NIH Public Access
Author Manuscript
Semin Immunol. Author manuscript; available in PMC 2011 February 1.

Published in final edited form as:
Semin Immunol. 2010 February ; 22(1): 3–9. doi:10.1016/j.smim.2009.11.005.

Those other mammals: The immunoglobulins and T cell receptors of marsupials and monotremes

Robert D. Miller
Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque, NM, 87110, USA, rdmiller@unm.edu, Tel: 1 (505) 277 2844, Fax: 1 (505) 277 0304

Abstract

This review summarizes analyses of marsupial and monotreme immunoglobulin and T cell receptor genetics and expression published over the past decade. Analyses of recently completed whole genome sequences from the opossum and the platypus have yielded insight into the evolution of the common antigen receptor systems, as well as discovery of novel receptors that appear to have been lost in eutherian mammals. These species are also useful for investigation of the development of the immune system in organisms notable for giving birth to highly altricial young, as well as the evolution of maternal immunity through comparison of oviparous and viviparous mammals.

Keywords

marsupials; monotremes; immunoglobulins; T cell receptors; evolution

1. Introduction

1.1 What are mammals?

What springs to mind when asked, “what is a mammal?” Do you picture a mouse, cat, cow or horse? For the more adventurous is it a whale, llama, bat, or armadillo? Maybe you picture yourself? They are all appropriate examples of mammals. But what do all these species have in common? Most relevant to this review is that they are all eutherians, or what are more commonly called placental mammals. A safe wager, unless you live in Australia, is when asked to name a mammal your first instincts are a eutherian example. This is not surprising since eutherian mammals are the most common, most geographically widespread, and most abundant of the mammalian lineages. Let’s face it, the mammals you run into on a daily basis, whether you ride them, pet them, study them, or eat them, are eutherians. That is of course unless you live in certain parts of the Americas or Australasia then they might not be eutherians. It might be an entirely different kind of mammal: maybe an opossum, kangaroo, or wombat. These are also mammals but definitely not eutherians. Rather they are metatherians, more commonly called marsupials, and they are a very distinct group. And how would you define what is a mammal? Is it having fur and feeding their young milk from mammary glands? They are two very good characteristics with which to define mammals, the latter being eponymous. What about giving birth to live young (viviparity) instead of laying eggs like birds (oviparity), is this a good definition of a mammal? No it isn’t. The platypus, a Prototherian, is a true mammal with fur and mammary glands but it reproduces by laying eggs.

There are three extant lineages of mammals: Eutherians, Metatherians, and Prototherians (or monotremes such as the platypus). They are phylogenetically distinct and each has their own unique characteristics. The eutherians and metatherians together are the Therians, a viviparous lineage that diverged from the oviparous Prototherians at least 165 million years (Myr) ago

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(Figure 1) [1]. Conservative estimates put the divergence of metatherians and eutherians from each other around 145 Myr, a timeframe that is supported by the fossil record [1]. Most of the defining characteristics distinguishing the three lineages are reproductive. In general, eutherians give birth to relatively precocial young that have completed much of their early development in the womb following a relatively long gestation. Metatherians (marsupials) give birth to relatively altricial young that are born after a short gestation and develop further while firmly suckling on a teat, sometimes in a pouch (the marsupium). Throughout this review the common terms marsupial and monotreme will be used for Metatheria and Prototheria. However, the term eutherian will be used rather than the common "placental" since marsupials also form placenta and the implications of placental versus aplacental may not be appropriate [2].

1.2 Marsupial and monotreme immunology

Comparative analyses of the immune systems from the different mammalian lineages can reveal both adaptation as well as the origins of uniquely mammalian characterstics. In the inaugural volume of the journal *Developmental and Comparative Immunology,* Ashman wrote an essay that raised the hope of a "brighter future" for marsupial immunology [3]. One question that certainly existed at the time, and still does, was: do the immune systems of marsupials and monotremes resemble that of eutherians in a common mammalian way? Or were the immune systems of marsupials and monotremes each distinctly different in ways that reflect differences in life history or evolutionary divergence? Unfortunately, the scarcity of marsupial and monotreme specific reagents and, more importantly, the absence of particular model species around which large communities of investigators focused, meant that the immunology of these species lagged behind that of eutherians. Fortunately model species have been developed and, over the past few years, molecular genetic resources and whole genome sequencing have occurred for a limited number of marsupial and monotreme species. The first complete genome sequence of a representative marsupial, the gray short-tailed opossum *Monodelphis domestica* was published in 2007 and was quickly followed by the first monotreme genome, the platypus *Ornithorhyncus anatinus* [4,5]. These resources have provided a wealth of data from which to analyze the genetics underlying evolution and novel adaptation in the different mammalian lineages. Such research holds the promise of a better understanding of the evolution of maternal immunity in mammals as well as potential unique adaptation to altricial birth in the marsupials and monotremes. In addition, the study of marsupials and monotremes helps fill an evolutionary gap between well-studied eutherians such as humans and mice and some of the traditionally studied non-mammalian species such as chickens and frogs. One example of where the study of the marsupial immune system has provided insights is in the structure and evolution of the Major Histocompatibility Complex (MHC). The opossum MHC is comparable to that of humans and mice in size and complexity, but its overall organization shares similarity to that of non-mammals [6]. Comparison of the opossum MHC to that of eutherians, for example, has revealed that a complex pattern of gene duplication and translocation that gave rise to the current organization in mice and humans occurred early in the evolution of the eutherians, but after their divergence from marsupials.

Here is reviewed what has been learned regarding immunoglobulin (Ig) and T cell receptor (TCR) biology in marsupials and monotremes over the past ten years, primarily through the analysis of the molecular genetics of these receptors. What has emerged is evidence of marsupials and monotremes being typically mammalian in many ways, with a high degree of conservation in the Ig and TCR. However, there are features of both the Ig and TCR in these two non-eutherian lineages that are absent in eutherians that suggest both novel adaptation and gene loss during the radiation of extant mammals

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2. The conventional T cell receptors

2.1 Genomic organization the conventional TCR genes

Homologues of the conventional α, β γ, and δ TCR chains have been characterized, at least at the cDNA level, for multiple marsupial and monotreme species [7–14]. However, complete genomic analyses and annotation of the TCR loci have only been performed for one marsupial species, the opossum *M. domestica* [14]. The results of these analyses revealed that the overall structure and complexity of the opossum TCR loci is similar to that of mice and humans. The total number of V, D and J gene segments at each locus, and therefore the potential receptor diversity, is comparable between opossums and well-studied eutherian species. Furthermore the general translocon-type organization of the opossum TCR loci is similar to that of humans and mice. In addition the chromosomal regions where these genes are located have a high degree of conserved synteny with eutherian mammals and other amniotes such as chickens [14]. This conserved synteny will become more significant later in subsection 3 of this review where the non-conventional TCR present in marsupials and its origins and evolution is considered.

