MHC Control of the Naive TCR α-Chain Repertoire[1]

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The naive T cell repertoire is shaped by interactions between developing thymocytes and thymic stroma. Both positive and negative selection involve the clonotypic TCR and MHC molecules carrying self-peptides. Except for the MHC-dependent effects of superantigens on TCR Vβ usage, there has been little evidence that the TCR structure of naive T cells varies with the selecting MHC products. To examine this point from another angle, in particular the TCR α-chain, we have analyzed α-chain usage in a system in which the vast majority of T cells express a transgene-encoded TCR β-chain, compatible with efficient T cell development on a wide range of MHC haplotypes. Endogenous TCR α-chains are thus selected without interference from the forces known to act on TCR-β, permitting us to observe MHC influences on α-chain selection. We have used Vα-specific Abs to quantitate α-chain usage in MHC congenic, MHC recombinant, and MHC transgenic mice and provide evidence that the naive TCR α-chain repertoire is under MHC control. The data demonstrate a direct impact of known MHC class II products but also reflect more complex influences, apparently involving other gene products within the MHC. Sequence analysis of differentially selected TCR suggests that selection acts on the entire α-chain, including Vα, Jα, and the junctional region. *The Journal of Immunology,* 1994, 153: 3005.

The diversity of the αβ T cell repertoire arises from the somatic rearrangement of multiple gene segments encoding TCR V, D, and J domains. During the joining of these segments, additional variability is contributed by the removal and/or template-independent addition of nucleotides. Structural considerations and experimental evidence suggest that the resulting V-(D)-J junction (which corresponds to the CDR3 region of Igs) directly contacts antigenic peptides in the MHC peptide binding site (for review and references, see Ref. 1). The V-(D)-J domains of both TCR α- and β-chains determine the fine specificity of MHC-restricted peptide recognition, and the selection of peripheral T cells during an immune response acts on both TCR α- and β-chains, often recruiting TCR with particular TCR Vα and Vβ segments and related junctional sequences (reviewed in Ref. 2). This is perhaps best illustrated by the preferential use of Vα11 and Vβ3, usually in combination with a limited set of J segments, by cytochrome *c*-reactive T cells in B10.A mice (1) and by the observation that exposure to Ag of TCR single chain transgenic mice can select from the endogenous repertoire TCR chains similar (or identical) to the original partner (3, 4).

That TCR α- and β-chains contribute in a similar fashion to the MHC-controlled generation of the naive TCR repertoire during thymic selection seems likely (5) but is not easily verified using current approaches: in most examples studied, the TCRs of interest are either defined by function not structure (e.g., Ref. 6) or represent monospecific transgenic systems with little flexibility beyond a selection/no selection outcome (reviewed in Ref. 7). Thymically expressed MHC molecules apparently can bias the fine specificity of T cells elicited by intentional priming (8), but the prediction that the CDR3 sequences responsible for these differences might be determined during thymic selection (9) could not be confirmed when TCR rearrangements were sequenced in naive mice (10).

Several TCR Vα families, including Vα3.2 (11, 12), Vα11 (12, 13), Vα2 (12, 14), and Vα8 (15), are preferentially expressed by either CD4 or CD8 T cells and their frequencies show mouse strain-dependent variations (12–14, 16). Although these observations have been taken to indicate that Vα families are subject to positive or negative selection by MHC class I and/or class II molecules (11–16), an important caveat to this conclusion stems from the variability of the TCR β-chains in these experimental

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setups: because of superantigens and other, less well-defined factors, TCR Vβ families are subject to strong selective forces, both positive (e.g., Ref. 17) and negative (e.g., Ref. 18); the resulting TCR-β repertoire changes may then affect α-chain selection indirectly, because not all TCR chains pair equally well (19, 20).

To circumvent this problem, we have analyzed the repertoire of rearranged TCR α-chains in a situation where the TCR β-chain is fixed, an approach conceptually similar to that of Jorgensen et al. (3) and Ivars (21), who used single chain TCR transgenic mice to investigate determinants of peptide recognition and of MHC class preference. We employ TCR single chain transgenic mice in which the vast majority of T cells share the expression of a β-chain chosen because it is compatible with the efficient development of CD4 single-positive thymocytes and peripheral T cells on all MHC haplotypes tested (see below). These features allow us to investigate the MHC dependence of TCR-α selection independent of selective forces acting on TCR-β. We find that the H-2^k class II region is associated with a striking overrepresentation of one TCR V gene family, Vα11, in MHC congenic and MHC recombinant strains. Using an Eα MHC transgene, we show that this effect is controlled, at least in part, by the expression of class II heterodimers. On sequence analysis, the selected TCR α-chains have distinctive features beyond shared Vα usage, suggesting that the MHC control of the naive TCR-α repertoire described in this study acts via MHC/peptide complexes rather than superantigen-like structures.

Materials and Methods

Mice

Animals were kept and treated according to EEC guidelines. Transgenic mice expressing only the TCR Vβ-chain (Vβ8.2, Jβ2.1) of the T cell clone V2.1, specific for PR8 influenza hemagglutinin 111–119 in the context of H-$2E^d$ (22), were derived as described (23). Founders (C57BL/6 × DBA/2) were crossed initially to C57BL/6 (B6, H-2^b) and B10.GD (H-2^{g2}) mice for two generations. H-2 homozygous progeny was identified by immunofluorescence staining (see below) of PBMCs (prepared from tail vein blood by Ficoll-Hypaque centrifugation; Pharmacia Biotech Inc., Uppsala, Sweden). Mice transgenic for TCR-β were crossed further to C57BL/6 (B6, H-2^b), B10 (H-2^b), B10.GD (H-2^{g2}), B10.G (H-2^q), B10.S (H-2^s), B10.A (H-2^a), B10.D2 (H-2^d), B10.BR (H-2^k), B10.A(4R) (H-2^{h4}), B10.A(5R) (H-2^{i5}), B10.A(3R) (H-2^{i3}), or B10.AQR (H-2^{y1}) strains (Harlan Olac, Bichester, UK). Data on the TCR Vα repertoire reported in this study are mostly from mice maintained on B6/B10 background for four to six generations.

