A unique T cell receptor discovered in marsupials

Zuly E. Parra*, Michelle L. Baker*, Ryan S. Schwarz*, Janine E. Deakin†, Kerstin Lindblad-Toh‡, and Robert D. Miller*§

*Center for Evolutionary and Theoretical Immunology and Department of Biology, University of New Mexico, Albuquerque, NM 87131; †Australian Research Council Centre for Kangaroo Genomics, Research School of Biological Sciences, Australian National University, Canberra, ACT 0200, Australia; and ‡Broad Institute, 320 Charles Street, Cambridge, MA 02141

Edited by Max D. Cooper, University of Alabama, Birmingham, AL, and approved April 20, 2007 (received for review October 14, 2006)

T cells recognize antigens by using T cell receptors (TCRs) encoded by gene segments, called variable (V), diversity (D), and joining (J), that undergo somatic recombination to create diverse binding specificities. Four TCR chains (α, β, γ, and δ) have been identified to date, and, as T cells develop in the thymus, they express exclusively either an αβTCR or a γδTCR heterodimer. Here, we show that marsupials have an additional TCR (TCRμ) that has V, D, and J that are either somatically recombined, as in conventional TCRs, or are already prejoined in the germ-line DNA in a manner consistent with their creation by retrotransposition. TCRμ does not have a known homolog in eutherian mammals but has features analogous to a recently described TCRδ isoform in sharks. TCRμ is expressed in at least two mRNA isoforms that appear capable of encoding a full-length protein, both of which are transcribed in the thymus and spleen. One contains two variable domains: a somatically recombined V and a prejoined V. This appears to be the dominant isoform in peripheral lymphoid tissue. The other isoform contains only the prejoined V and is structurally more similar to conventional TCR chains, however invariant. Unlike other TCRs, TCRμ uses prejoined gene segments and is likely present in all marsupials. Its similarity to a TCR isoform in sharks suggests that it, or something similar, may be present in other vertebrate lineages and, therefore, may represent an ancient receptor system.

evolution | immune system

Hallmarks of the adaptive immune systems in jawed vertebrates are cells (lymphocytes) that use somatic DNA recombination to assemble the genes that encode antigen receptors. This recombination provides the means to generate a large repertoire of receptors with diverse binding specificities (1). There are two classes of antigen receptors that are used by B and T cells, respectively: Ig and T cell receptor (TCR). Although there is variation in the isotypes of Ig present, to date, all jawed vertebrates appear to have the same four TCR homologs (2, 3): TCRα, -β, -γ, and -δ. All contain V domains that are encoded by the variable (V), diversity (D), and joining (J) gene segments; recombined V and J encode the V domain in TCRα and TCRγ, whereas the TCRβ and TCRδ chains use V, D, and J (4). A typical TCR locus is organized in a "translocon" arrangement in which an array of V segments is upstream of one or more D segments (in the case of TCRβ and TCRδ), followed by one or more J segments (4). In developing T cells, these gene segments undergo so-called VDJ recombination that is mediated, in part, by the recombination activating gene (RAG) recombinase system to assemble the exon encoding the V domain (1, 5). To date, the V, D, and J gene segments in TCR loci have always been found in a nonrecombined state in the germ-line DNA and require somatic cell recombination for expression. After recombination, T cells are then selected in the thymus to eliminate those that bind self-antigens (contributing to self-tolerance) and, in the case of αβTCR and some γδTCRs, for ability to bind molecules encoded by the MHC (4). αβTCR and some γδTCRs bind antigens that are presented on MHC molecules, whereas other γδTCRs bind antigen directly (6).

The marsupials are of a distinct mammalian lineage that is noted for highly altricial young that are comparatively less mature than that of typical eutherian ("placental") mammals (7–12). In contrast to most eutherian mammals, which have a thymus and other lymphoid tissues that develop *in utero*, the marsupial thymus is primarily undifferentiated epithelium at birth, and there are few or no circulating lymphocytes. T cell-dependent responses in marsupials are correspondingly absent or delayed in the first week of life, and the young are highly dependent on maternal protection (7–12).

The evolutionary time separating marsupials and eutherians, which last shared a common ancestor 170–180 Mya (13, 14), make it possible that marsupials have evolved immune strategies that are not found in more commonly studied eutherians. Here we report a locus in marsupials that encodes a TCR chain that is not found in eutherians and that undergoes VDJ recombination in the thymus. First isolated as an expressed sequence tag, this locus was thought to encode a divergent TCRδ (15). Several characteristics, however, support that this is a distinct TCR isotype, which we have named TCRμ (μ or M for marsupial) in recognition of its identification in marsupials. TCRμ has been found in three marsupial species to date: the gray short-tailed opossum *Monodelphis domestica*, the Northern brown bandicoot *Isoodon macrourus*, and the tammar wallaby *Macropus eugenii.* Given the distant phylogenetic relationship of these three species, TCRμ is likely to be common to all marsupials (15, 16).

