Accepted Manuscript

Marsupial and monotreme serum immunoglobulin binding by proteins A, G and L and anti-kangaroo antibody

Paola K. Vaz, Carol A. Hartley, Glenn F. Browning, Joanne M. Devlin

PII: S0022-1759(15)30057-0
DOI: doi: 10.1016/j.jim.2015.10.007
Reference: JIM 12099

To appear in: *Journal of Immunological Methods*

Received date: 21 August 2015
Revised date: 28 October 2015
Accepted date: 28 October 2015

Please cite this article as: Vaz, Paola K., Hartley, Carol A., Browning, Glenn F., Devlin, Joanne M., Marsupial and monotreme serum immunoglobulin binding by proteins A, G and L and anti-kangaroo antibody, *Journal of Immunological Methods* (2015), doi: 10.1016/j.jim.2015.10.007

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Marsupial and monotreme serum immunoglobulin binding by proteins A, G and L and anti-kangaroo antibody.

Paola K. Vaz[1*], Carol A. Hartley[1], Glenn F. Browning[1] and Joanne M. Devlin[1].

[1]Asia-Pacific Centre for Animal Health, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3010, Australia.

[*]Author for correspondence: Paola K. Vaz, ph: +61 3 83447368, email: pvaz@unimelb.edu.au

Carol Hartley, email: carolah@unimelb.edu.au

Glenn Browning, email: glenfb@unimelb.edu.au

Joanne Devlin, email: devlinj@unimelb.edu.au

[1]

ACCEPTED MANUSCRIPT

[1] **Abbreviations:** IgL = immunoglobulin light chains, IgH = immunoglobulin heavy chains, EGK = eastern grey kangaroo, WGK = western grey kangaroo, BTRW = brush-tailed rock wallaby, YFRW = yellow-footed rock wallaby, TW = tammar wallaby, SW = swamp wallaby, Red.K = red kangaroo, T. Dev = Tasmanian devil, LFP = long-footed kangaroo, NBTP = northern brush-tailed possum.

ACCEPTED MANUSCRIPT

Abstract

Serological studies are often conducted to examine exposure to infectious agents in wildlife populations. However, specific immunological reagents for wildlife species are seldom available and can limit the study of infectious diseases in these animals. This study examined the ability of four commercially available immunoglobulin-binding reagents to bind serum immunoglobulins from 17 species within the *Marsupialia* and *Monotremata.* Serum samples were assessed for binding, using immunoblots and ELISAs (Enzyme-linked immunosorbent assays), to three microbially-derived proteins - staphylococcal protein A, streptococcal protein G and peptostreptococcal protein L. Additionally, an anti-kangaroo antibody was included for comparison. The inter- and intra-familial binding patterns of the reagents to serum immunoglobulins varied and evolutionary distance between animal species was not an accurate predictor of the ability of reagents to bind immunoglobulins. Results from this study can be used to inform the selection of appropriate immunological reagents in future serological studies in these clades.

Keywords: Wildlife, marsupial, monotreme, immunoglobulin, ELISA, diagnostics

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1.0 Introduction

Early comparative studies on mammalian immunoglobulins have suggested that marsupials are evolutionarily closer to eutherian mammals than they are to monotremes (De Château et al., 1993; Erntell et al., 1986; Kirsch, 1968; Kronvall et al., 1970). This is consistent with evolutionary studies based on morphological data (e.g. fossil records, as reviewed in Clemens, 1970) and, more recently, on molecular phylogenetic studies (Bininda-Emonds et al., 2007; van Rheede et al., 2006). Monotremes are considered to have diverged from therian (marsupial and placental) mammals in the late Jurassic to early Cretaceous periods, at a time when mammalian immunoglobulin (Ig) classes and subclasses may not have fully emerged (Bininda-Emonds et al., 2007; Clemens, 1970). All vertebrate species examined to date, including members of the *Monotremata* and *Marsupialia,* possess molecules resembling the immunoglobulins from at least four of the five major immunoglobulin classes; IgM, IgG, IgA and IgE, with further subclasses detected in some species (Bell et al., 1974a; Bell et al., 1974b; Belov and Hellman, 2003; Marchalonis, 1969, 1971; Marchalonis and Edelman, 1968; Wang et al., 2009; Zhao et al., 2009). To date, IgD has not been described in any marsupial species examined, although a putative IgD homologue has been identified in the duck-billed platypus (*Ornithorhynchus anatinus*) genome. This putative IgD molecule appears to share greater structural homology with IgD from amphibians, reptiles and fish than IgD from eutherian mammals (Belov and Hellman, 2003; Zhao et al., 2009). Additionally, the platypus genome encodes a novel Ig isotype (IgO) that appears to be a structural intermediate between mammalian IgG and IgY from birds and reptiles (Zhao et al., 2009). All marsupials and monotreme species examined to date possess both κ and λ light chain (IgL) homologues, and in the grey short-tailed South American opossum (*Monodelphis domestica*) and common brush-tailed possum (*Trichosurus vulpecula*) the genomic organisation of the heavy (IgH) and IgL loci is very similar to that of eutherian mammals (Baker et al., 2005; Belov et al., 1999; Wang et al., 2009).