To introduce MHC transgenes, H-2^b V2.1 TCR β-chain transgenic mice (crossed to B6 at least twice) were crossed with Eα^k (Eα16, Ref. 24), or $A_\alpha{}^k$ $A_\beta{}^k$ transgenic lines (25) carried on the B6 background. Mice transgenic for the TCR β-chain of the 2B4 hybridoma (26–28) were maintained on a mixed B6/B10.BR background from a breeding stock kindly supplied by Dr. M. M. Davis.

Immunofluorescence staining and flow cytometry

Staining was conducted at 4°C in PBS, 0.2% BSA, and 0.1% sodium azide. Two washes in this buffer followed each step. For H-2 typing, PBMCs were stained as appropriate with anti-MHC class II mAbs (29): 2A2 (H-2A^b and others but not A^d), Y219 ($A^{b,d}$ and others but not A^q), MK-D6 (A^d but not $A^{b,k}$, cross-reactive with A^q), 14-4-4S (Eα), H116 ($A\beta^k$), 10.2.16 ($A\alpha^{k,s}$), and the anti-D^d-specific mAb H97.67.7 (30).

To determine the TCR-β transgene status and to analyze the TCR Vα repertoire, PBMCs, lymph node cells, or thymocytes were incubated with the rat mAbs KJ16 (anti-Vβ8.1/2; 31), RR3–16 (anti-Vα3.2; 11), RR8–1 (anti-Vα11.1/2 of $V\alpha^b$; 16), KT50 (anti-Vα8; 15), or B-20.1 (anti-Vα2; 14), followed by FITC-conjugated mouse anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or the hamster mAb 1.F2 (anti-Vα11, specific for a subset of cells reactive with RR8–1; 13) followed by FITC-conjugated goat anti-hamster Ig (Jackson ImmunoResearch). After blocking with 5% rat serum, anti-CD4-PE[3] (Caltag, San Francisco, CA) and biotinylated anti-CD8 (Caltag) were added, and biotin was revealed by streptavidin-conjugated tricolor (Caltag). Co-expression of TCR Vα families with Vβ8 by individual T cells was assessed in some experiments by staining with Vα mAbs (see above) and goat anti-rat Ig PE/Texas Red Duochrome (Southern Biotechnology Associates, Birmingham, AL) followed by anti-CD4-PE (Caltag), biotinylated F23.1 (Vβ8; the kind gift of Dr. R. Ceredig) (32), and streptavidin-FITC (Sigma Chemical Co., St. Louis, MO). Samples were analyzed on a FACScan (Becton Dickinson, Mountain View, CA) with appropriate gates set on forward and side scatter.

Cloning and sequencing of TCR Vα11 chains

LN cells from TCR β-chain transgenic and control mice (on both the H-2b or H-2 k haplotypes) were sorted for the expression of CD4 and Vα11. After addition of 5×10^5 HeLa cells to provide carrier RNA, RNA and cDNA were prepared as described (33) and Vα11 transcripts amplified by two successive rounds of PCR (34): portions of the cDNA synthesis reaction were completed to 50 μl with Taq polymerase buffer and amplified for 30 cycles with a primer derived from the Cα region (oligo NJ108, GGCCCCATTGCTCTTGGAATC) and a primer from the Vα11 region (PY144, GACAATAC *GCATGC*CAGGAACAAAGG AGAATGG). The cycles consisted of 30 s at 94°C, 30 s at 45°C, and 1 min at 72°C. Five microliters of the amplification mix were then reamplified for another 30 cycles with another nested Cα region primer (NJ110, TCTC *GAATTC*AGGCAGAGGGTGCTGTCC) and PY144 (cycles as above). The correct amplification products were then digested with *Eco*RI and *Sph*I (which cleave at the artificial sites engineered into PY144 and NJ110, underlined above), purified by gel electrophoresis and elution of the approximately 320-bp band, and cloned into the *Eco*RI/*Sph*I sites of M13 mp19. Recombinant clones were screened by plaque hybridization with a Cα region oligo (OE6, CACAGCAGGTTCT GGGTTCTG) and sequenced according to standard procedures. Precautions taken to guard against sample contamination were as described (33, 34).

Results

MHC control of TCR Vα usage

To analyze the MHC dependence of the naive TCR α-chain repertoire, we used transgenic mice in which the great majority of T cells (92 to 100% of $CD4^+$ and 88 to 99% of $CD8^+$ on all haplotypes tested) express the same β-chain (Vβ8.2, Jβ2.1) derived from the T cell clone V2.1. Pairing of this β-chain with the original α-chain (Vα4, Jα47-Jα designations used in this study are according to Ref. 35) would result in an E^d-restricted TCR specific for PR8 influenza hemagglutinin 111–119 (22). Although we did not find T cells reactive with the anti-clonotypic mAb 6–5 in H-2^d or any other haplotype, the transgenic β-chain was compatible with efficient $CD4^+$ T cell development in all haplotypes tested (see below). Vβ 8.2 is not subject to superantigen-driven deletion or overselection in C57Bl mice (33).

[3] Abbreivation used in this paper: PE, phycoerythrin.