Results

TCRμ Is Encoded at a Unique TCR Locus. FISH localizes the TCRμ genes to *M. domestica* chromosome 3, nonsyntenic to the conventional TCR located on chromosomes 1p (TCRα/δ), 8q (TCRβ), and 6q (TCRγ) [supporting information (SI) Fig. 5] (17). In the *M. domestica* genome, TCRμ is organized as six tandem clusters covering a 614-kb region. Each cluster contains one V (Vμ), two or three D (Dμ), and one J (Jμ) gene segment(s), along with the exons encoding constant (Cμ), connecting peptide, and transmembrane (Tm)–cytoplasmic (Ct) regions (Fig. 1*A* and SI Fig. 6). The Tm–Ct exon encodes basic amino acids (lysine and arginine) with positions that are conserved in the Tm region of conventional TCR chains and that participate in formation of the TCR-CD3 cell surface complex (18) (Figs. 1*B* and 2). Also present in the connecting peptide is a conserved cysteine that in conventional TCR chains participates in interchain disulfide bonds that form the TCR het-

Author contributions: Z.E.P. and R.D.M. designed research; Z.E.P., M.L.B., R.S.S., and J.E.D. performed research; M.L.B., R.S.S., and K.L.-T. contributed new reagents/analytic tools; Z.E.P., M.L.B., J.E.D., K.L.-T., and R.D.M. analyzed data; and Z.E.P. and R.D.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Abbreviations: C, constant; CDR, complementarity determining region; Ct, cytoplasmic; D, diversity; FR, framework region; J, joining; L, leader; NAR, nurse shark antigen receptor; RAG, recombination activating gene; TCR, T cell receptor; Tm, transmembrane; V, variable.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ979394–DQ979411 and EF503715–EF503723).

§To whom correspondence should be addressed at: Department of Biology, University of New Mexico, MSC03 2020, Albuquerque, NM 87131-0001. E-mail: rdmiller@unm.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0609106104/DC1.

