γ-^{32}P]ATP (25). The Northern blots were hybridized with oligonucleotide probes in 6X SSC, 1% SDS, 500 µg/ml salmon sperm DNA, 5X Denhart's, 10% Dextran at 39°C for 48 h.

Lysate Hybridization. Freshly prepared T cell hybridomas were first screened for antigen reactivity. The antigen-specific hybrids were transferred to a 96-well plate. Lysate hybridization was performed as previously described (36). Since the content of TCR mRNA is relatively low in hybridomas (0.01%), a cell number of 10^5/well or above was used for most hybridomas. After hybridizing with a variable region probe, the blot was checked with a constant region probe. Hybridomas that failed to hybridize with the constant region probe were not counted in the analysis. All hybridomas were screened by lysate hybridization before subcloning. In addition, it is difficult to maintain many hybridomas growing at proper density, as T cell hybridomas are less stable at high cell density. Thus, the results of lysate hybridizations should represent a lower limit of the total number of hybridomas that use the particular TCR variable gene.

Peptides. Moth cytochrome *c* peptide P93-103 (E-L-I-A-Y-L-K-Q-A-F-K) is a gift of Dr. R. Schwartz (National Institutes of Health, Bethesda, MD). λ repressor P12-26 and its analogs (P12-24, P15-26, [Phe^{22}]P12-26, and [His^{22}]P12-26), staphylococcal nuclease P81-100 (R-T-D-K-Y-G-R-G-L-A-Y-I-Y-A-D-G-K-M-V-N) were synthesized and purified as previously described (23, 37).

Results

Frequently, T cell responses of a particular inbred strain of mouse are reactive primarily to a limited region of the protein. The diversity of that T cell reactivity as measured by fine specificity analysis, however, suggests that there are many overlapping T cell epitopes present within the small peptide region to which the T cells are directed (for review see reference 38). By using truncated analogs of P12-26, five different reactivity patterns have been identified with P12-26-specific T cell hybridomas isolated from BALB/c mice and restricted by the I-A^d molecule (23). T cell-reactive peptides competition experiments (21, 22, 39, 40), as well as the recent determination of the class I MHC protein structure (41), have strongly supported our original hypothesis that a single binding site on class II MHC molecule exists. Therefore, it was suggested that diversity in T cell reactivity is a result of each T cell interacting preferentially with a different segment of the same peptide accommodated in the binding site of the class II molecule (23).

We thus reasoned that the TCR of the hybridoma 7B7.3, which recognizes the COOH-terminal part of P12-26 (Fig. 1), should be distinct from the antigen receptor of the hybridoma 8F8.10, which interacts primarily with the NH$_2$ terminus of P12-26 (Fig. 1, reference 23). There are, thus, likely to be at least two distinct types of TCRs recognizing P12-26 in the context of I-A^d. In contrast, the I-E^k-restricted T cell hybridomas all react in an identical way toward P12-24 and P15-26 as T cell hybridoma 8I (Fig. 1). This limited reactivity suggest a less heterogeneous population of TCRs amongst the I-E^k-restricted T cells.

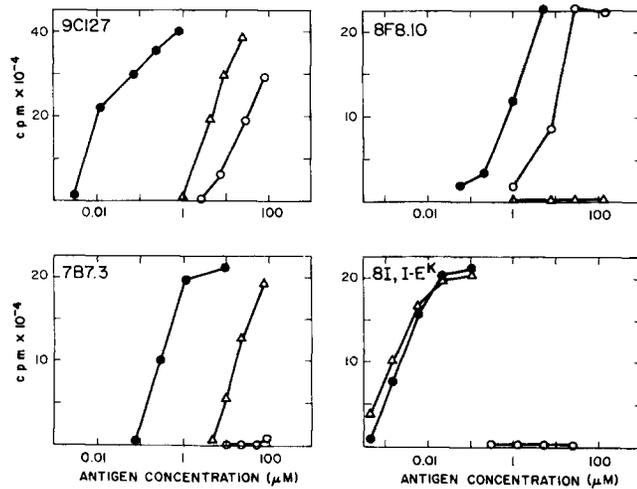A cDNA library was constructed from 8I T cell hybridoma and was screened with

FIGURE 1. (A) Reactivity patterns of I-A^d-restricted T cell hybridomas (9C127, 7B7.3, and 8F8.10) and of I-E^k-restricted T cell hybridomas (represented by 8I). Activities were measured by IL-2 production (23) in the presence of various peptides, P12-26 (●), P15-26 (△), and P12-24 (○) representing a portion of the λ repressor cI protein. Each point is the arithmetic mean of triplicate experiments. SD is <10% of the mean. (B) The sequence of P12-26, P12-24, and P15-26 of bacteriophage λ repressor cI protein.

