Molecular Analysis of the Influences of Positive Selection, Tolerance Induction, and Antigen Presentation on the T Cell Receptor Repertoire

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Summary

Immunization of both B10.A and B10.S(9R) mice with pigeon cytochrome *c* (pcc) elicits T cells capable of proliferating to pcc presented on I-E major histocompatibility complex (MHC) molecules. The T cell receptor (TCR) repertoire used by pcc-specific T cells from these two strains is markedly different, even for T cells recognizing very similar antigen/MHC complexes. Our current studies have been directed toward explaining this differential expression between MHC congenic strains of TCR gene elements capable of recognizing similar ligands. Analysis of the TCR repertoire of pcc-specific T cells from F_1[B10.A × B10.S (9R)] → parent radiation chimeras has demonstrated that much of this difference is a result of the positive selection of T cells for MHC restriction specificity. Further analysis of T cell lines from F_1 mice and from radiation chimeras stimulated in vitro with pcc on both B10.A and B10.S(9R) antigen-presenting cells has provided clear-cut examples of the influence of positive selection, tolerance induction and of both in vivo and in vitro antigen presentation on the shaping of the TCR repertoire for a protein antigen. This is the first molecular analysis of how positive selection, tolerance induction, and antigen presentation can combine to mold the TCR repertoire.

The murine T cell proliferative response to pigeon cytochrome *c* (pcc)[1] is restricted by molecules encoded by the I-Eβ region of the MHC and is specific for the COOH-terminal 24 amino acids of pcc (1–4). One unusual characteristic of this T cell response is its extreme crossreactivity; all T cell clones specific for pcc can recognize some form of the antigen in the context of several Eβ alleles (1, 2, 5). This MHC crossrecognition and the ready availability of chemical and phylogenetic variants of cytochrome *c* make possible a fine specificity analysis that allows finger printing of T cell recognition on a clonal level. Such fine specificity analyses of pcc-specific T cell clones from three MHC congenic strains have defined only seven reactivity patterns, revealing the pcc-specific T cell response to be phenotypically limited. Cells exhibiting each phenotype appear with a regular frequency in oligoclonal antigen-specific T cell lines derived from in vivo primed mice (1, 2, 5). We and others have taken advantage of this limited, predictable response to embark upon a thorough analysis of the TCR gene elements expressed by T cell clones from each functional phenotype (6–9). These studies revealed that very limited numbers of TCR gene elements are used to encode the receptors used by pcc-specific T cells. Virtually all (22 of 23) of the T cell clones examined from B10.A mice use the Vα11 family of genes (6, 7, 9). Vα usage among pcc-specific T cells from B10.S(9R) mice is similarly restricted, with the Vα10 family predominating (8). The Jα elements are also limited; T cells of each phenotype express one, or at most five, of the ~50 Jα elements available. In both strains of mice, the limitations on the gene elements used to encode the β chains of pcc-specific T cells are equally strict. Furthermore, there is a correlation between the Vβ-Jβ elements used by a pcc-specific T cell and the MHC antigens recognized by that particular clone, within the context of the Vα gene family characterizing the response in each of the two strains (6, 7, 9). These observations are summarized in Table 1. The limited TCR gene element usage among pcc-specific T cells is complemented by a similarly limited degree of junctional diversity, which is nevertheless known to be selectable by antigen (6, 7, 10).

The initial observation that served as the starting point for the current studies is as follows. T cell clones from B10.A and B10.S(9R) use different gene elements to encode receptors that recognize very similar antigens. Thus, T cells categorized as phenotype III (B10.A) and V[(B10.S(9R)] recognize antigen on both B10.A and B10.S(9R) APC but use TCR

[1] *Abbreviations used in this paper:* mcc, tobacco hornworm moth cytochrome *c*; pcc, pigeon cytochrome *c*.

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Table 1. *Antigen Reactivity Patterns and TCR Gene Element Usage in Cytochrome c-specific T Cells from B10.A and B10.S(9R) Mice*

Strain	Phenotype	Antigen/MHC reactivity*			TCR gene elements used	
		B10.A	B10.S(9R)	B10.A(5R)	α Chain	β Chain
B10.A	I	M, P	–‡	M >> P	$V_\alpha 11$ + 5 J_α	$V_\beta 3.1$ + $J_\beta 1.2,2.5$
B10.A	II	M, P	α-I-A^s	M >> P	$V_\alpha 11$ + $J_\alpha C7$	$V_\beta 3.1$ + $J_\beta 1.2$
B10.A	III	M, P	M ± P	–	$V_\alpha 11$ + $J_\alpha 84$	$V_\beta 16$ + $J_\beta 2.1$
B10.A	IV	P	P	P	$V_\alpha 11$ + $J_\alpha 28$	$V_\beta 15$ + $J_\beta 1.1,2.4$
B10.S(9R)	V	M, P§	M, P	M >> P	$V_\alpha 10$ + $J_\alpha C7$	$V_\beta 1$ + $J_\beta 1.2$
B10.S(9R)	VI	–	P	–	V_α unknown	$V_\beta 1$ + $J_\beta 1.2$
B10.S(9R)	VII	–	M, P	–	$V_\alpha 10$ + $J_\alpha 61$	$V_\beta 1$ + $J_\beta 2.1$

pcc-reactive T cell clones from B10.A and B10.S(9R) mice were typed according to antigen/MHC specificity by proliferation assays and TCR gene element usage by cDNA cloning and sequencing or by Southern analysis (6–8, 34). The numbers of independent T cell isolates represented by each phenotype are as follows: I = 6; II = 2; III = 5; IV = 4; V = 3; VI = 1; and VII = 3. The J_αs used in phenotype I clones are $J_\alpha 84$, 28, 2B4 and 2 distinct but unidentified J_α elements. The V_α gene used by the phenotype VI clone is unidentified, but known to be distinct from the V_α genes used by other cytochrome c-reactive T cells.

* Antigen/MHC reactivity was measured on peptide 88–104 from pcc (P) and peptide 88–103 from mcc (M) as presented on three APC: B10.A = E_β^k, B10.S(9R) = E_β^s, and B10.A(5R) = E_β^b.

‡ –, No detectable reactivity

§ Phenotype V clones from B10.S(9R) mice are also characterized by an anti-I-E_β^k alloreactive response measurable on B10.A APC in the absence of added antigen.

encoded by unrelated V and J elements (see Table 1 for summary). This difference in TCR gene usage between two MHC congenic strains is not due to differences in the pool of available germline genes. We and others have analyzed digested liver DNA from both strains with probes specific for all the relevant V_α and V_β region genes and found no differences (11; our unpublished observations). Furthermore, B10.A mice can express $V_\beta 1$, the V_β gene characteristic of phenotype V clones from B10.S(9R) mice (12).

