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J Immunol 1998; 160:259-265; ;
http://www.jimmunol.org/content/160/1/259
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When contrasted with information available for placental mammals, very little is known about the development of immunocompetence in marsupials. Marsupials, however, provide interesting immunology problems, since most appear to be born at a stage of development much less mature than that of placental mammals. To further understand the marsupial immune system, the Ig repertoire of the short-tailed opossum, Monodelphis domestica, was characterized. The majority of the VH clones were isolated in an unbiased manner by screening a spleen cDNA phage library, using C region probes, or anchored PCR, using C region-specific primers paired with vector specific primers. Analysis of 54 unique VH sequences from this marsupial revealed the presence of two VH families in the expressed Ig repertoire. The larger family, which contributed the majority of the clones identified, appears to be derived from 10 to 12 germline VH segments. The second family of clones is derived from a single germline VH. Both VH families are related to group III sequences described in other vertebrates. Unusual codon bias differences between the two families may result in very different patterns of somatic mutation within the opossum Ig repertoire.


Mature Ig genes are assembled by recombination of the germline gene segments V, D, and J in the Ig heavy chain (Igh) and V and J in the Ig κ and λ light chains. From comparative studies of Ig gene content and expressed V sequences, three distinct mechanisms for creating V segment diversity have been found to predominate in different species: 1) germline diversity, 2) gene conversion, and 3) somatic mutation. Germline diversity refers to the presence of a significant number of different, functional VH segments in the germline Igh locus, which V(D)J recombination utilizes to create a diverse Ig repertoire. The diversity of VH sequences is usually evident by the presence of divergent families of VH, which have been found in mice and humans as well as nonmammalian vertebrates including amphibians and fish (1–4). A VH family is defined as one in which the members share >80% nucleotide similarity. In mice, for example, VH segments have diverged into at least 14 families based on sequence homology (reviewed in Ref. 1).

In contrast, some species have been shown to lack germline VH diversity and rely on post-V(D)J rearrangement mechanisms for diversifying their Ig. The chicken Igh locus, for example, contains only a single functional VH segment and several related VH pseudogenes (ψVH), limiting the number of functional germline rearrangements (5). Variation in the expressed VH region is generated by modifying the single, functionally rearranged VH through gene conversion using the ψVH as donor sequences. A similar system has been described for the Igh locus in rabbits, in which the majority of B cells rearrange the most D proximal VH segment, which is then modified by gene conversion (6). Alternatively, sheep and swine appear to derive their expressed Ig repertoire from a few closely related VH that are modified by somatic mutation before Ag exposure (7, 8). So far, in species using either gene conversion or somatic mutation, the germline VH are similar to each other and essentially belong to a single VH family. In chickens, rabbits, and swine, the VH family present is most closely related to group III families found in mice and humans, whereas in sheep the expressed VH appear to be group II (7–9). It is interesting that the three mechanisms of VH diversity (germline diversity, gene conversion, and somatic mutation) appear not to be linked to phylogeny. Comparative studies have greatly increased our understanding of the evolutionary history of these mechanisms and may reveal as yet undiscovered ways of creating VH diversity.