● P12-26   Leu-Glu-Asp-Ala-Arg-Arg-Leu-Lys-Ala-Ile-Tyr-Glu-Lys-Lys-Lys

○ P12-24   Leu-Glu-Asp-Ala-Arg-Arg-Leu-Lys-Ala-Ile-Tyr-Glu-Lys

△ P15-26   Ala-Arg-Arg-Leu-Lys-Ala-Ile-Tyr-Glu-Lys-Lys-Lys

the constant region probes of TCR α and β chains. The functional TCR α gene sequence identified from the library and its predicted protein sequence is shown in Fig. 2. The V_α of 8I is a member of $V_\alpha 3$ family and shares 95% of its nucleotide sequence with the reported V_αTA39 gene sequence (42, Table I). In Northern analysis, 13 out of 14 I-E^k-restricted, P12-26-reactive T cells were found to express a V_α sequence crosshybridizing with $V_\alpha 8I$ (data not shown). By using $V_\alpha 8I$ as a probe, the expressed α chain of two additional T cell hybridomas (4I and 10I) were then isolated. 4I uses the same V_α as 8I, whereas, 10I uses a third member of the $V_\alpha 3$ family (Fig. 2). All three T cells utilize different J_α elements (Fig. 2). $J_\alpha 8I$ is identical to J_αTA39 (42). Southern analysis (Fig. 3) reveals eight different rear-
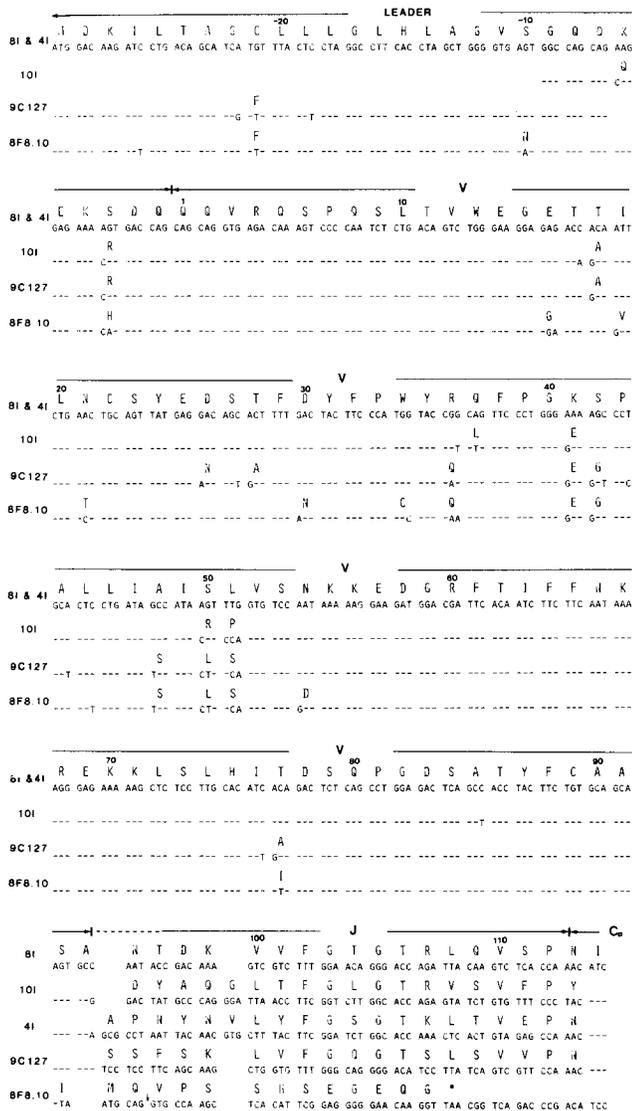

FIGURE 2. Nucleotide and predicted amino acid sequence of TCR α chain genes from λ repressor P12-26-specific T cell hybridomas. Amino acid residues are numbered according to reference 42, where TA39 (the first member of $V_\alpha 3$) is also reported. Spaces have been introduced to obtain proper alignment. Nucleotides identical to those of 8I are indicated by dashes. The boundary between J_α and V_α is uncertain. For each T cell, at least two independent cDNA clones were sequenced (see Materials and Methods). These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00810.

TABLE I

Sequence Similarity between Vα3 Family Genes

	Vα8I	Vα10I	Vα9C127	Vα8F8.10	VαTA39*
Vα8I	–	289/300	279/300	280/300	287/300
Vα10I	93/100	–	280/300	279/300	297/300
Vα9C127	88/100	90/100	–	283/300	281/300
Vα8F8.10	86/100	83/100	88/100	–	278/300
VαTA39	92/100	97/100	91/100	84/100	–

Comparisons were made between variable regions (coding sequences position – 9 to position 91 in Fig. 2). As the germline sequences remain unknown, the actual similarity may be slightly lower when residues 3' to the position 91 (nucleotide position 273) are included. Nucleotide sequences similarity is indicated above the diagonal line, protein sequence similarity is below the diagonal line. Similarity is expressed as number of identical residues per number of total residues.

* From Arden et al. (42).

ranged patterns of Vα genes among 13 A/J T cell hybridomas. Three groups of T cells (3I, 7II, and 24I; 14I and 14II; 22I, 26IV, and 31II) use identical Vα-Jα elements. The other five T cells each use a different Vα-Jα combination. To further distinguish Vα usage, oligonucleotides specifically hybridized to Vα8I or Vα10I were
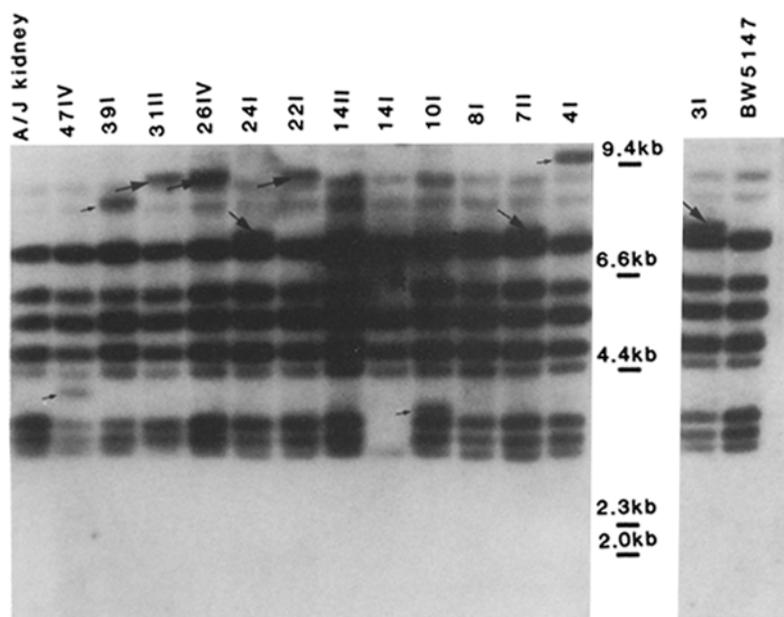