Several forces could contribute to the origin of this clear-cut difference in TCR gene element usage between two MHC congenic strains of mice with the same pool of germline TCR genes. In vivo presentation of cytochrome *c* or environmental antigens in the context of I-A^k/I-E^k in B10.A mice and I-A^s/I-E_β^s in B10.S(9R) mice could skew the peripheral TCR repertoire. The imposition of self tolerance, resulting in the functional deletion of K^k/I-A^k/I-E^k-reactive cells in B10.A mice and K^s/I-A^s/I-E_β^s-reactive cells in B10.S(9R) mice, could also skew the TCR repertoire of pcc-specific T cells. The anti-I-A^s alloreactivity of phenotype II clones and the anti-I-E^k alloreactivity of phenotype V clones from B10.S(9R) mice are pertinent examples in this regard, although deletion of T cells can occur in the absence of overt alloreactivity in vitro (13). And finally, a skewing of the functional TCR repertoire by the positive selection for self MHC restriction specificity during T cell differentiation in the thymus could account for our earlier observations. Clearly, these three forces are not mutually exclusive. In fact, the data reported below reveal how tolerance induction, antigen presentation, and positive selection each can combine to determine the very limited TCR repertoire expressed by T cells specific for pcc.

Materials and Methods

Mice. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or bred in the animal facilities of the National Institutes of Health (Bethesda, MD), the University of California (San Diego, CA), or the Research Institute of Scripps Clinic. Mice of either sex were used between the ages of 6 and 12 wk. Radiation chimeras were constructed by intravenous injection into lethally irradiated (950 rad) hosts of ~10^7 anti-Thy-1 plus complement-treated bone marrow (BM) cells from donors that had received a single intraperitoneal injection of 100 μl of anti-Thy-1 mAb 1 d before they were killed. F_1[(B10.A × B10.S(9R)] BM reconstituted, irradiated B10.A mice are referred to as F_1 → B10.A chimeras. Chimeras were immunized no sooner than 3 months after irradiation and reconstitution.

Antigens and Immunization. pcc type XIII was purchased from Sigma Chemical Co. (St. Louis, MO) and used as is at 30 μg per ml for the in vitro maintenance of T cell lines and clones. Antigen used for in vivo priming was first separated from polymeric and deamidated forms by using COOH-methyl Sephacryl chromatography as described previously (14). Animals were immunized in the hind footpads with a total of 100 μg of purified pcc emulsified 1:1 in CFA containing killed *Mycobacterium tuberculosis*, strain H37 Ra (Difco Laboratories, Detroit, MI). Both the tobacco horn worm moth cytochrome *c* 88–103 and the pigeon cytochrome *c* 88–104 peptides used for the proliferation assays were synthesized at the Protein and Oligonucleotide Facility at the University of California, San Diego and purified by reverse-phase HPLC using a C_4 resin (Vydac; The Separations Group, Hesperia, CA). These peptides were chosen on the basis of studies on the minimal peptide size required to stimulate pcc-specific T cell clones (15, 16).

T Cell Lines and Clones. T cell lines were established by a published protocol (5) from the popliteal and inguinal lymph nodes of mice primed 7–8 d previously with pcc. T cell clones were de-

rived by limiting dilution of these lines. Long-term antigen-specific T cell lines and clones were maintained as described previously (5) by repeated cycles of stimulation with APC plus antigen and expansion in 100 U/ml of rIL-2 (received through a kind grant from Cetus Corp., Emeryville, CA; reference 17). Due to the high degree of MHC crossreactivity that characterizes the proliferative T cell response to pcc, this antigen cannot be used to test whether the T cells from the radiation chimeras show the expected degree of self preference of MHC restriction specificity. Therefore, as a test of chimeric phenotype, T cell lines from each chimera were established on F_1 APC and the purified protein derivative of the tuberculin contained within the CFA. When tested for proliferation to this antigen presented on parental APC, each line demonstrated a minimum of a 20-fold preference for recognizing antigen on host-type APC.

Assays of T Cell Proliferation. Proliferation assays were performed as described previously (7). Briefly, in flat-bottomed 96-well plates, 2×10^4 responder T cells were cultured in triplicate with various doses of intact cytochrome *c* or synthetic peptide and 5×10^5 irradiated (3,000 rad) spleen cells as a source of APC. On day 3 of culture, wells were pulsed overnight with 1 μCi of [^{3}H]TdR (2.0 Ci/mM), harvested, and the incorporation of [^{3}H]TdR was measured by liquid scintillation spectroscopy.

cDNA Cloning and Sequencing. Total cellular RNA was extracted by the guanidinium thiocyanate procedure previously described (18), and enriched for poly(A)$^+$ RNA over oligo(dT) cellulose. Synthesis of oligo(dT)-primed, double-stranded cDNA was performed by the RNase H method described elsewhere (19). Homopolymer dG tails were added to cDNA that had been size selected on Sepharose 4BCL for a modal length of 1,700–1,900 nucleotides. Tailed cDNA was annealed to the dC-tailed vector pUC9 and transformed according to Hanahan (20). Approximately 30,000 original colonies were screened with ^{32}P-labeled nick-translated probes for C_β and C_α, and the longest cDNA clones were sequenced (21). All sequences were determined from both strands of DNA.

Southern Analysis. High molecular weight DNA was prepared as described previously (7), digested to completion with the specified restriction enzymes, and electrophoresed through 0.8% agarose for ~1,000 volt-hours. The DNA was transferred to nitrocellulose by the method of Southern (22) and hybridized at 42°C in 50% formamide as previously described (7) to DNA probes that were ^{32}P-labeled by nick translation or random priming. Washing the hybridized blots and stripping the probe from blots to be rehybridized were all performed as described elsewhere (7). V region–specific DNA probes were as follows: $V_\beta1$, a 350-bp fragment subcloned from 86T1 cDNA (23); $V_\beta3.1$, a 600-bp fragment subcloned from 2B4V_β (24); $V_\beta15$, a 350-bp fragment isolated from the C8β chain cDNA clone (7); $V_\beta16$, a 450-bp fragment from 4C3V_β (6); $V_\alpha8.8$, a 340-bp fragment isolated from the F4α chain cDNA clone described here; $V_\alpha10.3$, a 400-bp fragment subcloned from the D6α chain cDNA clone (8), and $V_\alpha11.1$, a 400-bp fragment subcloned from the 4C3α chain cDNA clone (6). To identify the sister clones among those T cells isolated from the same line, we used the $J_\beta1$, $J_\beta2$, $D_\beta1$, and $D_\beta2$ DNA probes described previously (7) to visualize all β chain gene rearrangements, whether aberrant or not. Only T cells with distinct DJ or VDJ rearrangements were considered independent and included in Tables 2 and 3. Furthermore, DNA samples from all clones found to have similar productive rearrangements were rerun on the same gel with DNA from the clone with the prototypical rearrangement. Thus, DNA samples from all the cells listed as having the 8.4-kb $V_\alpha11.1$ rearrangement were run together with DNA from clone C8 and found to share a rearranged band of the same electrophoretic mobility. DNA samples from cells listed as having small differences in the size of hybridizing bands (for example $V_\beta3.1$ hybridizing bands of 4.0, 4.3, 4.5 and 4.8 kb) were consistently distinguishable when run together on the same gel.

