RELATIVE Vβ TRANSCRIPT LEVELS IN THYMUS AND PERIPHERAL LYMPHOID TISSUES FROM VARIOUS MOUSE STRAINS

Inverse Correlation of I-E and Mls Expression with Relative Abundance of Several Vβ Transcripts in Peripheral Lymphoid Tissues

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Lymphocytes recognize foreign antigens via a variety of cell surface receptor glycoproteins. B lymphocyte antigen receptors can bind free antigens, in contrast to TCR, which recognize antigenic peptides in the context of molecules coded by the MHC. This phenomena is known as MHC restriction (1–3). Biochemical studies on antigen presentation indicate that MHC molecules present fragments of processed antigens (4), and recent x-ray crystallographic studies strongly suggest that the MHC molecules bind these antigens in a cleft formed on its external surface (5, 6). It is presumably this antigen-MHC complex that is the ligand for the TCR. The antigen-MHC recognition component of the TCR complex is usually formed by an α,β protein heterodimer in association with the CD3 complex of proteins (7–9). Like Igs, the TCR α and β chains have V and C region domains. The V region domains are encoded by multiple gene segments that somatically recombine to form functional transcription units. These rearrangements occur during T cell development in the thymus. The β chain V region domain is encoded by three separate gene segments; Vβ, Dβ, and Jβ. Similarly, the α chain V region domain consist of Vα and Jα gene segments. Over the last 5 yr, many genes for the α and β chain have been isolated and well characterized (reviewed in references 10 and 11).

Using Vβ-specific mAbs, several investigators have recently analyzed various factors influencing TCR repertoire. Experiments by Kappler, Marrack, and colleagues (12, 13) have demonstrated that a high frequency of TCRs that use the Vβ17a V region are specific for I-E, an MHC class II molecule, regardless of the other gene segments used to encode the antigen receptors. In mice that express cell surface I-E molecules, this reactivity leads to elimination of nearly all of the Vβ17a-bearing cells, presumably by the removal of self-reactive cells in the thymus (12, 13). Additional Vβ gene segment–restricted reactivity has been found in association with the Mls

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loci. The Mls loci code for determinants that can elicit very intense one-way mixed lymphocyte reactions between cells from mice that are identical at the MHC loci but disparate at one of the two known Mls loci (14, 15). T cells from Mls-1^b mice that use either Vβ6 or Vβ8.1 strongly react to Mls-1^a stimulator cells, and the peripheral receptor repertoire of Mls-1^a mice lack T cells expressing Vβ6 and Vβ8.1, likely due to elimination of self-reactive cells during T cell maturation (16, 17).

The mouse Vβ gene segments are unusual when compared with their homologues in the TCR α chain or to Igs, in that there are very few Vβ gene segments (<30) and they belong to single element families, with the exception of the Vβ5 and Vβ8 families, which have three crosshybridizing members (18–20). Also, there are very few interstrain polymorphisms (21), and unlike Ig V region genes, somatic mutations have not been documented. At this time, only a few Vβ gene segments can be identified using mAbs to their encoded proteins. To provide a broad assessment of Vβ repertoire expression during thymic development and in peripheral T cells, we have developed a highly specific, sensitive, and quantitative RNase protection assay that measures the relative abundance of transcripts for various Vβ gene segments. We found that in the thymus, relative Vβ transcript levels varied over a 20-fold range. For most of the Vβ gene segments, the relative levels of transcripts were nearly the same in both thymus and peripheral lymphoid tissues. An exception was observed with one Vβ gene segment, whose expression was strongly associated with the expression of I-E. The Mls haplotype was also associated with variations in peripheral transcript levels for six Vβ gene segments. By using the level of transcripts as a reflection of the number of cells using a particular Vβ gene segment, we have compared the relative frequency of Vβ gene segment used in thymocytes and peripheral T cells and found several factors that affect these frequencies.

Materials and Methods

Animals. The following mouse strains were used in these experiments: BALB/b, BALB/c, BALB/k, C57BL/10 Sn (B10), B10.M, B10.S, B10.BR, B10.D2, B10.A (3R), B10.A (18R), AKR/J, and CBA/J. The mouse strain B10.A (18R) was a generous gift of Dr. J. H. Stimpfling, Dr. P. Wettstein, and A. Reichert of the McLaughlin Research Institute, Great Falls, MT. All other mice were bred and maintained in our colony at Stanford University School of Medicine. Mice of 1–4 mo of age were used in all studies.

V and C Region Probes. Vβ1, Vβ2, Vβ3, Vβ4, Vβ5.1, Vβ8.1, Vβ8.2, Vβ10, and Vβ12 probes were obtained from Dr. Rick Barth, Cal. Tech (19). Vβ5.2, Vβ6, and Vβ14 probes were obtained from Dr. Mark M. Davis, Stanford University (18, 22). Vβ7 and Vβ9 probes were obtained from Dr. Dennis Loh, Washington University (20, 21). Vβ nomenclature follows that of Barth et al. (19). The C region probe was subcloned from a C6VL cDNA β chain TCR clone obtained from Dan W. Denney of this lab. All probes were subcloned into the pSPT672 vector (pSPT672 is identical to GEM-2) (Promega Biotec, Madison, WI), which contains SP6 and T7 RNA polymerase promoters flanking a region of unique restriction endonuclease sites.

Antisense ^{32}P-labeled RNA probes were made by in vitro transcription of linearized plasmids according to Melton et al. (23). The ^{32}P-labeled RNA probes were treated with DNase I, extracted with phenol/chloroform, and then purified by spin column chromatography using P-60 resin (Bio-Rad Laboratories, Richmond, CA). Analysis of the radioactive probes by acrylamide gel electrophoresis and autoradiography showed that >95% of the incorporated label was in full-length transcripts (data not shown).

RNA Preparation and Ribonuclease Protection Assay. Thymus and peripheral lymphoid tissues (spleen, mesenteric, brachial, axillary, inguinal, and cervical lymph nodes) were dissected

from each mouse strain. The tissues were homogenized in guanidine thiocyanate (Fluka Chemical Corp., Hauppauge, NY), and the RNA pelleted through a CsCl cushion according to the method of Chirgwin et al. (24). RNA samples were isolated from 3-10 mice to average individual animal variations.