Table I. *TCR Vα usage in MHC congenic mice* [a]

Strain	Percentage of CD4+ T Cells			
	B6	B10.A	B10.D2	B10.G
MHC haplotype	b	a	d	q
Vα2	18.5 ± 4.3 (6)	16.2 ± 2.8 (10)	22.2 ± 2.0 (4)	11.7 ± 1.0 (4)
Vα3.2	1.4 ± 0.8 (5)	2.5 ± 1.0 (8)	ND	1.9 ± 0.6 (5)
Vα8	3.7 ± 1.0 (11)	7.4 ± 1.3 (9)	4.2 ± 0.5 (4)	8.9 ± 0.9 (4)
Vα11	2.0 ± 1.3 (38)	13.7 ± 1.7 (19)	2.7 ± 0.1 (4)	3.5 ± 0.5 (5)

[a] Data were collected as described in Figure 1.

Our analysis of the TCR α-chain repertoire used the available mAbs specific for the TCR Vα families 2, 3.2, 8, and 11 (11–16). The frequency of $CD4^+$ cells that use these Vα regions was evaluated by flow cytometry of PBL. As shown in Table I, the usage of Vα11 was clearly haplotype dependent in the transgenic mice, with a sevenfold difference between H-2^b and H-2^a. MHC-dependent variations of Vα2, Vα3.2, and Vα8 frequencies were more limited, with variations of twofold or less. Differential selection of Vα11 also was observed in lymph node cells (data not shown) and was selective for the $CD4^+$ T cell lineage (Fig. 1). Simultaneous staining for CD4, Vα11, and Vβ8 confirmed that Vα11 frequently was used by TCR-β transgene-expressing $CD4^+$ T cells in H-2^a (13.5 ± 1.0%, n = 3) but not H-2^b mice (3.4%, n = 2), formally ruling out a decisive influence of the rare $CD4^+$ T cells expressing endogenous (rather than transgenic) TCR β-chains (data not shown).

The haplotype dependence of Vα11 usage also was observed in a comparison of CD4 single-positive thymocytes from H-2^a and H-2^b mice (Fig. 2). Note that although the transgenic TCR β-chain imposes a similar bias of thymocyte development toward the CD4 compartment in both haplotypes, the proportion of CD4 single-positive thymocytes that use Vα11 clearly is different.

In the backcrosses conducted to obtain MHC homozygous individuals, Vα11 frequencies segregated with the MHC only, indicating that Vα11 usage among $CD4^+$ T cells is under direct MHC control and not influenced by remaining genetic heterogeneity that might conceivably be present in the congenic strains. It is formally possible that the apparent positive influence of the MHC on Vα11 frequencies is compensatory, resulting, for example, from a hypothetical deletion event affecting other Vα families. Because the MHC-imposed overrepresentation applies to the Vα11, but not the Vα2, Vα3.2, or Vα8 families (Table I), this seems unlikely.

Analysis of 2B4 TCR β-chain (Vβ3) transgenic mice indicates that MHC control of the naive TCR-α repertoire is not limited to the V2.1 β-chain transgenic line (Table II). In 2B4β transgenic mice, Vα11 frequencies also correlate with the MHC haplotype, being low in H-2^{bb}, high in H-2^{kk}, and intermediate in H-2^{kb} mice. In contrast with V2.1 TCR β-chain transgenic mice, however, the 2B4 single chain transgenic mice suffer from complicating MHC effects on TCR β-chain selection: the 2B4 β-chain skews T cell development to the CD4 lineage on the H-2^k but not the H-2^b haplotype (27, 28). As reported previously, Vα11 frequencies showed similar but much weaker skewing in TCR-β nontransgenic mice (16; Fig. 3), suggesting that some endogenous β-chain(s)/(families) may fail to pair with Vα11 or that the resulting heterodimers are not efficiently selected.

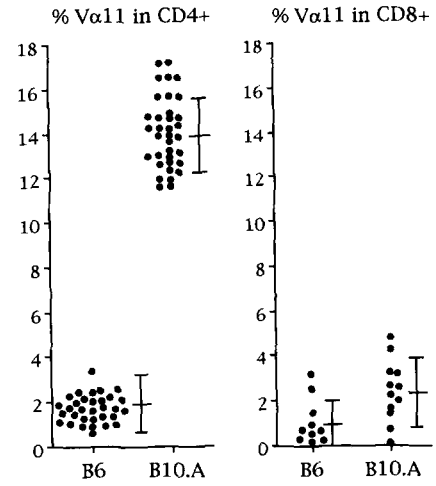

FIGURE 1. Comparison of Vα11 frequencies among peripheral T cells in B6 and B10.A mice. PBMCs were stained with the anti-TCR Vα11.1/2 mAb RR8.1 (13) followed by anti-rat IgG-FITC or anti-rat IgG-FITC alone as a control, anti-CD4, and anti-CD8, and analyzed by flow cytometry.

MHC loci controlling Vα11 usage

We next attempted to identify the genetic loci responsible for the MHC-linked differences in Vα11 usage. In addition to B6, Vα11 frequencies were low in V2.1 TCR-β transgenic $CD4^+$ T cells of the MHC congenic strains B10, B10.D2, B10.GD, B10.G, and B10.S (Fig. 3*A*). In contrast, B10.BR mice, which share with B10.A the k alleles in the class II region, showed a clear overselection of Vα11 (sevenfold compared with B6; Fig. 3*B*). F_1 mice between haplotypes with high and low Vα11 usage displayed intermediate to high Vα11 frequencies (Fig. 3*C*), reminiscent of the partially penetrant positive selection effects described in TCR transgenic mice (7, 27).

