



# Koala retrovirus epidemiology, transmission mode, pathogenesis, and host immune response in koalas (*Phascolarctos cinereus*): a review

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## Abstract

Koala retrovirus (KoRV) is a major threat to koala health and conservation. It also represents a series of challenges across the fields of virology, immunology, and epidemiology that are of great potential interest to any researcher in the field of retroviral diseases. KoRV is a gammaretrovirus that is present in both endogenous and exogenous forms in koala populations, with a still-active endogenization process. KoRV may induce immunosuppression and neoplastic conditions such as lymphoma and leukemia and play a role in chlamydiosis and other diseases in koalas. KoRV transmission modes, pathogenesis, and host immune response still remain unclear, and a clear understanding of these areas is critical for devising effective preventative and therapeutic strategies. Research on KoRV is clearly critical for koala conservation. In this review, we provide an overview of the current understanding and future challenges related to KoRV epidemiology, transmission mode, pathogenesis, and host immune response and discuss prospects for therapeutic and preventive vaccines.

## Introduction

Koala retrovirus (KoRV) is a source of great concern to conservationists, as well as a source of great interest to virologists, immunologists, and epidemiologists. KoRV threatens koala (*Phascolarctos cinereus*) populations, both in the wild in Australia and in zoos around the world. Koala conservation has become an even more pressing issue of late as populations of Australia's iconic marsupial species have been devastated in recent bush fires in that country, heightening the need to prioritize efforts against KoRV. However, lessons learned in the fight against KoRV will be applicable far beyond the field of koala conservation.

KoRV is a gammaretrovirus belonging to the family *Retroviridae* and was first identified in 1988 in a leukemic koala [1]. Subsequently, the KoRV genome was fully sequenced for the first time in 2000 [2]. KoRV has the typical morphology of a gammaretrovirus, with spherically shaped virions ranging from 80–100 nm in diameter [3]. KoRV has a positive-sense, single-stranded RNA genome approximately 8.4 kb in size, containing *gag*, *pol*, and *env* genes with long terminal repeat (LTR) regions at the 5' and 3' ends [2, 4, 5]. The KoRV genome encodes a reverse transcriptase and structural proteins such as the main core protein p27Gag and the envelope proteins gp70 and p15E [6].

KoRV is closely related genetically to gibbon-ape leukemia virus (GaLV) [2, 4], feline leukemia virus (FeLV), and porcine endogenous retrovirus (PERV) [3]. KoRV has been linked to immunosuppression, chlamydiosis, lymphoma, and leukemia and leads to poor outcomes for infected individuals [4, 7–10]. Progress in KoRV-related research may therefore shed light on issues that need to be tackled for any retrovirus.

A further interesting feature of KoRV is its existence in both exogenous and endogenous forms, in contrast to human endogenous retroviruses (HERVs), which exist entirely in the human genome but show few associations with pathologies. The endogenization process for KoRV started relatively recently (approximately 22,000 to 49,000 years ago) [11] and appears to be ongoing; KoRV thus presents a great opportunity to study the virus endogenization process in real

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time. By contrast, other mammalian retrovirus infections date back over millions of years of evolutionary history, during which initially exogenous retroviruses were integrated into the host germ line and became endogenous viruses that were solely transmitted vertically from parents to offspring [12]. KoRVs, on the other hand, can be transmitted both vertically and horizontally [5, 7, 13–16].

So far, nine subtypes of KoRV have been isolated (KoRV-A to KoRV-I; Table 1) [17]. This classification is based on sequence differences in the receptor-binding domain of the envelope gene [18, 19]. Of the nine subtypes, KoRV-A and KoRV-B are currently the most extensively characterized [7, 9]. KoRV-A is transmitted vertically, whereas KoRV-B is transmitted both vertically and horizontally [4, 9, 17]. KoRV-A and KoRV-B utilize different entry receptors: phosphate transporter (PiT1) in the case of KoRV-A and thiamine transporter 1 (ThTR1) in the case of KoRV-B [7, 20]. Also, many recombinant KoRV (recKoRV) variants have been recognized in koalas [15, 21, 22].

The prevalence of KoRV varies depending on the geographical location of a particular population [13]. Variations in KoRV proviral loads in koala genomes have been reported by us and others [5, 13, 16, 23–25]. These reports are indicative of the ongoing endogenization process that KoRV is undergoing.