The RNase protection assay was a modification of the method described by Melton et al. (23). The 16 C and V region RNA probes used in the assay were divided into three probe sets such that: (*a*) no two members of the same $V\beta$ gene family were in the same probe set, (*b*) $V\beta6$ and $C\beta T$ were present in all three probe sets, and (*c*) probe sets contained 5×10^5 Cherenkow counts of $V\beta6$ probe per hybridization reaction and equal molar amounts of the other probes. $V\beta$ gene segments probes belonging to the same $V\beta$ family were placed in separate probe sets to avoid hybridization of $V\beta$ mRNA to related but nonidentical probes. These mismatched hybrids would be degraded during the RNase digestion, resulting in inaccurately low measurements. The $V\beta6$ probe was placed in all three probe sets so that the values between the three probe sets could be adjusted for differences that occur from handling the samples, such as efficiency of precipitation and gel loading. $C\beta T$ probe was used as a second control to verify the procedure, and when $V\beta6$ transcript levels were low, $C\beta T$ values were used to normalize the $V\beta$ measurements between the probe sets.

Each probe set was hybridized to increasing concentrations of sample RNA. Thymus RNA samples were titered in increments of 5 μg from 0-30 μg of total cellular RNA. Peripheral lymphoid RNA samples were titered in increments of 25 μg from 0-150 μg of total cellular RNA. Yeast tRNA was added to the sample RNA so that the total amount of RNA was the same in each reaction. The cellular RNA and probes were hybridized together in 30 μl of 40-mM pipes, pH 7.6, 0.4 M NaCl, 1.0 mM EDTA, 80% formamide, and 10% DMSO, at 42°C for >12 h. 300 μl of an RNase solution containing 5 μg/ml RNase A (Pharmacia Fine Chemicals, Piscataway, NJ) and 0.85 U/ml RNase T1 (Pharmacia Fine Chemicals) in 10 mM Tris, pH 7.3, 0.3 M NaCl, 5 mM EDTA, and 10% DMSO was added to the samples and incubated at 50°C for 1 h to digest any single-stranded probe that had not hybridized to its complementary mRNA. After the digestion reactions, the RNases were inactivated with 0.3% SDS and extracted twice with phenol/chloroform (1:1). The RNase-resistant RNA-RNA duplexes were then resolved on 6% denaturing polyacrylamide gels containing 7.8 M urea and buffered with 1 × Tris-Borate-EDTA. The gels were dried onto 3M chromatographic paper and exposed to XAR-5 film. Gel pieces containing $V\beta$-protected probes were cut out and counted in liquid scintillant. Background values were determined by counting equal-sized gel pieces from above and below the probe bands. For each RNA concentration, the $V\beta6$ measurements from each of the three probe sets were averaged. The values for the other probes were then adjusted according to the change in $V\beta6$ for that probe set. The $V\beta$ measurements were also corrected for differences in sizes and sequences of the protected probes to reflect the molar amounts of each variable gene segment. A linear regression line was calculated for each V region probe by the least squares method and the midpoint value and standard deviation were determined. The midpoint values for the 15 variable gene segments were summed and the contribution of each V region to that sum was calculated. This value was termed percent of protected counts. The congenic BALB strains were analyzed in at least two separate experiments and the results averaged. For the other strains, only one measurement was taken.

Results

RNase Protection Is a Sensitive, Quantitative, and Specific Assay for Measuring Mouse $V\beta$ Repertoire Expression. To measure relative $V\beta$ transcript levels, we used an RNase protection assay on RNA samples from thymus and peripheral lymphoid tissues (Fig. 1). The RNase-resistant RNA-RNA hybrids for each $V\beta$ gene segment migrate on denaturing acrylamide gels as a unique band or bands whose size depends on the length of the $V\beta$ gene fragment subcloned into the transcription vector. The protected band(s) for each probe were determined by assaying the probes individually. Combining the probes into pools did not alter the sizes or relative amounts of the

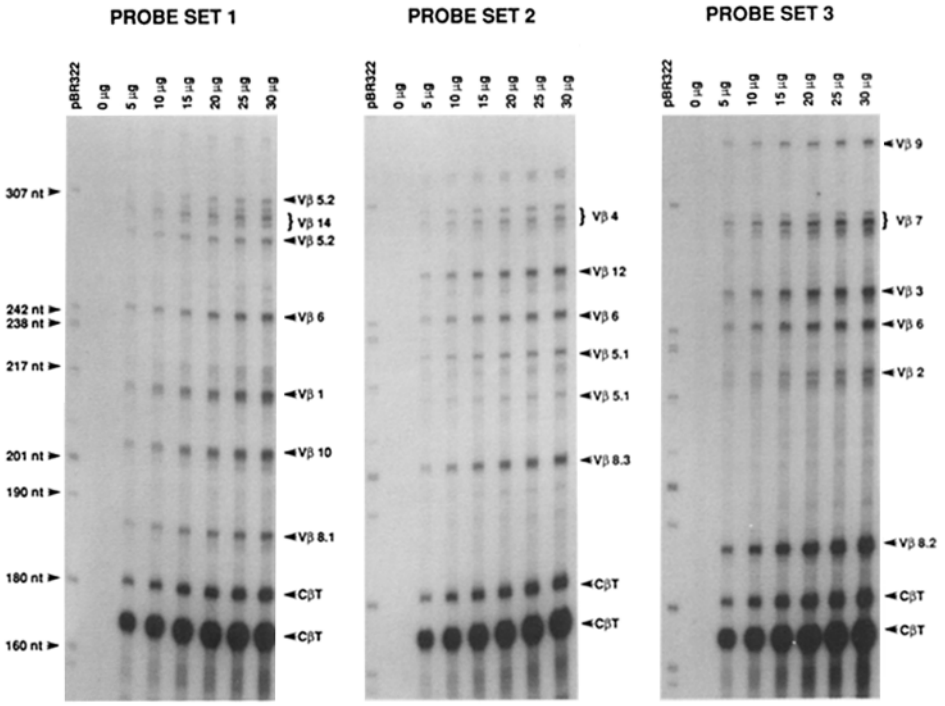

FIGURE 1. Ribonuclease protection assay on increasing concentrations of total RNA isolated from BALB/c thymus. Radiolabeled RNA probes to 15 Vβ gene segments and CβT were divided into three sets and hybridized to increasing concentrations of BALB/c thymus RNA. The samples were RNase digested, resolved on a 6% denaturing polyacrylamide gel, and exposed to x-ray film. Radiolabeled Msp I-digested pBR322 was used for size standards. Exposure was on Cronex film for 4 h at room temperature.

protected probes (data not shown). The amounts of protected probes were directly proportional to the amount of sample RNA assayed, indicating that the measurements were in the linear range of the assay. The regression coefficients for the titration curves were usually >0.97.

Because some Vβ gene family members are as much as 92% similar over the regions used as probes, the RNase protection assay had to be able to discriminate between probes hybridized with a perfect match and ones with a low percentage of mismatched base pairs. To assess the specificity of the RNase protection assay, the following experiment was done. Cold, sense RNA was transcribed in vitro from each member of the Vβ8 and Vβ5 gene segment family and then hybridized to the three probe sets. The samples were then RNase digested, resolved on a denaturing polyacrylamide gel, and exposed to XAR-5 film. Bands could be found only in the probe set containing the appropriate antisense ^{32}P-labeled probe. No bands were found in the two other probe sets containing related Vβ gene segments probes (data not shown). This experiment demonstrated that the signal in the protected band was due to exclusive hybridization of the probe to specific Vβ mRNA and not to mRNA from related Vβ gene segment family members.