Results

TCR Gene Elements Used by Crossreactive pcc-specific T Cell Clones from F_1→Parent Radiation Chimeras. We began investigating the origin of the strain-dependent expression of TCR gene elements by analyzing the receptor genes used by cytochrome *c*–specific T cells in F_1→parent radiation chimeras. In these animals, a single pool of F_1[B10.AxB10.S(9R)] hematopoietic stem cells differentiates in an environment of either B10.A or B10.S(9R) parental origin. In both sets of animals, functional T cells and APC are of F_1 origin. The intrathymic cells responsible for tolerance induction are also of BM origin, so that both groups of animals should be tolerant of both B10.A and B10.S(9R) antigens (25, 26). MHC restriction specificity, however, follows the origin of the thymic epithelium, and T cells from F_1→B10.A animals will be largely restricted to B10.A MHC, and vice versa for T cells from F_1→B10.S(9R) chimeras (27–30). Thus, by analyzing the TCR expressed by pcc-reactive T cell clones from F_1→parent chimeras, we can dissect out those differences in TCR expression due solely to antigen presentation or tolerance induction from those based on the imposition of MHC restriction specificity.

This part of the analysis focuses on the pcc-specific T cell clones (phenotypes III and V) that are capable of responding maximally to antigen on both B10.A and B10.S(9R) APC. To isolate crossreactive pcc-specific clones for analysis, T cells from in vivo primed F_1→B10.A and F_1→B10.S(9R) radiation chimeras were cloned in vitro in the presence of pcc and the nonthymic, parental APC. Three phenotype III clones were derived from individual F_1→B10.A radiation chimeras. Two clones derived from individual F_1→B10.S(9R) chimeras exhibited the crossreactive specificity pattern characteristic of phenotype V, with a noticeable lack of the anti-I-E^k reactivity that is typical of phenotype V clones derived from B10.S(9R) animals. Fig. 1 depicts the ability of one such phenotype III and one such phenotype V clone to proliferate in response to pcc and mcc presented on both B10.A and B10.S(9R) APC.

To determine the TCR gene elements used, DNA isolated from each of these five clones was digested with the relevant restriction enzymes and hybridized with labeled DNA probes specific for $V_\alpha10$, $V_\alpha11$, $V_\beta1$, $V_\beta3.1$, $V_\beta15$, and $V_\beta16$. As shown in Fig. 1 and Table 2, genomic DNA samples from the three phenotype III clones of F_1→B10.A chimeric origin show a rearrangement pattern identical to that characteristic of phenotype III clones from B10.A mice. In fact, the β chain of clone C6 is identical nucleotide for nucleotide to that used by the clone B10 of B10.A origin (7; and Sorger, S. B., personal communication). The two phenotype V clones from F_1→B10.S(9R) chimeras use the $V_\beta J_\beta$ elements used by clones of similar reactivity pattern from B10.S(9R) mice, and not those characteristic of B10.A clones (Fig. 1, Table 2). How-

Table 2. *TCR Gene Usage in Cytochrome c-specific T Cell Clones From Radiation Chimeras*

Phenotype	Origin	T Cell Clone	$V_\alpha 11$ HIII	$V_\beta 3$ HIII	TCR gene elements used	
					α Chain	β Chain
			kb	*kb*		
I	$F_1 \rightarrow A$	AD10	2.4	9.2	$V_\alpha 11.1 + J_\alpha 84$	$V_\beta 3.1 + J_\beta 1.2$
	$F_1 \rightarrow A$	A6A.5	8.4	4.5	$V_\alpha 11.1 + J_\alpha 28$	$V_\beta 3.1 + J_\beta (2.3)$
	$F_1 \rightarrow A$	A6A.7	7.4	4.3	$V_\alpha 11 + J_\alpha ?$	$V_\beta 3.1 + J_\beta 2.5$
	$F_1 \rightarrow A$	A6A.9	8.4	4.3	$V_\alpha 11.1 + J_\alpha 28$	$V_\beta 3.1 + J_\beta 2.5$
	$F_1 \rightarrow A$	A6A.10	4.3	9.2	$V_\alpha 11 + J_\alpha ?$	$V_\beta 3.1 + J_\beta 1.2$
	$F_1 \rightarrow A$	A6A.23	8.4	4.8	$V_\alpha 11.1 + J_\alpha 28$	$V_\beta 3.1 + J_\beta (2.2)$
III				$V_\beta 16$ RI		
	$F_1 \rightarrow A$	2F9	2.4	6.0	$V_\alpha 11.1 + J_\alpha 84$	$V_\beta 16 + J_\beta 2.1$
	$F_1 \rightarrow A$	C6	2.4	6.0	$V_\alpha 11.1 + J_\alpha 84$	$V_\beta 16 + J_\beta 2.1$
	$F_1 \rightarrow A$	G4	2.4	6.0	$V_\alpha 11.1 + J_\alpha 84$	$V_\beta 16 + J_\beta 2.1$
IV				$V_\beta 15$ HIII		
	$F_1 \rightarrow A$	A6A.12	8.4	3.1	$V_\alpha 11.1 + J_\alpha 28$	$V_\beta 15 + J_\beta 2.4$
	$F_1 \rightarrow 9R$	G7	8.4	3.1	$V_\alpha 11.1 + J_\alpha 28$	$V_\beta 15 + J_\beta 2.4$
	$F_1 \rightarrow 9R$	C2	8.4	3.1	$V_\alpha 11.1 + J_\alpha 28$	$V_\beta 15 + J_\beta 2.4$
V*			$V_\alpha 8$ HIII	$V_\beta 1$ RI		
	$F_1 \rightarrow 9R$	F4	1.7	4.0	$V_\alpha 8.8 + J_\alpha 84$	$V_\beta 1 + J_\beta 1.2$
	$F_1 \rightarrow 9R$	F11	1.7	4.0	$V_\alpha 8.8 + J_\alpha 84$	$V_\beta 1 + J_\beta 1.2$
VI			$V_\alpha 8, 10, 11$ HIII, RI, Bam	$V_\beta 1, 3, 15, 16$ HIII, RI, Bam		
	$F_1 \rightarrow 9R$	R6R.12	Germline	Germline	Unknown	Unknown
	$F_1 \rightarrow 9R$	R6R.17	Germline	Germline	Unknown	Unknown
	$F_1 \rightarrow 9R$	R6R.25	Germline	Germline	Unknown	Unknown
VII			$V_\alpha 10$ Bam	$V_\beta 3$ HIII		
	$F_1 \rightarrow 9R$	R6R.1	1.8	5.0	$V_\alpha 10 + J_\alpha ?$	$V_\beta 3.1 + J_\beta (2.1)$