MHC recombinant mouse strains were used to more precisely map the overselection of Vα11 mediated by the H-2^k class II region. Vα11 frequencies were high in V2.1 TCR β-chain transgenic B10.A(4R) mice (8.5-fold higher than in B6). Because B10.A(4R) shares A^k with B10.A and B10.BR but carries the defunct Eα allele of H-2^b, this result is compatible with a positive influence of the A^k region on Vα11 selection. However, Vα11 frequencies were also elevated, albeit not quite to the same levels, in the reciprocal recombinants: B10.A(3R) and B10.A(5R) express the A^b molecule and express E products from a

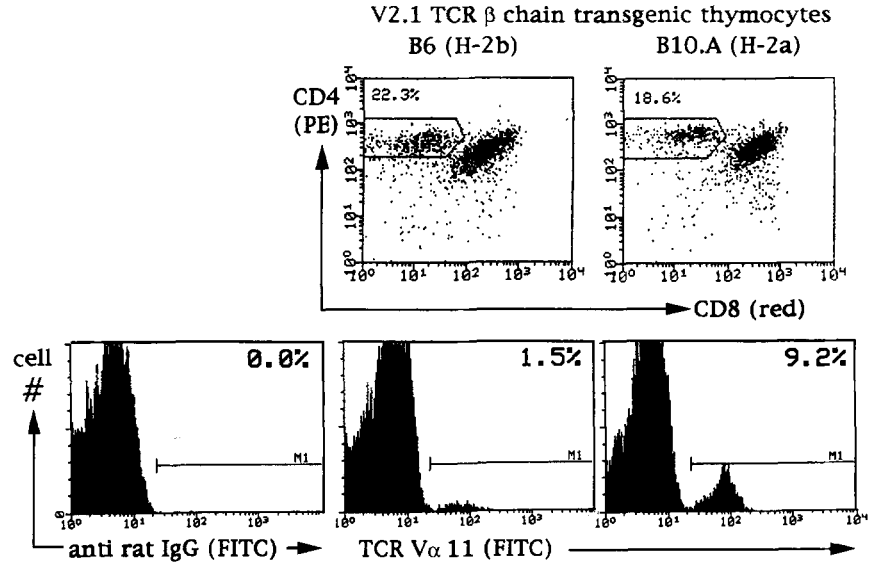

FIGURE 2. High frequeny of TCR Vα11 among CD4 single positive thymocytes in B10.A but not B6 mice. The mice analyzed were derived by mating B6 and B10.A females with a V2.1 TCR β-chain transgenic H-2^{g2} (B10.GD) homozygous male. TCR β-chain transgenic F_1 animals were backcrossed twice to B6 and B10.A, respectively. Thymocytes were stained as described in Figure 1. Histograms are gated on the CD4 single-positive population, as indicated in the dot plots. Data are representative of four thymi analyzed of each haplotype.

Table II. *Vα11 usage in 2B4 TCR β-chain transgenic mice* [a]

H-2	Vα11 Frequency Percentage of CD4 T Cells (*n*)
bb	1.8 ± 0.4 (17)
kb	7.6 ± 1.4 (9)
kk	10.5 ± 0.7 (6)

[a] The mice analyzed were produced by mating B6 and B10.BR females with H-2^{bk} 2B4 TCR transgenic mice (h-2^b and H-2^k derived from B6 and B10.BR, respectively.

functional Eα^k locus. They both have Vα11 frequencies 4.5-fold higher than H-2^b (Fig. 3*D*).

These results suggest that the A^k and E^k regions can both contribute to Vα11 overselection by the H-2^k class II region. We used class II transgenic mice to test directly the involvement of E^k and A^k molecules. First, the V2.1 TCR β-chain transgenic mice were crossed with the Eα^k transgenic line Eα16 (24). Vα11 frequencies in Eα16 transgenics were fourfold higher than in E-negative littermates (Fig. 3*E*), indicating that a single dose of the Eα16 transgene was sufficient to restore to B6 mice Vα11 usage comparable to homozygous B10.A(3R) and B10.A(5R) mice (Fig. 3*C*). High Vα11 usage was seen when the V2.1 and Eα16 transgenes were on the B6 background but also on the background of the Aβ° mutation in which the A^b molecule is absent. These data indicate that the EαEβ complex itself is responsible for the overselection of Vα11 seen in V2.1 transgenics on the 3R or 5R background.

Second, we crossed A^k-encoding transgenes with the V2.1 line. Aα^k and Aβ^k chains segregate independently in the α46β42 transgenic mouse lines used (25). Neither chain alone had an effect and only a twofold rise in Vα11 usage was seen in B6/A$\alpha^k\beta^k$ double transgenic mice (Fig. 3*F*). This modest increase clearly does not match the effects seen in B10.A(4R) mice. Among the possible explanations discussed below for this difference, Vα11 frequencies might be controlled, in addition to classical class II heterodimers, by other class II-like products or other genes in the MHC class II region (38). Candidates were the MA and MB loci, which have been shown to strongly influence MHC class II molecule structure and function by controlling peptide loading (39, 40). To map the centromeric boundary of the region involved in Vα11 selection, we crossed the V2.1 TCRβ transgene to B10.AQR mice. The A and E loci of this strain are of H-2^k haplotype but the region centromeric of the Tap-2 locus derives from H-2^q. At 12.6 ± 1.2%, Vα11 frequencies are high in B10.AQR (Fig. 3*G*). This analysis implicates a region of ~100 kb, defined by the q/k breakpoint of B10.AQR and the k/b breakpoint of B10.A(4R), in TCR α-chain selection (Fig. 4).

Additional features of TCR α-chain selection

To gain insights into what determines the MHC-dependent selection of Vα11-containing TCR α-chains, we analyzed the sequence composition of the V-J junctional regions of these TCR α-chains. MHC/peptide and MHC/superantigen represent two potential ligands driving overselection. For selection by superantigen-like ligands, we would expect (by analogy to superantigen effects on the TCR β-chain) little similarity beyond common Vα usage, whereas selection by MHC/peptide complexes would necessitate the involvement of the TCR-CDR3 region, possibly reflected in shared structure in the V-J junction. We addressed this issue by sorting Vα11-expressing $CD4^+$ T cells from V2.1 TCR β-chain transgenic B6 and B10.A mice (as well as nontransgenic littermates) and sequencing the TCR α-chains after amplification with Vα11-specific primers (Fig. 5).