Unfortunately, the RNase protection assay can not distinguish functional from

nonfunctional transcripts, such as those created by out-of-frame joining of V to D gene segments. For the C region probe, the assay is also unable to differentiate between full-length 1.3-kb transcripts from truncated 1.0-kb transcripts that lack Vβ region sequences (18). Because of possible disparate ratios between the 1.3- and 1.0-kb CβT transcripts in various RNA samples, the transcript levels for each Vβ gene segment are expressed relative to the total amount of transcripts for the 15 Vβ gene segments used in this assay, instead of a percentage of CβT transcripts.

Relative Abundance of Vβ Gene Segment Transcripts in Thymus and Periphery. The relative abundance of 15 Vβ transcripts was measured in the thymus and peripheral lymphoid tissues of BALB/c mice (Fig. 2). In the thymus, the relative Vβ transcript levels varied over a 20-fold range, with Vβ8.2 being the most abundant and Vβ9 the least abundant transcript. The difference between the most and least abundant Vβ transcripts was even greater in the periphery, due to very low levels of Vβ3 and Vβ5.2 transcripts. There were no obvious correlations between Vβ transcript levels to their physical order on chromosome 6 (22, 25, 26).

Comparisons were made between the relative abundance of each Vβ transcript in the thymus vs. peripheral lymphoid tissues (Fig. 2). For 10 of the 15 Vβ gene segments, differences in the relative levels were <2.0% between the two tissues. However, levels of Vβ3, Vβ5.1, and Vβ5.2 transcripts in the periphery were 25% or less of the levels seen in the thymus. Vβ2 and Vβ8.1 gene segments were expressed at levels that were higher in the periphery than in the thymus by 34% and 45%, respectively.

The Relative Abundance of Vβ3 and Vβ5.2 Transcripts in Peripheral Lymphoid Tissues Are Determined by MHC Haplotype in BALB Mice. Because the TCR repertoire is influenced by MHC during T cell development in the thymus (27), we next looked for possible differences in relative Vβ transcript levels that correlated with H-2 haplotype. RNA was analyzed from thymus and peripheral lymphoid tissues of BALB/c (H-2^d) and two H-2 congenic BALB mouse strains, BALB/b (H-2^b) and BALB/k (H-2^k). In the thymus, there were no striking differences among the three H-2 haplotypes in the relative amounts of all 15 Vβ transcripts (Fig. 3 *a*). In the periphery, however,

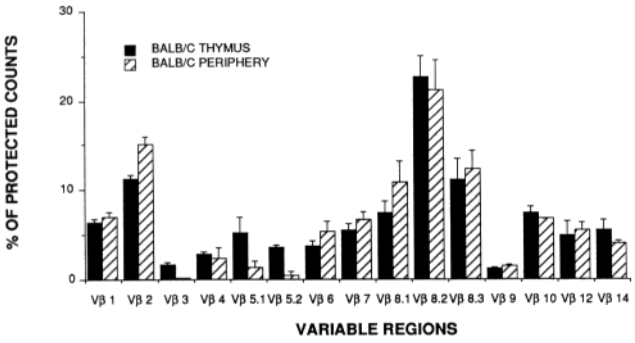

FIGURE 2. Relative transcript levels for 15 Vβ gene segments in RNA samples isolated from BALB/c thymic and peripheral lymphoid tissue. The relative amounts of Vβ gene segment transcripts were measured by an RNase protection assay and then adjusted to reflect the molar amounts of each V region transcript. The values are plotted as percentage of protected counts, which is the amount of counts for a particular Vβ gene segment divided by the sum of all 15 Vβ gene segments counts. The values represent the average of three separate experiments. The error bars denote 1 SD.