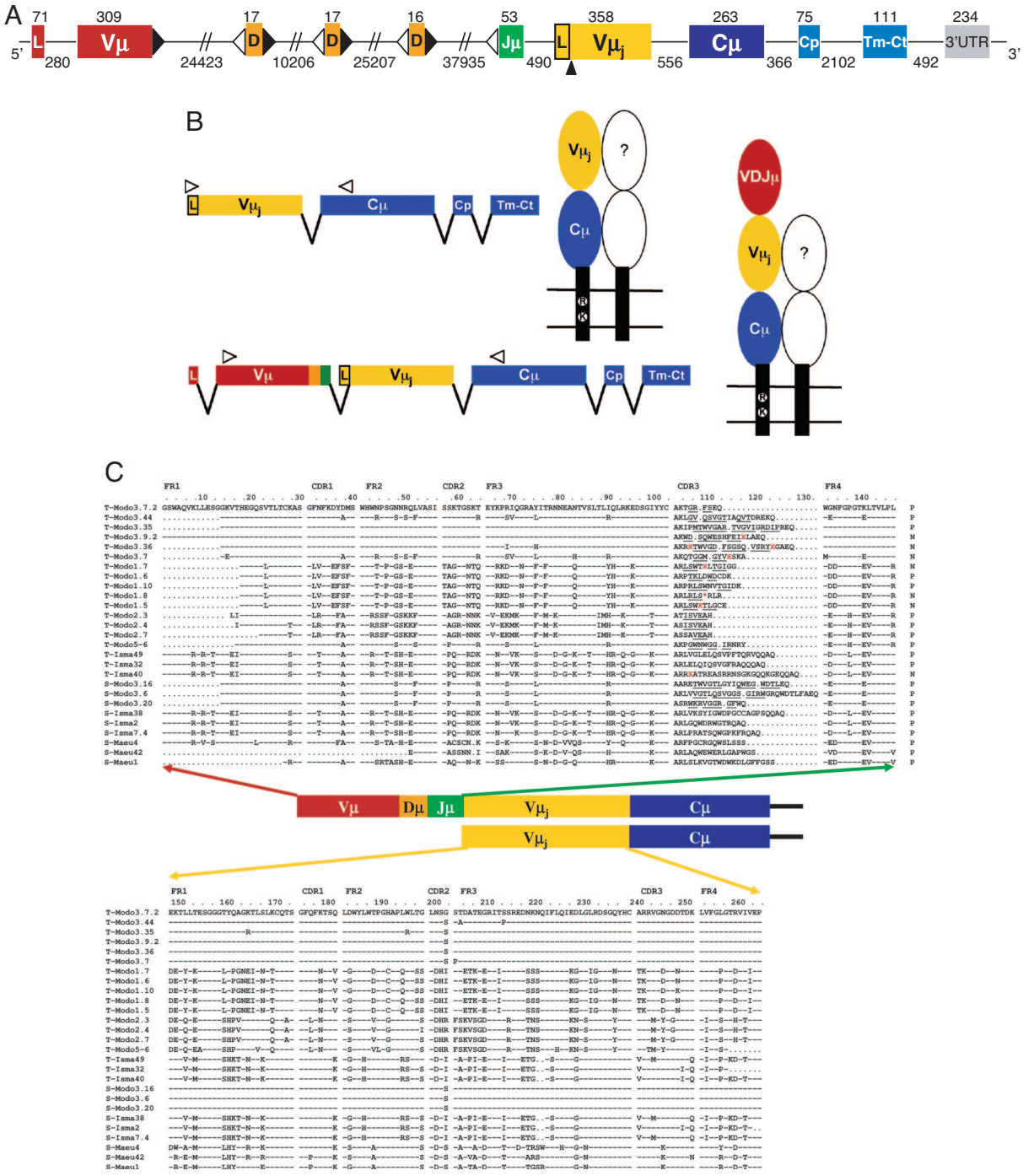

Fig. 1. Diagrams of cluster organization, alternative isoforms, and alignment of TCRμ2.0 sequences. (*A*) Representative TCRμ cluster. Closed or open triangles flanking the Vμ, Dμ, and Jμ gene segments indicate the presence of 23- or 12-bp spacer recombination signal sequences, respectively. The L sequence, connecting peptide (Cp), Tm–Ct, and 3′ UTR exons are indicated. The numbers are intron and exon length in nucleotides and are representative of cluster no. 3 (see SI Fig. 6). The predicted L sequence of Vμ_j is framed by a box, and an arrow below the exon indicates the location of the embedded mRNA splice site. (*B*) Model of the two TCRμ isoforms showing the mRNA splice forms and predicted protein organization, with the TCRμ1.0 isoform above and the TCRμ2.0 below. Arrows above the exons indicate the locations for the PCR primers used for RT-PCR. Conserved arginine (R) and lysine (K) in the Tm region are indicated. (*C*) Alignment of predicted amino acid translations of the V domains in the TCRμ2.0 isoform isolated from thymus (T) and spleen (S) cDNA libraries. Sequences are labeled by species, followed by a clone number. Modo, *M. domestica*; Isma, *I. macrourus*; Maeu, *M. eugenii*. Productive (P) or nonproductive (N) clones are indicated on the right; a red asterisk in the CDR3 region indicates in-frame stops, and a red letter X indicates frame-shifting nucleotide insertion/deletion in the nonproductive rearrangements. The residues corresponding to distinct Dμ segments are underlined for the *M. domestica* sequences.

erodimers. Vμ, Dμ, and Jμ are flanked by canonical recombination signal sequences consistent with being substrates for RAG-mediated VDJ recombination (Fig. 1*A*) (5). The recombination signal sequence flanking the Dμ gene segments are asymmetrical, with a 12-bp spacer on the 5′ side and a 23-bp spacer on 3′ side, a configuration that is typical of D segments in TCR (Fig. 1*A*) (19, 20). There are two additional partial clusters that lack Vμ and Dμ segments (SI Fig. 6), and at least

Fig. 2. Nucleotide alignment of genomic TCRμ sequences from *M. domestica*, *I. macrourus*, and *M. eugenii*. The region shown spans the Jμ through Tm–Ct exon. Complete intron sequences are not included for brevity. Modo-C1, C3, and C7 are three representative clusters in the MonDom4 assembly that are numbered according to their order along the chromosome 3. The Jμ in C7 is missing because of a gap in the current sequence assembly. The FGXG motif is underlined and in bold in Jμ and Vμ_j FR4. mRNA donor (blue/green) and acceptor (green/red) splice sites are highlighted. Highlighted in yellow are arginine and lysine residues in the Tm region that participate in CD3 assembly. The Isma and Maeu sequences were generated by sequencing PCR products amplified from genomic DNA. For Isma-3, the primers were located in the regions corresponding to Jμ and Vμ_j to confirm the absence of the intron. In Isma-6 and Maeu, the primers were located in Vμ_j and Cμ to confirm the germ-line-joined nature of the Vμ_j and its location upstream of Cμ in both species. RSS, recombination signal sequence.

one of which is used in VDJ recombination with Vμ and Dμ from upstream clusters (Fig. 1*C*).