In the present study, we describe the diversity of VH segments expressed in the Ig repertoire of a metatherian (marsupial) mammal, the South American short-tailed opossum (Monodelphis domestica). Very little is presently known about the molecular development of immunocompetence in metatherians, which diverged from eutherians (placental mammals) at least 130 million years ago during the Cretaceous (10). M. domestica is a member of the didelphid marsupials, which are believed to be among the oldest living mammals and separated early from the other marsupial orders (11, 12). The immunologic studies that have been done with marsupials have yielded interesting differences from placental mammals including the general lack of a MLR, typically poor responses, and unusual patterns of isotype switching (reviewed in Refs. 13 and 14). M. domestica is available as a laboratory-bred marsupial, developed over the last decade as a model didelphid marsupial, which has also provided an opportunity to study immunologic development in a species with the general marsupial characteristic of being significantly less developed at birth than a placental mammal (13). Newborn M. domestica are at a developmental point similar to a human or mouse embryo at 8 wk or 13 days of gestation, respectively (13). At birth, there are few...
detectable lymphocytes, and the thymic epithelium appears undifferentiated (17). Studies using *M. domestica*, or any marsupial for that matter, have been restricted by the lack of knowledge concerning the ontogeny of immunocompetence and the generation of immune responses. To develop reagents for such studies, we recently began to characterize the *M. domestica* homologues of genes important for lymphocyte development, including RAG1 (18) and terminal deoxynucleotidyl transferase (33). Here, we report the characterization of the expressed *Monoledphils* *V*<sub>H</sub> repertoire.

**Materials and Methods**

**DNA, libraries, and probes**

All genomic *M. domestica* DNAs were extracted from liver tissue. Size-selected genomic DNA for library construction was prepared by electrophoresing EcoRI- or SpeI-digested DNA through agarose and extracting the DNA in the selected size range either by melting the agarose and using phenol and chloroform or using a gel extraction kit following the manufacturer’s recommended protocol (QIAquick; Qiagen, Chatsworth, CA). For constructing the EcoRI library, the size range of genomic DNA fragments isolated and pooled was 1 to 10 kb. The *SpeI* library was made specifically to isolate a genomic *V*<sub>H</sub> fragment and was constructed using genomic DNA fragments of a size range of 3 to 5 kb. The extracted DNA was ligated to ZAPII phage arms, packaged, and plated for screening following the manufacturer’s recommended protocol (Stratagene, La Jolla, CA).

A premade, commercially available *M. domestica* spleen cDNA library, also in ZAPII, was also purchased (Stratagene). A plasmid clone (P83) containing an unprocessed germine *V*<sub>H</sub> segment from another didelphid genus, *Didelphis virginiana* (North American opossum) was generously provided by Dr. Roy Riblet (Medical Biology Institute, La Jolla, CA). All other DNA clones used are described in this report. All probes used in this study were prepared as DNA inserts excised from the plasmids and labeled with [32P]dCTP using the random primer method (Prime-It Kit, Stratagene). All DNA sequences reported were derived by sequencing both directions using an automated DNA sequencer (Perkin-Elmer) and the 5′ and 3′ coding sequences of the C<sub>μ</sub> and C<sub>a</sub> regions were identified. Six of these clones contained C<sub>μ</sub> regions homologous to other mammalian C<sub>μ</sub> region genes, while the other two were homologous to C<sub>a</sub> (not shown). Not unexpectedly, all *V*<sub>H</sub> sequences present in these clones had a high degree of sequence homology with the germine *V*<sub>H</sub> regions (sequences 10, 11, 22, 23, and 26–28 in Fig. 1A). The C<sub>μ</sub> and C<sub>a</sub> sequences are available as GenBank accession numbers AF012109 and AF012110, respectively; a more complete description of the *M. domestica* C<sub>μ</sub> region sequences will be made elsewhere (R. D. Miller and G. H. Rosenberg manuscript in preparation). To screen for novel *V*<sub>H</sub> sequences without bias, a probe containing the *M. domestica* C<sub>μ</sub> coding sequence and lacking *V*<sub>H</sub>, D<sub>H</sub>, J<sub>H</sub>, and C<sub>μ</sub> regions was derived from one of the full length cDNAs and used to screen the spleen cDNA library. A total of nine clones (sequences 94 and 96 in Fig. 1A are representative) were identified, and the *V*<sub>H</sub> regions were sequenced. Although identified solely on the basis of the presence of C<sub>μ</sub>, all nine clones contained *V*<sub>H</sub> regions that had >85% nucleotide similarity to the previously identified *V*<sub>H</sub> sequences. All of these clones, therefore, are by definition members of a common *V*<sub>H</sub> family that we have designated MdoV<sub>H</sub>1 (*M. domestica* *V*<sub>H</sub> family 1).