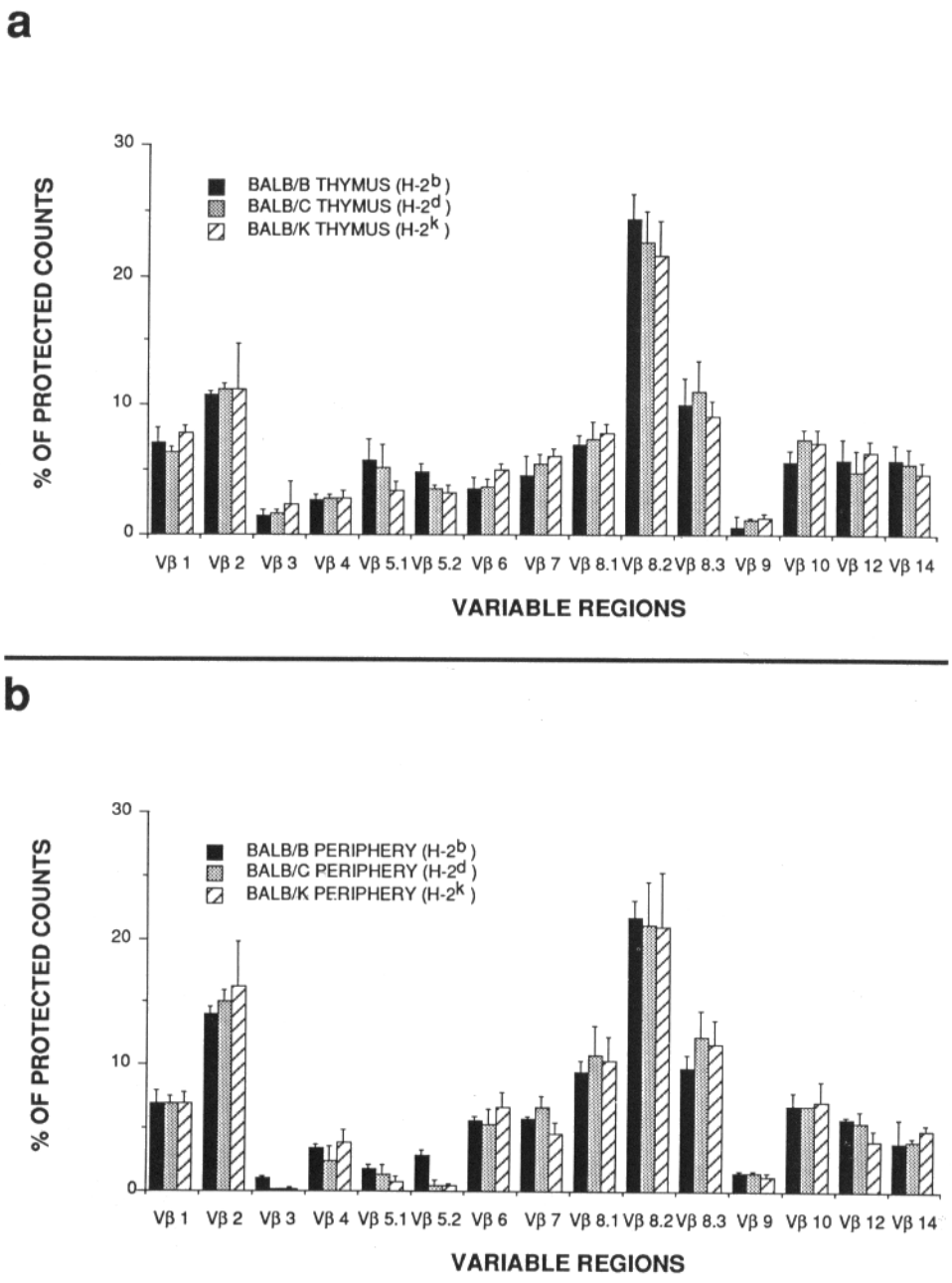

FIGURE 3. Comparison of the relative transcript levels of 15 Vβ gene segments among H-2 disparate BALB congenic strains. BALB/b (H-2^b) is I-E^-. BALB/c (H-2^d) and BALB/k (H-2^k) are I-E^+. The transcript levels were measured with an RNase protection assay. Vβ gene segment transcripts are measured relative to the sum of the 15 Vβ gene segments counts. The values represent the average of two to three separate experiments. The error bars denote 1 SD. (*a*) Measurements from RNA isolated from thymuses. (*b*) Measurements from RNA isolated from peripheral lymphoid tissues (spleen and major lymph nodes).

two Vβ gene segments, Vβ3 and Vβ5.2, were significantly different among the BALB H-2 congenic mouse strains (Fig. 3 *b* and Table I). Both Vβ3 and Vβ5.2 were expressed at fivefold greater levels in the periphery of H-2^b mice than in the H-2^d and H-2^k mice. The thymic transcript levels of Vβ2, Vβ5.1, and Vβ8.1 differed from their peripheral levels in all three H-2 congenic strains. Thus, these Vβ segments do not show any obvious correlations with H-2 haplotype.

Low Relative Abundance of Vβ3 and Vβ5.2 Peripheral Transcripts in BALB Segregates as a Dominant Trait. If the reduced relative amounts of Vβ3 and Vβ5.2 transcripts in H-2^d and H-2^k haplotypes were due to elimination of self H-2-reactive cells, then codominant expression of MHC proteins in a H-2 heterozygous mouse should result in a similar reduction of transcripts. To test this hypothesis, BALB/b mice were mated to BALB/c and BALB/k mice, and thymus and peripheral lymphoid tissue RNA samples from their offspring were analyzed. The relative thymic levels of all the Vβ transcripts in the F_1 progeny were similar to the three parental strains (data not shown). However, the relative levels of Vβ3 and Vβ5.2 transcripts in the periphery of the F_1 animals were greatly decreased from those measured in BALB/b (Figs. 4 *a* and 5). The peripheral levels of the other Vβ gene segments did not significantly differ among the F_1 or the parental strains (data not shown). This demonstrates that the low peripheral transcript levels of Vβ3 and Vβ5.2 in the H-2^d and H-2^k haplotypes segregates as a dominant trait.

The Relative Level of Vβ5.2 Transcripts Is Determined by I-E Expression. Low relative levels of peripheral Vβ3 and Vβ5.2 transcripts were found in mice of H-2^k and H-2^d haplotypes (Fig. 3 and Table I). An important difference between those two haplotypes and the H-2^b haplotype is the lack of surface I-E in the latter due to a defective E^b_α chain gene (28). This suggests that the reduced relative peripheral levels of Vβ3 and Vβ5.2 transcripts might be due to the elimination of T cells expressing those Vβ segments because of self-reactivity to I-E. To investigate the possible inverse correlation between I-E expression and peripheral Vβ3 and Vβ5.2 transcript levels, congenic B10 mouse strains were analyzed because of the numerous well-characterized H-2 recombinant strains. RNA samples from thymus and peripheral lymphoid tissues were assayed from B10 (H-2^b), B10.M (H-2^f), B10.S (H-2^s), B10.BR (H-2^k), and

TABLE I

Vβ3 and Vβ5.2 Expression in the Periphery Is Associated with MHC

	Alleles at H-2 loci*							I-E	Percent of protected counts due to:‡	
Strain	K	Aβ	Aα	Eβ	Eα	S	D	expression	Vβ3	Vβ5.2
BALB/b	b	b	b	b	b	b	b	$I\text{-}E^-$	1.3 ± 0.1	2.7 ± 0.4
BALB/c	d	d	d	d	d	d	d	$I\text{-}E^+$	0.1 ± 0.1	0.5 ± 0.4
BALB/k	k	k	k	k	k	k	k	$I\text{-}E^+$	0.2 ± 0.1	0.4 ± 0.2
B10	b	b	b	b	b	b	b	$I\text{-}E^-$	2.2 ± 0.4	3.0 ± 1.1
B10.BR	k	k	k	k	k	k	k	$I\text{-}E^+$	2.2 ± 0.1	0.6 ± 0.1
B10.A (3R)	b	b	b	b	k	d	d	$I\text{-}E^+$	1.4 ± 0.8	0.6 ± 0.2
B10.A (18R)	b	b	b	b	b	b	d	$I\text{-}E^-$	1.6 ± 0.4	2.2 ± 1.5

* From reference 32.

‡ Percent of protected counts is the amount of protected counts for either Vβ3 or Vβ5.2 divided by the total counts of the 15 Vβ gene segments used in the RNase protection assay.