Microbially-derived proteins A, G and L have immunoglobulin-binding properties, although their binding affinities vary between animal species (De Château et al., 1993; Erntell et al., 1986; Kronvall et al., 1970). Protein A and G bind primarily to the Fc region of IgG (Akerström and Björck, 1986; Björck and Kronvall, 1984), while protein L binds to some κ isotype subgroups of IgL and as a result has a broader immunoglobulin binding range (Akerström and Björck, 1989; De Château et al., 1993; Nilson et al., 1992). Early immunoprecipitation and precipitation inhibition studies measuring the immunoglobulin binding affinities of these proteins were performed on sera from a range of mammalian species. Sera from representative members of the class *Mammalia*, including monotremes, showed some reactivity with staphylococcal protein A, with the exception of the common opossum (*Didelphis marsupialis*), which showed no reactivity (neither

ACCEPTED MANUSCRIPT

precipitation nor inhibition). Only one other marsupial species (the red kangaroo, *Macropus rufus*) was included in this study, and sera from the red kangaroo inhibited precipitation. Geographical and, subsequently, evolutionary distance were proposed as factors that could account for the differences in reactivity of these two animal species, but only one marsupial species from each continent was included in the study (Kronvall et al., 1970). Similar studies have examined reactivity with protein L (including sera from the common opossum and the red kangaroo) and protein G (using sera from unknown marsupial species) and identified some reactivity with both proteins. No monotreme species were included in these studies (De Château et al., 1993; Erntell et al., 1986). Occasional species-specific studies conducted in other marsupial species have reported immunoglobulin-binding, such as binding of proteins A and G to immunoglobulin in koala sera (Wilkinson et al., 1991).

Studies in monotreme and marsupial species are frequently undertaken to examine exposure to infectious agents in individual animals in zoos and wild animal populations. These studies often incorporate serological techniques such as enzyme-linked immunosorbent assays (ELISAs) or neutralisation assays, with varying degrees of success (Banazis et al., 2010; Portas et al., 2014; Stalder et al., 2015). While common limitations in immunological assay development can include factors such as the selection of appropriate antigen targets, wildlife research can be further hindered by a lack of commercially available serological reagents. As a result, several published studies have utilised institutionally prepared reagents (Brown et al., 2011; Wilkinson et al., 1991), presenting a dual problem of limited availability of reagents and the potential for lack of consistency between reagents prepared for different studies. One commercially available reagent that has been successfully applied in multiple macropodid sero-prevalence studies is a polyclonal anti-kangaroo whole serum antibody (Bethyl Laboratories Inc.) (Banazis et al., 2010; Bennett et al., 2011; Potter et al., 2011). However, there is limited published data available describing the ability of this reagent to detect antibodies present in sera from other (non-macropodid) marsupial species.

This study aimed to examine the capacity of four commercially available immunoglobulin-binding reagents to bind serum immunoglobulins from a range of members of the *Marsupialia* and *Monotremata.* Serum samples were tested for binding to three recombinant microbially-derived Ig-binding proteins - staphylococcal protein A, streptococcal protein G and peptostreptococcal protein L. Additionally, the commercially available polyclonal anti-kangaroo whole serum antibody was included for comparison.

2.0 Materials and methods

ACCEPTED MANUSCRIPT

2.1 Animal sera and immunoglobulin-binding reagents

The serum samples used in this study were available in our laboratory archives or in those of Melbourne Zoo. Serum samples from 17 species of Australian marsupials and monotremes were used to test immunoglobulin-binding by polyclonal rabbit anti-kangaroo whole serum antibody (Bethyl Laboratories Inc., #BETHA140-105) and three recombinant horseradish peroxidase (HRP) conjugated Ig-binding proteins - protein A (Life Technologies, #10-1123), protein G (Life Technologies, #101223) and protein L (Southern Biotech, # 7500-05). Sera from distantly related eutherian mammals and avian species were included as control samples. Where possible, serum samples from multiple individual animals from each species were pooled for testing. The details of the animal sera used in this study are shown in Table 1.

2.2 Immunoblots

Dot blots were performed in order to initially test and compare the relative binding of the four reagents to immunoglobulin present in serum samples from all animal species listed in Table 1. For dot blotting polyvinylidene fluoride (PVDF, Immobilon-P membrane, Merck Millipore) membranes were treated and processed according to the manufacturer's instructions. Briefly, 4 µl of each member of a dilution series of each sera (diluted 1:50, 1:100, 1:200 and 1:400 in phosphate buffered saline, PBS) was applied to the membrane. Membranes were immersed in methanol for 15 secs and then allowed to air-dry on blotting paper for 15 mins. The membranes were then incubated for 60 mins with polyclonal rabbit anti-kangaroo antibody, or HRP-conjugated proteins A, G or L, all at a 1:4000 dilution in PBS containing 0.05% Tween 20 (PBS-T) and 5% w/v skim milk powder, without a prior blocking incubation. For assays using the anti-kangaroo antibody, membranes were then incubated in polyclonal swine anti-rabbit Ig-HRP (Dako, # P021702-2) at a 1:2000 dilution in PBS-T for 60 mins. Membranes were washed with PBS containing 0.05% Tween 20 for 20 mins between incubations and prior to visualisation. Binding was visualised using the Clarity Western ECL Blotting Substrate (BioRad).