**Results**

**Isolation of *M. domestica* *V*<sub>H</sub>**

The striking nucleotide conservation found in the first framework region (FR1) of group III *V*<sub>H</sub> gene segments from different species has facilitated using cloned *V*<sub>H</sub> from one species to cross-hybridize *V*<sub>H</sub> from another species (20). Using a cloned germine *V*<sub>H</sub> (P83) from *D. virginiana* as a probe, we were able to detect approximately eight faintly hybridizing bands, ranging in size from 1 to 10 kb, on a Southern blot of EcoRI-digested *M. domestica* genomic DNA (not shown). A phage library was constructed using size-selected EcoRI fragments from *M. domestica* genomic DNA, and the library was screened using P83 as a probe. Three unique phage clones were identified, isolated, and sequenced and found to contain partial *V*<sub>H</sub> segments with significant homology to other mammalian group III sequences (sequences 17, 18, and 39 in Fig. 1A).

Three clones contained the *χ* sequence in FR1. However, due to a common EcoRI site in the FR3 region, they lacked approximately 37 nucleotides of 3′ coding sequence and, since these clones are likely germine fragments, the recombination signal sequences also were missing.

**Isolation and characterization of *M. domestica* IgH cDNAs**

Using one of the germine *V*<sub>H</sub> clones (clone 17) as a probe, a spleen cDNA library from *M. domestica* was screened, and eight full length clones containing the *V*<sub>H</sub>, D<sub>H</sub>, J<sub>H</sub>, and C<sub>μ</sub> regions were identified. Six of these clones contained C<sub>μ</sub> regions homologous to other mammalian C<sub>μ</sub> region genes, while the other two were homologous to C<sub>a</sub> (not shown). Not unexpectedly, all *V*<sub>H</sub> sequences present in these clones had a high degree of sequence homology with the germine *V*<sub>H</sub> segments (sequences 10, 11, 22, 23, and 26–28 in Fig. 1A). The C<sub>μ</sub> and C<sub>a</sub> sequences are available as GenBank accession numbers AF012109 and AF012110, respectively; a more complete description of the *M. domestica* C<sub>μ</sub> region sequences will be made elsewhere (R. D. Miller and G. H. Rosenberg manuscript in preparation). To screen for novel *V*<sub>H</sub> sequences without bias, a probe containing the *M. domestica* C<sub>μ</sub> coding sequence and lacking *V*<sub>H</sub>, D<sub>H</sub>, and J<sub>H</sub> was derived from one of the full length cDNAs and used to screen the spleen cDNA library. A total of nine clones (sequences 94 and 96 in Fig. 1A are representative) were identified, and the *V*<sub>H</sub> regions were sequenced. Although identified solely on the basis of the presence of C<sub>μ</sub>, all nine clones contained *V*<sub>H</sub> regions that had >85% nucleotide similarity to the previously identified *V*<sub>H</sub> sequences. All of these clones, therefore, are by definition members of a common *V*<sub>H</sub> family that we have designated MdoV<sub>H</sub>1 (*M. domestica* *V*<sub>H</sub> family 1).