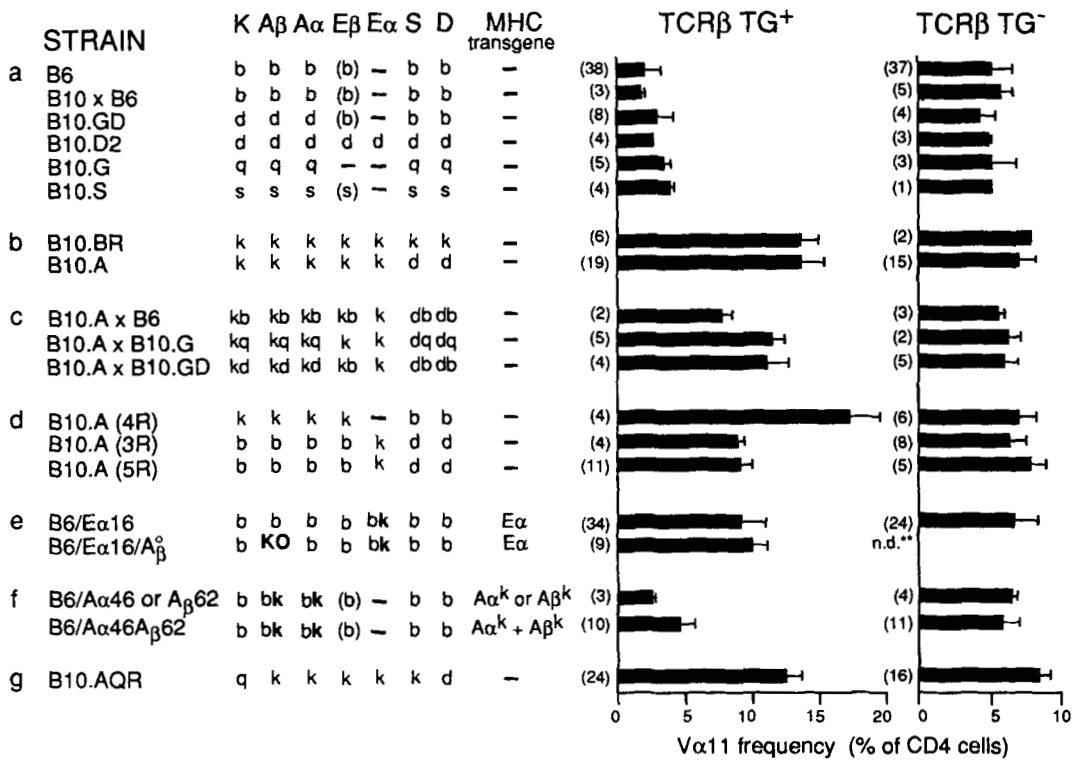

FIGURE 3. MHC control of the naive TCR α-chain repertoire. PBMC or lymph node cells from 3- to 12-wk-old mice were stained and analyzed as described in Figure 1. Background staining (second layer only) was usually >0.1%, and values were not subtracted. Data for males and females were pooled because sex did not affect Vα11 usage; the numbers of mice analyzed are given in brackets. MHC transgenes are in bold type. KO, a locus disrupted by gene targeting; n.d., not done.

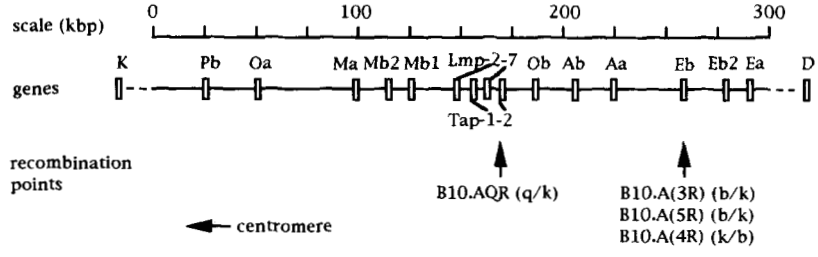

FIGURE 4. The mouse MHC class II region. The position but not the size of genes (open boxes) is roughly to scale (38).

The sequences derived from the five transgenic B10.A mice analyzed are dominated by a few TCR Jα regions, in particular Jα27 and 41. Together, they account for 17 of 36 sequences (24 of 46, including repeats). That Jα usage is more restricted in TCR-β transgenic mice than nontransgenic mice is perhaps not surprising, especially because we are analyzing representatives of only one Vα family. However, the dominant Jα segments occur primarily in the overselected sequences of B10.A but not B6 mice.

Certain sequence features can be discerned within each group. Seven distinct B10.A TCR β-chain transgenic sequences (12, counting repeats) use TCR Jα27, a gene segment used frequently, along with Vα11 by cytochrome *c*-specific T cells raised in B10.A mice (1). In each case, exactly four germ-line Jα nucleotides are removed during joining and in all but one sequence N nucleotides contribute a proline residue to the CDR3. Also, all chains but one have the same CDR3 length. A Vα11 germ line-encoded glutamic acid residue is conserved at the V end of each CDR3. Interestingly, this residue was identified as a direct contact point for MHC (E^k)-bound antigenic peptide in a related, B10.A-derived TCR Vα11.1/Jα27 chain (3). Jα27 occurs in only two α-chains from B6 TCR-β transgenic mice; the J segment in both starts at a different nucleotide from the one conserved in the B10.A sequences. One B6 sequence contains an *N*-encoded proline but here it replaces the Vα11-encoded glutamic acid found in all B10.A sequences containing TCR Jα27.