2.2 Germ-line contribution to αβ T cells early in opossum development

The altricial nature of the newborn marsupial makes it an ideal model to study early development in the immune system. At birth most marsupials including the opossum *M. domestica* lack a differentiated thymus, and their overall state of development has been likened to that of a human fetus at the eighth week of gestation [15–17]. The absence of apparent lymphoid tissue at birth, and its later development, usually by the second postnatal week, correlates well with early studies on the ontogeny of immuno-competence in newborn marsupials. Both cell mediated and humoral responses do not appear until the second postnatal week or later in most marsupial species [18–21].

One question that might be asked is if the newborn marsupial immune system is analogous to that of a fetal eutherian in its developmental state? In other words does postnatal development in a marsupial follow similar patterns, both in the order of appearance and diversity of specific cell types as in eutherians? So far this question has been addressed in only a limited way and the answer appears to be that marsupials and eutherians are similar in some ways and different in others. One way in which the opossum differs from humans and mice is in the order of T cell subset development. In eutherians the first cell type to appear during fetal development are γδ T cells [22]. In the opossum however, the earliest mature TCR transcripts detected encode αβ chains, which are detectable within the first 24 hours after birth [23]. Transcripts encoding TCRδ chains are also detected early, however TCRγ transcripts are not detected until the second postnatal week. Therefore, if the orders of TCR chain rearrangement and transcription is reflective of patterns of T cell subset ontogeny, it appears that in the opossum αβ and γδ T cell development is reversed relative to that of eutherians. The evidence of developing, and possibly functionally mature T cells, within the first 24 hours after birth in the opossum was somewhat unexpected since this is a time prior to any evidence of a functional thyms in this species [17,23]. Where such T cell development in the newborn opossum is occurring remains to be determined. It is possible that it is occurring at the site of the thymic rudiment and playing a role in thymus differentiation.

Analysis of the TCR V gene segments being used early in newborn opossums revealed limited diversity in both the TCRα and β chains [23]. It is possible that limited diversity is a reflection of limited cell numbers at an early stage in development. However this may not be the case since there is a recurring pattern to the diversity of the early TCRα and β transcripts. The selection of early Vβ gene use appears to be independent of position in the locus but dependent on the use of micro-homology between the V, D and J segments [23]. This is similar to what

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

has been seen in Ig and TCR repertoires in some fetal eutherians [24,25]. In contrast to TCRβ, the genomic position of V segments appears to have an influence in early TCRα rearrangements. There is a bias for the most 3′ or C proximal V genes in the newborn opossum [23]. The earliest TCRα rearrangements also show evidence of both P and N nucleotide additions in junctions of the V and J segments indicating that the earliest developing T cells are using terminal deoxynucleotidyl transferase (Tdt) to increase diversity.

3. The non-conventional T cell receptors

The discovery of the α, β, γ, and δ TCR chains over two decades ago solved a major unanswered question in immunology of how T cells specifically recognized antigen [26–28]. A decade later it was clear that all jawed vertebrates had homologues of TCR α, β, γ, and δ and until recently these were the only TCR isotypes known [29]. This made more remarkable recent discoveries of an unusual TCRδ isoform in cartilaginous fish called NAR-TCR and a fifth TCR chain in marsupials [30,31]. This chain has been designated TCRμ or M in recognition of its discovery in **m**arsupials, and this nomenclature appears to continue to be appropriate given that it is also found in **m**onotremes and is therefore ancient in the **m**ammalian lineage [14,31].

3.1 The discovery of TCRμ

While analyzing expressed sequence tag (EST) data from a marsupial, the Northern brown bandcoot *Isoodon macrourus*, Baker and colleagues noted clones that were clearly homologous to TCR chains and most resembled TCRδ [11,12]. The sequences however were quite distinct from the TCRδ genes already known at the time from other marsupial species [9]. Later, based on physical mapping, it was realized that the genes encoding this EST were not located on the same chromosome as the conventional TCR genes [31,32]. Nothing homologous to this EST could be identified in the available eutherian whole genome sequences and so this sequence was designated as a novel TCR chain [14,31]. Further analysis revealed TCRμ undergoes V(D)J recombination in thymocytes which, along with the sequence similarity, supported that this is in fact a TCR [31].

Using marsupial TCRμ constant (C) region sequences to search the genomic sequences available from more species, a homologue was identified in the recently completed platypus *O. anatinus* genome [5,14]. The homology was confirmed by a number of shared features discussed in more detail below in this section. These features include C region sequence identity, use of V domains that are more similar to Ig than TCR, and expression in multiple isoforms with similar overall structure. Homology based searches of the available chicken, frog, and Anolis lizard genomes did not uncover genes resembling TCRμ homologues, although the conventional TCR chain genes α, β, γ, and δ were easily identified [14]. So far only the platypus, and one other monotreme species, the short beaked echidna *Tacchyglossus aculeatus*, are the only species for which TCRμ homologues have been found outside of marsupials. Given their phylogenetic relationship, the presence of TCRμ in monotremes and marsupials would be consistent with this TCR chain's presence being ancient in mammals, and its apparent absence in eutherians being due to gene loss early in the evolution of this lineage (Figure 1) [14].

3.2 Genomics and evolution of TCRμ

Although TCRμ in marsupials and monotremes are clearly homologous there are differences between the two lineages that give insight into the origins of this locus (unpublished observations) [14,31]. The opossum *M. domestica* genome is sufficiently well assembled to provide a detailed view of the genomic organization of the TCRμ genes in this species [14]. Unfortunately, the platypus genome assembly is incomplete and unable to provide detailed

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

information on the overall organization of the TCRμ genes in this species, however it is available as a source of sequence data for individual gene segments [5].

