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Haematopoiesis in Marsupials

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ABSTRACT

Marsupials are a group of mammals that give birth to immature young lacking mature immune tissues at birth, and are unable to mount their own specific immune defence. Their immune tissues develop in a non-sterile ex-utero environment unlike that of eutherian mammals such as ourselves. Marsupials are therefore ideal models for studying the development of immune tissues, in particular haematopoiesis, yet relatively little has been investigated. Most studies have been restricted to histological or immunohistological studies, however some factors likely to be involved, based on eutherian studies in haematopoiesis, have been isolated and characterised, including a few key markers, and some cell signaling and regulation molecules, mostly involved in lymphocytopoiesis. However the role of many molecules in haematopoiesis is largely presumed. We currently lack much of the rudimentary information regarding time of appearance and expression levels of these molecules, and no functional studies have been conducted. This paper reviews our knowledge of marsupial haematopoiesis to date, and highlights the need for future research in marsupials to gain further insights into the evolution of haematopoiesis.

1. Introduction

Marsupials (metatherian mammals) are one of the three groups of mammals. They differ from their Prototherian counterparts (or monotremes) as they give birth to live young. Marsupials also differ from eutherian mammals, such as humans and mice, as they give birth to comparatively underdeveloped young, that lack histologically mature immune tissues (Ashman and Papadimitriou, 1975; Basden et al., 1996, 1997; Block, 1964; Old et al., 2003a, b), and are unable to mount specific immune defence (reviewed in Old and Deane, 2000).

As marsupials are born in such an underdeveloped state, immunologically, compared to eutherian mammals, they are excellent models for studying the development of the immune system and immunity in mammals. Studies describing the development of the immune tissues in marsupials have recently been reviewed and the gaps in our knowledge and understanding reported (Borthwick et al., 2014). One area that remains relatively elusive in terms of our knowledge is haematopoiesis. This paper is a review of the current knowledge of marsupial haematopoiesis and offers some avenues for future research in this relatively unexplored area of marsupial immunology.

Haematopoiesis in non-eutherian mammals (monotremes and marsupials) has been studied in only a few species to date (Table 1). In marsupials, the majority of these have focused on histological descriptions (Ashman and Papadimitriou, 1975; Basden et al., 1996; Block, 1964; Cisternas and Armati, 1999; Old et al., 2003a; 2003b; 2004a; Yadav, 1972). Even fewer studies on haematopoiesis in monotremes have been conducted. These studies are limited to histological descriptions of bone marrow and spleen from one adult platypus (*Ornithorhynchus anatinus*) (Tananka, 1986; Tanaka et al., 1988), and the histological and immunohistology of immune tissues with limited haematopoietic description in a further 15 adult platypuses (Connolly et al., 1999). There are no studies of the haematopoietic tissues of any echidna species or any developmental studies.

Histologically, marsupial haematopoiesis is similar to that of eutherian mammals, where it is initiated in the primitive yolk sac, and is continued by the liver, before being replaced by the bone marrow. As discussed earlier however, when compared to eutherian mammals, marsupials are born with no histologically mature immune tissues and the immune tissues must therefore develop externally in a non-sterile ex-utero environment. When born, unlike eutherian mammals, whereby the bone marrow is the active haematopoietic organ, in marsupials the liver is the active haematopoietic organ, and has been described in all marsupials studied (reviewed by Borthwick et al., 2014; and Old and Deane, 2000). The bones at birth in marsupials are limited to cartilaginous structures with medullary regions. Shortly after birth however the

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Table 1
: List of Marsupial and Monotreme species, their classification, and the haematopoietic tissues studied and methods used.