All vertebrates tested to date harbor endogenous retroviruses [26], which are genetic traces of past infections in which the viral genome was endogenized and then rendered inactive due to substitution mutations and deletions [27, 28]. In contrast, genetic analysis has revealed that endogenous KoRV contains a full-length replication-competent genome showing an infection pattern similar to that of exogenous retroviruses [29].

Although the pathogenesis of KoRV infections in koalas is poorly understood, KoRV-associated pathologies in koalas are considered a major threat to koala health and conservation. KoRV has been linked to neoplastic diseases, including

lymphoma and leukemia in wild and captive koalas, which are associated with high mortality [4, 7, 30].

As the information summarized above makes clear, much research has been undertaken on KoRV from a variety of perspectives (ranging across wildlife conservation, epidemiology, virology, microbiology, and immunology); however, our knowledge in all of these spheres is incomplete. An overview of what is known—and what is not known—about KoRV is particularly timely and will be of wide interest across the scientific community.

In this review, we outline the state of existing knowledge on KoRV and outline the challenges that remain. A proper understanding of the epidemiology and pathogenesis of KoRV infection and host immune responses against KoRV infection are critical for the development of effective preventative and therapeutic strategies for this virus. We focus on the epidemiology, transmission routes, pathogenesis, host immune response, and the issue of vaccine development in our survey of this interesting and challenging viral infection of koalas.