TCRμ Contains Prejoined V, D, and J Segments. Located between Jμ and Cμ in each cluster is an exon encoding a complete V domain (Fig. 1*A*). The sequence at the 5′ end of this exon, corresponding to framework region (FR)-1 through FR3 of V segments, is most similar to the nonrearranged Vμ and also the VH (Ig heavy chain V gene segments) (Fig. 3*A*). Furthermore, the sequence at the 3′ end corresponding to FR4 encodes a peptide motif (FGXG) that is conserved in J segments from TCR and Ig light chain genes (Fig. 2). Thus, this exon appears to contain V, D, and J

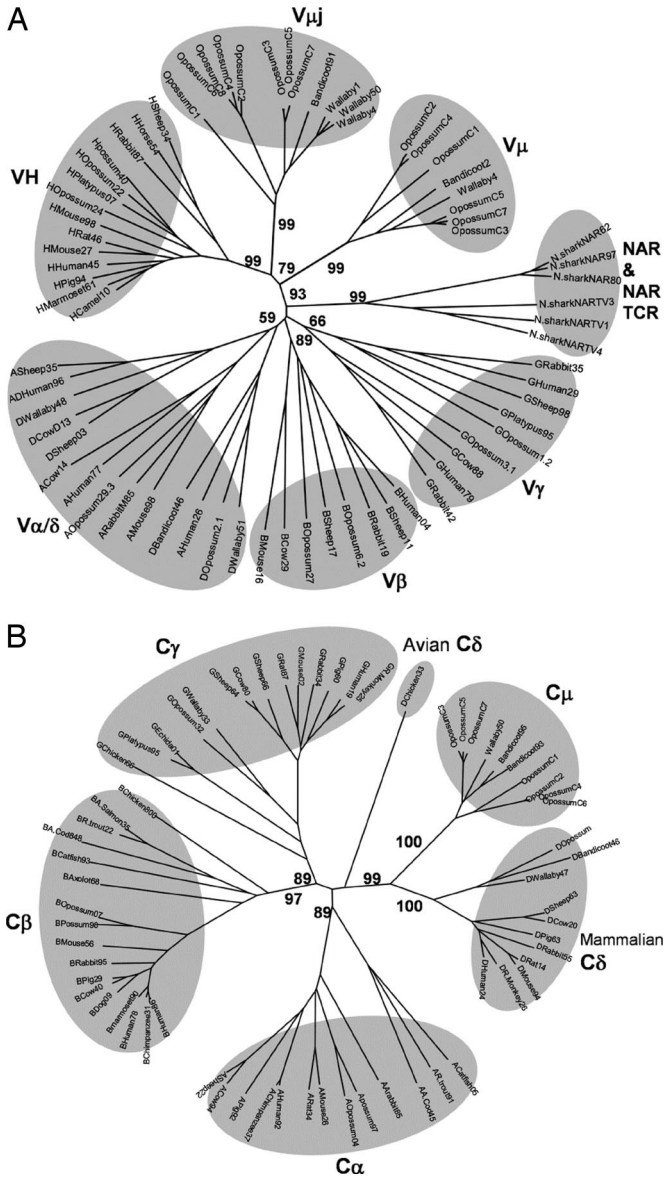

Fig. 3. Phylogenetic analysis of V (*A*) and C (*B*) regions from TCRμ, conventional TCR, and Ig. The trees shown were from nucleotide alignments analyzed by using the neighbor-joining method. Similar results were obtained by using both the maximum parsimony and minimum evolution. The numbers are the percentage of bootstrap support for each node based on 1,000 replicate trees.

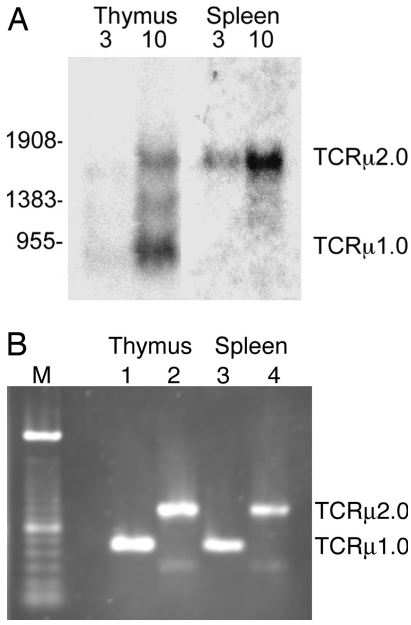

Fig. 4. Transcription of TCRμ isoforms in the opossum. (*A*) Northern blot of thymus and spleen whole RNA from 9- to 11-month-old *M. domestica* probed with the Cμ region of cluster 3 (SI Fig. 6). The bands corresponding to the TCRμ1.0 and TCRμ2.0 isoforms are indicated. The middle band in the thymus RNA corresponds to an out-of-frame splice variant that is generated by some clusters. (*B*) RT-PCR analysis of the TCRμ1.0 and TCRμ2.0 isoforms in thymus and spleen RNA of a 7-week-old *M. domestica*. Actin controls for the RT-PCR were performed and were positive in all cases (data not shown). M, marker lane. Lanes 1 and 3 are the result of primers specific for the TCRμ1.0 isoform, and lanes 2 and 4 are from primers specific for the TCRμ2.0 isoform. The primer locations are indicated in Fig. 1*B*.

segments already fused together in the DNA, hereafter referred to as a Vμ_j (joined Vμ). Vμ_j lacks the intron in the leader (L)-coding sequence normally found in Ig and TCR V gene segments and present in the nonrecombined Vμ (Figs. 1*A* and 2). Vμ_j, therefore, appears to be a processed gene, suggesting that its origin may have been by retrotransposition (21, 22).