Species	Classification	Tissues studied	Method of study	References
Platypus (*Ornithorhynchus anatinus*)	Prototherian Ornithorhynchidae	Bone marrow Spleen	Histology; Immunohistology	Tanaka, 1986; Tanaka et al., 1988; Connolly et al., 1999
Virginian opossum (*Didelphis virginiana*)	Metatherian Polyprotodont Didelphidae	Liver Bone marrow Spleen	Histology	Block, 1964; Cutts et al., 1973; Cutts and Krause, 1982
Marmosa mitis	Metatherian Polyprotodont Didelphidae	Liver Bone marrow	Histology	Bryant and Shrifrine, 1974
White-bellied opossum (*Didelphis albiventris*)	Metatherian Polyprotodont Didelphidae	Spleen	Histology; Immunohistology	Coutinho et al., 1995
Gray short-tailed opossum (*Monodelphis domestica*)	Metatherian Polyprotodont Didelphidae	Liver Spleen	Specific gene isolation; Cell signaling and regulation gene isolation; *In silico* investigation	Aveskogh and Hellman, 1998; Aveskogh et al., 1999; Belov et al., 1999c; Miller et al., 1998; Lucero et al., 1998; Miller et al., 1999; Guth et al., 1998; Baker et al., 2005; Parra et al., 2009; Wang et al. 2012; Miller and Rosenberg, 1997; Wong et al., 2011a,b
Quokka (*Setonix brachyurus*)	Metatherian Diprotodont Macropodidae	Liver	Histology	Ashman and Papadimitriou, 1975
Tammar wallaby (*Macropus eugenii*)	Metatherian Diprotodont Macropodidae	Liver Bone marrow Spleen	Histology; Immunohistology; Specific gene isolation; Cell signaling and regulation gene isolation; *In silico* investigation	Basden et al., 1996; Harrison et al., 1999; Harrison and Deane, 1999; Zuccolotto et al., 2000; Baker et al., 2001; Harrison et al., 2003; Old and Deane, 2003; Parra et al., 2008; Young and Harrison, 2010; Wong et al., 2006; 2011a; 2011b; Young, 2011; Alsemgeest et al., 2013
Brushtail possum (*Trichosurus vulpecula*)	Metatherian Diprotodont Phalangeridae	Liver Spleen Lymph node Thymus Lung	Specific gene isolation; Cell signaling and regulation gene isolation	Belov et al., 1998, 2001, 2002a; 2002b; Wedlock et al., 1996; 1999a; 1999b; Young et al., 2012
Northern brown bandicoot (*Isoodon macrourus*)	Metatherian Polyprotodont Peramelidae	Liver	Histology; Immunohistology	Cisternas and Armati, 1999
Stripe-faced dunnarts (*Sminthopsis macrourus*)	Metatherian Polyprotodont Dasyuridae	Liver Bone marrow Spleen	Histology; Immunohistology	Old et al., 2003b; Old et al., 2004a; Old et al., 2004b

liver start to develop its mature structure with a decrease in haematopoietic cells until only a few haematopoietic foci are evident, before ceasing, at which time the liver becomes mature in appearance and no longer exhibits any haematopoietic activity.

At the same time the liver is decreasing its haematopoietic activity, the bones start to become ossified, a medullary cavity forms, and haematopoietic foci start to appear. In all marsupial species studied to date, haematopoiesis in the medullary cavity continues for sometime until involution starts to occur as the animal reaches a certain level of maturity, and virtually disappears, being replaced by yellow adipose tissue (reviewed by Borthwick et al., 2014; and Old and Deane, 2000).

Few studies have built on these histological investigations in marsupials. Old and Deane (2003) and Old et al. (2004b) have confirmed that the bone marrow of stripe-faced dunnarts (*Sminthopsis macrourus*) and tammar wallabies (*Macropus eugenii*) lack mature T and B-cells (based on immunohistochemical studies). However a lack of antibodies to immature immunological cell types, and other antibodies likely to cross-react with cells in the bone marrow are currently unavailable. Likewise, no genetic expression studies have focused on expression of any immunological markers in bone marrow samples. The descriptions of the haematopoietic cells in marsupials is therefore solely based on their histological appearance, these being primarily of the erythrocytic, leucocytic and granulocytic lineages (Ashman and Papadimitriou, 1975; Basden et al., 1996; Block, 1964).

The cell types that have been described in haematopoietic tissues of marsupials are similar to that described in eutherians and prototherians. As marsupials are born with no mature immune tissues and are unable to mount specific immune responses (reviewed by Old and Deane, 2000), it is not surprising that at birth high levels of erythropoiesis and granulocytopoiesis occur, with lymphocytopoiesis relatively absent until shortly after birth.

2. Histological description of haematopoiesis

2.1. Liver

In eutherians, the liver has essentially ceased haematopoiesis prior to birth, however the liver is actively haematopoietic at the time of birth in marsupials. In *Didelphis*, the liver diverticulum begins to form from the foregut (McCrady, 1938). The liver then enlarges and begins haematopoietic activity prior to birth. Block (1964) described haematopoiesis in the developing Virginian