Polymorphism Within a TCRAV Family Influences the Repertoire Through Class I/II Restriction

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Polymorphism Within a TCRAV Family Influences the Repertoire Through Class I/II Restriction

Bee-Cheng Sim, Jay L. Wung, and Nicholas R. J. Gascoigne

Antibody-staining experiments have shown that closely related members of the TCRAV3 family are reciprocally selected into the CD4 or CD8 peripheral T cell subsets. This has been attributed to the individual AV3 members interacting preferentially with either MHC class I or MHC class II molecules. Single amino acid residues present in the complementarity-determining regions (CDR) CDR1α and CDR2α are important in determining MHC class specificity. We have now extended these observations to survey the expressed repertoire of the AV3 family in C57BL/6 mice. Three of the four expressed AV3 members are preferentially selected into the CD4+ subset of T cells. These share the same amino acid residue in both CDR1α and CDR2α that differ from the only CD8-skewed member. Preferential expression of an individual AV3 is not caused by other endogenous α- or β-chains, by any conserved CDR3 sequence, or by the usage of TCRAJ regions. This study shows that residues in the CDR1 and CDR2 regions are primary determinants for MHC class discrimination and suggests that polymorphism found within a TCRAV family has an important effect on the overall shaping of the T cell repertoire.


The T cell repertoire within an individual is biased toward the ability of the TCR to recognize MHC proteins. T cells that are positively selected on MHC class I protein develop into CD8+ peripheral T cells, and those that recognize MHC class II proteins become positively selected into CD4+ cells (1, 2). Transgenic studies show that the CD4 or CD8 phenotype of T cells will be in accordance with the specificity of the transgenic TCR for either MHC class I or MHC class II (3–6). All TCRAV regions analyzed to date with anti-TCRA Abs exhibit a remarkable preference to be “skewed” into either the CD4+ or CD8+ peripheral T cell subset. Vα3.2, Vα8, and human Vα12.1 are consistently found in a higher proportion in the CD8+ cells (7–9); while Vα3.1, Vα2, and Vα11.1/11.2 are more predominant in the CD4+ population of T cells (10–13). Because this phenomenon is generally independent of MHC haplotype, it has been strongly suggested that each of these Vα elements is positively selected by either MHC class I or MHC class II molecules. This view is consistent with reports that the TCR has an intrinsic ability to interact with MHC molecules (14, 15). In addition, the crystal structure of TCR-MHC class I complexes show the germline-encoded complementarity-determining regions (CDR) CDR1α and CDR2α of the TCR making significant contact with the MHC molecule (16, 17). In one case, the CDR1α and CDR2α contact the class I molecule, but the CDR1β and CDR2β have little or no interaction (17).

In C57BL/6 (B6) mice, the closely related Vα3.1 (AV3S5) and Vα3.2 (AV3S2) elements are reciprocally skewed into the CD4+ and CD8+ T cell subsets, respectively (7, 13). Comparison of the sequences of AV3S5 and AV3S2 shows that four amino acid residues differ between them. These are at positions 27, 51, 85, and 92. It has previously been shown that T cells expressing an AV3S5 transgene are skewed preferentially into the CD4+ subset (13, 18). Using a panel of mutant AV3S5 transgenes, we determined that residue 27 in the CDR1α region and residue 51 in the CDR2α region are sufficient for determining MHC specificity. When either of these residues in AV3S5 were mutated to the corresponding residue from AV3S2, the transgene expression was skewed to the CD8 cells (18).