Similarities in Vμ_j gene structure among the three marsupial species reveal a likely common origin for this exon early in marsupial evolution. The sequences corresponding to the complementarity determining region (CDR)3, created by the junction of the VDJ gene segments during RAG-mediated recombination, are identical in length, which is consistent with being from a common event (Figs. 1*C* and 2). Vμ_j is intronless in both *M. domestica* and *I. macrourus* (not determined for *M. eugenii*), and the sequences from all three species form a single phylogenetic clade (Fig. 3*A*). These results are consistent with a common origin for Vμ_j before the separation of the American and Australasian marsupials 60–70 Mya (23, 24). The origin of the TCRμ clusters may be older, however, because the Cμ sequences always form a single phylogenetic clade sister to marsupial and eutherian TCRδ C region sequences (Fig. 3*B*), supporting its emergence before the separation of marsupials and eutherians 170–180 Mya but after the separation of birds and mammals 310 Mya (13, 14). Because there are interspecific sequence differences in the Vμ_j, we tested for evidence of positive selection acting on this exon. The ratio of nonsynonymous (dN) to synonymous (dS) substitutions for Vμ_j were always <1 for both FR and CDR analyzed separately (dN/dS = 0.46 for FR; dN/dS = 0.41 for CDR) or together (dN/dS = 0.43), which is consistent with an absence of positive selection acting on Vμ_j. The same was true for the somatically recombining Vμ segments (dN/dS = 0.57 for FR; dN/dS = 0.52 for CDR). These results may indicate conservation of the protein sequence for either recognition or structural purposes rather than diversification at the germ-line level. The interspecific differences in the V regions, therefore, are likely due to the divergence times among the three species analyzed, a timeframe comparable with that separating humans and mice (13, 14).

TCRμ Is Expressed in Two Apparently Functional Isoforms. TCRμ mRNA of different sizes were detected in thymus and spleen by Northern blot and RT-PCR analyses (Figs. 1*B* and 4). When sequenced, the shortest form was found to encode a transmembrane protein composed of a single Vμ_j and Cμ (Fig. 1*B*), and the longer form encodes two V exons and Cμ. The C proximal V is always Vμ_j, and the distal V is a recombinant of the upstream Vμ, Dμ, and Jμ segments. These two isoforms are called TCRμ1.0 and TCRμ2.0 for the presence of one or two V domains, respectively. The TCRμ2.0 isoform uses an internal,

IMMUNOLOGY

canonical mRNA splice site in the $V\mu_j$ L sequence to splice the upstream, recombined Vμ–Dμ–Jμ to FR1 in $V\mu_j$ (Figs. 1*B* and 2). A third TCRμ isoform detected in thymus RNA by both RT-PCR (data not shown) and Northern blot analysis (middle hybridizing band in thymus RNA in Fig. 4*A*) is created by a splice between the recombined Vμ–Dμ–Jμ and a cryptic splice site in FR3 of some $V\mu_j$ (Fig. 2). This splice is always out of frame and would not encode a full-length protein. This variant is abundant in the thymus (Fig. 4*A*) but difficult to detect in the peripheral lymphoid organs (data not shown) and, because it is out of frame, may be selected against in cells entering the periphery.

There is junctional diversity in CDR3 in the distal V domain of TCRμ2.0 characteristic of RAG-mediated VDJ recombination (Fig. 1*C*). The CDR3 of many of the clones contain sequences corresponding to more than one Dμ being used during VDJ recombination (Fig. 2*C*). Thirteen of 21 TCRμ2.0 cDNAs (62%) from an *M. domestica* thymus library contained productive rearrangements (i.e., ORFs), and similar results were found for *I. macrourus* thymus cDNAs (Fig. 1*C*). Nonproductive rearrangements contained in-frame stops or were out of frame because of insertions/deletions in their CDR3 (Fig. 1*C*). In contrast, 100% of splenic TCRμ2.0 cDNA were productive (six of six from *M. domestica*, seven of seven from *I. macrourus*, and five of five from *M. eugenii*). These data support the notion that VDJ recombination occurs in the thymus and that there is selection for only those cells with productive rearrangements entering the periphery.