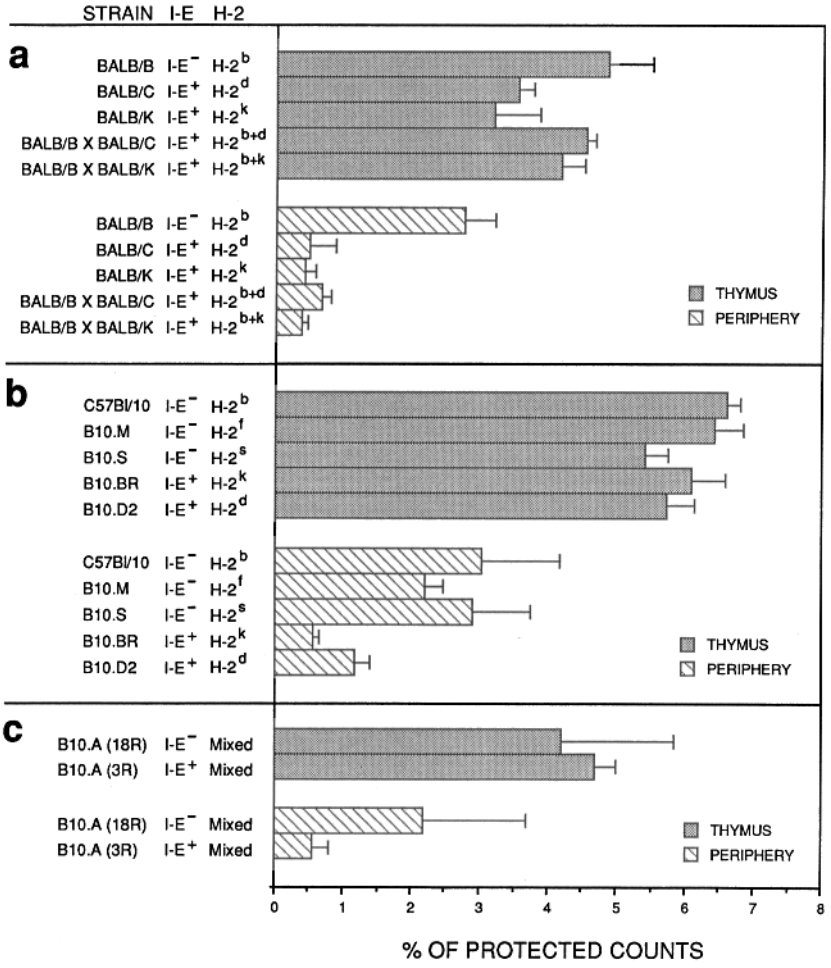

FIGURE 4. Comparison of Vβ5.2 gene segment transcript levels among the thymus and peripheral lymphoid tissue of I-E$^+$ and I-E$^-$ mice. RNase protection assay was used to determine the relative amounts of Vβ5.2 transcripts. The relative Vβ5.2 transcript levels are given as the amount of protected counts for Vβ5.2 divided by the sum of all the Vβ gene segments counts used in the protection assay. The RNA was isolated from the thymus or the spleen and major lymph nodes (periphery) of 4–6-wk-old mice. The error bar denotes 1 SD. (*a*) Vβ5.2 transcript levels in BALB mice and BALB F_1 crosses. (*b*) Vβ5.2 transcript levels in H-2 congenic B10 mice. (*c*) Vβ5.2 transcript levels in the recombinant inbred mice B10.A (3R) and B10.A (18R). B10.A (3R) and B10.A (18R) are syngeneic at the H-2 loci except at E_α (α chain of I-E) and S (a complement factor). B10.A (18R) is E^b_α and B10.A (3R) is E^k_α (see Table I for haplotype of the MHC loci).

B10.D2 (H-2^d) mice. The H-2 haplotypes b, f, and s are I-E$^-$, whereas the H-2 haplotypes k and d are I-E$^+$ (28–31). The relative thymic levels of the 15 Vβ transcripts in all the B10 congenic mice were similar to the BALB mice strains (data not shown). The relative peripheral Vβ5.2 transcript levels, however, varied among the different H-2 haplotypes (Fig. 4 *b*). Similar to the results obtained for BALB mice, peripheral Vβ5.2 transcript levels were decreased in I-E$^+$ H-2 haplotypes as compared with I-E$^-$ strains. The average level of Vβ5.2 transcripts in periphery of

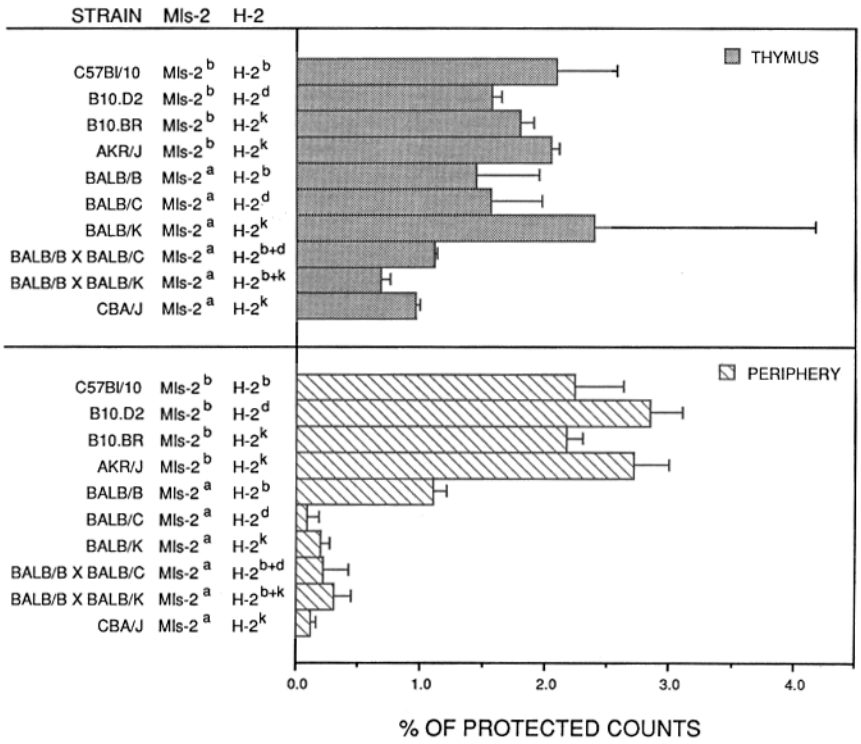

FIGURE 5. Comparison of Vβ3 relative transcript levels in the thymus and peripheral lymphoid tissue among Mls-2^a and Mls-2^b mice. The percent of protected counts represents the amount of protected counts in an RNase protection assay for the Vβ3 gene segment relative to the sum of the protected counts from a pool of 15 Vβ gene segments. The error bar denotes 1 SD.

I-E$^+$ mice was 0.87 ± 0.42% of the total protected counts compared with 2.71 ± 0.45% in I-E$^-$ strains. Unlike the BALB strains, the relative peripheral Vβ3 transcript levels were similar in all five B10 congenic strains, regardless of MHC haplotype or I-E expression (Fig. 5). Thus, the relative level of Vβ3 expression is dependent not only on MHC haplotype, but also on other gene(s) not linked to MHC.