The mouse TCRBV locus contains many more V regions than the TCRBV locus. Having undergone several rounds of gene duplication, most Vα families have several family members (19). Analysis of published Vα families shows that much of the within-family diversity is in the CDR1α and CDR2α regions (11, 20–23). With the presence of many closely related Vα genes with a high degree of polymorphism in CDR1α and CDR2α, which we know can result in biased usage of the various members of a Vα family in class I- or class II-restricted T cells, it is likely that the T cell repertoire will be affected. We therefore analyzed the expressed closely related members of the AV3 family in B6 mice. Only one of the four AV3 members observed in B6 is strongly over-represented in the CD8+ T cell subset, with the other three family members being biased to the CD4+ population to varying degrees. The three CD4-skewed members share similar amino acid residues in both the CDR1α and CDR2α and differ from particular residues found in the single CD8-skewed member. There appears to be no preference in TCRAJ usage in either T cell population. Taking into account the lack of obvious influence by endogenous β-chains, β-chains, and J regions, the unique residues present in the CDR1α and CDR2α are probably crucial for controlling selection into class I- or II-restricted T cells. Polymorphism within a TCRAV family will have a strong impact on MHC class discrimination which inevitably will contribute to the overall T cell repertoire.

Materials and Methods

Mice

The B6 mice were obtained from The Scripps Research Institute vivarium. The TCRA knockout mice, C57BL/6-J-Tcra<sup>tm1Mom</sup> (24) (TCRA<sup>−/−</sup>) were a seventh generation backcross to B6 and were purchased from The Jackson Laboratory (Bar Harbor, ME). The panel of Vα3 transgenic mice, namely...
Vo3.1, Vo3.1 m, CD1R m, and CD2R m, are described in detail elsewhere (18). Briefly, the α-chain from the T cell clone Ar-5 (25), AV3SSJ31, was used to generate the Vo3.1 transgenic mice. Specific point mutations were made in the wild-type AV3SS to resemble the closely related AV3S2 element giving rise to the three mutant transgenic lines. Therefore, the Vo3.1 m transgenic mice bear three mutations in the AV3SS gene at positions from serine (S27) to phenylalanine (F) (i.e., S27F, S51P, and S85W; the CD1R m transgenic mice carry the S27F single amino acid mutation in the CD1R α region, while the CD2R m mice have the S51P mutation in the CD2R α.

**FACS analysis and Abs**

Erythrocyte-free PBL were subjected to three-color FACS analysis. The wild-type Vo3.1 and CD2R m transgene-expressing cells were detected with an antiserum raised against AV3SS (13, 18), while lymphocytes expressing the Tα-TCR were expressed predominantly in the CD8 population.

In the Vo3β pairing experiments described in Figure 3, lymphocytes isolated from lymph nodes were subjected to four-color FACS analysis and sorted into the respective CD4+ and CD8+ cell subsets. Total RNAs from the sorted CD4+ and CD8+ population of T cells were extracted following standard procedures (26). First strand cDNA was reverse transcribed using a cDNA kit (Life Technologies, Grand Island, NY) and subjected to two rounds of PCR with nested AV3 primers. In the first round of PCR, the 5′ primer used was specific for the AV3 leader sequence (5′-TCCGAGCTCATGCCTGCGA CTCTGTGCA 3′), and the 3′ primer was specific for the 3′ end of the α-chain constant region (5′-TGGTATGCCAAGGCTGTTCAGGGAGAAGCCTTT 3′). The second round of PCR used a primer specific for the 5′ end of the variable region (5′-GATGCGAAGGCAGTCAAGT 3′) with the 3′ primer complementary to the 5′ end of the constant region (5′-CCGAGAAGGCTGCCTGCGATTGTGACGAGGGAGAAACCCTT 3′). Both 5′ and 3′ end primers will hybridize equally well to AV3-1, S2-, S3-, S5-, and S6 (no 5′ leader sequence is available for AV3S7). They will, however, not anneal to AV3S4 or AV3S8, which are very divergent members of the AV3 family, having ~75% identity at the amino acid level. It has previously been reported that AV3S4 was not very divergent members of the AV3 family, having

**Genomic Southern blot analysis**

Genomic DNA from the liver of B6 mice was isolated, subjected to complete restriction endonuclease digestion, separated on an 0.8% agarose gel, and blotted onto zeta-probe GT membrane (Bio-Rad, Richmond, CA) according to standard procedures (30). Hybridization was conducted at 68°C with a 220-bp AV3-specific probe, isolated from a genomic subclone of AV3SSJ31 (25). This probe spans part of the intron following the leader sequence and ~one-half of the variable region. The filter was washed in 3× SSC/0.1% SDS at 65°C with a final wash in 0.3× SSC/0.1% SDS. Under these fairly stringent conditions, no cross-hybridization to other Vα families is observed.