Only two of the eight opossum TCRμ clusters (clusters 1 and 7) contain the exons necessary to encode the TCRμ1.0 isoform because of in-frame stops in the L sequence in the other six clusters (data not shown). All six complete and one of the partial clusters (cluster 6) have been found to produce the TCRμ2.0 isoform, according to comparison of cDNA sequences with germ-line DNA (data not shown). Cluster 6, a partial cluster lacking Vμ and Dμ, achieves this by using the Vμ and Dμ segments from upstream cluster 5 (clone Modo5-6 in Fig. 1*C*). RT-PCR detects both the TCRμ1.0 and TCRμ2.0 forms in thymus and spleen RNA; however, only the TCRμ2.0 form was readily detected in spleen in a Northern blot (Fig. 4). This implies that the TCRμ2.0 form may be the most abundant, or primary, isoform in the peripheral tissues.

Discussion

TCRμ has several characteristics that merit its classification as a TCR isotype. First, it has all of the hallmarks of a locus that undergoes RAG-mediated VDJ recombination, and this recombination takes place in the thymus (i.e., in a thymus-dependent cell). Furthermore, the J and C sequences of TCRμ have greatest similarity to TCRs, and the recombination signal sequences are organized more like that of TCRs than Ig. TCRμ also has several characteristics that are consistent with its not being a paralogous copy of one of the conventional TCR isotypes (25). TCRμ maps to a distinct region of the *M. domestica* genome unlinked to TCRα/δ, TCRβ, and TCRγ and has a cluster style organization that is atypical for TCR loci (4, 17). This is not unheard of in TCR genomics, however, because mouse TCRγ genes are in clusters as well (6). However, a cluster organization is not the rule for all marsupial TCRs because the TCRα, -β, -γ, and -δ loci in *M. domestica* all have translocon organizations (Z.E.P., M.L.B., R.D.M., unpublished results). The phylogenetic analyses support an evolutionary relationship between TCRμ and both TCRδ and Ig heavy chain (Fig. 3). The Vμ gene segments are related to VH, whereas Cμ is related to Cδ. Collectively, these results support TCRμ having evolved from a recombination between an ancestral Ig heavy chain locus and a TCR locus, most likely TCRδ. TCRμ may form complexes similar to conventional $\alpha\beta$TCRs and $\gamma\delta$TCRs because of the presence of conserved residues in the Tm region known to mediate interactions with CD3 molecules (18). What other chains TCRμ associates with is not yet known. It is possible that there remains yet a sixth marsupial TCR chain to be discovered. Alternatively, TCRμ may form heterodimers with one of the conventional chains or perhaps homodimers with itself.

The organization of the TCRμ clusters and the transcripts isolated allow for the generation of at least two possible translated forms: the TCRμ1.0 form that would contain an invariant V domain and the TCRμ2.0 form that would contain somatically diversified extra V domain. Whether or not both are synthesized remains to be determined, and the difference in transcript level in the peripheral tissues suggests that the TCRμ2.0 form may be more common in extrathymic cells. Furthermore, the discovery that Vμ and Dμ from upstream clusters can be recombined to Jμ segments in downstream clusters, including recombination with the partial clusters, increases the potential number of VDJ combinations and may be a means to increase diversity of the TCRμ2.0 isoform.

Unlike the conventional TCRs in vertebrate lineages, TCRμ contains V genes that do not require somatic recombination. In mammals, this distinction applies not only to TCRs but also to Ig. The structure of $V\mu_j$ is consistent with an origin by retrotransposition, which would be a mechanism for generating joined V in the germ line. In this model, RAG-mediated VDJ recombination would have occurred, either in a T cell or possibly a germ cell, followed by retrotranscription and insertion of an intronless copy into the germ line by homologous recombination (22). Germ-line complete or partial VDJ fusions have been found in Ig genes of fish and birds, but, in contrast, they are thought to have been generated by RAG-mediated recombination directly in the germ-line DNA (26–28). It is possible that $V\mu_j$ was generated similarly; however, a precise excision of the intron by DNA deletion also would have been required to account for $V\mu_j$ appearing processed (22).

In many respects, TCRμ2.0 appears analogous to a TCRδ isoform that has been described recently in sharks [nurse shark antigen receptor (NAR)–TCR] and that contains a double V domain created by tandem VDJ recombination (29). Like TCRμ, the nurse shark TCRδ locus also appears to have evolved by recombination between an Ig locus (IgNAR) and TCRδ. In both NAR–TCR and TCRμ, the resulting distal V domain is most related to Ig V domains, suggesting that they may bind antigen directly like Ig and some $\gamma\delta$TCRs, rather than MHC-presented antigens such as $\alpha\beta$TCR (6, 29). TCRμ also shares similarity to shark IgNAR and mammalian TCRδ in by using multiple D segments in a single VDJ recombination (19, 20, 30). Collectively, these results support TCRμ as having an ancestral relationship with TCRδ. However, similarities between marsupial TCRμ and shark NAR–TCR appear to be convergent evolution. For example, TCRμ is encoded at its own locus, whereas NAR–TCR is encoded as part of the conventional TCRδ locus (29). Furthermore, TCRμ V sequences are more related to conventional VH, where the N-terminal V domain in NAR–TCR is related to Ig-NAR V genes, which do not have a known mammalian homolog (29). In NAR–TCR, the proximal V domain requires somatic VDJ recombination rather than recombination with prejoined V, as in TCRμ.