**Search for other *M. domestica* *V*<sub>H</sub> families**

To expedite and simplify the screening of large numbers of expressed *V*<sub>H</sub> sequences, anchored PCR was performed using the spleen cDNA library as target. Reverse primers complementary to the 5′ coding sequences of the C<sub>μ</sub> and C<sub>a</sub> regions were designed and paired in PCR with primers specific for cloning vector sequences in the ZAPII phage used to construct the cDNA library. PAGE of the amplified products revealed a diffuse band ~600 bp (not shown), which is close to the predicted product size for amplifying a complete V region domain, including the CDR3 region and the 5′ end of the constant region. Since the CDR3 region is created by the junction of the *V*<sub>H</sub>, D<sub>H</sub>, and J<sub>H</sub> segments and can contain variable numbers of nucleotides, variable length PCR products would be expected if amplification were polyclonal. Similar results were achieved using the C<sub>μ</sub> or C<sub>a</sub> primers paired with either the M13 or T3 primers in PCR. A total of 32 new, unique *V*<sub>H</sub> sequences were generated from this strategy (25 using the C<sub>μ</sub>
primer and 7 using the Ca primer). Most of these sequences show 80% similarity to the known MdoV H1 family (Fig. 1A, sequences 298, 338, 356, and 364 are representative). However, six clones were found to differ significantly from the MdoV H1 family (Fig. 1B). This second set of sequences shared, on average, 75% nucleotide homology with the MdoV H1 sequences. These differences, by convention, place the sequences shown in Figure 1B in a second VH family, which we have designated MdoV H2. Two of the six MdoV H2 clones were later found to be identical to the germline MdoV H2 sequence and are not shown. The cloning of the germline MdoV H2 gene segment is described in the next section.

FIGURE 1. Alignment of representative Monodelphis V\textsubscript{H} sequences from MdoV\textsubscript{H1} (A) and MdoV\textsubscript{H2} (B). The designation following the clone number refers to isolation by hybridization from the spleen cDNA library (c) or by anchored PCR using C\textmu-(m) or Ca (a)-specific primers. Clones 17, 18, and 39 in A (GenBank accession nos. AF012123, AF020794, and AF020795) and the sequence designated “germline” in B (GenBank accession no. AF012124) are sequences from genomic DNA clones. For alignment purposes, the intron sequence has been deleted from genomic sequences. The CDR3 regions are not shown. Dashes indicate similarity and periods indicate gaps or missing data.
found 74% similarity at the nucleotide level and 68% identity at the amino acid level in the coding region (Fig. 2). As shown in Figure 2, most of the differences are in CDR1 and CDR2, although there are stretches of nucleotides in both FR1 and FR3 which contribute to the differences between the V segments. All six MdoVH2 sequences isolated by PCR were generated using the Cm primer; none were found using Ca. Using clone 340 as a probe, the spleen cDNA library was screened for additional full length clones containing a VH from the MdoVH2 family. Screening the equivalent of approximately 8.3 x 10^4 phage clones detected only a single clone, an IgA, containing an MdoVH2 family sequence (sequence 511 in Fig. 1B).

Numbers of VH segments in the M. domestica genome

Using a representative VH clone from each of the two families as probes, a Southern blot containing M. domestica genomic DNA digested with various restriction enzymes was probed using either clones 356 (A, MdoVH1) or 340 (B, MdoVH2). The probes were derived from cDNAs to eliminate problems of repetitive DNA sequences found in the genomic clones. Restriction enzymes shown are: B, BamHI; E, EcoRI; EV, EcoRV; H, HindIII; P, PstI; S, SacI; and X, XbaI. Size markers are shown in kilobases.

However, we cannot rule out the possibility that some more intense bands represent multiple VH segments comigrating. The estimate of the total number of MdoVH1 gene segments is based on observing the number of bands present using a variety of restriction enzymes. In contrast, the MdoVH2 family probe (clone 340) hybridizes to only a single restriction fragment in most lanes, suggesting that the MdoVH2 family comprises only a single VH. The MdoVH2 family probe also hybridized a 4.3-kb SpeI fragment from M. domestica genomic DNA (not shown), which was cloned from a phage library constructed from SpeI-digested, size-selected genomic DNA to determine the germline sequence of this single-member VH family. This sequence is shown as the "germline" sequence in Figure 1B for comparison with the sequences generated by PCR amplification.

For purposes of alignment, only the coding sequence from the germline MdoVH2 gene segment are shown (Fig. 1B). The intron and recombination signal sequence (RSS) sequences for the germline MdoVH2 gene segment are available from GenBank as accession number AF012124.