**Statistics**

Student’s t test, ANOVA, and Mann-Whitney tests were performed using the program InStat v2.01 (Graphpad Software).

**Results**

**Reciprocal skewing of AV3 family members into the CD4 and CD8 T cell subsets**

The frequency of expression of AV3 family members in the CD4+ and CD8+ peripheral T cell subsets in B6 mice was determined by FACS analysis. The AV3S2 TCR was differentiated from the other AV3 family members by the Vα-TCRβ pairing experiments described in Figure 3, with 3′-labeled 5′ primer for the second-step PCR, as described (29). PCR was performed with Taq polymerase. The PCR product was run on a 6% DNA sequencing gel and analyzed using a PhosphorImager and ImageQuaNT software (Molecular Dynamics, Sunnyvale, CA).

**PhosphorImager and ImageQuaNT software (Molecular Dynamics, Sunnyvale, CA).**

**FIGURE 1.** Ab staining of AV3 family members in CD8+ and CD4+ peripheral T cell subsets. Lymphocytes were triple-stained for FACS analysis as described in Materials and Methods. The frequency of Vo3.2 (AV3SS2) present in B6 mice is presented as the percentage of RR3-16+ cells in the respective T cell subsets. For the other AV3 family members, data are calculated as a percentage of cells positive for the Vo3.1 antiserum minus the percentage of RR3-16+ cells in the same T cell population (*). Symbols represent individual mice.

**PhosphorImager and ImageQuaNT software (Molecular Dynamics, Sunnyvale, CA).**
Endogenous \( \alpha \)-chains do not influence preferential AV3 transgene skewing

In all previous studies, the preferential skewing of \( \alpha \) has been observed in the presence of other endogenous \( \alpha \) elements. It has been suggested that during thymic development and in mature T cells, functional allelic exclusion is maintained by competition between two \( \alpha \)-chains for a single \( \beta \)-chain (31, 32). It is therefore possible that in transgenic studies, endogenous \( \alpha \) elements may influence an individual \( \alpha \) to be over-represented in the CD4 or CD8 subset. To address this question, we bred our panel of AV3 transgenic mice to the TCRA\( ^{-/-} \) strain so as to limit the choice of \( \alpha \)-chain to that of the transgene (Materials and Methods) (18). Second- and third-generation backcross offspring typed as AV3 transgene\( ^{-/-} \) were analyzed for expression of the transgene in the CD4 and CD8 population. When present as the sole expressed \( \alpha \)-chain, the Vo3.1 (AV3S5J31) transgene consistently showed an over-representation of the transgene in the CD4\( ^+ \) subset compared with the CD8\( ^+ \) population (Fig. 2). The ratio of the transgene expressed in the CD4\( ^+ \) to that in the CD8\( ^+ \) population (CD4-CD8 ratio \( \pm \) SD) is 2.24 \( \pm \) 0.39. In contrast, the three transgenic lines carrying various mutant forms of the AV3 chains showed a relatively higher proportion of CD8\( ^+ \) cells expressing the transgene (Fig. 2). The AV3S2-like Vo3.1 m\( ^+ \)TCRA\( ^{-/-} \) mice had the highest frequency of the mutant Vo3.1 transgene expressed in the CD8\( ^+ \) population with a CD4-CD8 ratio of 0.73 \( \pm \) 0.36. The two transgenes that each had a single residue from the AV3S2 sequence had a higher CD4-CD8 ratio. The CDR1 m\( ^+ \)TCRA\( ^{-/-} \) mice had a CD4-CD8 ratio of 1.06 \( \pm \) 0.20 while that of the CDR2 m\( ^+ \)TCRA\( ^{-/-} \) was 1.41 \( \pm \) 0.30. Although the effect of skewing was less prominent on the TCRA\( ^{-/-} \) than on the wild-type B6 background, these data clearly show that endogenous \( \alpha \)-chains did not affect the preferential skewing of the individual transgenic \( \alpha \)-chains. The strength of selection for class I restriction by the AV3S2 residue appears to be marginally stronger for the CDR1 than for the CDR2 region, with an additive effect as reflected in the Vo3.1 m transgenic line. This is consistent with the notion of preferential interaction with MHC class I or class II transgenic \( \alpha \)-chain.