To confirm that the immunoglobulin-binding reagents were binding to proteins of the expected size of Ig molecules, proteins in 10 µl of undiluted sera from two representative species of Australian marsupials (quokka and wombat) and a positive control serum sample (human) were separated on a 8.75% w/v acrylamide gel as previously described (Baker et al., 2014), modified to reducing and non-denaturing conditions. The proteins were transferred to a PVDF membrane, blocked in PBS containing 0.05% Tween 20 (PBS-T) and 5% w/v skim milk powder for 60 mins at 37°C, and the membrane then incubated with the immunoglobulin-binding reagents, and binding detected, as described above.

ACCEPTED MANUSCRIPT

2.3 ELISAs

ELISAs were performed to further investigate the binding sensitivities of the four reagents to immunoglobulin in serum samples from selected animal species. Fifty microlitre volumes of half log_{10} serial dilutions of sera (1:50 to 1:15,811 or to 1:158,111 in 0.032 M Na_2CO_3, 0.068 M $NaHCO_3$, pH 9.6) were added to the wells of 96-well plates (Nunc MaxiSorp, Sigma-Aldrich) and the plates then incubated overnight at 4°C. The plates were then washed 3 times with PBS-T. Each well in the plate was blocked with 50 µl PBS-T (pH 7.4) containing 5% w/v skim milk powder and 10% w/v BSA fraction V (Sigma-Aldrich) for 2 hours at 37°C. The plates were then washed twice with PBS-T. Polyclonal rabbit anti-kangaroo antibody or HRP-conjugated protein A, G or L at a dilution of 1:4000 in ELISA diluent (PBS-T containing 2.5% w/v BSA fraction V and 2.5% w/v skim milk powder) was added to each well and the plates incubated for 60 mins at room temperature. Swine anti-rabbit HRP was used as a secondary antibody at a 1:2000 dilution in ELISA diluent in the assay using anti-kangaroo antibody. The plates containing this secondary antibody were incubated for 40 mins at room temperature. The plates were then washed 3 times with PBS-T. ABTS peroxidase (KPL) substrate was added to each well and the plates were read after 10 mins at room temperature. The absorbance of each well was read at 405 nM using a microplate reader (Labsystem Multiskan MS). Linear regression analysis of absorbance values was used to determine endpoint dilutions of the sera for each species for each Ig-binding reagent. The absorbance selected as the endpoint threshold was 0.2.

3.0 Results

3.1 Immunoblots

All marsupial serum samples tested reacted strongly with the anti-kangaroo antibody at all dilutions tested (Figure 1). Binding to monotreme sera, and to each of the control sera (eutherian and avian), was either weak or undetectable at the dilutions tested.

Binding to protein G was poor across the marsupial species tested, with only weak binding detected to sera from a small number of species at the lowest dilution of sera tested (long footed potoroo, Tasmanian devil and koala; 1:50 dilution). Similarly weak binding was detected to rat sera. No binding was detected to platypus sera but moderate binding was detected to the short-beaked echidna serum, at levels comparable to that seen with canine sera. Strong binding was detected to human and horse sera at all dilutions tested. No other binding to protein G was detected.

Binding to protein A was generally strong across the animal species tested, although weaker reactivity was seen with eastern bettong and rat sera. Importantly, the monotreme sera tested were

ACCEPTED MANUSCRIPT

both bound strongly. No binding was detected to koala or chicken sera at any of the dilutions tested.

Strong binding to protein L was detected across the marsupial species tested, as well as with human sera. Binding to sera from the monotreme species was either undetectable or weak, at levels comparable to those seen with rat, horse and canine sera. Binding to chicken sera was not detectable.

Western blots were performed on sera from three representative species using four immunoglobulin-binding reagents (Figure S1). Proteins G, A and L were all found to react with large proteins (approx. 100 – 170 kDa) in the wombat, quokka and human sera, consistent with the expected size of intact mammalian immunoglobulin. The anti-kangaroo antibody was found to react with large proteins (approx. 100 – 170 kDa) in the wombat and quokka sera, but also to smaller serum proteins (approx. 72 kDa).

3.2 ELISAs

Binding to half-log serial dilutions of pooled sera from selected marsupial and monotreme species was measured for each of the immunoglobulin-binding reagents using ELISAs. The highest dilution of sera yielding an endpoint absorbance value of 0.2 at 405 nM was determined for each species. These results are shown in Table 1. Graphs showing absorbance values for each dilution of sera are shown in the supplementary material (Figure S2).

The anti-kangaroo antibody bound to sera from all the marsupial species tested. Within the *Macropodidae* there was little variation between members, with endpoint values ranging from 103,500 (swamp wallaby) to 143,800 (eastern grey kangaroo). Similar endpoint values were observed within the *Potoroidae* and in other marsupial species, although a lower endpoint value was observed with koala sera (endpoint dilution = 1:69,596). Neither monotreme sera nor other control sera bound this antibody at detectable levels.

Protein G bound to sera from only two marsupial species (Tasmanian devil and koala, endpoint dilutions = 1:916 and 1:50, respectively) and one monotreme species (short-beaked echidna, endpoint dilution = 1:9,664).

More variation was observed between species using protein A. Sera from both monotreme species bound protein A strongly (endpoint dilution = 1:15,500 for platypus and 1:23,178 for short-beaked echidna) and binding was also detected to sera from the majority of the marsupial species tested (endpoint dilutions = 1:7,300 – 1:16,400). The three exceptions were sera from the two members of the *Bettongia* genus (eastern bettong and woylie) and koalas, to which no binding was detected.