FIGURE 3.   Southern blot analysis of TCR α chain gene rearrangement in I-E^k-restricted, λ repressor P12–26-specific T cell hybridomas. 25 μg of DNA from each hybridoma indicated and from A/J kidney were digested with Eco RI and analyzed as described in Materials and Methods. The Vα8I probe used is a 272-bp fragment (Tth31 sites at nucleotide position 16 to 284). The rearranged DNA fragment shared by more than one T cell is indicated by the heavy arrows (3I, 7II, 24I; 14I, 14II; 22I, 26IV, 31II). Similar patterns were observed with Hind III digested DNAs (not shown), in which a 1.3-kb rearranged DNA fragment is shared by 3I, 7II, and 24I, and a 6.9-kb rearranged DNA fragment is present only in 22I, 26IV, and 31II.

made and used to screen the collection of T cells. About equal numbers (six versus seven) utilize one or the other gene among I-E^k-restricted P12-26-reactive T cells (Fig. 4). Because V_α10I is identical to V_αTA39 in the oligonucleotide probe region, additional probes that distinguish V_α10I from V_αTA39 were synthesized and used to screen the collection. None of the P12-26-specific T cells hybridize with oligonucleotide of V_αTA39 in Northern analysis (data not shown).

In contrast to the observed predominant selection of the V_α gene family used, four different V_β genes are used by 13 T cells of I-E^k restriction. Fig. 5 lists the V_β gene usage and V-D-J region sequence of 8I, 10I, 4I, and 3I. V_β6 and V_β14 are used only by 8I and 4I, respectively. The V_β8 family gene was used by two T cell hybridomas (39I, 47IV) in addition to 10I. The different rearrangement pattern in Southern blots indicate different V_β8 or J_β elements are used by each of 10I, 39I, and 47IV (data not shown). The β chain of 3I contains V_β1, which is rearranged to J_β2.1 element rather than J_β2.5 expressed in BW 5147. Southern analysis demonstrates all the other I-E^k T cell hybridomas share the same extra rearranged DNA band as 3I (Fig. 6, arrow). From the known genomic restriction map of J_β2 region (9, 43), the size of the rearranged fragment (2.8 kb) indicates all these T cells utilize an identical V_β1-J_β2.1 combination. The 2.1-kb DNA fragment (Fig. 6, indicated by   ) represents the rearranged β chain of BW5147, as J_β2.5 is 700 bp 3' to J_β2.1 (43). This 2.1-kb V_β1 rearrangement is missing in 3I (Fig. 6), correlating with the observation that the β chain of BW5147 is absent in 3I cDNA library.

A clear correlation between the primary structures of the α and/or β chains expressed and the MHC restriction molecule has yet to be established. All I-E^k-restricted T cell hybridomas recognize P12-26 in the context of $E_\alpha^k E_\beta^b$. None of the 13 I-E^k T cells are alloreactive to I-A^s and I-A^b molecule. There is apparently much less heterogeneity in MHC specificity in comparison with I-E^k-restricted, cytochrome c-specific T cells (see below).

Even though I-E^k-restricted T cell hybridomas display a restricted reactivity towards P12-24 and P15-26, fine specificity differences can be used to distinguish fur-
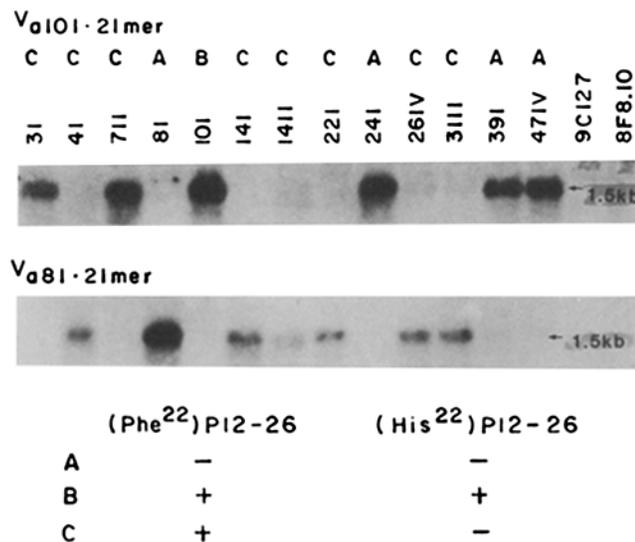

FIGURE 4. V_α8I and V_α10I usage among I-E^k-restricted, P12-26-specific T cells. The Northern blot was hybridizing with a γ-^{32}P-labeled oligonucleotide specific for the V_α10I gene (GGACACTGGACGTATGG-CTAT) or the V_α8I gene (GGACACCAAACTTATGG-CTAT). Because V_α10I is identical to V_αTA39 in this region, additional probes that distinguish V_α10I from V_αTA39 were made to screen the panel of cells. None of the P12-26-specific T cells hybridize with V_αTA39. Reactivity toward [Phe^{22}]P12-26 and [His^{22}]P12-26 is indicated with each T cell hybridoma.