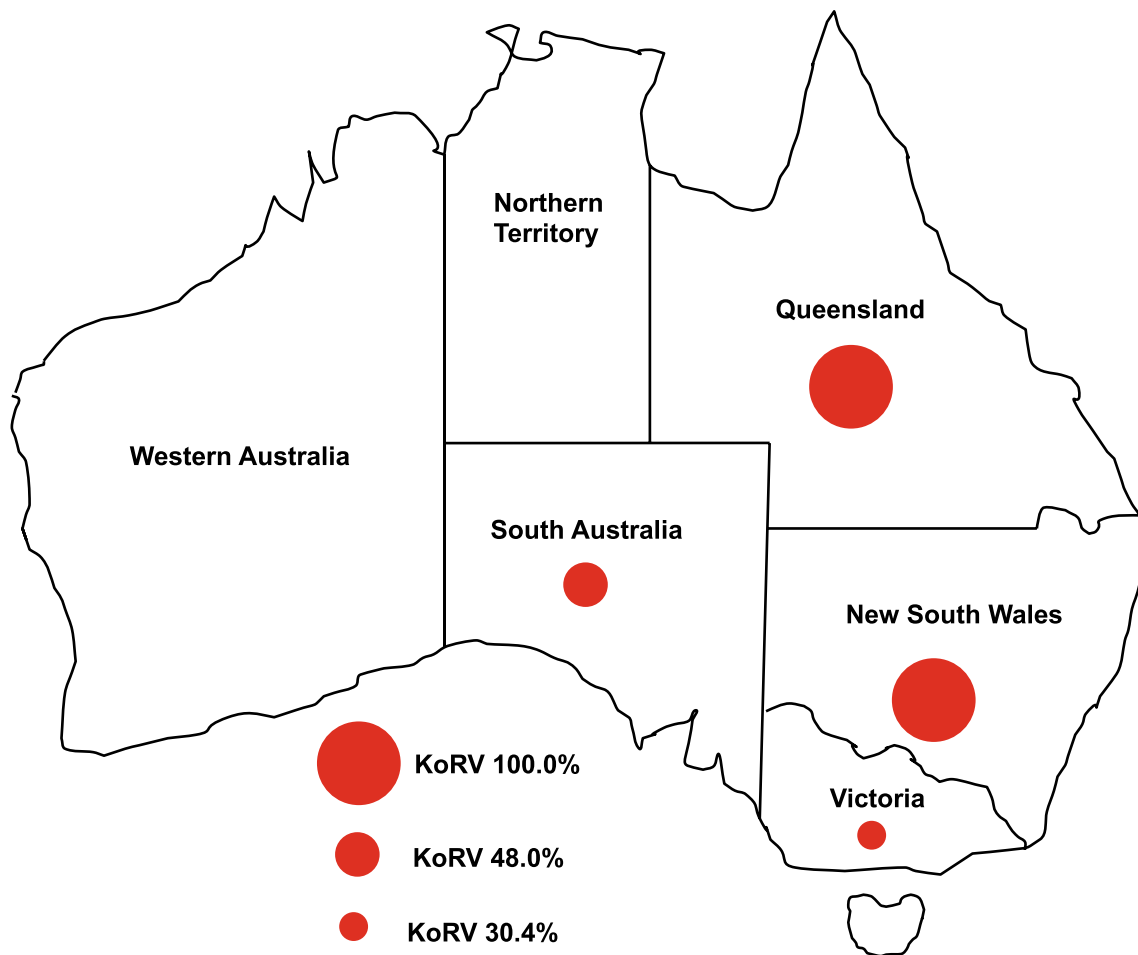
## Epidemiology of KoRV

KoRV is now endemic in both wild and captive koalas and represents a major threat to koala health [7, 9, 23, 31–33]. KoRV shows variations in its prevalence depending on the geographical location of the population and the virus subtype [13, 25, 33–35] (Fig. 1).

Epidemiological research on KoRV in the wild has tended to divide koalas broadly into two populations on the island continent of Australia: a northern population with habitats in Queensland and northern New South Wales and a southern population with habitats in Victoria and South Australia. Within the northern population, the prevalence of KoRV provirus is regarded as 100%, with every reported case of testing yielding positive evidence of the presence of a provirus [4, 9, 13, 33] (Table 2). The southern population

**Table 1** A summary of KoRV transmission modes

Subtype	Endogenous or exogenous	Transmission mode	Study area
KoRV-A	Endogenous	Vertical	Wild and captive Australian koala populations [4, 9, 13, 17, 54], koalas in a Japanese zoo [24]
KoRV-B	Exogenous, highly prevalent and associated with diseases	Both horizontal and vertical	Wild koala populations in Australia [9, 17], koalas in a US zoo [7], koalas in a Japanese zoo [14, 24]
KoRV-C	Exogenous with low prevalence	Horizontal and vertical	US zoo [57], Japanese zoo [16]
KoRV-D	Exogenous	Horizontal	Wild koala populations in Australia [15, 17]
KoRV-E	Exogenous with low prevalence	Horizontal	Wild koala in Australia [15], koalas in a US zoo [73]
KoRV-F	Exogenous	Horizontal	Wild koala populations in Australia [17, 18]
KoRV-G	Ongoing exogenous with low prevalence	Horizontal	Wild koala populations in Australia [18]
KoRV-H	Ongoing exogenous with low prevalence	Horizontal	Wild koala populations in Australia [18]
KoRV-I	Ongoing exogenous and low prevalent	Horizontal	Wild koala populations in Australia [18]



**Fig. 1** The geographical distribution of KoRV provirus in free-ranging koala populations in Australia. The KoRV provirus prevalence data shown on the map have been summarized from Table 2. The prevalence of KoRV provirus was shown to be 100% in koala popula-

tions of Queensland (805/805) and New South Wales (100/100), vs. 48.0% (268/558) and 30.4% (255/839) in koala populations of South Australia and Victoria, respectively. This map was generated from maps of Australia that are available through the internet

previously showed a much lower prevalence, reportedly ranging from 14.8 to 25% [8, 13, 34]; however, the three most recent studies have shown markedly higher figures, ranging from 42.4 to 99.0% [25, 33, 35] (Table 2).

Genetic diversity has also been addressed in other recent studies. The southern population appears to show lower absolute proviral copy numbers than the northern population [33]. A possible explanation advanced for this phenomenon concerns the relatively high replication efficiency of subtypes other than KoRV-A in the southern population [35]. Most of the data obtained so far concern KoRV subtypes A and B, and the data are far from complete for subtypes C to I. However, subtype expression has been addressed in one recent study, in which KoRV-A, -B, -D and -F were found to be the major subtypes present in wild populations and KoRV-D was found to be the significantly most prevalent subtype, with 10- and 100-fold greater expression than KoRV-A and KoRV-F, respectively [17].

The endogenous subtype, KoRV-A, has a prevalence rate of 100% in the northern population [4, 9, 13]. However, KoRV-A is also reportedly spreading within the southern population [13], with prevalence reaching 42.4% and 65.3% at the respective sampling points in the states of Victoria and South Australia, but these findings were not accompanied by the detection of KoRV-B [33] (Table 3). However, the prevalence of KoRV-B may be increasing in the northern population; a previous figure was given as 24% [9], with 48.5% as the most recently reported prevalence of KoRV-B in the northern population [35] (Table 3).