pcc-specific T cell clones from F_1[B10.AxB10.S(9R)]$\rightarrow$B10.A and from $F_1 \rightarrow$B10.S(9R) radiation chimeras are grouped into functional phenotypes as assessed by proliferation assays. Appropriately digested DNA from each clone was hybridized with $V_\alpha 8,10,11$ and $V_\beta 1,3,15$ and 16-specific DNA probes; only the rearrangements are noted for each clone. Restriction enzymes used were HindIII (HIII), EcoRI (RI), and BamHI (Bam). DNA samples from phenotype VI clones were digested with all three enzymes and hybridized with all seven probes. No rearrangements were seen, although all clones use the same member of the first J_β cluster in a potentially functional V_β-J_β rearrangement. TCR gene elements listed in brackets are estimated by the size of the rearranging fragment. All other elements correspond to rearrangements that have been previously identified by DNA sequencing. All clones labeled A6A were derived from the same chimera, as were all clones labeled R6R. All other clones were derived from separate individual chimeras. Only those clones shown to be independent by the criteria described in the Materials and Methods are listed. AD10, 2F9, C6, and G4 were cloned from lines derived from individual $F_1 \rightarrow$A chimeras and maintained in vitro on pcc and B10.S(9R) APC. All other clones of $F_1 \rightarrow$A origin were derived from the same line maintained on pcc plus B10.A APC. Clones G7, C2, F4, and F11 were derived from lines originating from individual $F_1 \rightarrow$B10.S(9R) chimeras and maintained in vitro on pcc and B10.A APC. All other clones of $F_1 \rightarrow$B10.S(9R) origin were isolated from the same line stimulated with pcc plus B10.S(9R) APC. Two independent isolates of A6A.5, A6A.10, A6A.12, R6R.12, and R6R.17 were analyzed as were seven isolates of clone R6R.1.

* Phenotype V with no anti-I-E_β^k reactivity.

Table 3. *TCR Gene Usage in Cytochrome c-specific T Cell Clones from F_1 Mice*

Clones maintained on:	T Cell Clone	Functional Phenotype	TCR gene elements used	
			α Chain	β Chain
B10.A APC	FAA.4	I	$V_\alpha 11$ + J_α?(2.2 kb HIII)	$V_\beta 3.1$ + $J_\beta 1.2$
	FAA.28	I	$V_\alpha 11$ + J_α?(3.7 kb HIII)	$V_\beta 3.1$ + $J_\beta 2.5$
	FAA.38	I	$V_\alpha 11.1$ + $J_\alpha C7$	$V_\beta 3.1$ + $J_\beta(2.7)$
	FAA.47	I	$V_\alpha 11.1$ + $J_\alpha 84$	$V_\beta 3.1$ + $J_\beta 2.5$
	FAA.2	IV	$V_\alpha 11.1$ + $J_\alpha 28$	$V_\beta 15$ + $J_\beta(2.1)$
	FAA.25	IV	$V_\alpha 11.1$ + $J_\alpha 28$	$V_\beta 15$ + $J_\beta 1.1$
B10.S(9R) APC	FAR.4	V	$V_\alpha 10$ + J_α?(1.8 kb Bam)	$V_\beta 1$ + $J_\beta 1.2$
	FAR.21	V	$V_\alpha 8$ + J_α?(12 kb HIII)	$V_\beta 1$ + $J_\beta 1.2$
	FAR.30	V	$V_\alpha 10$ + J_α?(1.8 kb Bam)	$V_\beta 1$ + $J_\beta 1.2$
	FAR.2	VII	$V_\alpha 8$ + J_α?(12 kb HIII)	V_β unknown + $J_\beta 2$
	FAR.10	VII	$V_\alpha 8$ + J_α?(12 kb HIII) $V_\alpha 11$ + J_α?(3.7 kb HIII)	V_β unknown + $J_\beta 2$
	FAR.11	VII	$V_\alpha 8$ + J_α?(12 kb HIII)	V_β unknown + $J_\beta 1$

Clones were isolated from lines derived from in vivo primed F_1[B10.A × B10.S(9R)] mice and maintained on parental APC plus pcc for four rounds of stimulation before cloning on F_1 APC. TCR gene usage was determined by Southern analysis of DNA digested with one of three enzymes and probed with the three V_α and four V_β genes known to characterize the parental pcc-specific T cell response. Only T cell clones known to be independent (as described in Materials and Methods) are listed. α Chain rearrangements not previously sequenced are noted in parentheses by the size of the fragment and the enzyme used for digestion. In FAR.10, two potentially functional V_α gene rearrangements were detected. The 3.7-kb HindIII fragment of FAR.10 DNA hybridizing with the $V_\alpha 11.1$ probe is also found in E2, a clone of B10.A origin (7). The 1.8-kb BamHI fragment hybridizing with the $V_\alpha 10$ probe is also found in clones of F_1→B10.S(9R) origin (Table 2). The J_β elements in parentheses are estimated by the size of the rearranging fragment. The phenotype VII clones use V_β elements distinct from $V_\beta 1$, 3.1, 15, and 16, and each has a potentially functional rearrangement to one of the two J_β clusters.

ever, $V_\alpha 10$, the variable region gene expressed by phenotype V clones from B10.S(9R) mice, is not expressed by the F_1→B10.S(9R) clones. To identify the V_α used by these T cell clones, a cDNA library was constructed using clone F4 poly(A)$^+$ mRNA as a template. Clones hybridizing to C_α (or to C_β) were selected and the longest ones were sequenced. The junctional regions of the α chain and β chain cDNA sequences from T cell clone F4 have been published (Fig. 2, *a* and *b* in reference 10). Clone F4 expresses $V_\alpha 8.8$, a new member of the $V_\alpha 8$ family (31–33), and $J_\alpha 84$, a J_α region commonly expressed by pcc-specific T cells from B10.A mice. $V_\alpha 8.8$ has a maximum of 23–25% amino acid homology to both $V_\alpha 10$ and $V_\alpha 11$ (10). The use of a new V_α gene, not closely related to either $V_\alpha 11$ or $V_\alpha 10$, may be a result of tolerance induction, that is the selection in F_1→B10.S(9R) chimeras against phenotype V clones recognizing B10.A APC in the absence of foreign antigen.

These results show that in F_1→parent chimeras, phenotype III and V clones, that are no longer distinguishable by the anti-B10.A alloreactivity of phenotype V clones from B10.S(9R) mice, nevertheless are "imprinted" with the TCR gene element usage characteristic of the parental host.

TCR Gene Elements Used by Noncrossreactive pcc-specific T Cell Clones from F_1→Parent Radiation Chimeras. By focusing on the TCR elements used by clones other than those belonging to phenotypes III and V, we hoped to identify the forces operating to mold the repertoire of TCR recognizing ligands peculiar to either B10.S(9R) or B10.A. As can be seen from Table 2, the pcc-specific clones derived from F_1→B10.A chimeras fall into the same phenotypic categories characteristic of B10.A clones, with the expected absence of anti-I-A^s-reactive phenotype II clones. Five of six clones isolated from lines of F_1→B10.A T cells maintained on antigen plus B10.A APC belong to phenotype I; the sixth is a phenotype IV T cell clone. A single phenotype I T cell was cloned from a F_1→B10.A line maintained first on B10.A and then on B10.S(9R) APC. Presumably, a small amount of reactivity to antigen on B10.S(9R) APC permitted the continued stimulation of this clone. The TCR gene elements encoding the receptors of these clones are indistinguishable from those used by B10.A T cell clones of the same phenotype. Phenotype I clones use mainly $V_\alpha 11.1$ and $J_\alpha 84$ or 28. Two new rearrangements have also been identified with the $V_\alpha 11$ probe, corresponding either to the use of a new member of the $V_\alpha 11$ family, a new J_α element, or both. The rearranged β chain genes of F_1→B10.A phenotype I clones are similarly familiar, $V_\beta 3.1$ plus $J_\beta 1.2$, 2.5, and two new J_β elements estimated to be $J_\beta 2.2$ and 2.3. Phenotype IV clones from F_1→B10.A chimeric mice are indistinguishable from those derived from B10.A parental animals, both in terms of TCR gene rearrangement and fine specificity of antigen/MHC recognition (data not shown, and Table 2). Thus, even in animals