The B10 recombinants B10.A (3R) and B10.A (18R) were used to more precisely map the MHC gene(s) responsible for the low level of Vβ5.2 transcripts within the H-2 complex. B10.A (3R) and B10.A (18R) are genetically identical except for the I-E$_\alpha$ and a closely linked complement (S) loci (32) (Table I). B10.A (3R) mice have the E$^k_\alpha$ gene and therefore are I-E$^+$, whereas B10.A (18R) mice have the E$^b_\alpha$ gene, which is defective, and thus are I-E$^-$. The relative thymic levels of the Vβ gene segments in B10.A (3R) and B10.A (18R) were similar to the other strains analyzed (data not shown). In the periphery, relative levels of all the Vβ gene segments, except Vβ5.2, were similar between the two strains. The relative peripheral Vβ5.2 transcript level in the I-E$^+$ strain, however, was less than one third the level seen in the I-E$^-$ strain (Fig. 4 *c* and Table I). This clearly maps the low level of peripheral Vβ5.2 transcripts to the expression of I-E.

The Levels of Vβ Transcripts Vary According to Mls-1 Haplotype. Recently, Vβ-specific mAbs have been used by several investigators to show the Vβ6$^+$ and Vβ8.1$^+$ T cells

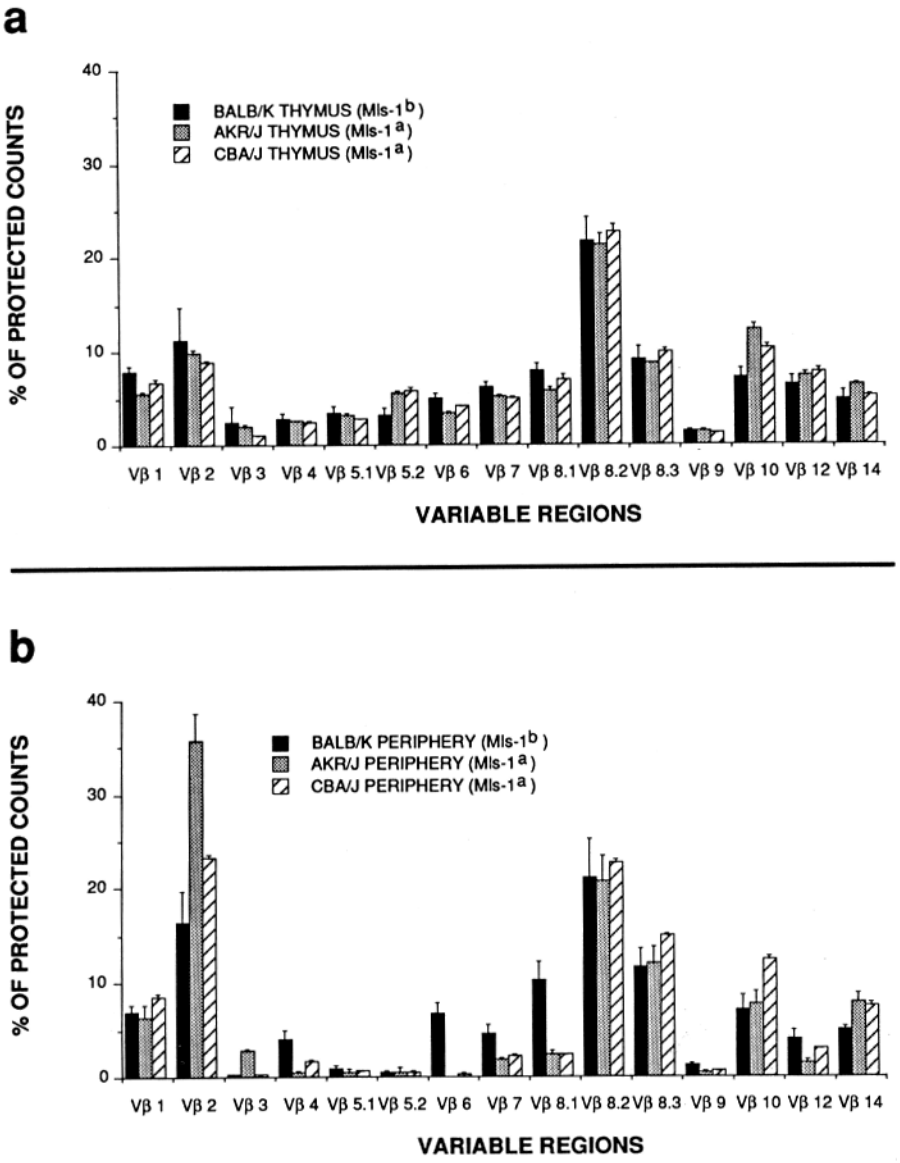

FIGURE 6. Relative transcript levels for 15 Vβ gene segments in RNA isolated from mice with differing Mls haplotypes. AKR/J and CBA/J are Mls-1^a, H-2^k mouse strains. BALB/k is a Mls-1^b, H-2^k mouse strain. The transcript levels are given as percent of protected counts, which is the amount of counts for a particular Vβ gene segment divided by the sum of all 15 Vβ segments counts. The error bar denotes 1 SD. (*a*) Measurements from RNA isolated from thymus. (*b*) Measurements from RNA isolated from peripheral lymphoid tissues (spleen and major lymph nodes).

are virtually absent in the periphery of Mls-1^a mice (16, 17). T cells using these particular Vβ gene segments appeared to be deleted in the thymus before they reach the periphery, presumably due to self tolerance. The vast majority of $V\beta6^+$ and $V\beta8.1^+$ T cells isolated from Mls-1^b mice were strongly stimulated by Mls-1^a stimu-

lator cells, suggesting that they recognized determinant(s) coded by the Mls-1^a gene. It was not established, however, whether T cells using other Vβ gene segments could recognize Mls-1^a determinants. To look for possible association between Vβ gene segments and Mls-1^a expression, RNA samples from thymus and peripheral lymphoid tissues of CBA/J (Mls-1^a, H-2^k) and AKR/J (Mls-1^a, H-2^k) were assayed for the levels of Vβ transcripts and compared with the H-2 identical mouse strain, BALB/k (H-2^k, Mls-1^b). The relative Vβ transcript levels in the thymus of AKR/J and CBA/J were very similar to that observed in the other strains of mice except for an increased level of Vβ10 from 44% to 72% (Fig. 6 *a*). However, several Vβ gene segments differed in their peripheral transcript levels between the Mls-1^a and Mls-1^b mice (Fig. 6 *b*). As expected from antibody studies, Vβ6 transcripts were barely detectable in either CBA/J or AKR/J mice, while in BALB/k mice, they represented 6.7 ± 1.2% of the protected counts. Also, Vβ8.1 transcript levels were reduced by greater than fourfold in both Mls-1^a strains compared with BALB/k.

Three additional Vβ gene segments not previously found to be associated with Mls-1^a reactivity had decreased peripheral transcript levels in Mls-1^a mice (Fig. 6 *b*). Vβ4, Vβ7, and Vβ12 had lower peripheral levels of transcripts in Mls-1^a haplotype mice compared with their thymic levels or to Mls-1^b haplotype mice. The decrease varied from 30% for Vβ12 in CBA/J mice to as much as 90% for Vβ4 in AKR/J mice.

