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Koala immunology and infectious diseases: How much can the koala bear?

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ABSTRACT

Infectious diseases are contributing to the decline of the iconic Australian marsupial, the koala (*Phascolarctos cinereus*). Infections with the obligate intracellular bacteria, *Chlamydia pecorum,* cause debilitating ocular and urogenital-tract disease while the koala-retrovirus (KoRV) has been implicated in host immunosuppression and exacerbation of chlamydial pathogenesis. Although histological studies have provided insight into the basic architecture of koala immune tissues, our understanding of the koala immune response to infectious disease has been limited, until recently, by a lack of species-specific immune reagents. Recent advances in the characterisation of key immune genes have focused on advancing our understanding of the immune response to *Chlamydia* infection, revealing commonalities in disease pathologies and immunity between koalas and other hosts and paving the way for the development of a koala *Chlamydia* vaccine. This review summarises these recent findings and highlights key aspects of the koala immune system requiring further attention with particular regard to their most prominent infectious diseases.

Contents

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1. Introduction

The koala (*Phascolarctos cinereus*), an arboreal, herbivorous marsupial and internationally recognised symbol of Australia's unique fauna is the last extant member of the family, *Phascolarctidae*. Once widespread from north to south-eastern Australia, koala populations are currently experiencing dramatic declines across the majority of their remaining free-living populations (McAlpine et al., 2015). The rapid decline of koala populations can be attributed to a range of natural and anthropogenic influences, including habitat fragmentation from land clearing (Melzer et al., 2000), bushfire, dog attacks (Lunney et al., 2007), motor vehicle traumas (Dique et al., 2003) and disease (Rhodes et al., 2011). While koala conservation strategies struggle to manage the range of factors threatening koala populations (McAlpine et al., 2015), modelling suggests that the control of disease is a key strategy to return peri-urban koala populations to stable levels (Rhodes et al., 2011).

Multiple aetiological agents of infectious disease have been reported in the koala including, an endogenous koala retrovirus (KoRV) (Tarlinton et al., 2005), a gammaherpesvirus (Vaz et al., 2011) and blood parasite, *Trypanosoma irwini* (McInnes et al., 2011). While KoRV has been implicated in immunosuppression of the host (Denner and Young, 2013), infection by the obligate intracellular bacteria, *Chlamydia pecorum*, is regarded as the most prominent infectious disease contributing to the decline and long-term viability of koala populations (Polkinghorne et al., 2013). Although, in most cases chlamydial infections are ubiquitous and asymptomatic (Polkinghorne et al., 2013), acute and/or persistent exposure to this pathogen may result in infertility, cystitis, debilitating blindness and/or respiratory disease in this host (Glassick et al., 1996; Jackson et al., 1999; Waugh et al., 2016b). The development of a koala-specific chlamydial vaccine is currently regarded as the most practical solution for the protection of diseased koala populations (Polkinghorne et al., 2013), with recent trials indicating promising results (Khan et al., 2016a,b; Waugh et al., 2016c). As with chlamydial infections in other hosts, a significant lack of understanding of the koala immune response to chlamydial infection otherwise hinders the development of this conservation tool (Mathew et al., 2014).

The koala, alongside other marsupials, provides excellent models for studying immunity (Belov et al., 2006) as they occupy a key position on the vertebrate phylogenetic tree, having diverged from eutherian (placental) mammals more than 148 million years ago (Bininda-Emonds et al., 2007). Importantly however, the mammalian immune system evolved before the divergence of the marsupial and eutherian lineages (Belov et al., 2007), so marsupials and eutherians share complex tissues, and the same genetic architecture of the mammalian innate and adaptive immune system (Siddle et al., 2010). Despite this, marsupials were once believed to have 'primitive' immune responses (Jurd, 1994). Early studies relied on the use of cross-reactive antibodies with variable levels of cross reactivity (Morris et al., 2014; Wong et al., 2006) contributing to the failure to detect immune responses that led to the suggestion that koalas are 'immunologically lazy' when compared to placental mammals (Wilkinson et al., 1992). With the development of species-specific immune reagents and advances in genomic knowledge (e.g (Hobbs et al., 2014). for the koala), we now know that the immune system of marsupials is significantly more complex than previously thought (Belov et al., 2007), with features as intricate and advanced as their placental counterparts (Belov et al., 2013). This also holds true for koalas, with pilot studies of the koala immune response to natural chlamydial infections and vaccination also revealing that animals can produce strong and long-lasting humoral and cell-mediated immune (CMI) responses to chlamydial antigens (Kollipara et al., 2012; Mathew et al., 2013a,b, 2014; Morris et al., 2014). The ongoing expansion of these studies to characterise the immune gene repertoire of the koala (Morris et al., 2014) have also revealed a few surprises, with koala-specific innovations apparent even within marsupial lineages (Morris et al., 2015).

In light of these recent advances, the purpose of this review is to provide an overview of current knowledge of the koala immune system, the koala response to its most important pathogens and to highlight key areas that should be the subject of further research.

2. Development of the koala immune system; from immunologically incompetent joeys to adults with mature immune tissues

The primary function of the immune system is to protect the host from pathogens (Chaplin, 2006). The koala, like all marsupials, lacks mature immune tissues at birth (Old and Deane, 2000; Belov et al., 2013). The newborns are unable to produce an adaptive immune response (Coutinho et al., 1995; Old and Deane, 2000; Edwards et al., 2012), and instead are reliant on innate immunity (Belov et al., 2007) as well as passive immunity through the mother's milk (Edwards et al., 2012). Young and Deane (2001) revealed that the major cellular components of koala milk are immune cells including neutrophils and macrophages. Recent transcriptomic and proteomic studies have expanded this analysis of immune compounds in the koala (Morris et al., 2015). The mammary gland transcriptome revealed that there are 851 genes with primary immune functions, representing approximately 9% of all genes expressed in the koala mammary gland (Morris et al., 2015). Immunoglobulins and Ig receptors were also identified in the koala mammary transcriptome and the most abundant proteins were well-characterised milk proteins, including β-lactoglobulin, lactotransferrin and trichosurin, a protein unique to marsupials (Morris et al., 2015). Additionally, anti-microbial peptides (AMPs), including four cathelicidins (which lyse pathogens) were present in the koala mammary gland transcriptome, and novel proteins with potentially antimicrobial roles were also identified (Morris et al., 2014).

2.1. Koala immune tissues

The development of immune competence in marsupials is facilitated by the maturation of immune tissues, which occurs after birth while the young is in the pouch (Borthwick et al., 2014). However, research on the development of marsupial immune tissues has been limited to a couple of species (Belov et al., 2013), and do not include the koala. Nevertheless, we can make inferences about the development of koala immune tissues as all previously studied marsupial species appear to follow a similar pattern of immune tissue development (Old and Deane, 2000; Belov et al., 2013).

2.1.1. The thymus

The thymus, a pivotal organ in immune function (Lynch et al., 2009; Old and Deane, 2000), is responsible for the maturation of T-cells derived from bone marrow (Schuurman et al., 1997). Koalas have similar thymic architecture to other marsupials and eutherians with well-defined cortical and medullary regions and the formation of Hassall's corpuscles in the histologically mature thymus (Canfield et al., 1996). While most marsupials possess both cervical and thoracic thymuses (Canfield et al., 1996), koalas normally only possess a cervical thymus (Haynes, 2001). However, research has shown there is no significant structural or functional differences between cervical and thoracic thymuses in those Australian animals possessing both (Canfield et al., 1996; Stanley et al., 1972).