8I

V$_{\beta 6}$ ⊣ ⊢D$_{\beta 2.1}$ ⊣ ⊢——— J$_{\beta 2.1}$ ———⊣⊢C$_{\beta 2}$
C A S S R G G A Y A E Q F F G P G T R L T V L E
-- TGT GCC AGC AGT AGG GGG GGC GCC TAT GCT GAG CAG TTC TTC GGA CCA GGG ACA CGA CTC ACC GTC CTA GAG

10I

V$_{\beta 8.3}$ ⊣ ⊢D$_{\beta u}$⊣ ⊢——— J$_{\beta 2.4}$ ———⊣⊢
C A S S E S G R J T L Y F G A G T R L S V L E
-- TGT GCC AGC AGT GAA TCA GGG AGG GAC ACC TTG TAC TTT GGT GCG GGC ACC CGA CTA TCG GTG CTA GAG

4I

V$_{\beta 14}$ ⊣ ⊢ D$_{\beta 2.1}$ ⊣ ⊢——— J$_{\beta 2.3}$ ———⊣⊢
C A W N T G G A E I L Y F G S G T R L T V L E
-- TGT GCC TGG AAT ACT GGG GGG GCT GAA ACG CTG TAT TTT GGC TCA GGA ACC AGA CTG ACT GTT CTC GAG

3I

V$_{\beta 1}$ ⊣ ⊢ D$_{\beta 2.1}$ ⊣⊢——— J$_{\beta 2.1}$ ———⊣⊢
C A S S Q E G G J Y A E Q F F G P G T R L T V L E
-- TGT GCC AGC AGC CAA GAG GGG GGG GAC TAT GCT GAG CAG TTC TTC GGA CCA GGG ACA CGA CTC ACC GTC CTA GAG

9C127

V$_{\beta 8.1}$ ⊣ ⊢ D$_{\beta 1.1}$ ⊣⊢——— J$_{\beta 1.4}$ ———⊣⊢C$_{\beta 1}$
C A S S P G Q G A E R L F F G H G T K L S V L E
-- TGT GCC AGC AGT CCG GGA CAG GGG GCC GAA AGA TTA TTT TTC GGT CAT GGA ACC AAG CTG TCT GTC CTG GAG

8F8.10

V$_{\beta 8.3}$ ⊣ ⊢ D$_{\beta 2.1}$ ⊣⊢——— J$_{\beta 2.7}$ ———⊣
C A S S D A R T G G A Y E Q Y F G P G T R L T V L E
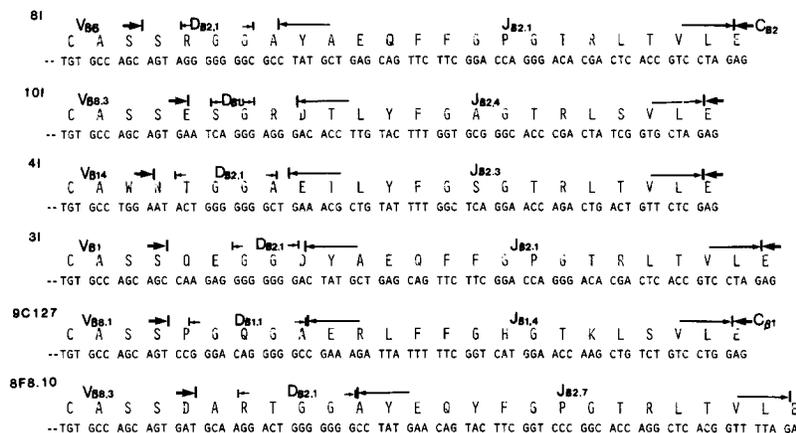-- TGT GCC AGC AGT GAT GCA AGG ACT GGG GGG GCC TAT GAA CAG TAC TTC GGT CCC GGC ACC AGG CTC ACG GTT TTA GA

FIGURE 5. Nucleotide and predicted amino acid sequence of the V-D-J region of the TCR β chain genes from λ repressor P12-26-specific T cells. The sequence of Vβ1, Vβ6, Vβ8.1, Vβ8.3, and Vβ14 have been reported (61-63). D and J regions are assigned according to the published germline sequences (9, 43, 64). Cβ2 is used by 8I, 10I, 4I, 3I, and 8F8.10, Cβ1 is used by 9C127. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00810.

ther between these T cells. It was of interest to determine if structure function correlations could be found amongst subsets of the collection of T cells. By comparing the reactivities with [Phe^{22}]P12-26 and [His^{22}]P12-26 (23), T cells derived from A/J mice are grouped into three different types (Fig. 4, *A-C*). A-type T cells, represented by 8I, are unresponsive to either [Phe^{22}]P12-26 or [His^{22}]P12-26. In contrast, 10I reacts well with both [Phe^{22}]P12-26 and [His^{22}]P12-26 (B type). C-type T cells, including 4I and 7II, do not recognize [His^{22}]P12-26, but react well with

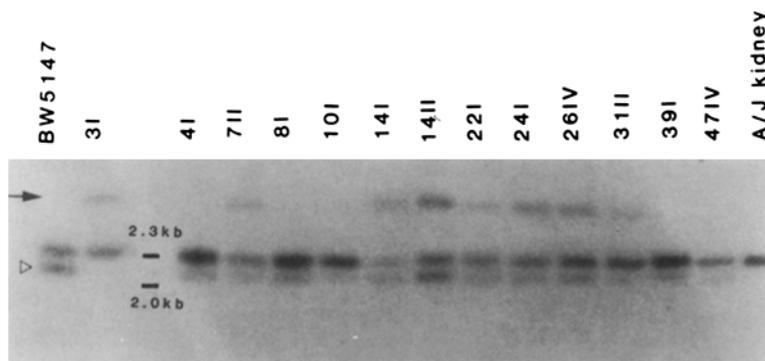

FIGURE 6. Southern blot analysis of TCR β chain gene rearrangements in I-E^k-restricted, P12-26-specific T cell hybridomas. DNA was digested with Eco RI as described (See Materials and Methods). The Vβ1 sequence was obtained from the β chain of BW5147 and contains 350 bp from the 5′ untranslated region to the Bam HI site within the V region. The 2.8-kib rearranged band of 3I (Vβ1-Jβ2.1) is indicated by the arrow. The 2.1-kb band ( ) in the BW5147 lane, not present in A/J kidney DNA (germline), is the rearranged β chain (Vβ1-Jβ2.5) of BW5147. This 2.1-kb band is absent in 3I. Five T cell hybridomas manifest an identical rearrangement as seen for BW5147; 8I expresses Vβ6; 4I uses Vβ14; 10I, 39I, and 47IV use Vβ8.