**Phylogenetic analysis of M. domestica VH sequences**

Both the MdoVH1 and MdoVH2 sequences consistently showed the highest match with human V3 family sequences (group III) when compared with the GenBank database using the BLAST algorithms. However, when compared with the Kabat database (21) at the amino acid level, MdoVH2 was most similar to mouse and human group II VH (not shown). To investigate these relationships further, a nucleotide alignment for mammalian VH was made using representative sequences for each of the 2 opossum, 14 mouse, and 6 human VH families so far identified, and the single known rabbit, swine, and sheep VH families available. The human VH7 family was not included in this analysis as it is closely related to human VH1. Included in the alignment was the only other known marsupial VH sequence, from the North American opossum D. virginiana. Using these alignments, a phylogenetic tree was generated (Fig. 4) that had similar topology to those reported by others (1, 9).
Interestingly, all of the marsupial V\textsubscript{H} sequences, including the North American opossum, clustered on a common branch, and this branch shared a common node with some of the human V3 sequences. For display purposes, the tree was rooted using the group I clan, which was chosen as the “outgroup.” Species designations or GenBank accession numbers are as follows: \textit{M. domestica}, this report; \textit{D. virgini-ana} (P83), provided by Dr. Roy Riblet; human, accession nos. M18510, M99646, M99686, U80145, U80147, U80148, U80162, X05714, and X64147; mouse, K01569, U04227, M21470, X03303, X55935, Z37145 (J558 (1)), M25465 (J558 (2)), X03398, M27021, J02576 (S107 (V1)), M16725 (S107 (V1)), M31285, X03301 M35502, M18510, M99646, M99686, U80145, U80147, U80148, U80162, X05714, and X64147; sheep, Z49180; rabbit, M93171; and pig, U15194.

**Discussion**

Defining the presence of V\textsubscript{H} families in \textit{M. domestica} is an important first step in understanding the development of immunocompetence in a species with developmental timing very different from mice or humans. How V\textsubscript{H} sequence diversity shapes the Ig repertoire has been best studied in mice and humans, both species possessing extensive V\textsubscript{H} sequence variation in their genomes. With the discovery of the limited available functional V\textsubscript{H} segments and the requirement for postrearrangement gene conversion to generate a diverse V\textsubscript{H} repertoire in chickens and rabbits (5, 6) and the \textit{V}\textsubscript{\lambda} of cattle (22), it is apparent that mouse or human Ig genetics are not a satisfying paradigm for all vertebrates. Studies of V\textsubscript{H} genes in sheep and pigs have found limited germline diversity in these species as well (7, 8). We report the first molecular analysis of a marsupial Ig repertoire and demonstrate that, in at least this one metatherian species, the available germline V\textsubscript{H} repertoire also appears to be limited in number and diversity. It is interesting to speculate that, as information is collected on more species, rodents and pri mates may turn out to be the exceptions among mammals rather than the rule by maintaining diverse V\textsubscript{H} families in their genomes.