The data reviewed above were obtained exclusively from wild populations, but some data are also available for zoo populations. We have previously reported a KoRV-A prevalence of 100% and a KoRV-B prevalence ranging from 50 to 63.6% in Japanese zoo populations (at Kobe Oji Zoo, Saitama Children's Zoo, and Hirakawa Zoological Park) [24]. The origins of these populations were not entirely

**Table 2** Variation of KoRV prevalence in northern (Queensland and New South Wales) and southern (Victoria and South Australia) koala populations in Australia

Reference	KoRV prevalence (%)			
	Queensland	New South Wales	Victoria	South Australia
[5]	n = 98 98/98 (100.0)	–	Ballart (n = 5) 3/5 (60.0) Raymond Island (n = 17) 5/17 (29.4)	Kangaroo Island (n = 26) 0/26 (0.0)
[13]	n = 277 277/277 (100.0)	n = 100 100/100 (100.0)	Mainland Victoria (n = 89) 65/89 (73.0); Four Victoria Islands (n = 80) 22/80 (26.6)	Kangaroo Island (n = 162) 24/162 (14.8) –
[34]	–	–	n = 648 160/648 (24.7)	–
[8]	n = 36 36/36 (100.0)	–	–	–
[9]	n = 290 290/290 (100.0)	–	–	–
[33]	–	–	–	Kangaroo Island (n = 170) 72/170 (42.4) Mount Lofty Ranges (n = 75) 49/75 (65.3)
[35]	n = 33 33/33 (100.0)	–	–	n = 28 27/28 (96.4)
[25]	n = 71 71/71 (100.0)	–	–	n = 97 96/97 (99.0)

clear. However, the three koalas targeted at one of these zoos (Hirakawa Zoological Park) had been born at a theme park in Queensland; hence, the findings that all three animals were KoRV-A positive while only two of three animals were KoRV-B positive are unsurprising [24]. In a recent study on coinfection in Japanese zoo-dwelling koalas, we found two animals to be positive for KoRV-A, KoRV-B, and KoRV-C [16]. Furthermore, in matching findings reported by us and other researchers, infectious virions have been produced from peripheral blood mononuclear cells (PBMCs) isolated from KoRV-positive koalas when co-cultured with HEK293T cells [23, 31]. In addition to human cells, cells from rats, cats, and mink can reportedly be infected with KoRV in vitro, although mouse cells cannot [6, 14, 20, 29]. However, the data from in vivo studies are limited; live rats reportedly can be infected with KoRV, but the pathogenicity remains unclear [6]. The data for zoo populations still falls short of the data available for wild populations, and the prevalence of KoRV subtypes C-I in koalas in Japanese zoo populations is still unclear.

### Transmission mode of KoRV

KoRV shows some resemblance to other retroviruses in terms of transmission. For example, HIV types 1 and 2 are known to jump species and infect new hosts [36, 37].

Similarly, KoRV is a potentially fatal gammaretrovirus that crossed the species barrier into koalas. The species from which the virus jumped into koalas is unknown. One candidate is a native Australian rodent, the grassland melomys (*Melomys burtoni*) [38, 39], and bats have also been suggested [40]. The argument for bats has been strengthened by the recently reported presence of KoRV-related gammaretroviruses in Australian and Asian bats, which suggests that bats may be an important reservoir of exogenous KoRV-related gammaretroviruses [40].

The endogenous form (KoRV-A) has infected germ cells and become a permanent resident of the koala genome. KoRV-A sequences are usually highly conserved among various isolates and lack the genetic diversity of an exogenous retrovirus [41]. However, researchers have observed that KoRV integrates into the koala genome at many locations, mostly unique to each individual koala, which suggests that integration is still recent and not uniform and fixed in the koala population [11, 21, 42, 43]. Transmission is understood to be vertical (dam-to-offspring) for the endogenous form [5, 9], but the mechanisms of horizontal transmission are not fully clear. Entry receptors have been reported for KoRV-A and KoRV-B [7, 20], but not for the other subtypes (KoRV-C to KoRV-I).