[Phe^{22}]P12-26. Fig. 4 clearly demonstrates that the usage of V$_\alpha$8I or V$_\alpha$10I is not correlated with this fine specificity toward antigen. Neither can the β chain usage be related to the reactivities toward [Phe^{22}]/His^{22}]P12-26, even though seven out of the eight T cells with V$_\beta$1-J$_\beta$2.1 rearrangement display C-type reactivities. For example, 10I, 39I, and 47IV all use V$_\beta$8 (and V$_\alpha$10I), but exhibit two distinct types of reactivities (A and B types). Furthermore, this antigen specificity can not be accounted for by the particular α-β chain combination used. Southern blot analyses indicates that the same V$_\alpha$-J$_\alpha$ and V$_\beta$-J$_\beta$ elements are used by 3I, 7II, and 24I (Figs. 3 and 6), but 24I reacts differently toward [Phe^{22}]P12-26 (Fig. 4). The fine specificity against [Phe^{22}]P12-26, therefore, may be attributed to the junctional diversity (including N regions) of α and/or β chains. Currently the TCR genes expressed in 7II and 24I are being isolated, and the sequences in the junctional regions will be determined.

By using peptides of different lengths surrounding residues 12-26, we have previously demonstrated that P12-26 contains complete immunodeterminants of the repressor protein when presented in the context of both I-A^d and I-E^k (23). This system provides a unique opportunity to study two populations of TCRs that interact with the same peptide presented by different Ia molecules. T cells derived from BALB/c were first screened with V$_\alpha$ and V$_\beta$ probes representative of expressed genes amongst I-E^k-restricted T cells. It was found that all I-A^d-restricted T cells, except 15C9.6 (the T cell with unusual reactivities [23]), express the V$_\beta$8 gene segment (Fig. 7 A). Four I-A^d-restricted T cells were shown to use a V$_\alpha$3 family gene
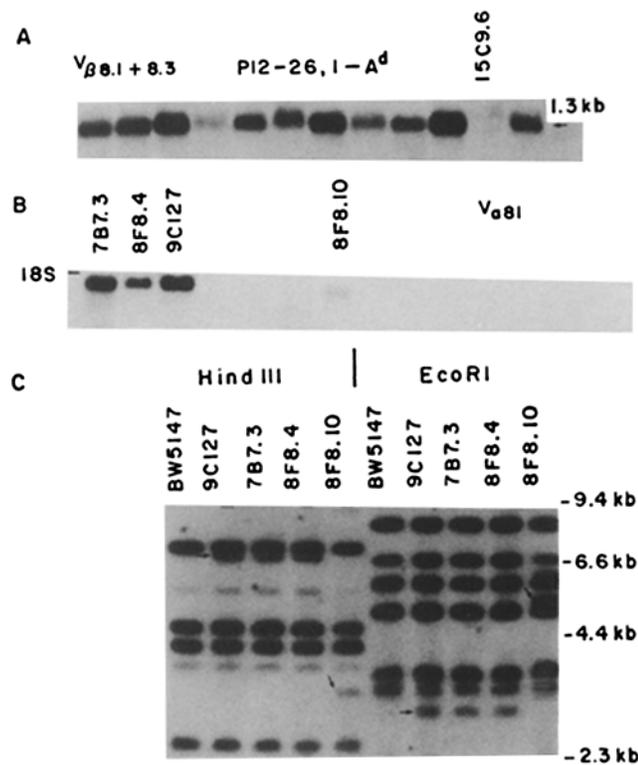

FIGURE 7.   (A and B) V$_\alpha$ and V$_\beta$ usage of I-A^d-restricted, P12-26-reactive T cell hybridomas. Nothern blot of I-A^d-restricted T cells (of all five different reactivities described in reference 23) were hybridized with (A) V$_\beta$8.1 plus V$_\beta$8.3 (B) V$_\alpha$8I probes. Only the T cell hybridomas of interest are identified. Separate Northern blots were used in A and B. (C) Southern blot analysis of TCR α chain rearrangements in 9C127, 7B7.3, 8F8.4, and 8F8.10. DNA is digested with either Eco RI or Hind III, subjected to electrophoresis, transferred, and hybridized with the V$_\alpha$8I probe. The arrow indicates the rearranged fragment.

(Fig. 7 B). Southern analysis (Fig. 7 C) indicates that hybridomas 9C127, 7B7.3, and 8F8.4 use an α chain of identical V_α-J_α rearrangement. The sequence of the α chain from 9C127 reveals that it contains a fourth member of the $V_\alpha 3$ family (Fig. 2). The same recombination of $V_\beta 8.1$-$D_\beta 1.1$-$J_\beta 1.4$ (Fig. 6) are also used by 9C127, 7B7.3 and 8F8.4 (Southern blot not shown). 7B7.3 (with 8F8.4) and 9C127 were grouped separately by reactivity (23), because 7B7.3 and 8F8.4 are almost nonresponsive to P12-24 (Fig. 1). The Southern analysis here suggests that the TCR α-β dimer may be identical for 7B7.3 (8F8.4) and 9C127. The difference between 7B7.3 and 9C127 is likely to reside in the potency of reactivity, as 9C127 recognizes P12-24 only at a concentration 5,000-fold greater than that of P12-26 (Fig. 1). Alternatively, different fine specificity may be attributed to sequence difference in the N region of TCR. The latter possibility will be examined at a latter time by cloning and sequencing the TCR of 7B7.3 and 8F8.4.