ACCEPTED MANUSCRIPT

Protein L bound to sera from all the marsupial species tested (endpoint dilution = 1:680 – 1:15,800), with the lowest endpoint value observed with sera from the yellow-footed rock wallaby. Binding was not detected to sera from either monotreme species.

4.0 Discussion

Sera from fifteen marsupial and two monotreme species were assessed for binding to four commercially available immunoglobulin-binding reagents using immunoblot and ELISA formats. Variation was observed within and between families, as well as between assay methods.

For marsupial species, the results indicated that the anti-kangaroo antibody, protein L or protein A would be suitable for most serological assays, although protein A and L may be preferred for immunoblotting as they can yield cleaner results on blots (Lal et al., 2005). The anti-kangaroo polyclonal antibody was raised against kangaroo whole sera and so most probably binds to Ig fragments and to multiple Ig classes, as well as to smaller serum proteins. This may have contributed to the broad-range of marsupial species to which binding was seen using this reagent. While this property may enable its use in the development of a pan-marsupial diagnostic serological tool, the ELISA and dot blot techniques used in this study did not discriminate between binding to immunoglobulin and to other protein components of sera. In most diagnostic serological techniques antigen is used to capture antigen-specific antibody prior to application of immunoglobulin-binding reagents. It is possible that the anti-kangaroo whole serum antibody may be less broadly reactive when other protein components in the sera are no longer present. This would need to be assessed in future studies. Protein G could detect proteins of the expected molecular weight of immunoglobulin in undiluted wombat and quokka sera in Western blots, but not in dot blots or ELISAs at the dilutions tested. Protein G also reacted weakly with Tasmanian devil sera in ELISAs, but in general protein G does not appear to be a good reagent for detection of Ig in marsupial sera, and in all cases better alternative reagents were identified.

Sera from two of the five extant monotreme species were tested in this study. The anti-kangaroo antibody did not detect sera from either species, but sera from both species consistently reacted strongly with protein A, indicating that protein A would be the preferred reagent for detection of immunoglobulin in these species. Interestingly, the short-beaked echidna serum was bound moderately by protein G in both assay platforms, while platypus serum was not. Neither the echidna nor platypus sera were bound by protein L in ELISAs, although some weak binding was detected to echidna serum in dot blots. As protein L only binds specific κ chain subgroups, the poor or undetectable binding of protein L to monotreme sera observed in this study is consistent with the reported predominance of expression of λ isotype IgLs in immunoglobulins (90%) in monotreme serum (Johansson et al., 2005). Additionally, there is a relatively limited repertoire of

ACCEPTED MANUSCRIPT

κ chain subgroups expressed in both duck-billed platypus (from four genomically encoded κ subgroups) and short-beaked echidna (from nine genomically encoded κ subgroups) (Nowak et al., 2004).

Sera from several species showed some reactivity with the immunoglobulin-binding reagents in dot blots, but did not have detectable levels of reactivity with the same reagents in ELISAs. This included eastern bettong and woylie serum reactivity with protein A, and short-beaked echidna serum reactivity with protein L. This does not necessarily exclude the use of these reagents in serological assays in these species, but does indicate their limited potential in some test formats. Our study did not detect significant binding of koala serum by protein A in dot blot or ELISAs, even though a previously published study used protein A to purify immunoglobulin from koala sera, although with less efficiency than protein G (Wilkinson et al., 1991). The apparent contrast may reflect the higher dilutions of serum used in our study (the serum was undiluted in the immunoglobulin purification study) and it is possible that binding may be detectable using protein A in undiluted koala sera in Western blots, as was seen with wombat sera in this study. However, the lack of detectable reactivity at higher dilutions suggests that protein A is unlikely to be a useful reagent for most serological diagnostic assays in koalas. Instead, protein L or anti-kangaroo antibody appears preferable. Future work to examine the immunoglobulin-binding capacity of non-commercial (institutionally prepared) marsupial immunoglobulin-binding reagents (Wilkinson et al., 1991) using serum from the marsupial and monotreme species included in this study is also indicated.

The confamilial variation observed in binding of sera to the different reagents could be explained by variation in the serum antibody composition in different animal species (Bell, 1977). Alternatively, the presence of inhibitors may also affect binding to the different reagents. Some reduction in binding was seen at lower sera dilutions in ELISAs (Figure S2), which may reflect the presence of such inhibitors. Several molecular immunogenetic studies on a small number of marsupial and monotreme species has identified novel immunoglobulin isotypes (IgO and a CH-rich IgD) (Zhao et al., 2009), T cell receptor (TCR) chains and chimeric Ig/TCRs (Miller, 2010; Parra et al., 2007; Parra et al., 2012) and it is not clear how these (or other as yet undiscovered immune proteins) may influence assays utilizing broad-spectrum immunoglobulin-binding reagents.

This study examined and compared binding of sera from a range of members of the *Marsupialia* and *Monotremata* to four commercially available immunoglobulin-binding reagents, using three conventional serological platforms. There was some variation in the patterns of binding between and within families, indicating that evolutionary distance is not an accurate predictor for selection

ACCEPTED MANUSCRIPT

of serological reagents in these subclasses of mammals. Further research to better characterise bound immunoglobulin may help define the role of novel immunoglobulin and receptors in monotreme and marsupial immunology and, by extension, serology. We anticipate that this study will aid wildlife researchers in the development of serological assays and diagnostic tests for detection of infections in marsupials and monotremes.