Because both marsupials and sharks possess a TCR expressing a double V configuration, it seems plausible that similar TCRs would be found in other vertebrate lineages as well. This raises the possibility of a class of TCRs that has gone unnoticed because of its absence in commonly studied eutherian mammals, such as humans and mice. Whether TCRμ is uniquely marsupial remains to be determined; however, its presence in three distantly related species supports the notion that it performs a role that has been conserved in this lineage of mammals. Furthermore, the absence of selection on the V genes of this locus suggests a conserved recognition role for this receptor.

Materials and Methods

***M. domestica* Whole-Genome Sequence.** The *M. domestica* whole-genome assembly used for this study (MonDom4) is available at GenBank under accession number AAFR03000000. The 614-kb genomic region containing the six complete and two partial TCRμ clusters spans coordinates 3.432520000 and 3.433134000.

RNA Analysis. RNA was extracted by using TRIzol (Invitrogen, Carlsbad, CA) and, when used for RT-PCR, was treated with TURBO DNA-free (Ambion, Austin, TX) to remove contaminating DNA. RT-PCR was performed by using a GeneAmp RNA PCR Core kit (Applied Biosystems, Foster City, CA) with Advantage-HF 2 PCR (BD Biosciences, San Jose, CA) under the following conditions: 94°C for 1 min, denaturation at 94°C for 30 sec, annealing/extension at temperatures ranging from 62°C to 66°C for 4 min, and a final extension period of 68°C for 5 min. PCRs were performed in 25-μl reactions. Actin primers used as controls were 5′-GGTTCAGGTGTCCAGAGGCC-3′ and 5′-CCAGGGCTGTGATTTCTTTTTG-3′ (64°C). All TCRμ cluster-specific primers pairs had an annealing/extension temperature of 62°C. *M. domestica* cluster C7 was as follows: Vμ, 5′-AAGGTGACACATGAGGGCC-3′; Vμ_j, 5′-CCACTCCTGCTCAATTTACTCCC-3′; and Cμ, 5′-GAAGTTCCTGATCAGGCAGGCG-3′. To amplify from genomic DNA, the *I. macrourus* Jμ to the Vμ_j the primers 5′-GGTCTTGGAACAGAAGTGACTGTAC-3′ and 5′-GGGAGCCTCCCCCTGACTCCATC-3′ were paired; to amplify from Vμ_j to Cμ, the primers 5′-CCTGACCCTCAAATGTGAAA-3′ and 5′-CTGGGTGACAGGTGTGCTTG-3′ were paired. To amplify the *M. eugenii* TCRμ cluster from genomic DNA, Vμ_j primer 5′-CTGGTATCTTTGGGCCCCTGGCC-3′ and Cμ primer 5′-TCCTGATCAGGCAGGCCACA-3′ were paired. Northern blots were prepared by using the NorthernMax formaldehyde-based system (Ambion), and probes were labeled by using a Strip-EZ DNA random primed StripAble DNA probe synthesis and removal kit (Ambion) according to the recommended protocols of the manufacturer.

Sequence Analyses. Sequences were analyzed by using Sequencher 4.1, compared with GenBank by using the BLAST algorithm, and aligned by using BioEdit by first aligning the protein translation and then converting back to the nucleotide sequence to retain codon position (31, 32). Phylogenetic analyses and nonsynonymous-to-synonymous substitution analyses were conducted by using MEGA version 3.1 (33). The GenBank accession numbers for sequences used in the V analysis are as follows: AY238451, M13726, DQ076246, M34198, M12885, D13077, D90014, J005903, D16113, AY238448, Z14996, U78035, M27904, AF030011, D17419, AF030017, D90129, M15616, D38135, M13429, Z12998, DQ011295, U73188, S60779, D38142, DQ022705, DQ022688, DQ022710, AY114762, AY114997, U18680, Z50034, DQ125454, AF434587, AF091140, AF012122, AF381307, AF012124, X03398, BC095846, U04227, U80145, U15194, AF298161, AJ245110, DQ979404, DQ979406, DQ979404, AY956350, DQ979403, and AY955291. The GenBank accession numbers for sequences used in the C analysis are as follows: U58505, U50991, AJ133845, M12885, AF133097, AY014504, AK131826, NM_001014234 (BC088274), X02592, XM_509837, AB087992, D10394, M55622, M12886, XM_527931, AF043178, U62990, D16409, D90140, AB079529, M13895, M11456, AF133098, AY014507, X70168, U39193, AJ133848, U18122, X97435, M37800, AY190025, X15019, L21160, D38134, X03802, Z27087, Z12966, X63680, Z12964, DQ499633, DQ499632, DQ011301, DQ011295, U22666, DQ076246, AY238447, Z12963, D90420, L21163, D26555, AF196214, M37694, AY190026, M21624, AY956350, AY955295, and AY955293. The sequences from MonDom4 used in the phylogenetic analysis are from locations 1.171502250, 1.171187281, 1.171051783, 8.200970738, 8.201195815, 6.236627382, and 6.236580558.