B10.A Transgenic

Vα11	N	repeats				TCRaJ	mouse#
2	1	2	CAAE	P	NTNKVVFGTGTRLQVLPNI	27	1
2	1	3	CAAE	P	NTNKVVFGTGTRLQVLPNI	27	2
1	1	3	CAAE	P	NTNKVVFGTGTRLQVLPNI	27	3
2	1		CAAE	P	NTNKVVFGTGTRLQVLPNI	27	3
2	2		CAAE	P	NTNKVVFGTGTRLQVLPNI	27	3
2	1		CAAE	A	NTNKVVFGTGTRLQVLPNI	27	4
?	4		CAAE	AP	NTNKVVFGTGTRLQVLPNI	27	1
?	2		CAAE	A	NTGYQNFYFGKGTSLTVIPNI	41	5
?	3		CAAE	A	NTGYQNFYFGKGTSLTVIPNI	41	5
?	3		CAAE	A	NTGYQNFYFGKGTSLTVIPNI	41	5
?	2		CAAE	G	NTGYQNFYFGKGTSLTVIPNI	41	5
2	3		CAAE	G	NTGYQNFYFGKGTSLTVIPNI	41	1
2	2		CAAE	G	NTGYQNFYFGKGTSLTVIPNI	41	1
?	3		CAAE	G	NTGYQNFYFGKGTSLTVIPNI	41	1
?	2	2	CAAE	S	NTGYQNFYFGKGTSLTVIPNI	41	4
?	2		CAAE	W	NTGYQNFYFGKGTSLTVIPNI	41	1
2	0	2	CAAE		GYQNFYFGKGTSLTVIPNI	41	1
2	0	4	CAA	G	NSNNRIFFGDGTQLVVKPNI	24	3
2	0		CAAE	G	NNRIFFGDGTQLVVKPNI	24	1
2	2		CAAE	G	NNRIFFGDGTQLVVKPNI	24	4
2	3		CAA	L	NAYKVIFGKGTHLHVLPNI	23	2
1	3		CAA	L	NAYKVIFGKGTHLHVLPNI	23	2
2	3		CAAD	T	TNAYKVIFGKGTHLHVLPNI	23	3
?	1		CAAE	D	TEGADRLTFGKGTQLIIQPYI	37	1
?	3		CAAE	D	TEGADRLTFGKGTQLIIQPYI	37	4
?	0		CAAE		NTEGADRLTFGKGTQLIIQPYI	37	4
2	5		CAA	G	SNTEGADRLTFGKGTQLIIQPYI	37	2
1	2		CAAD		ASSGSWQLIFGSGTQLTVMPDI	17	1
2	2		CAAE	A	NNNAGAKLTFGGGTRLTVRPDI	32	3
2	0		CAAE	D	NSGTYQRFGTGTKLQVVPNI	11	2
2	5		CAAE	A	ISGGSNYKLTFGKGTLLTVTPNI	45	5
?	5		CAA	GG	ASSSHSKLVFGNGTSLSVVPNI	42	4
2	4		CAAE	VH	NAPRFGAGTKLSVKPNI	35	3
1	1		CAAD		RGSALGRLHFGAGTNLIVIPDI	15	4
2	4		CAA	GL	SNYNVLYFGSGTKLTVEPNI	16	4
2	6		CAAE	A	GANTGNLTFGHGTILRVHPNI	44	4

B6 Transgenic

Vα11	N	repeats				TCRαJ	mouse#
2	3		CAAE	A	SNTNKVVFGTGRLQVLPNI	27	8
2	7		CAA	PR	SSNTNKVVFGTGRLQVLPNI	27	9
2	2		CAAE	G	GSALGRLHFGAGTQLIVIPDI	15	9
2	0		CAAE		GSALGRLHFGAGTQLIVIPDI	15	9
?	4		CAAE	DD	SGYNKLTFGKGSVLLVSPDI	09	8
1	4		CAAE	GD	SGYNKLTFGKGSVLLVSPDI	09	10
1	1		CAAE	P	SGSWQLIFGSGTQLTVMPDI	17	8
?	2		CAAE	P	SGSWQLIFGSGTQLTVMPDI	17	9
2	1	2	CAAE	A	SSGSWQLIFGSGTQLTVMPDI	17	9
1	6		CAAE	GS	SSGSWQLIFGSGTQLTVMPDI	17	9
?	2		CAA	L	NSGTYQRFGTGTKLQVVPNI	11	8
2	3		CAA	L	NSGTYQRFGTGTKLQVVPNI	11	8
2	2		CAAE	A	TNAYKVIFGKGTHLHVLPNI	23	10
2	7		CAA	VEA	NSNNRIPPGDGTQLVVKPAI	24	9
?	1		CAAE	A	NTGKLTFGDGTVLTVKPAI	21	9
?	3		CAAE	R	TGYQNFYFGKGTSLTVIPNI	41	8
2	1		CAA	P	GGSNAELTFGLGTKLSVKSNI	34	9
1	0		CAAE		DYSNNRLTLGKGTQVVVLPNI	07	10
2	4		CAAE	AT	NYNVLYFGSGTKLTVEPAI	16	9