Different transmission rates for KoRV-B—an exogenous subtype—have been reported. An annual transmission rate

**Table 3** Variation of KoRV subtype prevalence in northern and southern koala populations in Australia

Reference	KoRV subtype prevalence (%)		
	Queensland	Victoria	South Australia
[34]	–	n=166 KoRV-A 141/166 (88.8)	–
[8]	n=36 KoRV-A 36/36 (100.0) KoRV-B 9/36 (25.0)	–	–
[9]	n=290 KoRV-A 290/290 (100.0) KoRV-B 70/290 (24.1)	–	–
[33]	–	–	Kangaroo Island n=170 KoRV-A 72/170 (42.4) KoRV-B 0/170 (0.0) Mount Lofty Ranges n=75 KoRV-A 49/75 (65.3) KoRV-B 0/75 (0.0)
[35]	n=33 KoRV-A 33/33 (100.0) KoRV-B 16/33 (48.5)	–	n=28 KoRV-A 27/28 (96.4) KoRV-B 0/28 (0.0)

of 3% has been reported for adult-to-adult koala contact [9]. We previously reported on a case of sire-to-offspring transmission, differing from other reports where the transmission was from dam to offspring [7, 9]. Interestingly, we recently found a KoRV-B-negative offspring born of KoRV-B-positive parents [16]. Taken together, these results support the existence of a horizontal transmission mode for KoRV-B; however, little is known about the transmission modes for other subtypes, which require further investigation in order to achieve a complete understanding of how KoRV is transmitted. The current state of knowledge on KoRV transmission modes for different subtypes is summarized in Table 1.

### Pathogenesis of KoRV

Retroviruses are known to cause a range of diseases in their hosts, including neoplasia and central nervous system and immunosuppressive disorders [44–46]. Such causative associations have been postulated for KoRV, but the exact causal relationships between KoRV and a range of clinical diseases have yet to be established. Although direct associations

of KoRV and immunosuppression and neoplasia are not well documented, it has been shown that the KoRV viral load positively correlates with leukemia, lymphoma, and chlamydial disease in koalas [4, 47, 48], and the association with chlamydial disease in particular is regarded as a significant long-term threat to the survival of the species [4, 7–9].

KoRV-B was recently shown to be more pathogenic than KoRV-A and to have a significant association with chlamydial infection, although the exact molecular mechanism is not clear [8]. KoRV-B is also associated with neoplasia in koalas [7]. KoRV-B is an exogenous form of the virus, and exogenous KoRV infection has attracted recent attention for its role in periodontal disease, with speculation that exogenous KoRV may modulate the koala's immune response when they contract bacterial infections within the oral cavity [49].

KoRV-associated neoplasia may cause up to 60% of koala mortalities in captivity and at least 5% in the wild [2]. A high level of viremia is correlated with an increased risk of neoplasia and immunosuppression in koalas [47]. Lymphoma was recently reported for the first time in a South Australian KoRV-A-positive female koala, which was also affected by severe reproductive chlamydiosis [50].

As outlined above, koala populations in northern and southern Australia show differences in KoRV subtype prevalences and KoRV viral loads. This presents an interesting opportunity to investigate geographical variations in pathogenesis according to the characterization of viral disease in different populations; however, research on the full implications for lymphoma and chlamydial disease are still ongoing. Viral load was found to correlate positively with chlamydial disease severity in a southern population in a recent investigation looking for such northern-versus-southern patterns in viral load and disease pathology within Australia [48]. The same study also revealed that proviral and viral loads were positively correlated with lymphocyte and metarubricyte counts, and that viral load was positively correlated with splenic lymphoid area. These findings are suggestive of KoRV-induced oncogenesis [48].

Recent findings have enhanced our understanding of KoRV-induced pathogenesis in koalas, but much remains to be elucidated on the role of KoRV in neoplasms such as lymphoma and chlamydiosis. Extensive further investigations are required.

### Immune response against KoRV infection in koalas

Without a full understanding of how a host responds to a particular pathogen, prophylactic and therapeutic interventions cannot be developed for any disease, and immunological studies are essential to achieve such an understanding [51, 52]. The current situation with KoRV provides a good illustration of this point. Much remains unknown about how

koalas respond to KoRV, and studies of the immunomodulatory effects of KoRV in koalas are limited in number. This presents a challenge for those seeking to conserve koala populations. However, there have been promising developments in this field of research, as outlined below.