5.0 Acknowledgments

We thank Zoos Victoria, Michael Lynch, Pam Whitely, Kathryn Stalder and Jemima Amery-Gale for providing sera used in this study. Paola Vaz and Joanne Devlin are supported by the Australian Research Council.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

References

Akerström, B., Björck, L., 1986. A physicochemical study of protein G, a molecule with unique immunoglobulin G-binding properties. J Biological Chemistry 261, 10240-10247.

Akerström, B., Björck, L., 1989. Protein L: an immunoglobulin light chain-binding bacterial protein. Characterization of binding and physicochemical properties. J Biological Chemistry 264, 19740-19746.

Baker, L., Chitas, A.M., Hartley, C.A., Coppo, M.J., Vaz, P.K., Stent, A., Gilkerson, J.R., Devlin, J.M., Every, A.L., 2014. Recombinant herpesvirus glycoprotein G improves the protective immune response to *Helicobacter pylori* vaccination in a mouse model of disease. PLoS ONE 9, e96563.

Baker, M.L., Belov, K., Miller, R.D., 2005. Unusually similar patterns of antibody V segment diversity in distantly related marsupials. J Immunol 174, 5665-5671.

Banazis, M.J., Bestall, A.S., Reid, S.A., Fenwick, S.G., 2010. A survey of Western Australian sheep, cattle and kangaroos to determine the prevalence of *Coxiella burnetii*. Vet Microbiol 143, 337-345.

Bell, R.G., 1977. Marsupial immunoglobulins: the distribution and evolution of macropod IgG2, IgG1, IgM and light chain antigenic markers within the sub-class *Metatheria*. Immunol 33, 917-924.

Bell, R.G., Lynch, N.R., Turner, K.J., 1974a. Immunoglobulins of the marsupial *Setonix brachyurus* (Quokka). Characterization of three major serum classes, IgG2, IgG1 and IgM. Immunol 27, 1103-1115.

Bell, R.G., Stephens, C.J., Turner, K.J., 1974b. Marsupial immunoglobulins: an immunoglobulin molecule resembling eutherian IgA in serum and secretions of *Setonix brachyurus* (quokka). J Immunol 113, 371-378.

Belov, K., Harrison, G.A., Rosenberg, G.H., Miller, R.D., Cooper, D.W., 1999. Isolation and comparison of the IgM heavy chain constant regions from Australian (T*richosurus vulpecula*) and American (*Monodelphis domestica*) marsupials. Dev Comp Immunol 23, 649-656.

Belov, K., Hellman, L., 2003. Immunoglobulin genetics of *Ornithorhynchus anatinus* (platypus) and *Tachyglossus aculeatus* (short-beaked echidna). Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 136, 811-819.

Bennett, M.D., Woolford, L., Banazis, M.J., O'Hara, A.J., Warren, K.S., Nicholls, P.K., Sims, C., Fenwick, S.G., 2011. *Coxiella burnetii* in western barred bandicoots (*Perameles bougainville*) from Bernier and Dorre Islands in Western Australia. EcoHealth 8, 519-524.

ACCEPTED MANUSCRIPT

Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., MacPhee, R.D.E., Beck, R.M.D., Grenyer, R., Price, S.A., Vos, R.A., Gittleman, J.L., Purvis, A., 2007. The delayed rise of present-day mammals. Nature 446, 507-512.

Björck, L., Kronvall, G., 1984. Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. J Immunol 133, 969-974.

Brown, G.K., Kreiss, A., Lyons, A.B., Woods, G.M., 2011. Natural killer cell mediated cytotoxic responses in the Tasmanian devil. PLoS ONE 6, e24475.

Clemens, W.A., 1970. Mesozoic mammalian evolution. Annual Review of Ecology & Systematics 1, 357-390.

De Château, M., Nilson, B.H.K., Erntell, M., Myhre, E., Magnusson, C.G.M., Åkerström, B., Björck, L., 1993. On the interaction between protein L and immunoglobulins of various mammalian species. Scand J Immunol 37, 399-405.

Erntell, M., Myhre, E.B., Kronvall, G., 1986. Non-immune F(ab')2- and Fc-mediated interactions of mammalian immunoglobulins with *S. aureus* and group C and G Streptococci. Acta Pathologica Microbiologica Scandinavica Series B: Microbiology 94B, 377-385.

Johansson, J., Salazar, J.N., Aveskogh, M., Munday, B., Miller, R.D., Hellman, L., 2005. High variability in complementarity-determining regions compensates for a low number of V gene families in the λ light chain locus of the platypus. Eur J Immunol 35, 3008-3019.

Kirsch, J.A., 1968. Prodromus of the comparative serology of *Marsupialia*. Nature 217, 418-420.

Kronvall, G., Seal, U.S., Finstad, J., Williams, R.C., Jr., 1970. Phylogenetic insight into evolution of mammalian Fc fragment of gamma G globulin using staphylococcal protein A. J Immunol 104, 140-147.

Lal, A., Haynes, S.R., Gorospe, M., 2005. Clean western blot signals from immunoprecipitated samples. Mol Cell Probes 19, 385-388.

Marchalonis, J.J., 1969. Isolation and characterization of immunoglobulin-like proteins of the Australian lungfish (*Neoceratodus forsteri*). Aust J Exp Biol Med Sci 47, 405-419.

Marchalonis, J.J., 1971. Isolation and partial characterization of immunoglobulins of goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*). Immunol 20, 161-173.