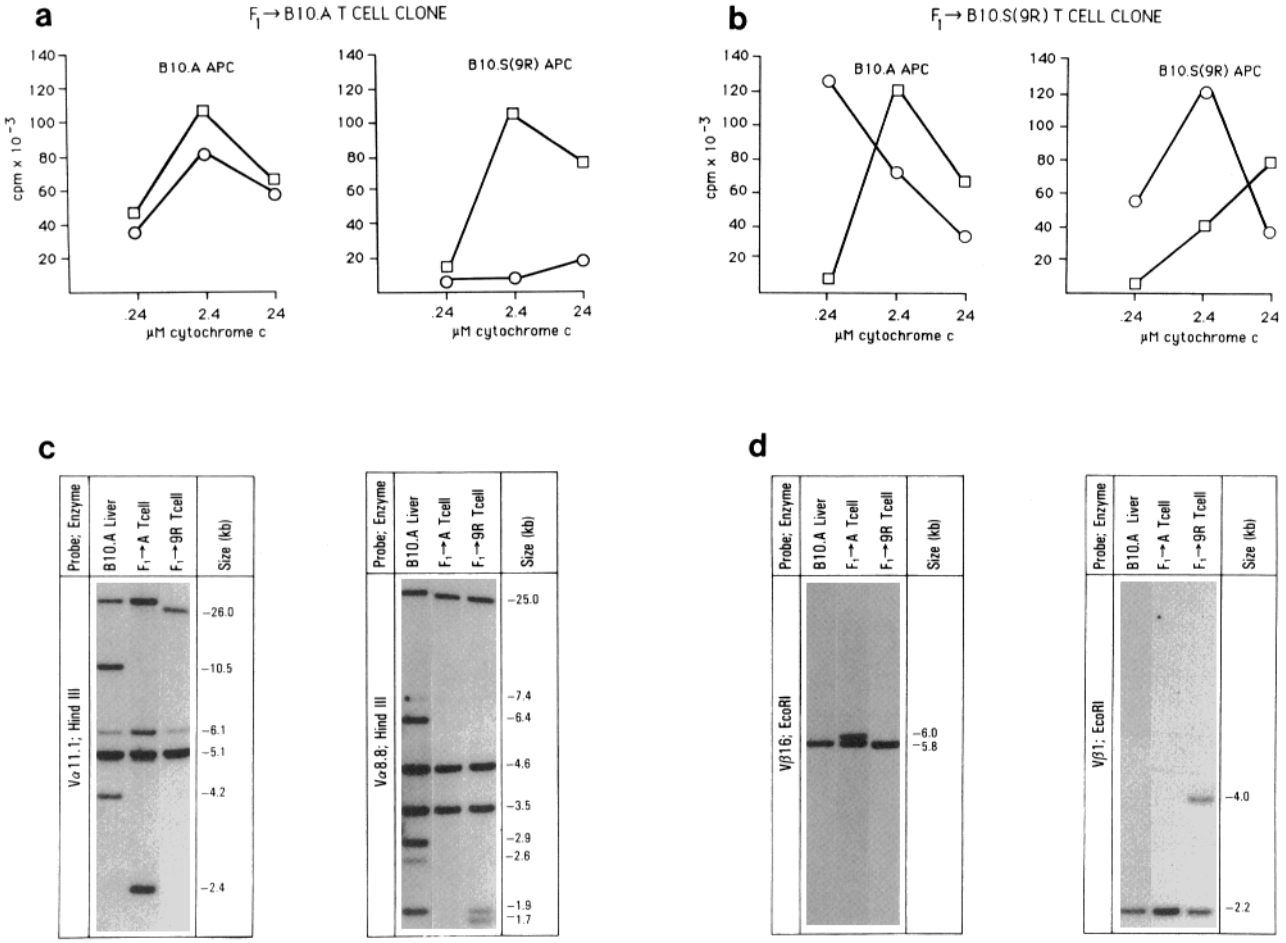

Figure 1. Antigen-specific proliferation and TCR gene rearrangements in crossreactive T cell clones from F_1→parent chimeras. (*A* and *B*) Proliferation of T cells in response to increasing doses of cytochrome *c* peptide: (□) pcc 88–103 and (○) mcc 88–104. Values plotted are cpm of [^{3}H]TdR incorporated in a 12–18-h pulse begun on the third day of coculture of 2×10^4 T cells with peptide and 5×10^5 irradiated B10.A or B10.S(9R) spleen cells as a source of APC. (*A*) Clone 2F9, a phenotype IIIA T cell clone of F_1→B10.A origin. Proliferation on B10.A APC in the absence of peptide = 1,860 cpm, to B10.S(9R) APC = 1,140 cpm. (*B*) Clone F11, a phenotype V clone (lacking anti-I-$E\beta^k$ reactivity) of F_1→B10.S(9R) origin. Proliferation to B10.A APC alone = 60 cpm; to B10.S(9R) = 140 cpm. (*C* and *D*) Southern analysis of genomic DNA from B10.A liver and the T cell clones 2F9 (F_1→B10.A origin) and F11 (F_1→B10.S(9R) origin). (*C*) DNA was digested with HindIII and hybridized with a V_α11-specific probe (*left panel*), stripped, and rehybridized with a V_α8.8 specific probe (*right panel*). Rearranged bands are a 2.4-kb V_α11.1 hybridizing band in the F_1→B10.A T cell and a 1.7-kb V_α8.8 hybridizing band in the F_1→B10.S(9R) T cell clone. (*D*) DNA was digested with EcoRI and hybridized with a V_β16-specific probe (*left panel*), stripped, and rehybridized with a V_β1-specific probe (*right panel*). Rearranged bands are a 6.0-kb V_β16 hybridizing band in the F_1→B10.A T cell and a 4.0 kb V_β1 hybridizing band in the F_1→B10.S(9R) T cell clone. Each panel is a composite figure of lanes taken from a full width gel. The noticeable difference in electrophoretic mobility of particularly the high molecular weight germline bands is due to a "frown" enhanced by the removal of irrelevant lanes.

that are tolerant of and can present antigen in the context of both I-$E\beta^k$ and I-$E\beta^s$, possession of an I-$E\beta^k$-bearing thymus imprints the B10.A pattern of TCR expression on cytochrome *c*–reactive T cells.