Lack of \( \beta \)-chain participation in \( \alpha \) skewing

Because TCR functions as an \( \alpha \beta \) heterodimer and the fact that certain \( \beta \)-elements have been reported to show skewed expression themselves (33–38), the effect of the \( \beta \)-chain on the preferential \( \alpha \) selection was investigated. Lymph node T cells from the panel of AV3 transgenic mice were sorted into transgene-expressing CD4\( ^+ \) and transgene-expressing CD8\( ^+ \) T cell populations and stained for various \( \beta \)-chains. With all nine anti-\( \beta \) Abs tested (V\( \beta \)3, -6, -7, -8, -9, -10, -11, -12, and -13), T cells expressing the Vo3.1, Vo3.1 m, CDR1 m, and CDR2 m transgenes paired with approximately equal frequency with any particular \( \beta \) element (Fig. 3). An ANOVA test showed no significant differences in these groups, with the possible exception of CD4\( ^+ \) V\( \beta \)12\( ^+ \) cells, where there was a weakly significant difference (\( p = 0.05 \)). Student’s \( t \) tests between each of the groups showed that there were significant differences between CDR2 m and both Vo3.1 m and CDR1 m (\( p = 0.0105 \) and 0.0113, respectively). It is unclear whether this is a real difference. If so, we would also have expected a difference between wild-type Vo3.1 transgenic and these transgenics.

The similar pairing of the Vo3 transgenes with different \( \beta \)s is observed in both the CD4\( ^+ \) and CD8\( ^+ \) subsets. For example, lymphocytes bearing the Vo3.1 transgene plus V\( \beta \)3 constitute \( \sim 1.70 \pm 0.01 \% \) (\( \pm \) SD) in the CD8 population; Vo3.1 m/V\( \beta \)3,
classes. Due to the lack of good anti-V

BALB/c mice, four AV3 family members have been

DNA bands for each endonuclease digestion and thus suggests that

ods

an AV3-specific probe derived from AV3S5 (Fig. 4). Hence, skewing of an individual

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is with V

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3, 2.65 ± 0.21%; Vα3.1 m/Vβ3, 3.17 ± 0.64%; CDR1 m/Vβ3, 2.83 ± 0.47%; and CDR2 m/Vβ3, 3.45 ± 0.35% (Fig. 3B). Hence, skewing of an individual α-chain is not influenced by endogenous β-chains within the T cell subsets. The pairing frequency of the panel of transgenic Vα3 chains for different Vβs is largely a reflection of the frequency of the Vβs expressed in B6 mice (33, 35, 38, 39). For example, all four transgenic Vα3 chains pair most frequently with Vβ8, the most common Vβ in B6 mice, accounting for an average of ~14% in both T cell populations. Some of the Vβ usage varies between CD4 and CD8 subsets. This difference is significant by t test for Vβ7 (p = 0.0001), Vβ9 (p = 0.0016), Vβ11 (p = 0.0014), and Vβ13 (p < 0.0001) skewing to CD8 cells and for Vβ6 (p = 0.0017) and Vβ3 (p = 0.0475) skewing to CD4 cells. The skewing of Vβ usage in pairing with the transgenic α-chains was similar to expression in the whole population (Refs. 35 and 39 and data not shown). The exception to this is with Vβ6, where the expression in CD4+ cells is increased in the Vα3+ population (8.5 ± 0.52%) compared with the level in the CD4+ population at large (4.6 ± 0.8%, p = 0.0016) (33, 39). Thus, this Vβ element may more frequently pair with the Vα transgenes to make a class II-restricted heterodimer than some other Vβs. Vβ6 has been noted as being positively selected on the class II molecule I-E (33, 34).