B10.A Non-Transgenic

Vα11	N	repeats				TCRaJ	mouse#
2	2		CAA		GTGGYKVVFGSGTRLLVSPDI	10	6
1	0		CAAE		TGGYKVVFGSGTRLLVSPDI	10	6
2	0		CAAE		TGGYKVVFGSGTRLLVSPDI	10	6
1	1		CAA		GTGGYKVVFGSGTRLLVSPDI	10	6
2	0	2	CAAE		TGGYKVVFGSGTRLLVSPDI	10	6
?	6		CAA	G	NTNTGKLTFGDGTVLTVKPNI	21	6
2	4		CAA	G	NTNTGKLTFGDGTVLTVKPNI	21	6
1	2		CAAE	HN	TNTGKLTFGDGTVLTVKPNI	21	6
?	4		CAA	AF	RGSALGRLHFGAGTQLIVIPDI	15	7
2	4		CAA	GF	RGSALGRLHFGAGTQLIVIPDI	15	7
2	11		CAA	TESR	SNYNVLYFGSGTKLTVEPNI	16	7
1	5		CAAE	SR	SNYNVLYFGSGTKLTVEPNI	16	7
2	0		CAA	A	SSSFSKLVFGQGTSLSVVPNI	42	7
2	3		CAA	AP	SSSFSKLVFGQGTSLSVVPNI	42	6
?	0		CAA	E	SSSFSKLVFGQGTSLSVVPNI	42	6
1	3		CAAE	E	NNRIFFGDGTQLVVKPNI	24	6
2	4		CAAE	AG	NNRIFFGDGTQLVVKPNI	24	6
2	4		CAAE	AR	NSNNRIFFGDGTQLVVKPNI	24	6
1	0		CAA		DSNYQLIWGSGTKLIIKPDI	26	7
2	5		CAA	GTM	DSNYQLIWGSGTKLIIKPDI	26	6
?	1		CAAE	E	TTASLGKLQFGTGTQVVVTPDI	19	6
2	6		CAA	RG	GSNAKLTFGKGTKLSVKSNI	34	6
?	0		CAA		TSGGNYKPTFGKGTSLVVHPYI	06	6
1	4		CAAE	AS	GSFNKLTFGAGTRLAVCPYI	04	6
2	1		CAAE	K	GGSAKLIFGEGTKLRVSSYI	48	6
1	4		CAAE	AA	SGSWQLIFGSGTQLTVMPDI	17	6
?	8		CAA	TY	GNTGKLIFGLGTTLQVQPDI	30	6

B6 Non-Transgenic

Vα11	N	repeats				TCRαJ	mouse#
?	3		CAA	AM	SNYNVLYPGSGTKLTVEPNI	16	11
?	1		CAAE	P	NVLYFGSGTKLTVEPNI	16	12
?	1		CAAE	A	GGYKVVFGSGTRLLVSPAI	10	11
1	1		CAAE	R	TGGYKVVFGSGTRLLVSPAI	10	13
?	2		CAA	G	NNNAPRFGAGTKLSVKPNI	35	13
1	4		CAAE	ED	NNAPRFGAGTKLSVKPNI	35	13
2	1		CAA	F	NTGNYKYVFGAGTRLKVIAHI	33	13
2	2		CAAE	S	TGNYKYVFGAGTRLKVIAHI	33	13
1	0		CAA		NSNNRIFFGDGTQLVVKPNI	24	13
1	4		CAAE	S	NNRIFGDGTQLVVKPNI	24	13
?	6		CAAE	AG	QGGSAKLIFGEGTKLTVSSTI	48	11
1	1		CAAE	T	NSGTYQRFGTGTKLQVVPNI	11	12
2	2		CAAE	G	GNTGKLIFGLGTTLQVQPDI	30	13
2	4	2	CAAE		GYQNFYFGKGTSLTVIPNI	41	13
2	5		CAA	R	GADRLTFGKGTQLIIQPYI	37	13
2	6		CAAE	AG	QGGRALIFGTGTTVSVSSNI	12	13
1	3		CAAD	D	TNAYKVIFGKGTHLHVLPNI	23	13
2	0		CAAD		TGANTGKLTFGHGTILRVHPNI	44	13
2	3		CAAE	A	DSNYQLIWGSGTKLIIKPDI	26	13
?	1		CAAE		SAGNKLTFGIGTRVLVRPDI	14	12

FIGURE 5. Sequences of Vα11 TCR α-chains selected on different MHC backgrounds. Lymph node cells from individual V2.1 TCR-β transgenic or nontransgenic B10.A or B6 mice were stained with anti-CD4 and anti-Vα11.1/2 and sorted for co-expression of both markers using a fluorescence-activated cell sorter. Five TCR-β transgenic B10.A mice, three TCR-β transgenic B6 mice (three independent sorts), and two B10.A and two B6 nontransgenic control mice (two independent sorts) were analyzed. Unique sequences are listed, and α-chains of identical amino acid sequence differ either in nucleotide sequence, Vα11.1 vs 11.2 subfamily usage, or stem from independent experiments. For α-chains that are indistinguishable by these criteria, only the number of repeats is shown. Repeats occurred mostly in B10.A TCR-β transgenic samples in which Vα11 CD4 T cells were most abundant and are, therefore, probably derived from different cells.

Another J segment frequently used with Vα11 in V2.1 TCR β-chain transgenic B10.A mice is TCR Jα41. As described above for TCR Jα27, the glutamic acid residue at the beginning of the CDR3 is conserved in all cases. TCR Jα41 usage begins at the same amino acid throughout and the length of the CDR3 is virtually invariable. TCR Jα41 appears in one sequence from a TCR-β transgenic B6 mouse, but as described above for sequences containing TCR Jα27, the initial J-derived amino acid differs from all the B10.A sequences. Overall, it seems that differential selection of Vα11 TCR α-chains includes particular CDR3 structures.

Discussion

Current views of thymic selection are biased by the dramatic impact of endogenous and exogenous superantigens on the TCR Vβ repertoire. On the other hand, little is known about the role of TCR α-chains in the selection of the naive TCR repertoire. We have investigated the naive TCR α-chain repertoire in mice of different MHC haplotypes. To minimize variables other than the MHC, we used MHC congenic, MHC recombinant, and MHC transgenic mice in which the majority of T cells express identical, transgene-encoded TCR β-chains. Mice transgenic for the Vβ8.2/Jβ2.1 chain derived from the H-2E^d-restricted, influenza hemagglutinin 111–119-specific T cell clone V2.1 are particularly informative because CD4 T cells expressing this transgene can be efficiently selected on multiple MHC haplotypes, thus eliminating TCR-α repertoire variations secondary to selective pressure on the TCR β-chain.