The neat picture painted so far by the analysis of F_1→B10.A clones is not reproduced in the analysis of clones derived from F_1→B10.S(9R) chimeras. Prototypical phenotype IV T cells have not been cloned from B10.S(9R) mice, nor have the TCR rearrangements characteristic of this phenotype been detected, even in oligoclonal pcc-reactive lines from B10.S(9R) animals (34). Nevertheless, T cells of this phenotype carrying the predicted V_α11.1 and V_β15 rearrangements have been cloned from F_1→B10.S(9R) chimeras (Table 2). The greater reactivity of these clones to antigen on B10.A APC (7, 34), a fact which prompted us to categorize these cells as "noncrossreactive," leads us to suspect their induction requires the in vivo and/or in vitro presentation of antigen in the context of I-$E\beta^k$. This requirement would be met in F_1 (see below) and F_1→B10.S(9R) animals, but not in the B10.S(9R) parent. Thus, the full potential of positive selection may be limited by the conditions of immune induction.

Our analysis of phenotype VI clones from F_1→B10.S(9R) chimeras has revealed that neither the α nor β chain gene

rearrangements resemble any so far identified for pcc-specific T cells from either B10.A or B10.S(9R) parent. Each of these clones has, however, a potentially productive rearrangement to the same member of the first J_β cluster (data not shown). And finally, the TCR gene elements rearranged in the single phenotype VII clone of $F_1 \rightarrow$B10.S(9R) origin are distinct from those used by clones from B10.S(9R) mice (Table 2). A different member of the $V_\alpha 10$ family and/or different J_α element is used in each case. More surprisingly, this clone uses a new rearrangement of $V_\beta 3.1$, a V_β element previously confined to pcc-specific clones from B10.A mice. This combination of $V_\alpha 10$ and $V_\beta 3$ rearrangements encodes a receptor that does not appear to recognize pcc on I-E_β^k. Furthermore, these rearrangements are selected against in lines of T cells from $F_1 \rightarrow$B10.S(9R) animals maintained first on B10.S(9R) APC and then on B10.A APC (see below). At least some of this "atypical" behavior among phenotype VI and VII clones could be due to the small number of such clones analyzed from both B10.S(9R) and $F_1 \rightarrow$B10.S(9R) mice. However, the appearance both of clones of unexpected phenotype and of clones of predicted phenotype with unexpected rearrangements of TCR genes indicates that the rules of TCR expression within the cytochrome *c* system are not solely dependent upon the thymus in which T cells differentiate, and therefore a function of MHC restriction specificity. The overlying influences of tolerance induction and antigen presentation require further analysis.

TCR Gene Elements Used by pcc-specific T Cell Clones from F_1 Mice. The data presented in Table 3 provide a clear example of how in vitro antigen presentation can skew the TCR repertoire of the population of responding T cells. pcc-specific clones derived from lines of F_1 T cells maintained on antigen plus B10.A APC belong to phenotypes I and IV and exhibit TCR gene rearrangements typical of B10.A T cell clones of the same phenotype. Of the 35 clones initially screened, none exhibited the response pattern characteristic of phenotype III, a finding for which we have no good explanation. We have, however, seen expression of $V_\beta 16$ in oligoclonal lines of cytochrome *c*–specific T cells from F_1 mice (data not shown). Clones derived from antigen-specific lines maintained on B10.S(9R) APC belong to phenotypes V and VII. The phenotype V clones from F_1 mice rearrange $V_\beta 1$ to $J_\beta 1.2$, as do clones of the same phenotype from both B10.S(9R) and $F_1 \rightarrow$B10.S(9R) animals. The α chain used by these clones involves either $V_\alpha 10$ or $V_\alpha 8$. Two of the phenotype VII clones from F_1 mice use the same rearrangement of $V_\alpha 8$ found in an F_1 phenotype V clone, whereas a third has both this $V_\alpha 8$ rearrangement and a rearrangement of $V_\alpha 11$ (see Table 3, clone FAR.10). We have not determined which of these rearrangements is productive. The V_β gene element(s) used by all three of these clones has not been identified.

These results demonstrate that in vivo, pcc-primed F_1 T cells maintained in vitro on antigen and B10.A APC generate clones that resemble B10.A T cells in terms of functional phenotype and TCR gene element usage. That same pool of cells maintained in vitro on antigen and B10.S(9R) APC resemble the B10.S(9R) response in these two properties. Thus, cytochrome *c*–specific T cells from F_1 mice can express each of the $V\alpha$ and $V\beta$ gene elements expressed by T cells from parental animals. The frequency of cells bearing particular rearrangements is drastically altered by the origin of the APC on which the T cells are maintained in vitro. These data should be contrasted with those obtained from T cells derived from chimeric animals and maintained on either parental type APC (see Table 5 below). T cells from $F_1 \rightarrow$B10.A chimeras maintained on B10.S(9R) APC do not follow the B10.S(9R) pattern of antigen specificity and TCR expression. These clones belong instead to phenotype III, with the $V_\alpha 11.1 + J_\alpha 84$, $V_\beta 16 + J_\beta 2.1$ rearrangements typical of phenotype III T cells from B10.A mice. And vice versa, $F_1 \rightarrow$B10.S(9R) T cells maintained on B10.A APC are either phenotype IV (with the TCR gene rearrangement found in all mice examined so far) or phenotype V, using B10.S(9R)-like TCR gene elements.

TCR Gene Elements Used by Oligoclonal Lines of pcc-reactive T Cells from F_1 and Chimeric Mice. Lines of antigen-reactive T cells were analyzed for TCR gene element usage to determine whether those conclusions drawn from data gathered from pcc-specific clones are generalizable or pertinent only for those specific clones analyzed. Aside from substantiating our earlier data that the TCR repertoire of pcc-specific T cells from $F_1 \rightarrow$B10.A animals resembles that of the B10.A parent, and vice versa for T cells from $F_1 \rightarrow$B10.S(9R) chimeras, these analyses demonstrate that B10.S(9R) APC are able to select in vitro for cells expressing $V_\beta 15$ (Table 4). Thus, $V_\beta 15$ expression is markedly enhanced in 3 out of 3 lines of T cells from primed $F_1 \rightarrow$B10.A chimeras maintained in vitro for four rounds of stimulation on pcc plus B10.A APC and then for three to four rounds of stimulation on pcc plus B10.S(9R) APC. Similarly, one of two $F_1 \rightarrow$B10.S(9R) lines maintained on pcc plus B10.S(9R) APC expresses $V_\beta 15$, as does one of two F_1 lines similarly maintained (Table 4). In the three cross-stimulated $F_1 \rightarrow$B10.A lines this enhancement of $V_\beta 15$ expression is associated with an increase in reactivity to pcc on B10.A(5R) APC, the hallmark of phenotype IV T cells (Table 4). It thus seems likely that in vitro presentation of pcc on B10.S(9R) APC can activate phenotype IV, $V_\beta 15$-expressing T cells. This activation may be inefficient, thus explaining why some lines (including the primary source for the clones analyzed above) do not demonstrate this activation.