Whole-genome sequencing has recently been achieved for koalas, and this is expected to greatly facilitate studies of immune responses in this species [22]. Fiebig et al. [6] observed increases in expression of interleukin-6 (IL-6) and IL-10 in human PBMCs incubated with KoRV, and these increases are suggestive of inflammation and immunosuppression, respectively, as shown in Fig. 2. The KoRV transmembrane envelope protein p15E has been found to contain an immunosuppressive domain that is highly conserved in different retroviruses [53]. The sequence of the KoRV p15E protein is highly conserved among different KoRV subtype proviruses, including those reported to be exogenous [54].

In a study with 16 koalas, Fiebig et al. observed that koalas did not produce antibodies against KoRV-A [55], but in a recent study using 235 koalas, Quigley et al. analyzed the antibody response and found that most koalas produced anti-KoRV antibodies [56]. This finding is very important for developing a therapeutic KoRV vaccine. In a previous study, we detected a high copy number of KoRV RNA (102,473 copies/mL) in the plasma of one koala, which then became negative when tested again several months later [23]. This might have been due to the induction of immune response [56].

Full-length sequences have been reported for important immune response genes in koalas, and diverse major histocompatibility complex (MHC) genes with 34 SNPs have been identified in a study using transcriptome analysis [57]. CD4 and CD8 $\beta$  expression were found to be down-regulated when expression profiles of several immune genes were investigated in mitogen-stimulated lymphocytes isolated from KoRV-A-positive koalas (Fig. 2) [58]. The CD4:CD8 gene expression ratio is a potential biomarker for

immunological evaluation [59, 60]. In a recent study, Maher et al. characterized the immune response to KoRV infection and found immunophenotypic changes in free-ranging Victorian koalas with significantly lower levels of IL17A and IFN- $\gamma$  gene expression accompanying a significantly decreased ratio of CD4:CD8 gene expression relative to that seen in KoRV-negative koalas (Fig. 2) [10]. Decreased levels of IL17A and IFN- $\gamma$  may render koalas more susceptible to bacterial and chlamydial infections, respectively [10]. Epitopes on the KoRV envelope protein that can be recognized by antibodies have been identified recently, showing that anti-KoRV IgG can be induced and decrease the viral load of KoRV [61].

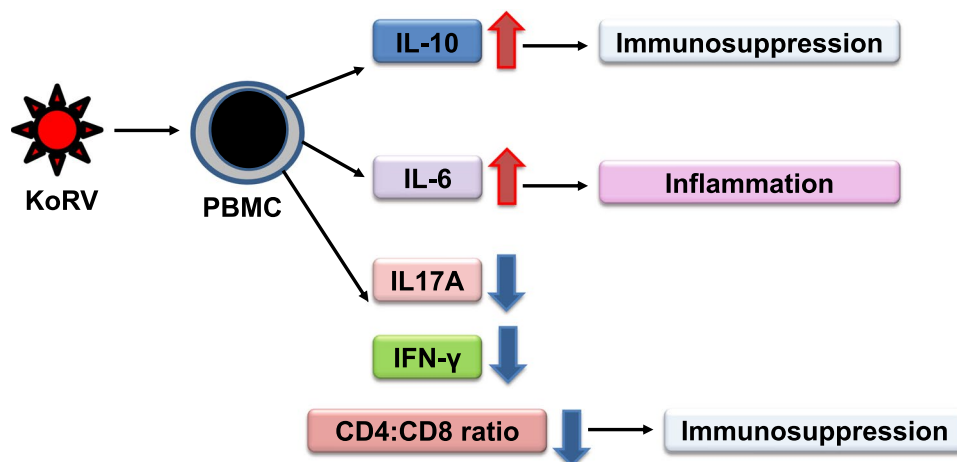
Thus, recent advances have been made in our understanding of the immune response to KoRV infection in koalas. These advances will play a crucial role on the road to developing an effective vaccine that can protect koalas.

## Vaccines

Currently, no licensed vaccine or effective treatment is available for KoRV or its associated neoplastic diseases. The majority of koalas are infected with KoRV, and therapeutic vaccines urgently need to be developed as we seek to curb the fatalities in koalas. A range of studies have demonstrated that therapeutic vaccines can enhance immunogenicity, reducing the viral or bacterial load in both human and animal infections, and that they can reduce pathogen-associated morbidity and mortality [62–66].

