Research paper

Molecular characterisation of the CD79a and CD79b subunits of the B cell receptor complex in the gray short-tailed opossum (*Monodelphis domestica*) and tammar wallaby (*Macropus eugenii*): Delayed B cell immunocompetence in marsupial neonates

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ABSTRACT

The B cell receptor (BCR) is a multiprotein complex that is pivotal to antigen recognition and signal transduction in B cells. It consists of an antigen binding component, membrane Ig (mIg), non-covalently associated with the signaling component, a disulphide-linked heterodimer of CD79a and CD79b. In this study, the gene and corresponding cDNA for CD79a and CD79b in the gray short-tailed opossum, as well as the cDNA sequences for CD79a and CD79b in the tammar wallaby, are described. Many of the structural and functional features of CD79a and CD79b were conserved in both marsupials, including the ITAM regulatory motif in the cytoplasmic tails of both subunits. The marsupial CD79 sequences shared a high degree of amino acid identities of 76% (CD79a) and 72% (CD79b) to each other, as well as 60–61% (CD79a) and 58–59% (CD79b) with their eutherian counterparts. RT-PCR analysis of CD79a and CD79b transcripts in the immune tissues of tammar pouch young revealed CD79a transcripts in the bone marrow, cervical thymus and spleen at day 10 postpartum. CD79b transcripts were detected in the bone marrow and cervical thymus at day 10 but were not detected in the spleen until day 21 postpartum. These results suggest that a functional BCR may not be assembled until day 21 postpartum and the tammar neonate may not be capable of mounting an effective adaptive immune response until this time. The molecular information presented here will allow further investigation of the role of the CD79 subunits in marsupial B cell signaling, especially during ontogeny and disease.

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1. Introduction

The mammalian CD79a (Ig- α) and CD79b (Ig- β) membrane proteins are expressed on B cells as a disulphide-linked heterodimer that associates non-covalently with membrane Ig (mIg) to form the B cell receptor (BCR) complex, a multiprotein structure that is pivotal to antigen recognition and signal transduction in B cells (reviewed in

DeFranco, 1993; Reth, 1992). Whilst the mIg component is responsible for binding antigen, the cytoplasmic tail of the mIg heavy chain is very short and therefore incapable of transmitting activating signals to the inside of the cell. Thus, it is the CD79 subunits of the BCR that mediate these intracellular signaling events (Ales-Martinez et al., 1991; Reth et al., 1991). Like other signaling proteins (i.e. CD3 receptors and Fc receptors), the cytoplasmic tail of CD79a and CD79b contain an immuno-receptor tyrosine-based activation motif (ITAM) (Reth, 1989). In B cells, antigen-induced clustering of the BCR triggers the phosphorylation of the ITAM tyrosine residues on the CD79a and CD79b sub-

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units. This activity promotes the recruitment and activation of an array of effector molecules, of which Syk tyrosine kinase and BLNK (B cell linker protein) are most notable, forming a highly complex cascade of signaling pathways (reviewed in Dal Porto et al., 2004; Gold, 2002). In addition to its role in signaling, the CD79 subunits are essential for the transport and display of mlg molecules on the B cell surface (Matsuuchi et al., 1992).

In humans, the CD79a and CD79b glycoproteins are encoded by separate genes, namely *mb-1* and *B29*, and are localized on chromosome 19 and chromosome 17, respectively (Hashimoto et al., 1994b; Wood et al., 1993). Both of these genes encode proteins with a single extracellular Ig-like domain, a transmembrane region and a cytoplasmic tail. CD79 transcripts have been identified in a few mammals, including humans (CD79b, Hashimoto et al., 1993; CD79a, Yu and Chang, 1992), mice (CD79b, Hermanson et al., 1988; CD79a, Sakaguchi et al., 1988), rats (CD79b, Nakazato et al., 1998), cows (CD79a, Youn et al., 1996) and pigs (CD79a, Lee et al., 2008). In addition, a CD79b homolog has been identified in chickens (Katsukura et al., 2001), whilst recently, CD79a and b sequences have been isolated in some fish species (Gusel'nikov et al., 2003; Sahoo et al., 2008). Although antibodies raised against human CD79a and b have identified cross-reactive proteins in marsupial tissues (Hemsley et al., 1995; Jones et al., 1993), nothing is known of these marsupial proteins or the genes encoding them.