Four TCR Vα families (Vα2, Vα8, Vα3.2, and Vα11) were studied and the representation of one, Vα11, showed striking MHC dependence. Preferential Vα11 usage was observed in MHC haplotypes containing the H-2^k class II region and was linked to MHC by backcross analysis. Consistent with a thymic event, Vα11 overselection was visible among CD4 single positive thymocytes and peripheral T cells. Vα11 frequencies were elevated in F_1 crosses between overselecting and underselecting haplotypes, reminiscent of the partial positive selection effects in F_1 crosses between positively selecting and supposedly neutral haplotypes in TCR-αβ transgenic mice (7, 27). Interestingly, the differences we found tended to mirror the variations in Vα11 usage described by Jameson et al. in nontransgenic mice (although in that instance the variations were very subtle compared with the differences observed here). Also reinforcing our conclusions on MHC-driven selection of Vα is the observation of similar effects with other TCR-β transgenic mice (Table II).

MHC recombinant mouse strains revealed two apparently independent effects of the H-2^k class II region, mapping to the A and E subregions. Strong overselection was seen in B10.A(4R) mice (A^k, no expressed E) but was reproduced only very partially in A^k transgenic mice (Fig. 3). Because we know that the A^k transgenes are expressed in a correct cell-specific and tissue-specific fashion and allow the selection and activation of A^k-restricted T cells (25), we consider two possible explanations for this observation. First, A^b (expressed in A^k transgenic mice with a B6 background but not in B10.A(4R) mice) may antagonize Vα11 overselection by A^k. Indeed, B6 × B10.A(4R) or B6 × B10.A F_1 animals have intermediate Vα11 frequencies, consistent with a negative influence of A^b. However, A^b did not seem to interfere with Vα11 overselection by E^k, because its elimination in the Aβ° mice still leaves the intermediate level of Vα11 usage in Eα16/V2.1 double transgenic mice (Fig. 3*E*). This rules out a simple A^b-mediated deletion of Vα11-expressing T cells. It does leave open the possibility of a more complex interference between A^b and A^k in Vα11 selection.

A second, perhaps more interesting explanation for the failure of A^k transgenes to fully restore Vα11 overselection is the involvement of other genes in the MHC class II region. Ob, Lmp-2, and Lmp-7 as well as Tap-1 and Tap-2 map centromeric of Ab (38); further away are the Ma and Mb genes, the products of which affect MHC class II peptide loading (39, 40). Vα11 frequencies were high on the B10.AQR background, which would tend to exclude Ma and Mb and place candidate genes for MHC class II-mediated TCR α-chain selection between the k/b breakpoint of B10.A(4R) and the q/k recombination in B10.AQR. Given the uncertainty on the exact position of the recombination event in B10.AQR (41), this stretch could include Ob, for which sequence analysis does not predict any difference between the k and b alleles at the protein level (42), and Tap-2, a gene appreciated more for effects on the physiology of MHC class I than MHC class II (38).

In B10.A(3R) and B10.A(5R) mice that express E products as a result of a functional Eα locus, Vα11 frequencies were 4.5-fold higher than in H-2^b. This overselection was reproduced by introducing into B6 mice an Eα transgene that supports the positive and negative selection of a functional T cell repertoire (43). The data obtained with transgenic and recombinant mice are thus fully consistent in this instance and formally demonstrate that MHC-encoded class II heterodimers can control the selection of specific TCR α-chains. There is actually precedent for a preferential interaction of Vα11 with the E molecule, which includes the predominance of this V gene family in the E-restricted response to cytochrome *c* (1) and the frequent Vα11 usage by E^k alloreactive T cell hybridomas (9).

Our results suggest that E^k (and perhaps A^k) can contribute to Vα11 overselection, a phenomenon reported previously for the apparently MHC-restricted recognition of superantigens by some T cell hybridomas (44), raising the issue of whether Vα11 selection might be mediated by superantigen-like ligands, although superantigens that act directly on TCR Vα selection have not yet been described. Known superantigens interact with multiple allelic forms of the E molecule, which would make it difficult to explain the different Vα11 frequencies found in B10.D2 (2.7%) and other E-expressing mice (8.3 to 13.7%; Fig. 3). This

haplotype specificity of Vα11 selection also contrasts with the overselection of Vβ6 in E-expressing strains (17), therefore this hypothesis would seem rather unlikely.

Additional evidence against a role for superantigens in our observations are the data from sequence analysis of Vα11 $CD4^+$ T cells isolated from TCR V2.1 β-chain transgenic and control mice. These revealed features extending beyond shared Vα usage, suggesting that selection operates at the level of the entire TCR α-chain. These features include 1) preferential and haplotype-dependent utilization of Jα segments coupled with preferred CDR3 lengths, 2) conservation of the first Jα-encoded amino acid in $H\text{-}2^a$- but not $H\text{-}2^b$-selected CDR3 sequences, and 3) frequent occurrence of a single N nucleotide-encoded residue, preferentially proline in the case of TCR Jα27. In addition to these sequence data, we have found that staining with the mAb 1.F2, specific for a subset of Vα11-expressing TCRs and apparently sensitive to Jα usage (16), is haplotype dependent, with a 10-fold difference between $H\text{-}2^b$ (B6) and $H\text{-}2^a$ (B10.A) V2.1 TCR β-chain transgenic mice (data not shown). Overall, these effects of preferential selection on the CDR3 loop would be expected for α-chain selection mediated by MHC/peptide complexes rather than superantigens as we know them.

In summary, this study provides direct evidence for MHC control of the naive repertoire of TCR α-chains independent of β-chain influence, and our analysis is most consistent with a scenario in which selection acts at the level of the whole receptor chain rather than on the Vα region in isolation.

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