Amino acid sequences in the CDR1α and CDR2α regions of the TCR influence MHC class discrimination

Southern blot analysis of genomic liver DNA from a wild-type B6 mouse digested with EcoRI, BamHI, and HindIII was probed with an AV3-specific probe derived from AV3S5 (Materials and Methods). This Southern analysis yielded four distinct AV3-hybridizing DNA bands for each endonuclease digestion and thus suggests that four genes exist for the AV3 family in this strain (Fig. 4). In BALB/c mice, four AV3 family members have been identified (23).

As shown in Figure 1, closely related members of the AV3 family are observed to undergo selection by different MHC classes. Due to the lack of good anti-Vα Abs that can differentiate between members of the same family, we have extended our anal-

ysis of the expressed repertoire of the AV3 family in B6 mice using DNA sequencing. mRNA from sorted CD4+ and CD8+ T cell subsets was extracted from B6 TCRA hemizygous mice (i.e., F1 mice derived from a cross between a B6 and a TCRA−/−) to avoid the problem of expression of two α-chain mRNAs (31, 32). By means of reverse transcription followed by two rounds of PCR, the variable regions of the expressed members of the AV3 family were cloned, sequenced, and compared with published data (23, 27, 40). Consistent with the Southern analysis, only four members of the AV3 family were detected by this strategy (Fig. 5): AV3S2 (known previously as Va3.2); AV3S3 (Va3.3); AV3S5 (Va3.1); and AV3S9. Identical in amino acid sequences except at position 67, AV3S5 and AV3S9 are two independent genes in B6 with other silent mutations throughout the nucleotide sequences. Both AV3S2 and AV3S9 have been reported elsewhere and have been established as B6 and bm12 TCRAV3 family members, respectively (23, 41, 42). AV3S3 was originally sequenced from the closely related strain, C57BL/10 (23, 41). AV3S5, previously reported from (BALB/c × A/J) TCRAα mice (25), was also expressed frequently in B6 mice. On the other hand, AV3S4 (40), originally derived from B6 and virtually identical with AV3S8 (derived from C.B20; a BALB/c-derived TCRAα strain congeneric for the IgH locus) (27), was not detected in our study. This is likely due to the relatively low homology in nucleotide and amino acid sequences of this member to the others in the AV3 family. AV3S4 and AV3S8 are as divergent from the rest of the AV3 family as AV3 is from the AV9 family (27). As shown in the sequence alignments, the B6 TCRAV3 family members displayed very limited sequence diversity at the nucleotide and amino acid levels, sharing >95% sequence homology (Fig. 5). Such strong sequence conservation suggests that these AV3 family members are the result of a series of recent gene duplication events. Importantly, the few differences observed between members are often to be found in the CDRs.

Of the AV3 α-chain cDNAs from the CD4+ and CD8+ populations that were randomly picked and sequenced in two independent experiments, a trend in the AV3 family member’s distribution into the CD4 and CD8 T cell subsets was apparent (Table I). Analyzing only clones that differ in the CDR3 region (i.e., independent clones), AV3S9, AV3S5, and AV3S3 were observed to be more frequently expressed in the CD4+ than the CD8+ peripheral T cell subset in both experiments, although to varying degrees. In contrast, AV3S2 is the only AV3 member that is strongly skewed into the CD8+ and is the least represented in the CD4+ population. This finding is consistent with FACS staining experiments performed with the antisera and RR3-16 (Fig. 1) and further confirms our transgenic studies (18).

Residues 27 and 51 that lie within the CDR1α and CDR2α regions, respectively (see Fig. 7), are important in determining the specificity for MHC restriction (18). The CD4-skewed TCRAV3 members, AV3S9, AV3S5, and AV3S3, are identical with each other in the CDR2α regions (Table I). In the CDR1α segment, AV3S9 and AV3S5 are similar to each other while AV3S3 bears an alanine residue in position 28 that differs from the glycine residue found in AV3S9 and AV3S5. In spite of this, all three CD4-biased members share the same amino acid residue in both CDR1α and CDR2α that differs in the single CD8-skewed member. In AV3S2, a phenylalanine residue in position 27 and a proline in position 51 replace the serine residue in both positions in the CD4-skewed members. It is therefore likely that the CDR1α and CDR2α residues that are unique to AV3S2 are responsible for its strong selection into the class I-restricted population. In contrast, the alanine in position 28, carried by the CD4-skewed AV3S3, is not sufficient or necessary to alter the selection of this Vα3 protein
from a class II-preferred to a class I-restricted phenotype. However, the conservative nature of the glycine/alanine change may mask an important role for residue 28 in the MHC interaction.