The marsupial TCRμ genes are organized as clusters and the number of clusters appears to vary with species. The opossum genome, for example, contains eight tandem TCRμ clusters [14]. Complete clusters contain a single V gene segment (Vμ) that can undergo V(D)J recombination, multiple D segments, a single J segment and the exons encoding the complete transmembrane form of the C region (Figure 2) [14,31]. Each cluster also contains an exon that contains V, D, and J segments already pre-joined (Vμj) in the germ-line DNA, located immediately upstream of the C region exons. Based on phylogenetic comparison of the eight opossum TCRμ clusters, they appear to have evolved by at least two rounds of complete cluster duplications [31].

Based on the current assembly it is not possible to tell if the platypus TCRμ genes are also organized in clusters, however some aspects of the genomic organization can be inferred from cDNA sequences available (Figure 2). The platypus TCRμ genes necessary to encode a complete extracellular form of the chain must include, at a minimum, two V gene segments and two J gene segments, all of which undergo recombination activating gene (RAG) mediated V(D)J recombination. To account for the structure of the platypus TCRμ cDNA sequences, the gene segments are likely organized as (V-J)-(V-J)-C where V segments are recombined to the J segment immediately down stream (Figure 2) (unpublished observations). None of the platypus gene segments appear as being pre-joined in the germ-line as in the marsupials. Rather, V and J gene segments that are flanked by canonical recombination signal sequences (RSS) in the germ-line account for all TCRμ cDNA sequences corresponding to the V domains isolated so far. The V genes are flanked by RSS containing a 23 basepair (bp) spacer and the J genes with a 12 bp spacer RSS similar to marsupial TCRμ and typical TCR genes (unpublished observation).

The V gene segments in both marsupial and monotreme TCRμ are more closely related to Ig heavy chain V genes than to TCR V domains based on sequence similarity [31, unpublished observations]. The C regions on the other hand are more closely related to TCRδ C region genes. Collectively these relationships have led to speculation that TCRμ is genetically an Ig-TCR hybrid, presumably derived from recombination between an IgH and a TCRδ [14]. This model however is difficult to prove one way or the other since there is no evidence in the opossum genome of such a recombination. In contrast the syntenic regions surrounding both the IgH and the TCRα/δ loci are highly conserved and stable across tetrapod vertebrates [14, 33]. Although homologues of TCRμ have not been found outside of mammals, is possible that it is a more ancient locus, predating the evolution of mammals. It is possible that any evidence of its origins have been lost or that it involved ancestral Ig and TCR loci that no longer exist, or have yet to be discovered in more distantly related species.

3.3 TCRμ isoforms in marsupials and monotremes

One of the more unconventional features of TCRμ is the predicted structure of the extracellular chains encoded by cDNA clones isolated. Both marsupial and monotreme TCRμ encodes an extracellular form that contains two V type and one C type domains (TCRμ2.0) (Figure 3). In the case of the marsupial TCRμ the somatically recombined V, D, and J segments encode the N-terminal V domain. This V domain has a high level of clonal diversity and contains long complementarity determining region-3 (CDR3) due to the inclusion of multiple D segments [31]. In addition, V genes from upstream clusters can be recombined to D and J segments in downstream clusters providing for additional V(D)J combinations and diversity taking advantage of the tandem array nature of the clusters [31]. The pre-joined V exon, Vμj, encodes the second V domain and, by its germ-line nature, is non-variable and also contains a relatively short CDR3 region (Figure 3) [31]. All Vμj genes sequenced so far contain CDR3 of identical

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

length, even when from distantly related marsupial species, suggesting that they are all derived from a single common ancestral V(D)J event [31]. There is a second TCRμ isoform found in marsupials containing only single V and C domains (TCRμ1.0), and is therefore structurally more similar to a conventional TCR chains (Figure 3). This isoform utilizes the Vμj gene segment and is therefore non-variable. This isoform is predominantly found in the thymus and can only be detected in peripheral lymphoid tissues at very low levels [31]. Whether this isoform is translated into a functional protein remains to be determined.

The platypus TCRμ isoform with double V domains is analogous in structure to that of marsupials, but is generated differently. The N-terminal V domain (Vμ1 in Figure 3) is encoded by an exon generated by a V to J recombination. This recombinant also contains a long CDR3, but the nucleotides encoding this region, which can be 30 to 60 bp in length, cannot be accounted for in the platypus genome. So far no sequences resembling TCRμ D segments have been found in the platypus genome. Rather the long CDR3 appears to be due solely to N nucleotide additions, added presumably by Tdt (unpublished observation). The second V domain (Vμ2 in Figure 3) is also encoded by an exon produced by somatic V to J recombination, and like marsupial Vμj has a short CDR3. In the case of Vμ2 however the short CDR3 is due to a near direct V to J join that includes few additional nucleotides and, in contrast to Vμ1, has low clone-to-clone variability. Therefore in the platypus this domain is encoded by an exon that appears to be assembled through somatic V(D)J recombination, whereas in marsupials it is encoded by pre-joined V gene [31]. In either case it shows little variability and appears to be acting almost as a second C domain.

There are many unanswered questions regarding the structure of TCRμ and the receptor complexes that contain this chain. For example, TCRμ presumably forms a heterodimer with another chain. What chain this might be remains to be determined. Many of the amino acids residues found to participate in heterodimer formation and interactions with CD3 are conserved in TCRμ [31]. Expression of the TCRμ2.0 in ontogeny correlates closely with the earliest expression of TCRγ, and based on TCRμ's similarity to TCRδ it is possible that they form a γμ TCR, however this is only speculation at this point [23,31].

3.4 Speculations on TCRμ's function

The presence of this novel TCR chain in marsupials and monotremes, along with its apparent absence in eutherians, raises the logical question of whether it was an adaptation to the altricial nature of birth in these lineages. In other words, was TCRμ lost in the eutherian lineage due to the evolution of longer gestation times, more developed placental structures, and birth of well-developed young? One prediction of this hypothesis would be that TCRμ expression would occur early in ontogeny. However this is not the case. Rather, TCRμ is the last of the TCR chains to be expressed in ontogeny in the opossum, at least in the case of the mature TCRμ2.0 isoform that is thought to be the functional isoform [23]. This is nearly two weeks after the first αβ TCR transcripts are detected and, thus, it appears unlikely that TCRμ is a novel adaptation specifically for the protection of atrical young.