Marchalonis, J.J., Edelman, G.M., 1968. Phylogenetic origins of antibody structure. 3. Antibodies in the primary immune response of the sea lamprey, *Petromyzon marinus*. J Exp Med 127, 891-914.

Miller, R.D., 2010. Those other mammals: the immunoglobulins and T cell receptors of marsupials and monotremes. Seminars in immunology 22, 3-9.

ACCEPTED MANUSCRIPT

Nilson, B.H., Solomon, A., Björck, L., Akerström, B., 1992. Protein L from *Peptostreptococcus magnus* binds to the kappa light chain variable domain. J Biological Chemistry 267, 2234-2239.

Nowak, M., Parra, Z., Hellman, L., Miller, R., 2004. The complexity of expressed kappa light chains in egg-laying mammals. Immunogenetics 56, 555-563.

Parra, Z.E., Baker, M.L., Schwarz, R.S., Deakin, J.E., Lindblad-Toh, K., Miller, R.D., 2007. A unique T cell receptor discovered in marsupials. Proc Natl Acad Sci U S A 104, 9776-9781.

Parra, Z.E., Lillie, M., Miller, R.D., 2012. A model for the evolution of the mammalian t-cell receptor alpha/delta and mu loci based on evidence from the duckbill platypus. Mol Biol Evol 29, 3205-3214.

Portas, T., Fletcher, D., Spratt, D., Reiss, A., Holz, P., Stalder, K., Devlin, J., Taylor, D., Dobroszczyk, D., Manning, A.D., 2014. Health evaluation of free-ranging eastern bettongs (*Bettongia gaimardi*) during translocation for reintroduction in Australia. J Wildl Dis 50, 210-223.

Potter, A.S., Banazis, M.J., Yang, R., Reid, S.A., Fenwick, S.G., 2011. Prevalence of *Coxiella burnetti* in western grey kangaroos (*Macropus fuliginosus*) in Western Australia. J Wildl Dis 47, 821-828.

Stalder, K., Vaz, P.K., Gilkerson, J.R., Baker, R., Whiteley, P., Ficorilli, N., Tatarczuch, L., Portas, T., Skogvold, K., Anderson, G.A., Devlin, J.M., 2015. Prevalence and clinical significance of herpesvirus infection in populations of Australian marsupials. PLoS ONE 10, e0133807.

van Rheede, T., Bastiaans, T., Boone, D.N., Hedges, S.B., de Jong, W.W., Madsen, O., 2006. The platypus is in its place: nuclear genes and indels confirm the sister group relation of monotremes and therians. Mol Biol Evol 23, 587-597.

Wang, X., Olp, J.J., Miller, R.D., 2009. On the genomics of immunoglobulins in the gray, short-tailed opossum *Monodelphis domestica*. Immunogenetics 61, 581-596.

Wilkinson, R., Allanson, M., Kolega, V., Lawrence, D., Neville, S., 1991. Purification and initial characterisation of koala immunoglobulins. Vet Immunol Immunopathol 29, 189-195.

Zhao, Y., Cui, H., Whittington, C.M., Wei, Z., Zhang, X., Zhang, Z., Yu, L., Ren, L., Hu, X., Zhang, Y., Hellman, L., Belov, K., Li, N., Hammarstrom, L., 2009. *Ornithorhynchus anatinus* (platypus) links the evolution of immunoglobulin genes in eutherian mammals and nonmammalian tetrapods. J Immunol 183, 3285-3293.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Sera used in this study and binding to HRP-conjugated immunoglobulin binding proteins (G, A and L) and to anti-kangaroo whole serum antibody as measured by ELISA.

Family	Scientific name	Common name	n*	Dilution of serum#			
				a-Kanga^	Protein G	Protein A	Protein L
Macropodidae	*Macropus giganteus*	Eastern grey kangaroo	4	143,885	< 50	14,077	14,313
	Macropus fuliginosus	Western grey kangaroo	1	133,611	< 50	7,300	9,821
	Macropus rufus	Red kangaroo	3	133,611	< 50	13,525	11,713
	Petrogale xanthopus	Yellow-footed rock wallaby	4	132,030	< 50	9,506	680
	Petrogale penicillata	Brush-tailed rock wallaby	4	129,660	< 50	10,215	11,003
	Macropus eugenii	Tammar wallaby	4	132,821	< 50	14,239	11,082
	Wallabia bicolor	Swamp wallaby	4	103,580	< 50	9,743	8,954
	Setonix brachyurus	Quokka	4	124,918	< 50	9,270	6,748
Potoroidae	*Potorous longipes*	Long-footed potoroo	3	111,483	< 50	15,022	8,718
	Bettongia gaimardi	Eastern bettong	4	105,950	< 50	< 50	4,541
	Bettongia penicillata	Woylie	4	121,757	< 50	< 50	12,816
Phalangeridae	*Trichosurus arnhemensis*	Northern brush-tailed possum	2	120,966	< 50	16,466	12,264
Dasyuridae	*Sarcophilus harrisii*	Tasmanian devil	4	114,644	916	11,870	13,131
Phascolarctidae	*Phascolarctos cinereus*	Koala	4	69,596	50	< 50	8,166
Vombatidae	*Vombatus ursinus*	Wombat	4	157,320	< 50	14,234	15,811
Ornithorhynchidae	*Ornithorhynchus anatinus*	Duck-billed platypus	4	< 50	< 50	15,574	< 50
Tachyglossidae	*Tachyglossus aculeatus*	Short-beaked echidna	1	< 50	9,664	23,178	< 50
Equidae	*Equus ferus caballus*	Horse	3	na	na	na	na
Canidae	*Canis lupus familiaris*	Dog	3	na	na	na	na
Hominidae	*Homo sapiens*	Human	2	< 50	28,873	23,304	17,379
Muridae	*Rattus norvegicus*	Rat	3	< 50	50	< 50	128
Phasianidae	*Gallus gallus*	Chicken	4	< 50	< 50	< 50	< 50