TCRAJ region usage and length of CDR3α

The TCRα-chain is generated by rearrangement of V and J gene segments, thereby making the α-chain J usage an additional factor that can affect the T cell repertoire. As shown in Table II, TCRAJ usage by the numerous α-chain cDNA clones in the CD4 and CD8 populations shows no clear pattern. Of the 21 TCRAJ segments used in 18 CD8-derived sequences and 22 CD4-derived sequences, only 4 were used in both groups. These are J7, J27, J37, and J39. Of the five most frequently used J regions (J27 was used five times, J21 and J37 were used four times, J34 and J22 were used three times), two were found only in CD8s (J21 and J22), one was seen only in CD4s (J34), and two were shared (J27 and J37). Thus, the overselection of the respective AV3 into the CD41 or CD81 peripheral T cell population is independent of a restricted set of Jα segments. The CDR3α results from VJ joining and N-region nucleotide addition during rearrangement of the TCR. The length of the CDR3 can be used as a measure of diversity. Minute changes in one amino acid in the CDR3 region can produce significant changes in overall TCR structure and alter recognition properties (43). In this study, the actual boundaries of the CDR3α have been determined from the mouse 2C TCR crystal structure (16). CDR3α starts at residue 93 and continues to two residues before the phenylalanine in the FGXG J-region motif (or before the leucine residue in the LGXG motif in the case of J7) (16). Although the AV3 clones found in the CD8 subset generally had slightly longer CDR3s than those present in the CD4s (medians of 9 and 10 amino acids, respectively) (Table II), the difference was not statistically significant. Spectratype analysis of CDR3 length showed no significant difference in length between AV3 family members expressed in the CD4 or CD8 subset (Fig. 6).

Table I. Skewed expression of AV3 family in B6 hemizygous mice into the CD4 and CD8 subsets

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Sequence</th>
<th>Representation</th>
</tr>
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<tbody>
<tr>
<td>AV3S9</td>
<td>VA3.1'</td>
<td>6/14 (42.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/22 (36.4%)</td>
</tr>
<tr>
<td>AV3S5</td>
<td>VA3.1</td>
<td>2/14 (14.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/22 (27.3%)</td>
</tr>
<tr>
<td>AV3S3</td>
<td>VA3.3</td>
<td>5/14 (35.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/22 (31.8%)</td>
</tr>
<tr>
<td>AV3S2</td>
<td>VA3.2</td>
<td>1/14 (7.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/22 (4.5%)</td>
</tr>
</tbody>
</table>

* Column 1 denotes the old and new nomenclatures (27) of the four expressed AV3 members. To differentiate the two variant forms of VA3.1, VA3.1' is used for AV3S9. Column 2 shows the differences in amino acid sequence in the CDR1 and CDR2 regions between the AV3 members. The appropriate residue change in each member is indicated. The frequency of expression of the different AV3 members in the CD4 and CD8 T cell populations are presented in the last column. The number of clones sequenced containing a particular AV3 with respect to total clones analyzed in two separate experiments is shown, with the calculated percentages in parentheses. Data from experiment 1 are indicated above that of experiment 2.
the corresponding CDR3 length of each individual clone from experiment 2 are listed. The lack of selec-
tivation specific for a rat class I molecule, that preferentially affect one Vα element, differ mainly by differential TCRAJ usage and CDR3 composition. Positive repertoire selection is believed to be independent of any specific CDR3/\(\beta\) requirements, while allo-
reactive responses require a more stringent CDR3/\(\beta\) composition to ensure a stronger MHC interaction (44, 45). The length distribution of a given CDR3 region has been used to predict recognition properties of TCR-\(\gamma\delta\), TCR-\(\alpha\beta\), and Igs and as a measure for diversity (29, 43). We did not find a significant difference in CDR3 length between the CD4 and CD8 populations expressing AV3. Overall, the TCR \(\alpha\) skewing into the CD4 or CD8 subset is due mainly to the Vα segment and is not achieved by differential TCRAJ element usage or contributed by a restricted CDR3\(\alpha\) sequence.