Further evidence that TCRμ, or the role it plays, might not be a novel adaptation in marsupials and monotremes comes from its similarity to a TCR discovered in cartilaginous fish. In sharks there is an isoform of the TCRδ chain, called NAR-TCR, which like TCRμ produces a double V structure [30]. NAR-TCR achieves this by undergoing double V(D)J recombination events, much like the platypus TCRμ. As in TCRμ, the N-terminal V domain of NAR-TCR is more similar to Ig V domains than TCR V domains. In the case of NAR-TCR the Ig V genes appear to be derived from an unusual light-chainless form of antibody unique to cartilaginous fish called the novel antigen receptor (IgNAR). The second V domain and C region is from the conventional TCRδ locus. Therefore, like TCRμ, NAR-TCR appears to be clearly the result of a recombination between an Ig-like locus and the TCRδ locus [30]. Given NAR-TCR uses

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

IgNAR type V domains and TCRμ uses more conventional VH type V domains it appears that their similarities are more likely to be the result of convergent evolution rather than homology by descent [31].

Flajnik and colleagues have speculated that the structure of the terminal V domain in receptors such as NAR-TCR may indicate direct antigen binding like Ig, rather than recognition of antigen-MHC complexes like conventional TCR [30]. Perhaps receptors such as shark NAR-TCR and mammalian TCRμ will reveal a whole new type of antigen recognition and T cell activation that does not necessarily involve antigen processing and presentation. In this way they may be more analogous to the style of direct antigen recognition that has been described for some eutherian γδ TCR [34]. Why analogous receptors have been lost in the eutherian lineage, however, remains to be determined.

4. Origins of germ-line joined V genes in marsupials

Complete or partial germ-line joined V genes have been described previously for Ig of cartilaginous and boney fish, and birds [35–37]. The discovery of TCRμ in marsupials was the first example of a mammalian pre-joined V gene and the first to be found in a TCR.

The origins of Vμj in marsupials are potentially complex. The gene lacks an intron separating the leader sequence from the exon encoding the extracellular V domain, indicative of having undergone transcription, mRNA processing and retro-transposition back into the genome [14,31]. In other words it appears to be a “processed” gene. If this model for the generation of Vμj is correct, then re-insertion into the genome is likely to have involved homologous recombination that would have targeted the TCRμ locus, replacing non-recombined gene segments with the recombined one [14]. If this took place in a germ cell it would have required the activation of RAG mediated V(D)J recombination at the TCRμ locus and active transcription of the locus. There is prior evidence of ectopic expression of the RAG recombinase system in non-lymphoid cells such as germ cells which is thought to have generated the germline joined Ig V genes in species such as the cartilaginous fishes, and more recently even in marsupials as discussed further in subsection 5.2 [33,38]. There is an alternative model for the origin of Vμj in marsupials that does not require retrotransposition however. Rather it is based on the evidence that some processed genes are generated through direct intron deletion [39]. In this model, V(D)J recombination of TCRμ gene segments could have taken place in the germ-line followed by selective deletion of the intron separating the leader exon from the V exon. Both models require what seem to be multiple improbable steps making it hard to determine which is the most parsimonious [14].

The V segments in the platypus TCRμ locus that correspond to marsupial Vμj are not germ-line joined, indicating that this receptor has followed a different evolutionary path (Figure 2). At the very least, the events leading to the evolution of Vμj occurred after the Therian-Prototherian split 175 Myr ago. It is worth noting that the opossum genome contains among the largest percentage of retroelements of any vertebrate sequenced so far [4]. Therefore retroelements such as the long interspersed elements (L1 or LINE1) and endogenous retroviral elements (ERV) are present and abundant to provide the enzymatic machinery for retrotransposition. In contrast retroelements are rare to non-existent in the platypus genome [5,40].

5. Immunoglobulins of marsupials and monotremes

Detailed genomic analysis and annotation of the Ig heavy and light chain loci is also available for only a single marsupial species, the opossum *M. domestica*, and for no monotreme species. The opossum genes are un-remarkable, having the typical translocon type organization [33]. The Ig heavy chain locus contains a number of insertions of retroelements, including long

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

interspersed and endogenous retrovirus elements, some of which may have contributed to duplications and deletions within the locus [33]. However, since the opossum has the highest percentage of retroelements in its genome of any vertebrate sequenced so far it is not clear whether the presence of retroelements has directly influenced the evolution of the Ig heavy chain locus or if there have been secondary insertions [4].

5.1 The heavy and light chain isotypes

Analyses of serum immunoglobulins (Ig) in both marsupials and monotremes confirmed early on that Ig isotypes similar to that known from eutherian mammals were likely to be present [42–45]. cDNA and genomic analysis of marsupial species such as *M. domestica* and *Trichosurus vulpecula* as well as monotremes such as *O. anatinus* and *Tachyglossus aculeatus* have confirmed the presence of IgM, IgG, IgE and IgA in these species. The presence of IgG and IgE in both marsupials and monotremes confirmed that the duplication that gave rise to these two important Ig isotypes occurred early in mammalian evolution [46–48]. Like many eutherian species, the platypus *O. anatinus*, a monotreme, has multiple Ig heavy chain subclasses. Specifically there are at least two IgG and two IgA sub-isotypes in the platypus [48]. In contrast, a marsupial, the opossum *M. domestica*, has only one functional copy of each of the heavy chain isotypes [33]. There is a second IgM in the opossum but it clearly appears to be a pseudogene and is not expressed. The discovery of only a single IgG in the genome of the opossum was surprising given earlier evidence of what appeared to be at least two IgG subclasses in this species and other marsupials [45]. It is possible that co-purification of multiple heavy chain isotypes gave a false impression of multiple IgG types were present in the serum Ig of marsupials. Alternatively there may be post-translational modifications, such as glycosylation, that result in different species of IgG heavy chain proteins being produced.

So far no IgD has been described in a marsupial and the genomic region predicted to encode the IgD constant regions appears to have been replaced by an insertion of retroelements in the opossum [33]. Whether these insertions deleted the IgD genes in this species or if they are universally absent in marsupials is not known. However a monotreme IgD homologue appears to be present in the platypus but has not been extensively characterized [33]. The presence of IgD in the platypus, along with most eutherian and some non-mammalian species suggests that this isotype is clearly ancient and was lost in at least the opossum and perhaps other marsupial lineages [33,49].