[#] Highest dilution of serum yielding an ELISA absorbance value of 0.2 at a wavelength of 405 nm. A half $\log_{10}$ dilution series of pooled sera (1:50 to 1:15,811 or to 1:158,111) were prepared for each species.
[*] Number of individual serum samples pooled for testing.
na = species sera not tested by ELISA
[^] a-Kanga = anti-kangaroo whole serum antibody

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure 1. Immunoblots of sera from Australian marsupial and monotreme species. Dot blots of serially diluted pooled sera showing binding to anti-kangaroo antibody and to HRP-conjugated proteins A, L and G. Sera from distantly related mammalian and avian species were also included as controls. EGK = eastern grey kangaroo, WGK = western grey kangaroo, Red. K = red kangaroo, YFRW = yellow-footed rock wallaby, BTRW = brush-tailed rock wallaby, TW = tammar wallaby, SW = swamp wallaby, LFP = long-footed potoroo, NBTP = northern brush-tailed possum, T. Devil = Tasmanian devil.

ACCEPTED MANUSCRIPT

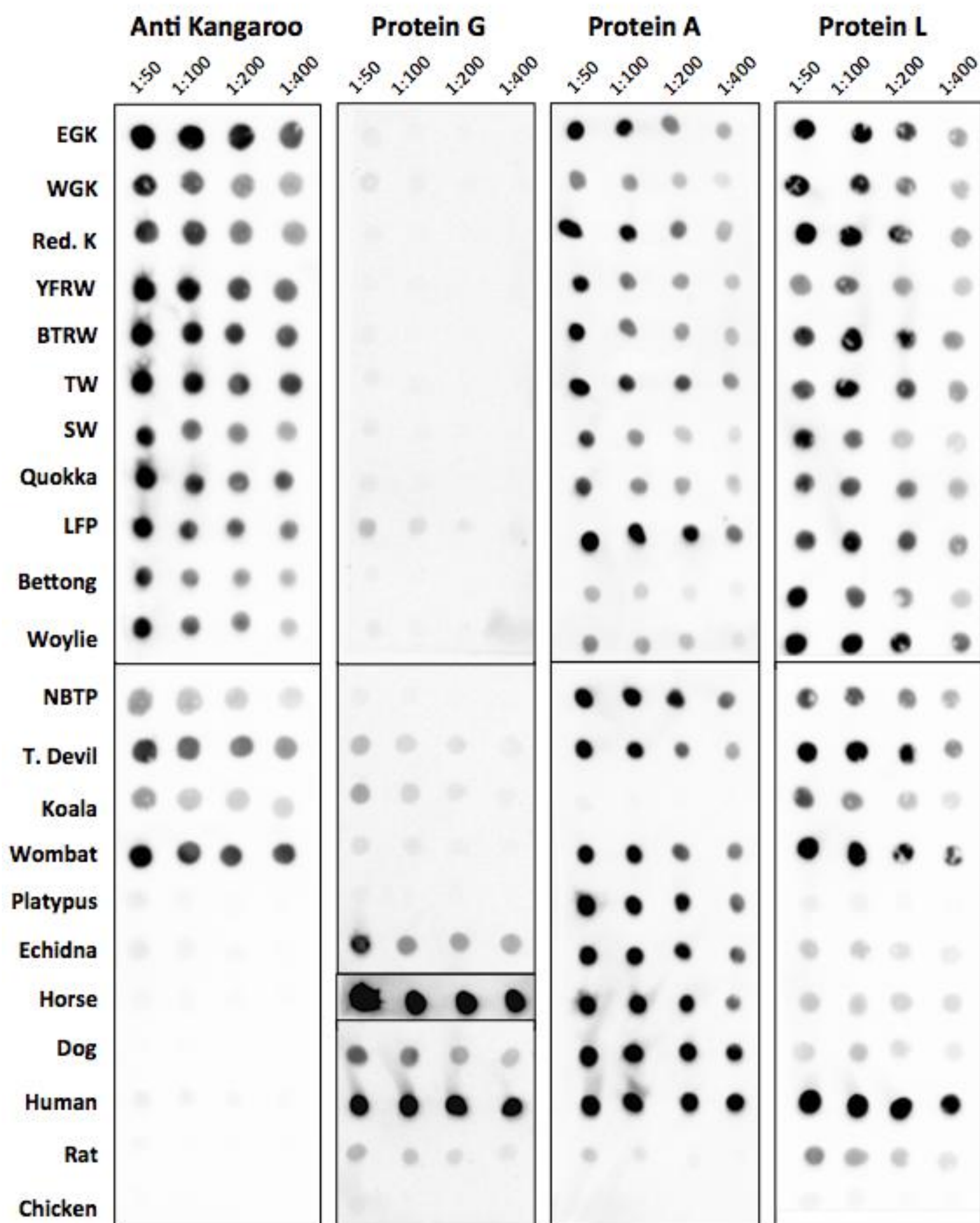
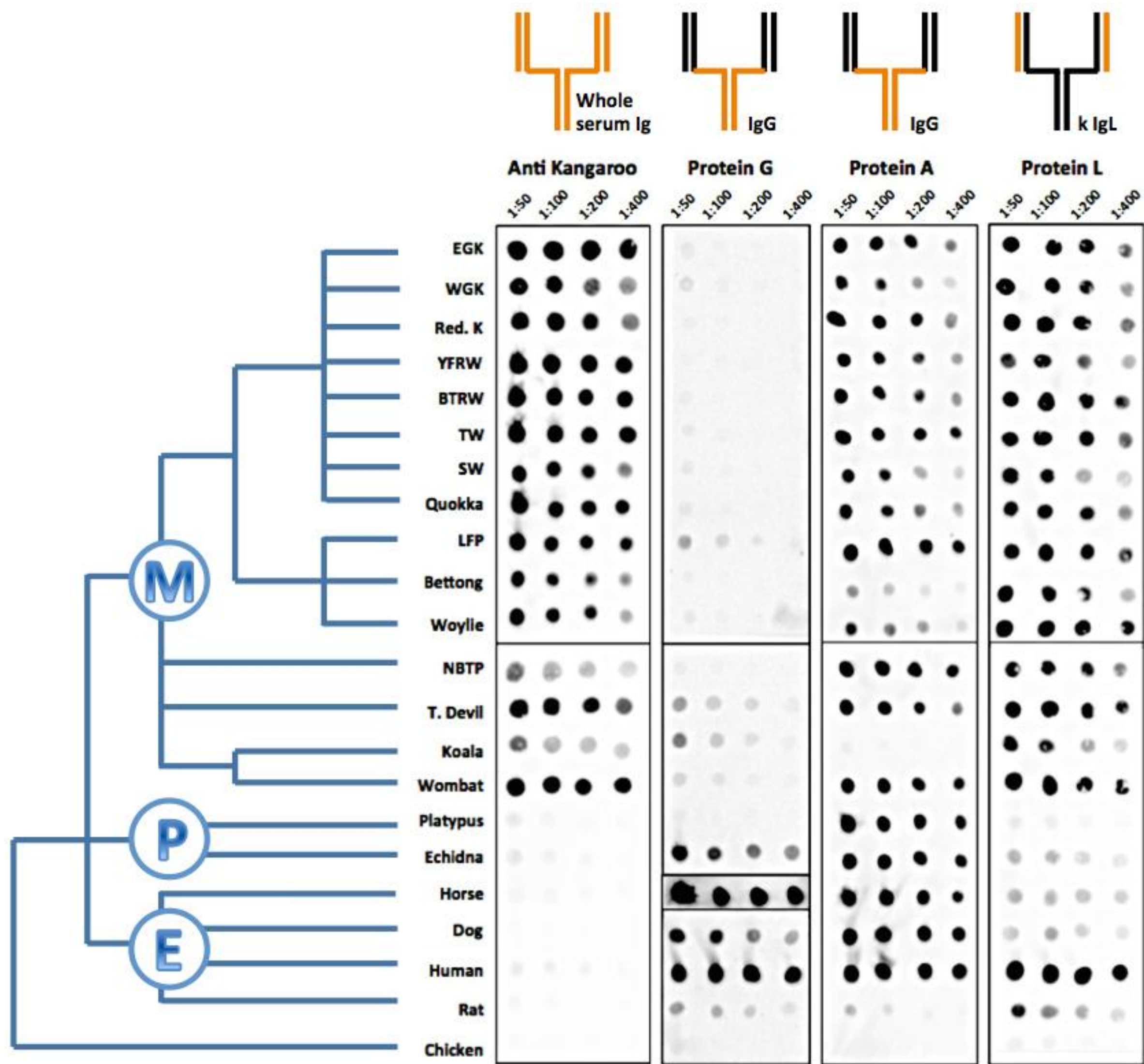


Fig. 1



Schematic representation of species taxonomy. M= Metatheria P= Prototheria E= Eutheria k IgL= Ig kappa light-chains

Graphical abstract

ACCEPTED MANUSCRIPT

Highlights

* Binding of four Ig-binding reagents to sera from 17 marsupial or monotreme species was studied
* Evolutionary distance between host species did not accurately predict Ig binding capacity
* Monotreme sera reacted strongly with protein A
* Marsupial sera reacted with protein A, protein L and polyclonal anti-kangaroo antibody
* The results can be used to inform the selection of appropriate serological reagents

ACCEPTED MANUSCRIPT

University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Vaz, PK; Hartley, CA; Browning, GF; Devlin, JM

Title:
Marsupial and monotreme serum immunoglobulin binding by proteins A, G and L and anti-kangaroo antibody

Date:
2015-12

Citation:
Vaz, P. K., Hartley, C. A., Browning, G. F. & Devlin, J. M. (2015). Marsupial and monotreme serum immunoglobulin binding by proteins A, G and L and anti-kangaroo antibody. Journal of Immunological Methods, 427, pp.94-99. https://doi.org/10.1016/j.jim.2015.10.007.

Persistent Link:
http://hdl.handle.net/11343/57454

File Description:
Accepted version