From analysis of published Vα sequences, it is difficult to assign sequence polymorphisms observed as allelic differences or differences between family members (11, 27, 48). AV3S4, previously isolated from B6 and designated as an AV3 member based on amino acid and nucleotide sequence homology to the family, was not detected in the present study. AV3S4 is more homologous to AV3S8 (98.6%). At the amino acid level, AV3S4 is as similar to AV3S1 and AV3S5 as AV3S5 is to AV9S1 and AV9S2 (75–80%). It is as similar to AV3S2, AV3S3, AV3S6, and AV3S7 as AV3S6 and AV3S7 are to AV9S1 and AV9S2 (70–75%). At the amino acid level, AV3S3 is more similar to AV9S1 and AV9S2 (75–80%) than it is to AV3S4 (70–75%). At the nucleotide level, AV3S3 is 75 to 80% identical with AV9S1 and AV9S2, but is 80–90% identical with V3S4. Based on the criteria that V gene segments that show >75% similarity at the nucleotide level are considered members of the same family (49), AV3S4 has been

discussion

It is remarkable that the five anti-Vα Abs (one of which is an antiserum) available in the mouse system show that each Vα segment is preferentially selected into either the CD4 or CD8 subset of peripheral T cells. This phenomenon is not only evident in mouse strains but is also observed in rats and humans (9, 44, 45).

**Table II.** TCRAJ usage and CDR3 length in the CD4 and CD8 subsets

<table>
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<tr>
<th>AV3</th>
<th>J usage</th>
<th>CDR3 length</th>
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<td>AV3S9</td>
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**Note:** The TCRAJ usage was deduced by comparison with published nucleotide se-
quences and adopting the nomenclature within (28). The CDR3 length has been ex-
trapolated from the 2C TCR crystal structure (16) where it is determined to start from

Discussion

It is remarkable that the five anti-Vα Abs (one of which is an antiserum) available in the mouse system show that each Vα segment is preferentially selected into either the CD4 or CD8 subset of peripheral T cells. This phenomenon is not only evident in mouse strains but is also observed in rats and humans (9, 44, 45).

Such prevalent skewing that is minimally affected by H-2 alleles is also observed in rats and humans (9, 44, 45). The second-round PCR included \(^{32}P\)-labeled 3'-primer. The PCR products were electrophoresed on a 6% DNA sequencing gel and exposed to a PhosphorImager screen. Analysis of the bands in the CD4 and CD8 lanes showed no significant difference in the length distribution.

**FIGURE 6.** Spectratype analysis of the CDR3\(\alpha\) lengths of AV3 mRNAs in the CD4 and CD8 subsets. mRNA from CD4\(^+\) and CD8\(^+\) TCRA hem-
izygous mice was subjected to RT-PCR as described in Materials and
Methods. The second-round PCR included \(^{32}P\)-labeled 3'-primer. The PCR
products were electrophoresed on a 6% DNA sequencing gel and exposed
to a PhosphorImager screen. Analysis of the bands in the CD4 and CD8
lanes showed no significant difference in the length distribution.
TCRAV3 FAMILY POLYMORPHISM INFLUENCES MHC RESTRICTION

FIGURE 7. Positions of the various amino acid residues that differ between AV3S5 and the other AV3 members. The 2C TCR structure is presented (protein database ID 1TCR) (16) viewed with the program RasMol. The α-chain is closer to the viewer and is shown in ribbon form, with the β-chain shown as strands. The molecule is oriented with the V domains above and the C domains below; thus the MHC-peptide recognition site is at the top of the structure. The residues that are different between AV3S5 and AV3S2 (A), AV3S3 (B), and AV3S9 (C) are shown, with side chains, in space-filling form. For clarity, the differences at positions 91 and 92 are not shown. These residues are involved in internal packing and do not form part of the CDR3.