All marsupial and monotreme species examined so far have homologues of both Igκ and Igλ [50–55]. Like heavy chains, the genomic organization of the light chain loci in *M. domestica* is un-remarkable and highly conserved with that of eutherian mammals [33].

5.2 The germline contributions to Ig diversity

One unusual feature of the Ig heavy V gene segments in marsupials is that all species examined so far not only have limited germ-line diversity, they have nearly the same limited diversity. It is as if all marsupials contain the same VH subgroup [56,57]. This is not the result of recent divergence of marsupial species since species such as the brushtail possum *Trichosurus vulpecula* and the opossum *M. domestica* are separated by at least 75 million years [1,56]. Rather it appears as if marsupial VH genes underwent a genetic bottleneck early in marsupial evolution, leaving the entire lineage with limited VH diversity. In contrast to marsupial heavy chain V genes, marsupial Ig light chain Vκ and Vλ are more complex and appear to have retained extensive ancient diversity [50,51,56]. It appears as if a common rule amongst marsupials is that germ-line Ig light chain diversity may contribute more to overall antibody diversity than does heavy chain. This contrast between the complexity of V genes in marsupial Ig heavy and light chains also means marsupials don’t appear to follow a rule that has emerged from studies of other vertebrate species. In most cases Ig heavy and light chain V genes appear

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

to be co-evolving in a way that results in those species having high levels of heavy chain V diversity also have high levels light chain V diversity [58,59]. Likewise, those species with limited heavy chain V diversity also have limited germ-line light chain V diversity. Marsupials as a group appear to be exceptions to this rule since they have limited heavy chain but complex light chain V diversity. Interestingly, monotremes more closely follow the co-evolutionary rule. The platypus has limited VH diversity and more limited light chain V diversity [54,55, 60]. The platypus appears to compensate for the limited germ-line diversity in both heavy and light chains with extensive CDR3 diversity [55,60]. A related monotreme, the echidna *T. aculeatus* has a more diverse VH repertoire and also has diverse light chain V genes [48,54, 55]. It is noteworthy that the platypus and the echidna are more divergent in their germ-line contributions to antibody diversity in spite of being more closely related than are the more distantly related marsupial families that are more similar in their style of antibody diversity [48,56].

One of the unexpected discoveries from detailed analysis of the opossum Ig heavy chain locus was a previously unknown V gene segment that appears to be a V to D germ-line joined gene [33]. This gene segment, annotated as VH3.1, has a complete gene structure and open reading frame, with an exon encoding the leader sequence separated by an intron from the exon encoding the extracellular V domain. In spite of appearing functional VH3.1 does not appear to contribute to the antibody repertoire. So far no transcripts or somatic B cell DNA containing rearranged VH3.1 have been found [33]. It is likely that VH3.1 is functionally dead since its use would require a developing B cell to perform an out of order VD to J rearrangement [41]. This gene segment does however provide further evidence for ectopic RAG expression in germ cells.

6. Concluding remarks

The analysis of marsupial and monotreme Ig and TCR has provided two contrasting views of the evolution of these receptors in mammals. On one hand the Ig and conventional TCR appear to be highly conserved and demonstrate that many of the uniquely mammalian characteristics that were discovered first in humans and mice, such as the duplication that gave rise to IgG and IgE, are in fact ancient in the mammalian lineage, predating the rise of the eutherians. On the other hand the discovery of the atypical TCRμ reinforces that there may be more receptor types out there waiting to be discovered, even in the jawed vertebrates. The presence of analogous TCR chains with atypical double V domain structures in species as distantly related as sharks, monotremes and marsupials suggests that such TCR may turn out to be more common across vertebrates than is currently realized. It was their absence from species such as humans and mice that lead to the lateness of their discovery.

Acknowledgments

The author acknowledges support from the U.S. National Science Foundation.

References

1. Bininda-Emonds ORP, Cardillo M, Jones KE, MacPhee RDE, Beck RMD, Grenyer R, et al. The delayed rise of present-day mammals. Nature 2007;446:507–12. [PubMed: 17392779]
2. Renfree MB. Life in the pouch: a womb with a view. Reprod Fert Develop 2006;18:721–34.
3. Ashman RB. Marsupial immunology: A brighter future? Dev and Comp Immunol 1977;1:283–4. [PubMed: 608496]
4. Mikkelsen TS, Wakefield MJ, Aken B, Amemiya CT, Chang JL, Duke S, et al. Genome of the marsupial *Monodelphis domestica* reveals lineage-specific innovation in coding sequences. Nature 2007;447:167–78. [PubMed: 17495919]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