The Vβ2 gene segment had higher peripheral transcript levels in the Mls-1^a mice (AKR/J and CBA/J) than the Mls-1^b (BALB/k) mice. Expression of Vβ2 transcripts in AKR/J mice was 3.7-fold greater in the periphery as compared with the thymus, and AKR/J also showed a 2.2-fold greater peripheral expression of Vβ2 transcripts than that found in the BALB/k strain. In the other strains assayed, the peripheral levels of Vβ2 transcripts were always greater than thymic levels, but the differences were much greater in AKR/J and CBA/J mice than previously measured.

The transcript levels of two Vβ gene segments were reduced in the periphery when compared with the thymus but in a manner independent of the Mls-1 haplotype. CBA/J and AKR/J mice are H-2^k and thus are I-E$^+$. Accordingly, relative peripheral Vβ5.2 transcript levels were greatly reduced in these mice, in agreement with the observations found in BALB and B10 congenic mice. Because of the I-E reactivity, it was not possible to look for Vβ5.2-associated Mls-1^a reactivity. There were no simple correlations between peripheral Vβ3 transcript levels and H-2 or Mls-1 haplotype (Fig. 6 *b*). CBA/J mice were similar to BALB mice with low peripheral Vβ3 transcript levels. In contrast, AKR/J mice were similar to B10 mice with peripheral Vβ3 transcript levels equal to their thymic levels (Fig. 5).

Discussion

In this study, we directly compared the relative transcript levels for 15 of the 22 known Vβ gene segments using an RNase protection assay. By assaying RNA isolated from primary tissues and directly measuring Vβ transcripts, many potential biases inherent in other methods used to measure Vβ frequency were avoided (18–20, 22, 33). Since there were no in vitro manipulations of the primary tissues before Vβ transcript measurements, problems associated with selective amplification were not present at either the cellular or molecular level. Such biases are present in assays dependent upon in vitro culturing, T cell clones, T cell hybridomas, or cDNA libraries (18–20, 22). Another problem associated with these methods is that the relatively

small sample size increases the risk of over- or underestimating the actual frequency of Vβ usage. Although there are some significant differences between previously reported values and our measurement, the relative frequency of use determined by most of these methods are roughly consistent with our findings.

The percentage of T cells expressing certain Vβ regions have been measured using antibodies that recognize particular or closely related Vβ regions. So far, five such antibodies have been reported in the literature (13, 34–36). Three mAbs recognize member(s) of the Vβ8 gene family; F23.1 recognizes T cells using any Vβ8 gene segments (37); KJ16 recognizes Vβ8.1 and Vβ8.2 gene segments (35); and F23.2 recognizes only the Vβ8.2 gene segment (16). Two antibodies, KJ23 and 44-22-1, recognize T cells expressing Vβ17a and Vβ6, respectively (13, Hengartner, H. et al., manuscript in preparation). The RNase protection assay measurements agree very well with much of the antibody studies. The percentage of $KJ16^+$ and $F23.1^+$ peripheral T cells in BALB/c mice are 18% and 25%, respectively, thus giving a KJ16/F23.1 ratio of 0.72 (35, 38). The corresponding values determined by RNase protection have an equivalent ratio of 0.72 (31.9:44.1) (Fig. 2). Also, the protected counts of Vβ8.1 and Vβ8.2 compared with the CβT-protected counts (when corrected for aberrant DJC transcripts) are approximately what would be expected from the percentage of $KJ16^+$ staining T cell (data not shown). The low peripheral Vβ6 and Vβ8.1 transcript levels in $Mls\text{-}1^a$ mice correlate well with the low percentage of T cells that stain with the corresponding antibodies (16, 17) (Fig. 6 *b*). The RNase protection values do not, however, agree with the ratio of 44-22-1/KJ16-staining cells in BALB/c. The antibody ratio (11:20 = 0.55) is much greater than the corresponding values determined by RNase protection (5.3:31.9 = 0.17) (Fig. 2).

A second possible cause for differing relative Vβ transcript levels is varying amounts of Vβ message per cell. Message stability or promoter strength can influence the amount of transcripts in a cell. Vβ8.2 is an example of a single Vβ8 gene segment with two promoters (39) that could lead to a higher level of transcripts per cell. Assay bias may also influence transcript measurements. All of the probes are specific for different V regions, and thus, they differ in size and in sequence. This may influence the rate of hybridization and stability of the RNA-RNA hybrids. These factors may account for the discrepancy between the RNase protection and the 44-22-1 staining data.

The different levels of Vβ transcripts suggest Vβ regions are used by T cells at very different frequencies. Since each V region is only represented once in the genome, gene copy number can not be the reason for the different amounts of Vβ transcript. Unlike immature B cells, which preferentially use V_H gene segments encoded nearest to the J_H region (40), there are no obvious correlations between the genomic order of the Vβ genes and their relative amounts of transcripts.

There are several stages during T cell maturation where the Vβ repertoire may be influenced. The initial Vβ gene segment recombination event in immature T cells could be biased for particular Vβ gene segments. The ability to associate with an α chain and form a functional TCR could vary among the different Vβ gene segments, and TCR that are encoded by particular Vβ gene segments may be subject to positive and/or negative selection. All of these factors may contribute to the appearance of the Vβ repertoire. Our work demonstrates that negative selection alters the peripheral Vβ repertoire.

Most of the Vβ transcripts in thymus RNA samples are from thymocytes that will never reach the periphery (41), whereas the peripheral Vβ transcripts are from T cells that have passed through the thymic selective processes. When relative abundance of thymic Vβ transcripts are compared with peripheral Vβ transcripts, most of the Vβ gene segments have similar levels (Figs. 2 and 3). Therefore, it is probable that thymic selective processes do not specifically recognize the Vβ region component of the TCR, but more likely elements of both the β and α chains. One exception to this rule is the inverse correlation between Vβ5.2 transcript levels and the expression of I-E. Peripheral Vβ5.2 transcript levels are low in the I-E$^+$, BALB/c, and BALB/k mice, compared with I-E$^-$, BALB/b mice (Figs. 3 and 4). As expected from codominant expression of H-2 molecules in heterozygous animals, there are relatively few peripheral Vβ5.2 transcripts in the progeny of BALB/b mice mated with either BALB/c or BALB/k mice (Fig. 4 *a*). The inverse correlation between Vβ5.2 and I-E expression is further strengthened by data from the H-2 congenic B10 mice. In I-E$^+$ B10 mice, peripheral Vβ5.2 message levels are three- to fivefold lower, compared with I-E$^-$ B10 mice (Fig. 4 *b*). Comparisons between the recombinant strains B10.A (3R) and B10.A (18R) strongly suggest that the relative low abundance of peripheral Vβ5.2 transcripts is due to the presence of surface I-E. These two strains genetically differ only in the haplotypes of I-E$_\alpha$ and the S locus (a complement component) (Table I). B10.A (18R) mice are I-E$^-$ and their peripheral Vβ5.2 transcript level is threefold greater than the peripheral level found in B10.A (3R) mice that are I-E$^+$ (Fig. 4 *c*). It is very unlikely that the complement factor haplotype alters the Vβ5.2 transcript levels. The two additional I-E$^+$ strains assayed, CBA/J and AKR/J, also lacked significant Vβ5.2 transcripts in their peripheral lymphoid tissues (Fig. 6 *b*). Unfortunately, it is not possible to determine from our data whether the low Vβ5.2 transcript levels are due to direct interactions between I-E and Vβ5.2 containing TCRs or if I-E expression causes some indirect changes that result in low Vβ5.2 transcript levels.