The 8F8.10 T cell, the other I-A^d-restricted hybrid containing a crosshybridizing $V_\alpha 3$ gene (Fig. 7 B), has a reactivity pattern distinct from that of 7B7.3 and 8I (Fig. 1). While the latter two react strongly with P15-26, 8F8.10 is completely nonresponsive toward P15-26. The V_α of 8F8.10 is a fifth member of $V_\alpha 3$ (Fig. 2). Table I lists the similarity in nucleotide and amino acid sequence between these five different $V_\alpha 3$ genes (four from this study and V_αTA39 from reference 42). The J_α element used by 8F8.10 has been reported before (TA37 in reference 42, F3.1 in reference 44), but a single nucleotide deletion (Fig. 2, arrow) creates a stop codon in the middle of the J_α region. The $V_\alpha 3$-containing α chain transcript is thus nonfunctional. Current attempts are made to identify the functional α chain of 8F8.10.

To further characterize the $V_\alpha 3$ usage in the T cell response, A/J mice were immunized separately with different antigens (λ repressor P12-26, moth cytochrome c P93-103,and staphylococcal nuclease P81-100). The expression of the $V_\alpha 3$ sequence in T cell hybridomas specific for each antigen was studied by lysate hybridization (36). In contrast to the high frequency usage of $V_\alpha 3$ in P12-26-specific T cells (Fig. 8 A), very few T cells specific for cytochrome c or nuclease were found to use $V_\alpha 3$ (Fig. 8, B and C). In another panel of P12-26-specific T cell hybridomas produced from BALB/c mice, 5 out of the 38 T cells hybridized with $V_\alpha 3$ (Fig. 8 D).

Discussion

To elucidate the molecular interaction of the TCR with the P12-26-Ia complex, the primary structures of the TCR α and β chains in T cells specific for P12-26 have been determined. A limited number of α and β chain variable genes were used among the population of T cells studied. A very interesting observation is that 13 independent T cell hybrids (derived from 12 A/J mice) use two members of a single crosshybridizing V_α family gene. This is a similar finding to the predominant usage of $V_\alpha 11$ gene in cytochrome c-specific T cells (45-47). Such a preferential usage of a particular variable gene is contrary to the random use of variable genes in insulin-specific (44) and hapten-specific (48) T cells. It is interesting to note that both λ repressor P12-26-specific and cytochrome c P93-103-specific T cells are I-E^k restricted. There is a possibility that such a preferential use of a specific V_α family is unique for I-E^k-restricted system. This may imply that there is a limited manner in which the I-E^k-antigen peptide complexes can be recognized by TCRs. Further characterization of the TCR from other systems will help answer this question.

A   P12-26,   I-E^k ✦ = 54

C   Nase,   I-E^k ✦ = 76

B   Cytochrome-c, I-E^k ✦ = 50
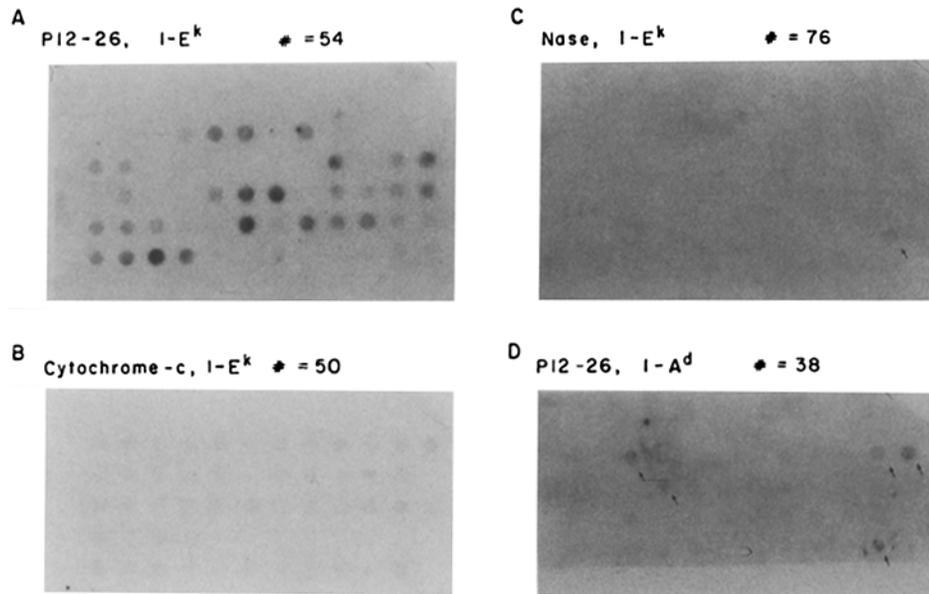
D   P12-26,   I-A^d ✦ = 38

FIGURE 8.   Lysate hybridization of T cell hybridomas specific for different antigens. T cell hybridomas were produced in A/J mice by immunization with (A) λ repressor P12-26, (B) moth cytochrome c 93-103, (C) staphylococcal nuclease P81-100, or in BALB/c mice with (D) λ repressor P12-26. Antigen-specific T cell hybridomas were transferred to a 96-well plate and lysed. The lysate was transferred onto a nitrocellulose membrane according to reference 36. Nitrocellulose filters were then hybridized with the V$_\alpha$8I probe and exposed for 1 wk with intensifying screens. The clones which hybridized with V$_\alpha$8I are indicated by arrow in C and D.

