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Review

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

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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.

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 us 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 is not. 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 (Fig. 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 characteristics. 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

* Tel.: +1 505 277 2844; fax: +1 505 277 0304.
E-mail address: rdmiller@unm.edu.

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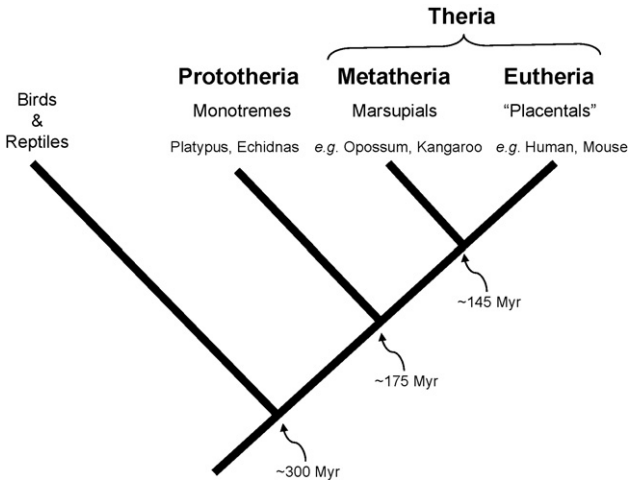

Fig. 1. Graphic representing the relationships of the three living lineages of mammals. Timing of divergence points based on work of Bininda-Emonds et al. [1].

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 10 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.

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 Section 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 h 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 h 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