5. Warren WC, Hillier LW, Graves JAM, Birney E, Ponting CP, Grützner F, et al. Genome analysis of the platypus reveals unique signatures of evolution. Nature 2008;453:175–83. [PubMed: 18464734]
6. Belov K, Deakin JE, Papenfuss AT, Baker ML, Melman SD, Siddle HV, et al. Reconstructing an ancestral mammalian immune supercomplex from a marsupial. MHC PLoS Biol 2006;4:e46.
7. Zuccolotto PD, Harrison GA, Deane EM. Cloning of marsupial T cell receptor α and β onstant region cDNAs. Immunol Cell Biol 2000;78:103–9. [PubMed: 10762409]
8. Baker ML, Rosenberg GH, Zuccolotto P, Harrison G, Deane EM, Miller RD. Further characterization of T cell receptor chains of marsupials. Dev Comp Immunol 2001;25:495–507. [PubMed: 11356229]
9. Harrison GA, Taylor CL, Miller RD, Deane EM. Primary Structure and Variation of the T-Cell Receptor α Chain from a Marsupial, *Macropus eugenii*. Immunol Letters 2003;88:117–25.
10. Belov K, Miller RD, Ilijeski A, Hellman L, Harrison GA. Isolation of monotreme T cell receptor alpha and beta chains. Immunogenetics 2004;56:164–9. [PubMed: 15133646]
11. Baker ML, Osterman AK, Brumburgh S. Divergent T cell receptor delta chains from marsupials. Immunogenetics 2005;57:665–73. [PubMed: 16160827]
12. Baker ML, Indiviglio S, Nyberg AM, Rosenberg GH, Lindblad-Toh K, Miller RD, Papenfuss AT. Analysis of a set of Australian northern brown bandicoot expressed sequence tags with comparison to the genome sequence of the South American grey short tailed opossum. BMC Genomics 2007;8:50. [PubMed: 17298671]
13. Parra ZE, Arnold T, Nowak MA, Hellman L, Miller RD. TCR gamma chain diversity in the spleen of the duckbill platypus (*Ornithorhynchus anatinus*). Dev Comp Immunol 2006;30:699–710. [PubMed: 16303181]
14. Parra ZE, Baker ML, Hathaway J, Lopez AM, Trujillo J, Sharp A, Miller RD. Comparative genomic analysis and evolution of the T cell receptor loci in the opossum *Monodelphis domestica*. BMC Genomics 2008;9:111. [PubMed: 18312668]
15. Ashman R, Keast D, Stanley NF, Waring H. The immunological responses of marsupials. Am Nat 1975;15:155–66.
16. Deane, EM.; Cooper, DW. Immunological development in pouch young marsupials. In: Tyndale-Biscoe, CH.; Janssens, PA., editors. The Developing Marsupial. Berlin: Springer-Verlag; 1988. p. 190-9.
17. Hubbard GB, Saphire DG, Hackleman SM, Silva MV, VandBerg JL, Stone WH. Ontogeny of the thymus gland of a marsupial (*Monodelphis domestica*). Lab Anim Sci 1991;41:227–34. [PubMed: 1658459]
18. Kalmutz SE. Antibody production in the opossum embryo. Nature 1962;193:851–3. [PubMed: 14453360]
19. La Via MF, Rowlands DT, Block M. Antibody formation in embryos. Science 1963;140:1219–20. [PubMed: 17837507]
20. Rowlands DT, Lavia MF, Block MH. The blood forming tissues and blood of the newborn opossum (*Didelphys virginiana*). II. Ontogenesis of antibody formation to flagella of *Salmonella typhi*. J Immunol 1964;93:157–64. [PubMed: 14214381]
21. Baker ML, Gemmell E, Gemmell RT. Ontogeny of the immune system of the brushtail possum, *Trichosurus vulpecula*. Anat Rec 1999;256:354–65. [PubMed: 10589022]
22. Allison JP, Havran WL. The immunobiology of T cells with invariant gamma delta antigen receptors. Ann Rev Immunol 1991;9:679–705. [PubMed: 1832874]
23. Parra ZE, Baker ML, Lopez AM, Trujillo J, Volpe JM, Miller RD. TCRμ recombination and transcription relative to the conventional TCR during postnatal development in opossums. J Immunol 2009;182:154–63. [PubMed: 19109146]
24. Feeney AJ. Predominance of VH-D-JH junctions occurring at sites of short sequence homology results in limited junctional diversity in neonatal antibodies. J Immunol 1992;149:222–9. [PubMed: 1607655]
25. Zhang Y, Cado D, Asarnow DM, Komori T, Alt FW, Raulet DH, Allison JP. The role of short homology repeats and TdT in generation of the invariant γδ antigen receptor repertoire in the fetal thymus. Immunity 1995;3:439–47. [PubMed: 7584135]
26. Hedrick SM, Cohen DI, Nielsen EA, Davis MM. Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. Nature 1984;308:149–53. [PubMed: 6199676]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

27. Brenner MB, McLean J, Dialynas DP, Strominger JL, Smith JA, Owen FL, Seidman JG, Rosen SF, Krangel MS. Identification of a putative second T-cell receptor. Nature 1986;322:45–9.
28. Chien YH, Iwashima M, Kaplan KB, Elliot JF, Davis MM. A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. Nature 1987;327:677–82. [PubMed: 2439914]
29. Rast JP, Anderson MK, Strong SJ, Luer C, Litman RT, Litman GW. α, β, γ, and δ T cell antigen receptor genes arose early in vertebrate phylogeny. Immunity 1997;6:1–11. [PubMed: 9052832]
30. Criscitiello MF, Saltis M, Flajnik MF. An evolutionarily mobile antigen receptor variable region gene: Doubly rearranging NAR-TcR genes in sharks. Proc Natl Acad Sci USA 2006;103:5036–41. [PubMed: 16549799]
31. Parra ZE, Baker ML, Schwarz R, Deakin JE, Lindblad-Toh K, Miller RD. Discovery of a new T cell receptor in marsupials. Proc Natl Acad Sci USA 2007;104:9776–81. [PubMed: 17535902]
32. Deakin JE, Parra ZE, Graves JAM, Miller RD. Physical Mapping of T cell receptor loci (TRA@, TRB@, TRD@ and TRG@) in the opossum (*Monodelphis domestica*). Cytogenet Genome Res 2006;112:342K.
33. Wang X, Olp JJ, Miller RD. On the genomics of immunoglobulins in the gray, short-tailed opossum. Monodelphis domestica. In review.
34. Chien YH, Konigshofer Y. Antigen recognition by gamma delta T cells. Immunol Rev 2007;215:46–58. [PubMed: 17291278]
35. Kokubu F, Litman R, Shamblott MJ, Hinds K, Litman GW. Diverse organization of immunoglobulin VH gene loci in a primitive vertebrate. EMBO J 1988;7:3413–22. [PubMed: 3145194]
36. Reynaud CA, Dahan A, Anquez V, Weill JC. Somatic hyperconversion diversifies the single Vh gene of the chicken with a high incidence in the D region. Cell 1989;59:171–83. [PubMed: 2507167]
37. Ventura-Holman T, Lobb CJ. Structural organization of the immunoglobulin heavy chain locus in the channel catfish: the IgH locus represents a composite of two gene clusters. Mol Immunol 2002;38:557–64. [PubMed: 11750657]
38. Lee SS, Fitch D, Flajnik MF, Hsu E. Rearrangement of immunoglobulin genes in shark germ cells. J Exp Med 2000;191:1637–48. [PubMed: 10811858]
39. Roy SW, Gilbert W. The evolution of spliceosomal introns: patterns, puzzles and progress. Nat Rev Genet 2006;7:211–21. [PubMed: 16485020]
40. Kordis D, Lovsin N, Gubensek F. Phylogenomic analysis of the L1 retrotransposons in deuterostomia. Syst Biol 2006;55:886–901. [PubMed: 17345671]
41. Sekiguchi, J.; Alt, FW.; Oettinger, M. The mechanisms of V(D)J recombination. In: Honjo, T.; Alt, FW.; Neuberger, M., editors. Molecular Biology of B Cells. London: Academic Press; 2004. p. 62-82.
42. Atwell JL, Marchalonis JJ, Ealey EH. Major immunoglobulin classes of the echidna (*Tachyglossus aculeatus*). Immunol 1973;25:835–40.
43. Bell RG, Lynch NR, Turner KJ. Immunoglobulins of the marsupial *Setonix brachyurus* (quokka). Immunol 1974;27:1103–15.
44. Bell RG. Marsupial immunglobulins: the distribution and evolution of macropod IgG2, IgM and light chain antigenic markers within the sub-class metatheria. Immunol 1977;33:917–24.
45. Shearer MH, Robinson ES, VandeBerg JL, Kennedy RC. Humoral immune response in a marsupial Monodelphis domestica: anti-isotypic and anti-idiotypic responses detected by species-specific monoclonal anti-immunoglobulin reagents. Dev Comp Immunol 1995;19:237–46. [PubMed: 8595822]
46. Aveskogh M, Hellman L. Evidence for an early appearance of modern post-switch isotypes in mammalian evolution; cloning of IgE, IgG and IgA from the marsupial *Monodelphis domestica*. Eur J Immunol 1998;28:2738–50. [PubMed: 9754561]
47. Miller RD, Grabe H, Rosenberg GH. The V_H Repertoire of a Marsupial: *Monodelphis domestica*. J Immunol 1998;160:259–65. [PubMed: 9551979]
48. Belov K, Hellman L. Immunoglobulin genetics of *Ornithorhynchus anatinus* (platypus) and *Tachyglossus aculeatus* (short-beaked echidna). Comp Biochem Physiol, Part A Mol Integr Physiol 2003;136:811–9.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