Discussion

One of the first steps along the pathway leading from incoming thymic immigrant to exiting mature thymocyte is the rearrangement of the genes encoding the TCR (35, 36). Possible incompatibilities in pairing between α and β chains aside, it is thought that the population of immature thymocytes expresses the full combinatorial range of α and β chains encoded in the genome. Achieving a peripheral lymphoid population of self MHC-restricted, self tolerant, immunocompetent T cells involves a careful molding of this "universal repertoire" by influences operating both intra- and extrathymically (25–29). Precisely how the thymus can select both *for* cells that can recognize self MHC plus a foreign peptide

Table 4. *B10.S(9R) APC Can Stimulate $V_\beta 15$ Expressing Cytochrome c–specific T Cells in Vitro*

Line	Origin and history	Reactivity* A	Reactivity* 9R	Reactivity* 5R	$V_\beta 15$ rearrangement‡ HIII band	$V_\beta 15$ rearrangement‡ Intensity
A5A4	$F_1 \rightarrow$A; 4 stimulations on A	M = P	M > P	M > P	3.7 kb	+
		+ + +	+ +	+	3.1 kb	+
A5A4R4	$F_1 \rightarrow$A; 4 stimulations on A	M > P	M > P	M > P	3.7 kb	+ +
	4 stimulations on 9R	+ + +	+ +	+ +	3.1 kb	+ +
A7A4	$F_1 \rightarrow$A; 4 stimulations on A	M > P	M > P	M >> P	Germline	–
		+ + +	+	+ +		
A7A4R4	$F_1 \rightarrow$A; 4 stimulations on A	M > P	M > P	M > P	2.8 kb	+ + +
	4 stimulations on 9R	+ + +	+ +	+ +		
A8A4	$F_1 \rightarrow$A; 4 stimulations on A	M = P	M = P	M > P	Germline	–
		+ + +	+ +	+ +		
A8A4R3	$F_1 \rightarrow$A; 4 stimulations on A	M = P	M = P	M > P	8.5 kb	+ +
	3 stimulations on 9R	+ + +	+ + +	+ + +	2.4 kb	+ +
R5R4	$F_1 \rightarrow$9R; 4 stimulations on 9R	M	M = P	M	8.5 kb	+
		+/–	+ +	+/–	6.0 kb	+/–
					3.1 kb	+/–
FCR	F_1(A × 9R); 9 stimulations on 9R	P > M	P > M	M = P	8.5 kb	+ + +
		+/–	+ + +	+/–		

Lines of T cells were established from pcc-primed mice and maintained on pcc and the indicated type of APC. The lines derived from $F_1 \rightarrow$B10.A chimeras are listed as pairs of parent and daughter lines, the latter having been cross-stimulated on B10.S(9R) APC for the indicated number of rounds. Each parent and daughter pair was derived from separate individual chimeras.

* Lines were tested for antigen/MHC reactivity as for Table 1, where A, 9R, and 5R refer to B10.A, B10.S(9R), and B10.A(5R) APC respectively. The pluses refer to the level of reactivity on the indicated APC.

‡ $V_\beta 15$ rearrangement was assessed by Southern analysis of HindIII-digested DNA isolated from the same pool of cells assayed for proliferation. The DNA samples were all run on the same gel, and quantitated both by ethidium bromide staining and C_β hybridization. The pluses refer to the relative intensity of the bands, following hybridization with the ^{32}P-labeled $V_\beta 15$-specific DNA probe.

and *against* cells that can recognize self MHC alone is a "central enigma" of immunology which has yet to be satisfactorily resolved. However, recent studies have confirmed that these selective forces operate on the basis of TCR specificity. Thus, cells expressing V_β genes whose products are known to confer reactivity to I-E, Mls-1^a, and Mls-2^a are deleted intrathymically in mice expressing these antigens (37–46). Similarly, in mice transgenic for rearranged TCR α and β chain genes, expression of the antigen/MHC ligands recognized by those combinations of α and β chain gene products results in depletion of the pool of TCR-expressing thymocytes (47, 48). Studies using unmanipulated, I-E transgenic or TCR transgenic mice have similarly provided evidence at the molecular level for the positive selection, or the rescue from programmed cell death, of self MHC-restricted cells (47–56). While such studies have provided unequivocal examples of positive and negative selection, they have tended to analyze these influences in isolation. The present study extends these analyses to the complete repertoire of T cells specific for a non-"superantigen" from mice that have not been genetically manipulated. Our results provide further molecular evidence for the positive and negative selection of cells on the basis of TCR expression, and also demonstrate, for the first time, the influence of both in vivo and in vitro antigen presentation on TCR expression.

Positive Selection Molds the Cytochrome c–specific TCR Repertoire. To focus on the role played by the positive selection for MHC restriction specificity on the shaping of the TCR repertoire, we have compared the TCR genes rearranged in pcc-specific T cells from F_1[B10.A × B10.S(9R)]$\rightarrow$B10.A and $F_1 \rightarrow$B10.S(9R) radiation chimeras. Both groups of animals are tolerant of and present antigen in the context of both B10.A and B10.S(9R) MHC gene products. However, these two sets of chimeras differ in the MHC antigens expressed by radioresistant (epithelial) components of the thymic microenvironment, those components known to im-

pose self MHC restriction specificity (30). Our data, summarized in Table 5, show that crossreactive phenotype III/V clones from $F_1 \rightarrow$B10.A mice use the TCR genes used by B10.A and not B10.S(9R) mice. In contrast, phenotype III/V clones of $F_1 \rightarrow$B10.S(9R) origin use the β chain genes used by B10.S(9R) and not B10.A mice. Thus, three of three phenotype III clones of $F_1 \rightarrow$B10.A origin rearrange Vβ16, as do five of five B10.A T cell clones of the same phenotype. Both phenotype V clones of $F_1 \rightarrow$9R origin use Vβ1 as do three of three phenotype V clones of B10.S(9R) origin. It is interesting that while the induction of tolerance eliminates the anti-B10.A reactivity of phenotype V clones, blurring the distinction in antigen/MHC recognition between phenotypes III and V, the selection of TCR gene expression in clones of these phenotypes from radiation chimeras is as stringent as it is in the parental animals.

Negative Selection Molds the Cytochrome c-specific TCR Repertoire. In searching for an example of how negative selection or tolerance induction influences TCR used by antigen-specific T cells, we compared the TCR repertoire of alloreactive,

Table 5. *Summary of TCR Gene Usage in Cytochrome c-specific T Cells From Parental, F_1, and Chimeric Mice*

Phenotype	TCR gene element	B10.A	B10.S(9R)	F_1[B10.A × B10.S(9R)]		$F_1 \rightarrow$B10.A		$F_1 \rightarrow$B10.S(9R)	
		A APC	9R APC	A APC	9R APC	A APC	9R APC	A APC	9R APC
I	V_α	11		11		11	11.1‡		
	J_α	84, 28, 2B4, ?	nf*	C7, 84, ??	nf	28, ??	84	nf	nf
	V_β	3.1		3.1		3.1	3.1		
	J_β	1.2, 2.5		1.2, 2.5, 2.7		1.2, 2.2, 2.3, 2.5	1.2		
III	V_α	11.1					11.1		
	J_α	84	nf	nf(l)§	nf	nf	84	nf	nf
	V_β	16					16		
	J_β	2.1					2.1		
IV	V_α	11.1		11.1		11.1		11.1	
	J_α	28	nf	28	nf(l)	28	nf(l)	28	nf(l)
	V_β	15		15		15		15	
	J_β	1.1, 2.4		1.1, 2.1		2.4		2.4	
V	V_α		10.3		10, 8			8.8	
	J_α	nf	C7	nf	??	nf	nf	84	nf(l)
	V_β		1		1			1	
	J_β		1.2		1.2			1.2	
VI	V_α	nf	?	nf	nf	nf	nf	nf	?
	J_α		?						?
	V_β		1						?
	J_β		1.2						1.X
VII	V_α	nf	10.4	nf	8(11)				10
	J_α		61		?	nf	nf	nf	?
	V_β		1		?				3.1
	J_β		2.1		1.X, 2.X				2.1

Summary of TCR gene usage in pcc-specific T cells of the indicated phenotypic categories derived from F_1, parental, and chimeric mice and maintained in vitro with pcc and the indicated APC. Data are summarized from Tables 2–4.