Although the available germline V\textsubscript{H} repertoire appears limited in \textit{M. domestica}, a significant number of sequence differences separate the V\textsubscript{H} into two divergent families, designated here as MdoV\textsubscript{H}1 and MdoV\textsubscript{H}2. By convention, V\textsubscript{H} sequences have been grouped into a common family when they share at least 80% nucleotide homology. Initially, this cut-off was established as the limit of cross-hybridization between V\textsubscript{H} sequences on Southern blots using standard hybridization stringency (23). The separation of the \textit{M. domestica} V\textsubscript{H} into two distinct families fits these criteria, with <75% similarity shared at the nucleotide level between MdoV\textsubscript{H}2 and any of the MdoV\textsubscript{H}1 sequences found so far. It should be cautioned that since many of the sequences presented in Figure 1 were generated by PCR, some nucleotide differences may be cloning artifacts. However, the significant sequences differences found between MdoV\textsubscript{H}1 and MdoV\textsubscript{H}2 cannot be accounted for by cloning artifacts. Based on nucleotide sequence homology and phylogenetic analysis, both families fall into group III, and most of the segments contain the \textit{\chi} sequence (5’-GCTGGTGG) in FR1, a characteristic found to be conserved across group III families in all vertebrates (20). The MdoV\textsubscript{H}1 and MdoV\textsubscript{H}2 family sequences are further separated by a two- and sometimes three-codon deletion in the CDR2 region of MdoV\textsubscript{H}1, which would correspond to amino acid residues 54, 55, and 56 in MdoV\textsubscript{H}2. The MdoV\textsubscript{H}1 sequences shown in Figure 1A were chosen to represent the diversity of sequences found in this family. Based on FR, CDR, and leader sequence differences, 6 would be a conservative estimate of the number of independent V\textsubscript{H}1 segments representative of the 10 to 12 germline segments seen by Southern blot analysis. A comparison of sequence 11 with sequences 338 (codon deletion in CDR2), 364 (codon insertion in FR3), 10, and 57 (distinct leader sequences) suggest that they are derived from five unique V\textsubscript{H}1 segments. Sequence 26 has a significantly different FR1 sequence and likely represents a sixth germline V\textsubscript{H}1 segment. All of the sequences shown have significant nucleotide differences and probably represent other distinct germline V\textsubscript{H}1 members, but at this time, without all of the germline MdoV\textsubscript{H}1 having been sequenced, cloning artifacts and somatic mutations in rearranged V\textsubscript{H} segments cannot be ruled out. Some of the germline MdoV\textsubscript{H}1 segments are likely to be pseudogenes that are not expressed. The recent completion of the entire human \textit{Igh} locus found that 43 of 87 V\textsubscript{H}1 segments were pseudogenes (24, 25). How many of the MdoV\textsubscript{H}1 members are functional will be determined when all germline members are sequenced, which will also provide the opportunity to analyze the role of gene conversion in V\textsubscript{H} diversity in this species. Most of the expressed V\textsubscript{H} repertoire is derived from the MdoV\textsubscript{H}1 family members, which may simply reflect MdoV\textsubscript{H}1 having multiple members while MdoV\textsubscript{H}2 has only a single member. V\textsubscript{H} families with one or only a few members have been described in the \textit{Igh} locus of other species as well. The mouse V\textsubscript{H}12 and V\textsubscript{H}3609N families (26, 27) and the human V\textsubscript{H}6 (28) family are examples. Any duplications, functional or not, which might have occurred during the evolution of MdoV\textsubscript{H}2 in the marsupial \textit{Igh} have not been maintained in the \textit{Monodelphis} genome. Since additional copies of MdoV\textsubscript{H}2 family members appear not to exist in \textit{Monodelphis}, it seems unlikely that...
gene conversion would contribute to diversification of expressed MdoVH2 sequences. However, it is possible that, as in sheep (7), somatic mutation before Ag selection may contribute to the diversity of a primary MdoVH2 repertoire. Further analysis of rearrangements using MdoVH2 during early B cell ontogeny in this species is needed to address this question. Whether MdoVH2 homologues exist in other marsupial species also remains to be determined.