Effective prophylactic vaccines against the exogenous subtypes are also urgently needed. Virus-neutralizing antibody responses and T cell responses are both important for vaccines that can effectively protect against retroviral infection [67]. The recent failure of an experimental AIDS vaccine in a phase IIb study highlights the need for a balanced immune response consisting of not only cellular immunity but also a broad and potent antibody

**Fig. 2** A schematic representation of the immune response to KoRV infection. Entry of KoRV into PBMCs induces the upregulation of IL-10 and IL-6, which may induce immunosuppression and inflammation. KoRV infection can also decrease IL-17A, IFN- $\gamma$  and the CD4:CD8 ratio, which can also induce immunosuppression



response, which is essential for preventing retrovirus infection [68]. Inducible neutralizing antibodies show great promise for preventing retrovirus infections, and these antibodies have envelope proteins as their main targets [69]. Notably, an effective vaccine against FeLV infection is available, and this vaccine contains an envelope protein as an immunogen [70]. Neutralizing antibodies against KoRV were reportedly raised after immunization with a recombinant transmembrane envelope protein rp15E [6]. Specifically, in that study, neutralizing antibodies were raised in significantly higher numbers after immunization with KoRV gp70 than rp15E. Furthermore, PERV was neutralized with antisera against p15E and gp70 of KoRV and PERV [6]. Therefore, it seems likely that KoRV envelope proteins can be utilized for the development of effective preventive or therapeutic vaccines for protecting koalas from infection. Currently, studies associated with the development of vaccines against KoRV are underway [30, 61, 71]. In one of these studies, a recombinant KoRV envelope protein vaccine candidate induced a significant humoral immune response and production of neutralizing antibodies in both KoRV-infected and KoRV-free koalas [61]. However, the abundant genetic diversity of KoRV encompasses nine KoRV subtypes [18], and this is a potentially critical point to consider for researchers seeking to prevent and control KoRV through vaccination. A number of recent studies have thrown further light on KoRV subtypes and have implications for research on KoRV vaccines. Hobbs et al. found KoRV-D and KoRV-E subtypes to be defective viruses, with significant deletions in the *gag* and *pol* genes [15]. Notably, recKoRV variants have been widely reported [15, 21, 22], and these may have been generated by recombination events due to retrotransposition or exogenous infection with KoRV [15]. Löber et al. demonstrated that recombination with an ancient koala retroelement disables KoRV, and this occurs frequently at an early point in the invasion process [21]. However, events involving recombination between endogenous KoRV and exogenous variants may occur, and researchers cannot rule out the possibility of a new variant with greater virulence emerging as the result of such recombination events.

Vaccine development necessitates the establishment of animal models of infection, but none have been established so far. To solve this problem, we are investigating tupaia (*Tupaia belangeri*) as a model of KoRV. We have successfully infected tupaia cells with KoRV [24]. This research provides evidence that tupaia are a potential new animal model for several viral infections [72]. Although more studies are required, the initial vaccine-induced specific immune responses appear promising and offer hope that koalas can be protected from KoRV infection.

## Conclusions

The current knowledge about KoRV is not complete, and progress has been hampered by, for example, an insufficient understanding of the immune response in koalas due to a lack of analytical tools. However, the recent publication of the whole koala genome sequence may lead to a range of new analytical tools such as antibodies and PCR assays, and the prospects of achieving a better understanding of KoRV pathogenesis and the immune response in koalas are good. Such understanding will be crucial for the development of more-effective therapeutic and prophylactic strategies. Additional research is needed to throw light on areas such as KoRV genetic diversity, the evolution of endogenous and exogenous subtypes, and the associations of subtypes with diseases that threaten long-term koala survival. As such research progresses on a range of fronts, we can anticipate remedying the current situation where no treatment or vaccine is available for KoRV infection. The development of urgently needed prophylactic and therapeutic strategies against KoRV will clearly benefit koala conservation, but the knowledge gained could be applicable for scientists seeking to combat similar retroviral disease across a wider range of species.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing financial interests.

**Ethical approval** This study was done in accordance with institutional committee protocols.

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