49. Ohta Y, Flajnik M. IgD, like IgM, is a primordial immunoglobulin class perpetuated in most jawed vertebrates. Proc Natl Acad Sci USA 2006;103:10723–28. [PubMed: 16818885]
50. Lucero JE, Rosenberg GH, Miller RD. Marsupial light chains: Complexity and conservation of Igl in the opossum *Monodelphis domestica*. J Immunol 1998;161:6724–32. [PubMed: 9862702]
51. Miller RD, Bergemann ER, Rosenberg GH. Marsupial light chains: Igk with four V families in the opossum *Monodelphis domestica*. Immunogenetics 1999;50:329–35. [PubMed: 10630297]
52. Belov K, Harrison GA, Miller RD, Cooper DW. Characterisation of the kappa light chain of the brushtail possum (*Trichosurus vulpecula*). Vet Immunol Immunopathol 2001;78:317–24. [PubMed: 11292532]
53. Belov K, Harrison GA, Miller RD, Cooper DW. Molecular cloning of four lambda light chain cDNAs from the Australian brushtail possum (*Trichosurus vulpecula*). Eur J Immunogenet 2002;29:95–9. [PubMed: 11918633]
54. Nowak MA, Parra ZE, Hellman LH, Miller RD. The complexity of expressed kappa light chains in the egg-laying mammals. The complexity of expressed kappa light chains in the egg-laying mammals. Immunogenetics 2004;56:555–63. [PubMed: 15448942]
55. Johansson J, Salazar JN, Aveskogh M, Munday B, Miller RD, Hellman L. High variability in complementarity-determining regions compensate for a low number of V gene families in the lambda light chain locus of the egg-laying mammals. Eur J Immunol 2005;35:3008–19. [PubMed: 16143985]
56. Baker ML, Belov K, Miller RD. Unusually similar patterns of antibody V segment diversity in distantly related marsupials. J Immunol 2005;174:5665–71. [PubMed: 15843567]
57. Aveskogh M, Pilström L, Hellman L. Cloning and structural analysis of IgM (μ chain) and the heavy chain V region repertoire in the marsupial *Monodelphis domestica*. Dev Comp Immunol 1999;23:597–606. [PubMed: 10579388]
58. Butler JE. Immunoglobulin gene organization and the mechanism of repertoire development. Scand J Immunol 1997;45:455–62. [PubMed: 9160087]
59. Sitnikova T, Su C. Coevolution of immunoglobulin heavy- and light- chain variable-region gene families. Mol Biol Evol 1998;15:617–25. [PubMed: 9615443]
60. Johansson J, Aveskogh M, Munday B, Hellman L. Heavy chain V region diversity in the duck-billed platypus (*Ornithorhynchus anatinus*): long and highly variable complementarity-determining region 3 compensates for limited germline diversity. J Immunol 2002;168:5155–62. [PubMed: 11994470]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