We report here the isolation of CD79a and CD79b in two distantly related model marsupials, the gray short-tailed opossum (*Monodelphis domestica*) and the tammar wallaby (*Macropus eugenii*). Like all marsupials, these species are born after a short gestation of only 15 days in the opossum and 26 days in the tammar (Tyndale-Biscoe, 2005). Marsupials are highly altricial at birth and have no mature lymphoid tissues (Deane and Cooper, 1988). The ability of the neonate to survive outside the sterile uterine environment with no functional immune system is of great interest. Identifying the collection of immune related genes that regulate host immunity in marsupials may shed light on the processes involved in protecting the vulnerable neonate, information that is not available from their eutherian counterparts. Our work in this area has been facilitated by the release of the first marsupial genome sequence data of the opossum (Mikkelsen et al., 2007). Using this information, we have focused on the characterisation of key molecular components of the adaptive immune system in marsupials. We recently reported the cloning of the T cell markers, CD4 (Duncan et al., 2007) and CD8 (Duncan et al., 2009), in the opossum and the tammar wallaby. These studies revealed conservation of many of the hallmarks of their eutherian homologs, including a regulatory motif and the presence in the marsupial promoter regions of several potential transcription factor-binding sites known to be important for promoter function in the eutherian CD4 and CD8 genes.

In order to further our analysis of lymphoid cell marker genes in marsupials we have cloned the B cell markers CD79a and CD79b. To this end, the opossum genome sequence data was used to (i) identify the genomic organization of the opossum CD79a and CD79b genes, (ii) isolate

the full-length CD79a and CD79b cDNAs in the opossum as well as in the tammar wallaby and (iii) analyse the expression of CD79a and CD79b gene transcripts in opossum (adult) and tammar tissues from pouch young to adulthood. The information reported here provides the first gene sequence information for B cell markers in marsupials and will assist in the elucidation of the molecular processes involved in B cell signaling in marsupials, a process known to be crucial for the differentiation, proliferation and survival of B cells in their eutherian relatives.

2. Materials and methods

2.1. Opossum CD79a and CD79b gene annotation

The *M. domestica* whole genome sequence data released from the Broad Institute was accessed through the Ensembl database (www.ensembl.org/Monodelphis_domestica/index.html). The putative opossum CD79a gene sequence was obtained from the MonDom0.5 Ensembl assembly (the preliminary assembly available temporarily on the Ensembl Pre-build site) and the putative opossum CD79b gene sequence was obtained from the MonDom5.0 Ensembl assembly by BLASTN search (using the default search parameters) using the human CD79a and CD79b cDNA sequences (GenBank accession nos. M74721 and S52229, respectively) as query. The putative opossum CD79a and CD79b Ensembl gene models were used to design primers for PCR analysis and cloning of the opossum and tammar CD79a and CD79b cDNA sequences.

2.2. Animal tissues and cDNA preparation

Opossum tissues (spleen, liver, kidney, lung, heart, brain and gut) from a 4-month-old male animal were supplied by the Department of Pharmacology, University of Melbourne, Victoria, Australia. Tammar wallaby tissues from adult (spleen, liver, kidney, lung, heart, brain, gut and blood) and pouch young (bone marrow, cervical thymus, spleen, lung, gut, and blood) were collected opportunistically from animals housed at the Macquarie University Fauna Park (North Ryde, NSW, Australia) and CSIRO, Sustainable Ecosystems (Canberra, Australia) that had been euthanized as part of other approved experimental work or as approved by the Macquarie University Animal Ethics committee (Approval Nos. 2006/002, 2007/030). The age of the tammar pouch young was estimated from head length measurements (Poole et al., 1991). Total RNA was isolated using TRI reagent (Molecular Research Centre, OH, USA) following the manufacturer's instructions. cDNA for RT-PCR was synthesised using SuperScript II reverse transcription system using oligo(dT) primers (Invitrogen, CA, USA) and RACE-ready first-strand cDNA was synthesised from opossum and tammar spleen tissue using the SMART RACE cDNA amplification kit (BD Biosciences Clontech, CA, USA) according to the manufacturer's protocol.

2.3. Cloning of opossum and tammar CD79a and CD79b cDNA

To confirm the putative opossum CD79 sequences, opossum CD79a and CD79b cDNA sequences were iso-

Table 1
Primer sequences.