* nf, Not found.

‡ Only a single clone was analyzed in this category, with minimal reactivity to pcc plus B10.S(9R) APC.

§ nf(l), Prototypical gene rearrangements have been found in lines but not clones of cells. The lines were maintained as follows: F_1 on pcc plus first B10.S(9R) then B10.A APC for phenotype III TCR rearrangements; F_1 on pcc plus B10.S(9R), $F_1 \rightarrow$B10.A on pcc plus B10.A then B10.S(9R) APC, and $F_1 \rightarrow$B10.S(9R) on pcc plus B10.S(9R) APC for phenotype IV rearrangements; $F_1 \rightarrow$9R on pcc plus B10.S(9R) APC for phenotype V TCR rearrangements.

phenotype V clones from B10.S(9R) mice with that of phenotype V clones tolerant of B10.A from F_1 and $F_1 \rightarrow$B10.S(9R) mice. While the gene elements encoding the β chains of the two groups of phenotype V T cell clones are the same, the α chain genes are not (see Table 5 for summary). The phenotype V T cell clones from $F_1 \rightarrow$B10.S(9R) chimeras rearrange $V_\alpha 8.8$ to $J_\alpha 84$, a rearrangement not seen either in lines or clones of B10.S(9R) T cells. $V_\alpha 8.8$ expression in pcc-specific T cells is correlated both with thymic expression of B10.S(9R) MHC antigens and with tolerance to both B10.A and B10.S(9R) antigens. However, $V_\alpha 10$ (and in particular, $V_\alpha 10.3$ + $J_\alpha C7$) is expressed even in tolerant F_1 and chimeric animals (Table 5 and data not shown). Thus, cells expressing α chains similar to those expressed by alloreactive phenotype V T cells are not deleted in tolerant animals. The subtlety in these examples of the influence of tolerance induction on TCR gene expression relative to previously published findings (37–48, 57) is not surprising. In these latter cases, V_β expression, regardless of V_α J_α or J_β expression, is correlated with recognition of "superantigens" such as Mls and staphylococcal enterotoxin, and it is the expression of this V_β alone that is highly regulated (40–44, 46, 57). Among T cells recognizing I-E + pcc, the expression of all gene elements is highly regulated, suggesting that both α and β chains are crucial in the recognition of this ligand (6–10). Thus, selection against pcc-reactive T cells that also recognize MHC (plus a self peptide?) would be expected to operate not solely at the level of V_α or V_β expression, but on the combination of all gene elements and junctional regions. The observation that the loss of anti-B10.A reactivity is associated with more significant changes in the α chain than in the β chain of the TCR of phenotype V T cells is interesting in light of our previous findings that anti-I-A^s alloreactivity in phenotype II T cells is correlated with $J_\alpha C7$ expression (6). This provides another example of the relationship between α chain expression and alloreactivity among T cells specific for cytochrome *c*.

Antigen Presentation In Vitro Molds the Cytochrome c-specific TCR Repertoire. Concrete examples of this type of influence are most simply derived by comparing the repertoire of pcc-reactive F_1 T cells maintained in vitro on B10.A APC to that of T cells maintained on B10.S(9R) APC. With the noticeable absence of phenotype III cells, clones derived from F_1 lines maintained on B10.A APC resemble B10.A clones in phenotype and TCR usage. Prototypical phenotype III reactivity and TCR usage have been seen in oligoclonal lines of F_1 cells maintained first on B10.S(9R) and then on B10.A APC (data not shown). Clones derived from F_1 lines maintained on B10.S(9R) APC instead resemble parental B10.S(9R) clones in phenotype and TCR usage. These findings are also of interest in demonstrating that the TCR positively selected by the thymic epithelium (plus a self peptide?) can be distinguishable from those selected by APC plus pcc. A similar type of antigen-driven selection at the level of TCR gene usage in cytochrome *c*–specific T cells from B10.A and B10.S(9R) mice has been documented previously in the cytochrome *c* system (7, 8, 34).

Antigen Presentation In Vivo Molds the Cytochrome c-specific TCR Repertoire. Documenting an example of this type of influence requires an analysis of the appearance of phenotype IV clones, that recognize pcc on I-$E_\beta^{k,s,b}$. Clones of this phenotype, expressing the prototypical $V_\alpha 11$ + $J_\alpha 28/V_\beta 15$ TCR, have been isolated from B10.A mice and from F_1, $F_1 \rightarrow$B10.A, and $F_1 \rightarrow$B10.S(9R) T cells maintained on B10.A APC. The absence of such clones from B10.S(9R) animals and from T cells from the latter three sets of animals maintained on B10.S(9R) APC suggests either that these cells require stimulation in vitro by the presentation of pcc on B10.A APC, or that their induction requires in vivo presentation on B10.A APC. The latter explanation is made more likely by our observations that lines of T cells from F_1 and chimeric animals maintained in vitro on B10.S(9R) APC do express $V_\beta 15$, and that phenotype IV clones can be maintained for at least several rounds of stimulation by pcc and B10.S(9R) APC (unpublished observations). Our failure to clone such cells may reflect the relative inefficiency of their stimulation by pcc presented in the context of I-E_β^s. It may be possible to resolve this issue more clearly by priming F_1 T cells to cytochrome *c* by adoptive transfer into parental hosts expressing either I-E_β^k or I-E_β^s (58). The observation that a B10.S(9R) thymus can positively select $V_\beta 15$-expressing phenotype IV cells that can either be induced or not by immunization with antigen illustrates how in vivo antigen presentation can "override" a thymic influence. Thus, while positive and negative intrathymic selection are currently well recognized to influence the TCR repertoire, antigen selection forms a no less significant facet of this process.

Our data demonstrate that even for the T cell response to cytochrome *c*, a response that is highly restricted both by functional phenotype and TCR usage, the generation of the TCR repertoire is a highly complicated process. The forces that combine to influence the generation of such a repertoire include the rescue from programmed cell death of cells bearing receptors with appropriate affinity for self MHC, the depletion of cells bearing receptors capable of inappropriate recognition of MHC plus self peptide, and the clonal expansion of immunocompetent cells by the presentation of exogenous antigens in the context of self MHC. It is only by analyzing the entire antigen-specific TCR repertoire that the interplay of these forces can be appreciated.

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