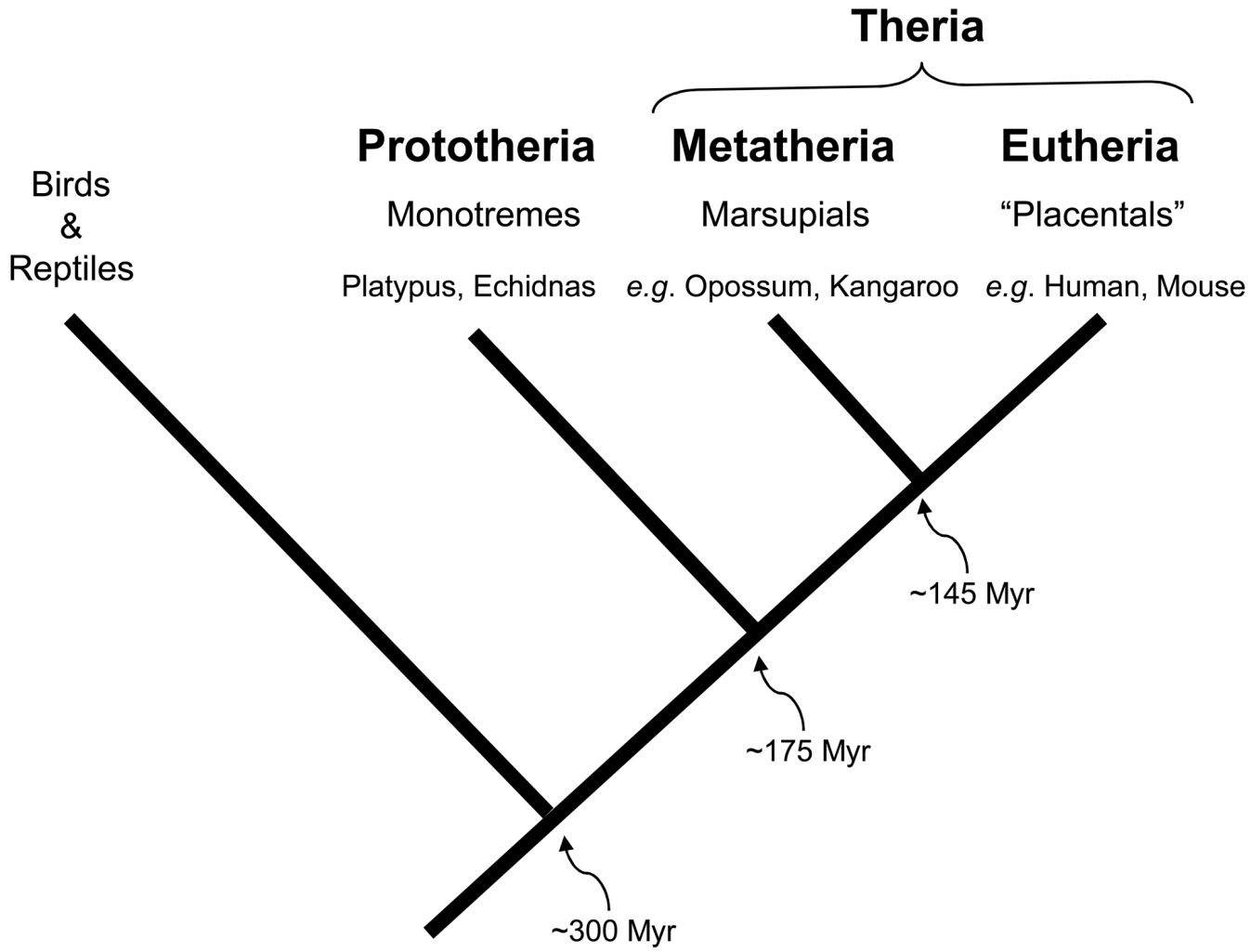

Figure 1.
Graphic representing the relationships of the three living lineages of mammals. Timing of divergence points based on work of Bininda-Emonds et al. [1].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

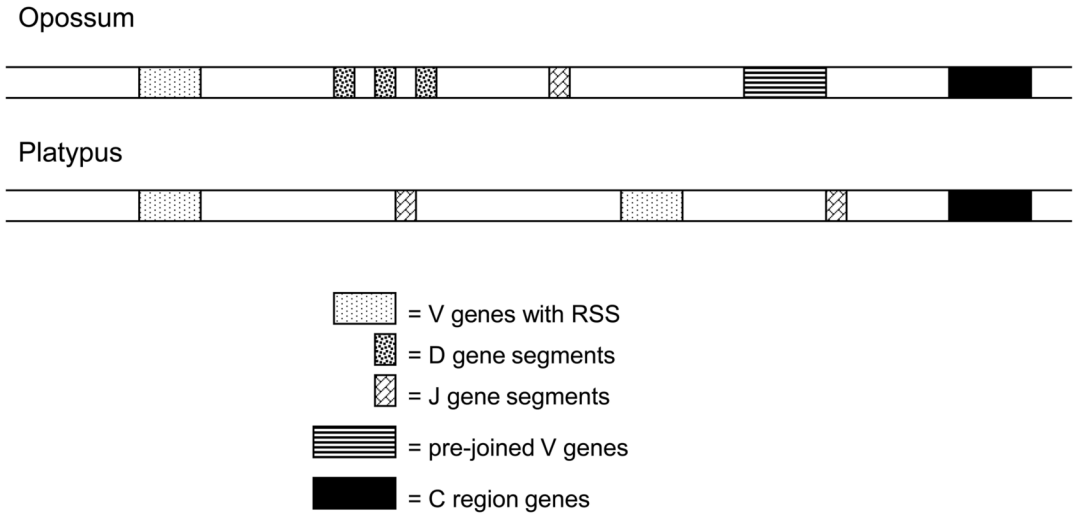

Figure 2.
Diagram of a representative opossum TCRμ cluster based on the *M. domestica* genome assembly [4,31] and a hypothetical platypus TCRμ locus interpreted from available genomic and cDNA data.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

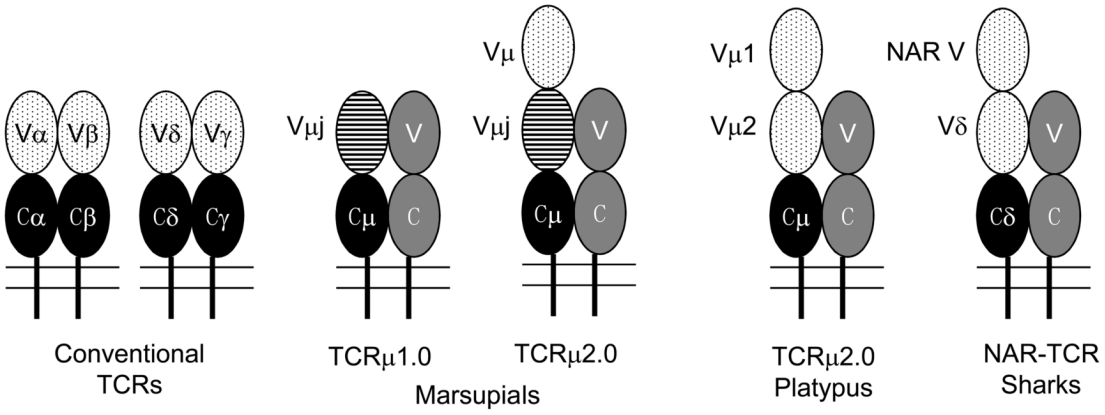

Figure 3.

Schematic of the predicted TCRμ chains from marsupials and monotremes. Shading corresponds to that shown in Figure 2. Variable domains encoded by exons that undergo somatic V(D)J recombination are shaded with polka-dots. V domains encoded by exons where the V, D and J segments are pre-joined in the germ-line are shaded with horizontal stripes. Hypothetical chains shaded gray represent the unknown partners for TCRμ.