Primer name	Sequence 5'–3'	Location ^a	Application
OpCD79aF1	CCAGTGTGCGGTCTCCATCAGGG	Opossum 335–357	CD79a 3' RACE PCR
OpCD79aR1	CCCTGATGGAGACCCGACACTGG	Opossum 335–357	CD79a 5' RACE PCR
OpCD79aF2	AGTGTGCGGTCTCCATCA	Opossum 337–354	Tissue expression
OpCD79aR2	AGCGTATTTCTCATCTCGCCA	Opossum 528–548	Tissue expression
TamCD79aF1	TCCTGYGGBACBTACCTCMG	Tammar 392–411	CD79a RT-PCR
TamCD79aR1	GMRCAGTCTCTVAGGTTMAG	Tammar 611–630	CD79a RT-PCR
TamCD79aF2	CGTGAGCCAATACCCAGACCTTC	Tammar 416–439	CD79a 3' RACE PCR
TamCD79aR2	GAAGGTCTGGGTATTGGCTCAG	Tammar 416–439	CD79a 5' RACE PCR
TamCD79aF3	ACTGCGAACACGATGGGA	Tammar 198–215	Tissue expression
TamCD79aR3	GGTCTGGGTATTGGCTCAC	Tammar 417–435	Tissue expression
OpCD79bF1	CTCATCATTTGCTTCATCACGGTCCC	Opossum 544–569	CD79b 3' RACE PCR
OpCD79bR1	GGGACCGTGATGAAGACAATGATGAG	Opossum 544–569	CD79b 5' RACE PCR
OpCD79bF2	CAAAGAAGGGCTGGGTGTG	Opossum 66–84	Tissue expression
OpCD79bR2	CCGTGATGAAGACAATGATGAG	Opossum 544–565	Tissue expression
TamCD79bF1	AGCRGMGGAACACVCTGAA	Tammar 517–535	CD79b RT-PCR
TamCD79bR1	TCYTGGCTGGRTGCTCDC	Tammar 730–748	CD79b RT-PCR
TamCD79bF2	CTCATCATTTGCTTCATCACCGTCCC	Tammar 564–589	CD79b 3' RACE PCR
TamCD79bR2	GGGACGGTGATGAAGACAATGATGAG	Tammar 564–589	CD79b 5' RACE PCR
TamCD79bF3	ACACCGCCACTCTCATCATC	Tammar 367–386	Tissue expression
TamCD79bR3	CCACCTTGCTCTCATCCTTATC	Tammar 606–627	Tissue expression

Note: Y = C/T, B = C/G/T, M = C/A, R = A/G, V = G/A/C, D = T/G/A.

^a Primer location in opossum CD79a cDNA (FJ817077), tammar CD79a cDNA (FJ817078), opossum CD79b cDNA (FJ817079) and tammar CD79b cDNA (FJ817080).

lated by RACE PCR using gene-specific primers that were designed based on the annotated opossum CD79 sequences. The primers used for amplifying the tammar CD79a and CD79b cDNA sequences were degenerate and were designed from an alignment of known eutherian CD79 sequences and the annotated opossum CD79a and CD79b genomic DNA. The primer sequences are listed in Table 1. Initially tammar cDNA fragments were amplified from spleen tissue using primers TamCD79aF1 and TamCD79aR1 or TamCD79bF1 and TamCD79bR1 for the CD79a and CD79b sequences, respectively. The PCR amplifications were performed using a touchdown protocol as follows: initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, an annealing temperature commencing at 55 °C and then decreasing by 0.5 °C per cycle down to 40 °C, and extension at 72 °C for 30 s. A final elongation step was carried out at 72 °C for 5 min.

The opossum and tammar CD79a RACE products were amplified using the primers OpCD79aF1 or TamCD79aF2 for 3' RACE and OpCD79aR1 or TamCD79aR2 for 5' RACE PCR. Similarly, the opossum and tammar CD79b RACE products were amplified using the primers OpCD79bF1 or TamCD79bF2 for 3' RACE and OpCD79bR1 or TamCD79bR2 for 5' RACE PCR. PCR reactions were performed in conjunction with RACE PCR primers provided with the kit and BD Advantage 2 polymerase mix using a touchdown program as follows: 5 cycles of 94 °C for 30 s and 72 °C for 2 min followed by 5 cycles of 94 °C for 30 s, 70 °C for 30 s and 72 °C for 2 min, then 30 cycles of 94 °C for 30 s, 68 °C for 30 s and 72 °C for 2 min. A final elongation step was carried out at 72 °C for 7 min. PCR products were purified using the Wizard SV gel and PCR clean-up system (Promega, WI, USA) and cloned into the pCR2.1 vector (Invitrogen, CA, USA). Clones were sequenced on a 3130xl DNA Capillary Sequencer (Applied Biosystems, CA, USA) at Macquarie University.