Both P12-26- and cytochrome c-specific T cells use a very limited number of β chains. The β chains of a few (but not all) cytochrome c-specific T cells have been related to their MHC specificity (45, 46, 49). Contrasting with the heterogeneous MHC reactivity of cytochrome c-specific T cells, no difference in the Ia specificity has been identified with P12-26-specific T cells. Despite the use of four different β chains (Fig. 6), all P12-26-reactive, E$_\alpha^k$E$_\beta^k$-restricted T cells are also restricted by E$_\alpha^k$E$_\beta^b$ and are not alloreactive to I-A^b or I-A^s (the alloreactivities found in cytochrome c T cells). It should be noted that in the latter system (45, 47, 50), normal T cell lines were used as opposed to hybridomas in this study. For unknown reasons, alloreactivities are much more difficult to detect among T cell hybridomas. The comparison between cytochrome c- and P12-26-specific T cells of similar MHC reactivities reveals little similarity on the β chain sequence. Cytochrome c-specific T cell of E$_\alpha^k$E$_\beta^b$ restriction (group I and IV in reference 47) use V$_\beta$3-J$_\beta$1.2 or J$_\beta$2.5 and V$_\beta$C10-J$_\beta$2.4, whereas, none of the P12-26-reactive T cell hybridomas use V$_\beta$3, V$_\beta$C10, J$_\beta$1.2, or J$_\beta$1.2. There is a possibility that different β chain elements are selected because of different strains of mice were analyzed (B10.A for cytochrome c, A/J for P12-26). It is however, more likely that MHC reactivity is encoded by a combination of V$_\alpha$-J$_\alpha$ and V$_\beta$-J$_\beta$ elements, which has recently been demonstrated by Matis et al. (50). Little sequence similarity would be expected between β chains pairing with V$_\alpha$3-J$_\alpha$ (P12-26 specific) and β chains pairing with V$_\alpha$11-J$_\alpha$ (cytochrome c specific).

Except for 9C127, 7B7.3, and 8F8.4, the primary structure of TCR α chain of P12-26-specific, I-A^d-restricted T cells have yet to be determined. It is not known if another predominant use of a V$_\alpha$ will also be found among I-A^d-restricted T cell hybridomas. Interestingly, there is a preferential expression of V$_\beta$8 in I-A^d-restricted, P12-26-reactive T cells (Fig. 7 A). It may be noted that V$_\beta$8 is also used by I-A^d-restricted, OVA-specific T cells DO-11.10, 7DO-286.2, 8DO-201.1 (51), and 3DO54.8 (data not shown). It is not known if the V$_\beta$8 gene family is selected because of I-A^d specificity. Because V$_\beta$8 genes are also used by I-E^k-restricted T cells (10I, 391, 47IV), and no crossreactivity at the Ia level can be found between T cells of I-A^d restriction and of I-E^k restriction (23), the adoption of V$_\beta$8 apparently is not sufficient to confer a I-A^d reactivity in the association with P12-26. The only I-A^d-restricted T cell hybridoma that does not use V$_\beta$8 is 15C9.6, which displays a unique reactivity toward various P12-26 analogs (23). For example, 15C9.6 is the only I-A^d-restricted T cell that reacts well towards [His^{22}]P12-26 and recognizes P12-24 much better than P12-26. Previous studies have concluded that the complete immunodeterminant of 15C9.6 includes the region extended beyond the NH$_2$ terminus of P12-26 (23). It is reasonable to expect that the TCR of 15C9.6, which interacts with a peptide different from P12-26 (e.g., P9-24), is composed of a different V$_\beta$ element.

The TCR D-J regions have been shown to be critical in the antigen/MHC recognition. In addition to the MHC reactivities defined from junctional diversities (50), two cytotoxic T cells react with the same antigen but have different MHC restrictions were shown to use identical J$_\alpha$-J$_\beta$ regions (48). In contrast, TCRs with identical variable elements but different D and J elements display unrelated antigen/MHC specificities (52). More recently, Kuo and Hood (18) have demonstrated that the pairing of the TCR α chain from cytochrome c-specific cells with a β chain of identical V$_\beta$, but different D$_\beta$-J$_\beta$ region, fails to reconstitute the cytochrome c/MHC recognition (18). Furthermore, a few amino acids differing at the D region (46) or N region (47) of the TCR can account for the fine specificity difference seen when testing cytochrome c analogs. In the present study, the fine specificity differences noted between the I-E^k-restricted T cells toward [Phe^{22}]P12-26 probably is related to the V-J junctional diversity, as T cells (3I, 7II, 24I) with identical V-J rearrangements display two distinct specificities. The few amino acid residues in the junctional region of TCR may therefore be involved in the direct contact with the antigen peptide presented by an Ia molecule. Of special interest is the failure of A-type T cells to recognize [Phe^{22}]P12-26. Since phenylalanine differs from tyrosine by only a hydroxyl group, the unresponsiveness to [Phe^{22}]P12-26 indicates an extremely restricted interaction between Tyr^{22} of P12-26 and the V-J region of the TCR on A-type T cells. The substitution of phenylalanine does not affect the binding of the peptide to I-E^k molecule, since [Phe^{22}]P12-26 is an effective inhibitor of 8I T cell activation (23). Considering that there are known to be multiple residues contacting the TCR on antigenic peptides (53, 54), the observation that the deletion of a hydroxyl group dramatically disrupts the apparent peptide-TCR affinity is intriguing. The sequences of the V-J regions of the TCR from these T cells may provide useful information towards understanding this specific interaction.

The fine specificities generated by the junctional diversities of the TCR and the observations that the TCR of the same antigen/MHC restriction may be formed

by the combination of different V_α and V_β gene segments (44, 46, 48) clearly demonstrate that there is no simple correlation between variable gene usage and specificity. The three-dimensional structure modeling of the TCR suggests a binding site created at the interfaces of β sheets contributed by the V domains of the two chains (14, 55), in a way very similar to an antibody. The recently determined crystal structure of antibody-antigen complex demonstrated that there is an extensive surface contact area between an antigen and an antibody in which almost all six hypervariable regions of antibodies are in contact with the antigen epitope (56, 57). Even though a TCR may recognize only the variation on the same general structure (i.e., a few residues of the peptide presented by a MHC molecule), such an interaction with peptide-MHC complex is likely to involve the V_α and V_β regions (containing four hypervariable regions) of TCR. It is possible that the prominence of TCR junctional diversity is due to the closer approximation of the third hypervariable regions of α and β chains to the peptide epitope when TCR binds to peptide-MHC complex. Furthermore, if the TCR interacts with antigen-Ia in an "induced-fit" mode as proposed by Coleman et al. (57), the interaction of V_α and V_β to create the proper binding site is essential for any antigen-MHC recognition.