Evolution of marsupial V\textsubscript{H} genes

All Monodelphis V\textsubscript{H} found so far are most similar to the group III-related sequences from other species; however, the degree of nucleotide differences between MdoVH\textsubscript{1} and MdoVH\textsubscript{2} suggests that they are not recent duplications. Phylogenetic analysis of MdoVH\textsubscript{1} and MdoVH\textsubscript{2} places these two V\textsubscript{H} families on a common branch within the group III V\textsubscript{H} families, most closely to some of the human V3 gene segments. This is not unusual given that the divergence times of some of the V\textsubscript{H} families predates mammalian radiation (9, 20, 29). The only other known marsupial V\textsubscript{H} is from another didelphid, D. virginiana, which is also a group III member and is most closely related to MdoVH\textsubscript{1}. From the phylogenetic tree, it is impossible to determine whether the separation of MdoVH\textsubscript{1} and MdoVH\textsubscript{2} occurred before or after the divergence of marsupials from placentals 130 million years ago, but it probably did occur before the radiation of the didelphid marsupials (i.e., Monodelphis from Didelphis), which has been estimated to have occurred anywhere between 15 to 50 million years ago (11).

It is curious that MdoVH\textsubscript{1} and MdoVH\textsubscript{2} are so closely related phylogenetically given that they share <75\% nucleotide similarity on average. In contrast, the human V3 sequences share >75\% nucleotide similarity, yet V3–15 is separated phylogenetically from the other V3 members in the alignment shown in Figure 4. The sequence dissimilarity between MdoVH1 and MdoVH2 is apparent in the phylogenetic tree by the long branch length for MdoVH2. Phylogenetic relatedness with a low overall sequence similarity can be explained by the pattern of nucleotide sequence distribution and distinctly different codon usage in the CDR1 and CDR2 regions of MdoVH2 as compared with MdoVH1. As described below, this may reflect a different pattern of somatic mutation in MdoVH2 compared with MdoVH1.

Codon usage and the likelihood of somatic mutation

Targeting somatic mutation to the CDRs has been associated with nonrandom distribution of nucleotide sequences in V region gene segments. Rogozin and Kolchanov (30) reported finding that two sites, RGY and TAA, are more likely to show a mutation than other sequences, with the mutation most likely occurring at the underlined position. The most notable mutational “hot spot” is the serine codon AGY, and an unusual pattern of codon bias for serines is found in variable regions, with the nonmutable serine codon, TCA, predominating in FRs and the mutable AGY in CDRs (31, 32). All V\textsubscript{H} families in mammals demonstrate this pattern, and the MdoVH\textsubscript{1} family sequences are no exception, further demonstrating the conserved nature of this mechanism for directing muta-
tion to the CDRs. However, while the CDRs of most MdoVH\textsubscript{1} sequences are serine rich, the CDR1 and CDR2 regions of MdoVH\textsubscript{2} are strikingly devoid of serines, as can be seen in the alignment shown in Figure 2. This suggests that the MdoVH2 gene segment may be under very different selection pressure(s) than the MdoVH1 family members. For example, in the MdoVH1 germline sequence from clone 17 (Fig. 1A), there are 13 serines, 8 in the FRs and 5 in the CDRs. Of the FR serines, 6 of 8 use the TCA codon and the other 2 use AGY. Four of the 5 serines present in the CDRs use the AGY codon and the other uses TCA. It is likely then that the members of the MdoVH1 family are good targets for somatic mutation directed toward the CDRs by maintaining bias toward AGY in the CDRs. In contrast, the sequence “AGY” is noticeably absent from the MdoVH2 CDR1 and CDR2 regions in any reading frame, although other potential mutation hot spots are still present. The codon “GTT” encoding glycine satisfies the RGY motif and TAA is present in a CDR as GTA ATA, although because of the reading frame, changing the first A in the sequence would result in a silent mutation. Whether MdoVH2 demonstrates an unusual pattern of somatic mutation or is devoid of somatic mutations remains to be seen through further analysis. All of the Ig cDNA clones that contain MdoVH2 have been, so far, IgM clones; an immediate goal will be to search for the use of this V\textsubscript{H} family in other isotypes to analyze patterns of somatic mutation presumably following Ag activation and affinity maturation.

Acknowledgments

The authors thank Drs. Ann Feeney, Tom Kepler, and Roy Riblet for helpful discussions and Dr. David Swofford for allowing us the use of the new test version of PAUP.

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