2.4. Sequence data analysis

The opossum CD79a genomic sequence was obtained by megaBLAST of the *M. domestica* trace archive at NCBI using the opossum cDNA sequence as query whilst the opossum CD79b genomic sequence was obtained from the Ensembl database. The genomic organisation of the opossum CD79a and CD79b genes were determined by aligning the genomic sequence and the experimentally derived cDNA sequence using the Spidey program at NCBI (<http://www.ncbi.nlm.nih.gov/spidey/>). These opossum CD79a and CD79b genomic sequences also contained the 5' flanking region of the gene for promoter analysis. The region upstream of the tammar CD79a and CD79b genes were obtained by megaBLAST of the *M. eugenii* trace archive at NCBI using the tammar cDNA sequences as query. These sequences were used for promoter analysis. The putative promoter region for the opossum and tammar CD79a and CD79b genes were analysed using TFsearch (www.cbrc.jp/research/db/TFSEARCH.html). The opossum and tammar CD79a and CD79b cDNA sequences were analysed using a number of programs available through the BioManager database at the Australian National Genomic Information Service (ANGIS). Multiple sequence alignments were performed using ClustalW and displayed using Genedoc (psc.edu/biomed/genedoc). Putative signal peptide cleavage sites for the amino acid sequences were predicted using the SignalP program (www.cbs.dtu.dk/services/SignalP) and domain boundaries were predicted using Interproscan (www.ebi.ac.uk/interproscan). Phylogenetic trees were drawn from the amino acid sequence alignment using the PHYLIP group of programs (Felsenstein, 1989), accessed via BioManager. The phylogenetic tree was displayed using the Treeview program (Page, 1996) and the bootstrap method was used to indicate confidence values for tree nodes based on 100 replicates.

2.5. Expression of CD79a and CD79b transcripts in opossum and tammar tissues

Total RNAs and cDNAs from adult opossum and tammar tissues as well as tammar pouch young tissues (days 10, 21, 60, and 120 postpartum) were prepared. For analysing expression in opossum tissues, the primer sequences were OpCD79aF2 and OpCD79aR2 or OpCD79bF2 and OpCD79bR2 for CD79a and CD79b, respectively. The expression of tammar CD79a and CD79b was analysed using the primer sequences TamCD79aF3 and TamCD79aR3 or TamCD79bF3 and TamCD79bR3, respectively. The primer sequences are listed in Table 1. Expression of the house-keeping gene G3PDH was documented as an internal control. The RT-PCR reactions were performed using 2.5 mM MgCl₂ and Flexi-Taq DNA polymerase (Promega, WI, USA) under the following conditions: initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at 60 °C for opossum and tammar CD79a and CD79b and 55 °C for G3PDH, and elongation at 72 °C for 30 s. A final elongation was carried out at 72 °C for 5 min.

3. Results and discussion

3.1. Marsupial CD79a and CD79b cDNA sequences

The full-length opossum CD79a cDNA sequence (Fig. 1A, GenBank accession no. FJ817077) and the tammar CD79a cDNA sequence (Fig. 1B, GenBank accession no. FJ817078) consisted of 1041 and 1195 nucleotides, respectively. Both sequences included an identical polyadenylation signal (AATAAA). In both marsupial sequences, the 660 nucleotide long open reading frame translated into a predicted polypeptide of 219 amino acid residues, initiated from the first 5'-proximal ATG codon (Kozak, 1983). The CD79a protein had a predicted molecular weight of 24 kDa in both marsupials. Cleavage of the signal peptide, predicted to be 23 amino acids long in both marsupials, would generate a mature polypeptide of 196 residues.

The full-length opossum CD79b cDNA sequence (Fig. 2A, GenBank accession no. FJ817079) and the tammar CD79b cDNA sequence (Fig. 2B, GenBank accession no. FJ817080) consisted of 1431 and 1420 nucleotides, respectively and included an identical polyadenylation signal (AATAAA) in both sequences. The open reading frame which is 696 nucleotides in length in both marsupial sequences, translated into a predicted polypeptide of 231 amino acid residues, initiated from the first 5'-proximal ATG codon (Kozak, 1983). The marsupial CD79b polypeptides both had a predicted molecular weight of 26 kDa. Cleavage of the signal peptide, predicted to be 29 residues in the opossum and 34 residues in the tammar, would generate a mature polypeptide of 202 residues and 197 residues, respectively.

3.2. Genomic organisation of the opossum CD79a gene

A putative opossum CD79a homolog was identified by BLASTN search of the Ensembl MonDom genome sequence data using the human CD79a cDNA sequence as the query. Although a putative opossum CD79a gene model was first

identified on the Ensembl MonDom0.5 assembly, attempts to identify this sequence data on ensuing *Monodelphis* genome assemblies (versions 2.0, 4.0, 5.0) were unsuccessful. The putative MonDom0.5 opossum CD79a gene model contained 606 nucleotides which defined 4 exons that were homologous to exons 2–5 of the human CD79a gene. Interestingly, analysis of the subsequent MonDom assemblies using the experimentally derived opossum CD79a cDNA sequence revealed only a small section of the opossum CD79a gene (exon 1 and a part of intron 1) that was located in a region of unaligned contigs that have not been anchored to a chromosome in the final assembly. Although the syntenic relationship of the opossum CD79a gene to its eutherian homologs could not be determined, it is interesting to note that exon 1 of the opossum CD79a gene was located directly upstream of the gene *RPS19* (40S ribosomal protein S19) as is the human CD79a gene on chromosome 19q13 (Hashimoto et al., 1994b).