To further characterize the $V_\alpha3$ usage in the T cell response, A/J mice were immunized separately with λ repressor P12-26, moth cytochrome P93-103, and staphylococcal nuclease P81-100. Assuming there are 10 different V_α families, the random usage of a V_α gene family member will result in ~10% of T cells containing messenger RNA crosshybridizing with a $V_\alpha3$ probe. If a high frequency usage of $V_\alpha3$ (as in P12-26-specific T cells) is due to the limited V_α repertoire available in A/J mice, $V_\alpha3$ should also be expressed at higher proportions in T cells specific for other antigens. Lysate hybridization of T cell hybridomas specific for each antigen (Fig. 8, A-C) clearly demonstrates that $V_\alpha3$ is expressed at very low frequency on T cells specific for cytochrome c and staphylococcal nuclease. The selection of $V_\alpha3$ is likely to be antigen specific, as all three peptides are $I-E^k$ restricted. As a control, $V_\alpha11$ is used by cytochrome c-specific T cells (data not shown), even though these clones are of A/J origin instead of B10.A previously reported (45-47). In another observation, $V_\alpha3$ is also expressed in $I-E^k$-restricted, P12-26-specific T cells produced from B10.BR mice (S. Roy, MIT, Cambridge, MA, unpublished results). The selection of $V_\alpha3$ (or $V_\alpha11$) is therefore biased toward antigen independent of the genomic background of $I-E^k$-bearing mice.

$V_\alpha3$ is also used by a few P12-26, $I-A^d$-restricted T cells, as confirmed by the use of lysate hybridization (Fig. 8 D). Of special interest is the analogy between the reactivities of $V_\alpha3$-expressing, $I-A^d$-restricted T cells with those of $I-E^k$-restricted T cells. P12-24 is poorly recognized by 7B7.3, 8F8.4, and 9C12.7; whereas, P15-26 is a good stimulator of these $I-A^d$-restricted T cells. Such a discrimination between P12-24 and P15-26 is not found in other $I-A^d$-restricted, P12-26-specific T cell hybrids, instead, this is more characteristic of $I-E^k$-restircted T cells (see 8I of Fig. 1). The usage of $V_\alpha3$, therefore, may confer a similar reactivity to T cells of different MHC restrictions. This support a specific role for antigen in the selection of a particular V_α family in the T cell response.

Within the small number of T cells analyzed (Fig. 4), different members of the $V_\alpha3$ family are used by T cells derived from BALB/c and A/J mice. Since cross-hybridization bands in Southern blot analysis are the same for BALB/c and A/J

germlines (data not shown for the BALB/c germline), $V_\alpha 3$ repertoire should be the same for either strain of mice. The expression of $V_\alpha 8I/10I$ in A/J mice and of $V_\alpha 9C127$ in BALB/c mice (and the nonexpression of $V_\alpha TA39$) in constituting the T cell response toP12–26 could be results of a specific selection. It is not known if different members of $V_\alpha 3$ are required to pair with different V_β ($V_\beta 8.1$ in $I\text{-}A^d$ restriction; $V_\beta 1$, $V_\beta 6$, $V_\beta 8.3$, $V_\beta 14$ in $I\text{-}E^k$ restriction) to form a functional TCR, or if $V_\alpha 9C127$ and $V_\alpha 8I/10I$ are also selected with respect to MHC restriction. In addition, can the usage of different $V_\alpha 3$ genes account for the difference in reactivity between 8I and 7B7.3 (such as P15–26 is as active as P12–26 in 8I, but not in 7B7.3, [23])? The answers to those questions, as well as further characterization of the present system, will provide a good understanding on the molecular interaction between the TCR, the antigen peptide, and the Ia molecule.

Summary

The T cell response to the λ repressor cI protein is directed to the same region of the protein (residues 12–26) in both BALB/c and A/J mice. A panel of T cell hybridomas specific for P12–26 in the context of either $I\text{-}E^k$ or $I\text{-}A^d$ have been isolated (23). To further understand the molecular interaction between the TCR and the Ia-P12–26 complex, the primary structures of the TCR of five T cell hybridomas have been determined. Southern and Northern analyses indicate that two members of the $V_\alpha 3$ gene family are used by 13 out of 14 $I\text{-}E^k$-restricted T cells. Four different V_β genes are used by these T cell hybridomas, while the majority (8 out of 13) express $V_\beta 1$ in combination with the $J_\beta 2.1$ element. No clear correlation can be seen in this system between gene usage and MHC restriction. In addition, the fine specificity of $I\text{-}E^k$-restricted T cells to a single amino acid substitution $[Phe^{22}/His^{22}]P12–26$ is not attributed to the usage of particular V_α and V_β elements. The $V_\alpha 3$ family gene is also used by a few $I\text{-}A^d$-restricted T cells. Interestingly, these $I\text{-}A^d$ T cells share a reactivity pattern more similar to that of $I\text{-}E^k$-restricted T cells than other $I\text{-}A^d$-restricted T cells. The nonrandom selection $V_\alpha 3$ is also demonstrated by the fact that $V_\alpha 3$ is used by P12–26-specific, but not by cytochrome c- or staphylococcal nucleas-specific, $I\text{-}E^k$-restricted T cells. This suggests that although antigen specificity may not be accounted for by either chain of the TCR, the members of $V_\alpha 3$ genes may be selected by the antigen (P12–26).

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