This work was supported by grants from the National Institutes of Health Institutional Development Award program of the National Center for Research Resources (to M.L.B. and R.D.M.) and the National Science Foundation (to R.D.M.).

1. Schatz DG (2004) *Immunol Rev* 200:5–11.
2. Flajnik MF (2002) *Nat Rev Immunol* 2:688–698.
3. Rast JP, Anderson MK, Strong SJ, Luer C, Litman RT, Litman GW (1997) *Immunity* 6:1–11.
4. Davis MM, Chein Y-H (2003) in *Fundamental Immunology*, ed Paul WE (Lippincott, Philadelphia), 5th Ed, pp 227–258.
5. Schlissel MS (2003) *Nat Rev Immunol* 3:890–899.
6. Hayday AC (2000) *Ann Rev Immunol* 18:975–1026.
7. Deane EM, Cooper DW (1988) in *The Developing Marsupial*, eds Tyndale-Biscoe CH, Janssens PA (Springer, Berlin), pp 190–199.
8. Hubbard GB, Saphire DG, Hackleman SM, Silva MV, VandBerg JL, Stone WH (1991) *Lab Anim Sci* 41:227–232.
9. Samples NK, VandeBerg JL, Stone WH (1986) *Am J Reprod Immunol Microb* 11:94–97.
10. Kalmutz SE (1962) *Nature* 193:851–853.
11. Rowland DT, Dudley MA (1969) *Immunol* 17:969–975.
12. Rowland DT, Blakeslee D, Lin H-H (1972) *J Immunol* 108:941–946.
13. Kumar S, Hedges SB (1998) *Nature* 392:917–920.
14. Woodburne MO, Rich TH, Springer MS (2003) *Mol Phylogenet Evol* 28:360–385.
15. Baker ML, Osterman AK, Brumburgh S (2005) *Immunogenetics* 57:665–673.
16. Baker ML, Wares JP, Harrison GA, Miller RD (2004) *J Mammal Evol* 11:1–16.
17. Deakin JE, Parra Z, Graves JAM, Miller RD (2006) *Cytogenet Genome Res* 112:342K.
18. Call ME, Wucherpfennig KW (2005) *Ann Rev Immunol* 23:101–125.
19. Elliott JF, Rock EP, Patten PA, Davis MM, Chein Y-H (1988) *Nature* 331:627–631.
20. Carroll AM, Slack JK, Mu X (1993) *J Immunol* 150:2222–2230.
21. Weiner AM, Deininger PL, Efstratiadis A (1986) *Annu Rev Biochem* 55:631–661.
22. Roy SW, Gilbert W (2006) *Nat Rev Genet* 7:211–221.
23. Kirsch JA, Lapointe FJ, Springer MS (1997) *Aust J Zool* 45:211–280.
24. Nilsson MA, Arnason U, Spencer PBS, Janke A (2004) *Gene* 340:189–196.
25. Brenner MB, McLean J, Dialynas DP, Strominger JL, Smith JA, Owen FL, Seidman JG, Rosen F, Krangel MS (1986) *Nature* 322:145–149.
26. Kokubu F, Litman R, Shamblott MJ, Hinds K, Litman GW (1988) *EMBO J* 7:3413–3422.
27. Reynaud C-A, Dahan A, Anquez V, Weill J-C (1989) *Cell* 59:171–183.
28. Ventura-Hohman T, Lobb CJ (2001) *Mol Immunol* 38:557–564.
29. Criscitiello MF, Saltis M, Flajnik MF (2006) *Proc Natl Acad Sci USA* 103:5036–5041.
30. Roux KH, Greenberg AS, Greene L, Strelets L, Avila D, McKinney EC, Flajnik MF (1998) *Proc Natl Acad Sci USA* 95:11804–11809.
31. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) *Nucleic Acids Res* 25:4876–4882.
32. Hall TA (1999) *Nucleic Acids Symp Ser* 41:95–98.
33. Kumar S, Tamura K, Jakobsen IB, Nei M (2001) *Bioinformatics* 17:1244–1245.