classified as a member of the AV3 family. However, in our Southern blot analysis, only four Vα3-hybridizing bands were detected, which suggests the existence of four AV3 family members. The four different classes of nucleotide sequences obtained from our “library” of AV3 clones did not include sequences resembling AV3S4. With a >75% sequence similarity, AV3S4 should have generated an intense hybridization signal in our Southern analysis. Using a similar AV3 probe isolated from the Ar-5 T cell clone, Tan et al. (23) had also failed to isolate an “allelic form” of AV3S4 from BALB/c, leading them to exclude AV3S4 as a AV3 family member.

Due to the lack of availability of anti-Vα Abs that can differentiate between all members of the same AV family, the present strategy of nucleotide sequencing of expressed V regions from TCRA hemizygous mice was used for the AV3 family. This study provides strong evidence that the composition of the TCR CDR1α and CDR2α regions have a major role in selection on MHC class I and II. Single residues can markedly affect selection. Parallel observations are drawn for the TCRAV11 family (manuscript in preparation). The TCR has evolved toward having a strong intrinsic ability for binding to MHC molecules (14, 15). The mouse TCRAV locus is large, and most Vα genes have several family members (19, 50–54). Analysis of published Vα families show that family members tend to have significant variation in their CDR1α and CDR2α regions (11, 20–23, 27). Crystal structures of TCR in complex with their MHC/peptide ligands in both mouse and human models have also revealed significant contact points between the CDR1α and CDR2α with the MHC molecules (16, 17). In one of these cases, MHC interactions are provided by both CDR3α and β loops and by CDR1 and CDR2 of Vα but not by Vβ (17). It is possible that a Vα element can interact better with most alleles of either MHC class I or II because potential TCR contact sites along the α-helices are remarkably conserved within the MHC class. In the structure of Garboczi et al. (17), CDR1α contacts six class I residues of which four are conserved. The CDR2α makes contact with three class I residues, all of which are conserved in different haplotypes. The class II residues in the positions analogous to these TCR contact residues are also conserved within class II alleles, but the residues are different amino acids from those in class I. Therefore, significant structural differences exist between class I and II MHC proteins that can result in class distinction. If each member is biased to recognize either class I or II protein as governed by their CDR1α and CDR2α sequences, the relative size and composition of the CD4 and CD8 T cell repertoires will be affected. These data and the conservation of skewed selection across different MHC haplotypes could well explain the maintenance of large families of closely related Vα genes and the concentration of within-family diversity in the CDRs.

Figure 7 shows the respective positions of the amino acid residues that differ between the AV3 family members with respect to AV3S5. As shown in Figure 7A, CDR1α residue 27 and CDR2α residue 51, but not residue 85 in AV3S2 that differ from the CD4-skewed AV3 members, are poised for interaction with the MHC molecules. It is therefore not surprising that these residues are critically involved in MHC class selection. Residue 85 lies at the base of β strand F and is not necessary for MHC class specificity (18). In AV3S3, CDR1α residue 28, although well positioned for MHC interaction (Fig. 7B), was not sufficient or crucial in altering MHC restriction. This could still be an important residue for MHC contact, but with the glycine to alanine change not making a significant difference. The glycine to serine change at residue 61 and the arginine to glutamine change at residue 76 are fairly conservative and do not change the selection. While CDR1α and CDR2α undoubtedly play a major role in MHC class specificity, residues located outside these regions may have an effect on the degree of skewing. AV3S5 is less skewed into the CD4 population than AV3S9. Positioned on the fourth variable loop (CDR4) (16), serine 67 in AV3S5 may be less interactive than the bulky isoleucine carried by AV3S9 (Fig. 7C). Residues from the CDR4 lie over the MHC-peptide complex (17). A recent study has revealed that N-terminal residues, i.e., not only the canonical TCR CDR regions, can affect TCR engagement with MHC-peptide complex (55). We are currently addressing this question with transgenic mouse studies.
References