The inverse correlation between I-E and Vβ5.2 gene segment expression is very similar to the association between I-E and Vβ17a defined by Kappler et al. (12, 13). In mice that are I-E$^-$, between 4% and 14% of peripheral T cells express Vβ17a. When these mice are mated to I-E$^+$ mice, their progeny have a very low percentage of Vβ17a$^+$ T cells. The difference in the percentage of Vβ17a$^+$ T cells in I-E$^-$ and I-E$^+$ mice is similar to the difference in peripheral Vβ5.2 transcripts. Using the Vβ17a-specific mAb, KJ23, it was not possible to determine whether the absence of Vβ17a$^+$ T cells in I-E$^+$ mice was due to elimination of T cells expressing Vβ17a or whether those cells were KJ23$^-$ because their TCR had been masked, capped, and internalized, or blocked before translation. Our data demonstrate directly that the absence of Vβ17a$^+$ T cells in the periphery of I-E$^+$ mice is not due to post-transcriptional regulatory events, and therefore, support the hypothesis that it is due to the elimination of Vβ17a$^+$ T cells.

A second example of changes in peripheral Vβ transcript levels was observed among mice with differing Mls-1 haplotype. Five Vβ transcript levels (Vβ4, Vβ6, Vβ7, Vβ8.1, and Vβ12) were markedly reduced in the periphery of Mls-1^a mice compared with their levels in their thymus and to H-2-matched, Mls-1^b mice (Fig. 5). The amount of decrease varied from near 100% for Vβ6 to 30% for Vβ12. The decrease in Vβ6 transcript levels was approximately the same as the decrease in the percent of T

cells that bound the Vβ6-specific mAb 44-22-1 (17). There was a large decrease in the level of Vβ8.1 transcripts as well, but not the 30-fold decrease that Kappler et al. (16) found when they compared the percentage of $V\beta8.1^+$ T cells from Mls-1^a with Mls-1^b mice strains. The level of decrease that we observe was approximately fivefold, which is close to the percentage of $V\beta6^+$ hybridomas that are reactive to Mls-1^a (16). Although we have not directly demonstrated the involvement of T cells expressing Vβ4, Vβ7, and Vβ12 with Mls-1^a reactivity, the decrease in peripheral message levels for these Vβ regions in Mls-1^a mice suggests such a correlation exists.

Although Vβ3 transcript levels are influenced by H-2 haplotype, as demonstrated in BALB H-2 congenic mice (Figs. 3 and 5, Table I), they are not determined solely by the H-2 haplotype. Vβ3 transcript levels are low in BALB mice with the K and D haplotype of H-2, but not the B10 mice with the same H-2 haplotypes. CBA/J (H-2^k), like BALB/k, shows a reduction of Vβ3, whereas AKR/J (H-2^k) is similar to B10. One possibility for this observation is that a polymorphic antigen in BALB and CBA/J mice associates with H-2^k or H-2^d MHC molecules resulting in the elimination of T cells expressing Vβ3. One polymorphic locus that matches the pattern of Vβ3 expression is the Mls-2 locus (Fig. 5). The Mls-2 locus, like Mls-1 locus, codes for determinant(s) that elicit intense one-way mixed lymphocyte reactions between cells from mice that are identical at the MHC loci but disparate at Mls loci. BALB/c and CBA/J are Mls-2^a where B10 and AKR/J are Mls-2^b. We suggest that determinant(s) coded by Mls-2^a locus in the context of H-2^d or H-2^k, but not H-2^b, causes elimination of Vβ3-expressing cells. As noted earlier, H-2^d and H-2^k differ from H-2^b with the presence of a functional I-E molecule (28), suggesting that the Mls-2^a reactivity may be I-E restricted. Recently, two other groups have reported similar results for the association between Vβ3 and Mls-2^a (42, 43).

Our findings support the hypothesis that self tolerance to certain transplantation antigens results from late thymic (and/or peripheral) deletion of T cells bearing TCR β chains responsive to those antigens, but perhaps the most striking finding in this study is that the thymic Vβ repertoire does not reflect the frequency of genomic Vβ genes. Furthermore, there is no obvious correlation between the published 5′-3′ genomic order of Vβ genes (22, 25, 26) and their relative amounts of transcripts. It shall be important to determine whether these differences in Vβ expression reflect the initial frequency of Vβ gene rearrangements during thymic lymphocyte development and/or whether it is the result of intrathymic selection of T cells after surface Vβ expression.

Summary

We have measured the relative levels of transcripts for 15 of the 22 known Vβ gene segments. The level of transcripts for the highest and lowest expressed Vβ gene segment differed by >20-fold in the thymus and an even larger difference was observed in the periphery. The levels of expressions were unrelated to the order of the Vβ genes on the chromosome. For most of the Vβ gene segments, the relative transcript levels were the same in the thymus and periphery, suggesting that thymic selection in general does not act solely upon the Vβ gene segment. One Vβ gene segment in the BALB and B10 mice strains was an exception to this rule. Vβ5.2 expression in the periphery of BALB and B10 mice inversely correlated with the expression of the MHC class II molecule I-E. Five Vβ gene segments had reduced transcript levels

in the periphery of Mls-1^a mice compared with their thymic levels or to the levels found in Mls-1^b mice. The peripheral level of Vβ3 transcripts vary with MHC and Mls-2 haplotypes. The observation that certain Vβ transcript levels are reduced in the periphery when compared with the thymus favors the hypothesis that self tolerance at the T cell level results in the elimination of self-reactive T cells, rather than paralysis by a block at some post-transcriptional step. Finally, the wide variability of Vβ gene segment expression in the thymus suggests mechanisms exist to import an early bias to the repertoire. Whether this bias results from differential Vβ segment rearrangement rates, differential Vβ expression rates, or events occurring after TCR-α/β expression on immature/nonmature thymocyte cell surfaces is yet to be determined.

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