Even though the full-length genomic sequence was missing from the Ensembl genome assemblies, analysis of the trace archive at NCBI showed full alignment of the entire opossum CD79a cDNA sequence with the traces, and this data was used to determine the putative genomic structure of the opossum CD79a gene (shown in detail and schematically in Figs. 1A and 3A, respectively). The opossum CD79a gene displayed remarkable similarity in exon structure to its human homolog (Ha et al., 1994). Both genes contained 5 exons that were identical in size (except exon 1 which is 7 residues shorter in the opossum) and were conserved in their sequences and locations of their exon/intron boundaries. Introns 1 and 2 contained the type I (also known as phase I) splice motif and introns 3 and 4 were the type 0 (phase 0) motif. This is in agreement with the introns of the CD79a gene in humans (Ha et al., 1994), mice (Kashiwamura et al., 1990) and cows (Youn et al., 1997). As in the eutherian CD79a genes, each exon of the opossum CD79a gene roughly encoded the structural domains of the predicted polypeptide with the exception of the cytoplasmic tail, which is encoded by two exons (Fig. 3A). In summary, exon 1 encoded the first 19 amino acids of the leader peptide whilst exon 2 coded for the remainder of the leader peptide (4 amino acids) and most of the Ig domain (96 amino acids). The rest of the Ig domain (17 amino acids), the entire transmembrane region (21 amino acids) and 2 amino acids of the cytoplasmic tail are encoded on exon 3 and the remainder of the cytoplasmic tail is divided between exons 4 and 5 (23 amino acids and 37 amino acids, respectively).

3.3. Genomic organisation of the opossum CD79b gene

The putative opossum CD79b gene identified on Chromosome 2 (at location 211,162,801 bp) of the Ensembl MonDom5.0 assembly was situated at a distance of 17.6 kb upstream of the *GH* gene cluster (growth hormone) and 12.4 kb downstream of the *SCN4A* gene (skeletal muscle Na-channel), arranged in a head-to-tail configuration in the same transcriptional orientation. This chromosomal arrangement showed highly conserved synteny with human chromosome 17q23 (Bennani-Baiti et al., 1998), though the *GH* and *SCN4A* genes are more closely posi-

(A)	1	ACGGGCAGACGGCACACTCTTTTCCCAAGCACTGTGCCAGCCGGG																						
45	ATG	ATG	CCC	GGC	CTG	AGC	CTG	CCC	TGC	GCC	CTT	CTC	CTC	GTC	TCT	GCC	CTG	AGC	CCT	G	<i>gtaggt...</i>			
-23	M	M	P	G	L	S	L	P	C	A	L	L	L	V	S	A	L	S	P		<i>I (unk)</i>			
103	<i>ccag</i>	GC	TCC	CAG	ACC	TGG	GCT	CTG	CAG	GTG	AAT	CCT	GGC	CCA	CCC	TCA	GTG	ACG	GTG	CCC	GTA	GGG		
-4	G	S	Q	T	▲	W	A	L	Q	V	N	P	G	P	P	S	V	T	V	P	V	G		
165	GGC	ATA	GCC	CAG	CTG	CCC	TGT	GAA	CAC	GAC	GGG	GGC	CCC	AAT	GCC	AAC	GTG	ACT	TGG	TGG	CGA	GTC		
18	G	I	A	Q	L	P	Ⓢ	E	H	D	G	G	P	N	A	<u>N</u>	<u>V</u>	<u>T</u>	W	W	R	V		
231	ATT	TTG	GGC	AAC	TTC	TCA	TGG	CCC	AAA	GTC	CAG	GTG	GCG	AAG	GAG	TCT	GAG	CTC	AAT	GGG	ACC	CTG		
40	I	L	G	<u>N</u>	<u>F</u>	<u>S</u>	W	P	K	V	Q	V	A	K	E	S	E	L	<u>N</u>	<u>G</u>	<u>T</u>	<u>L</u>		
297	ATC	CTC	TGG	GGA	GTG	AAA	AAG	AGC	GAT	GGA	GGC	GTC	TAC	CAG	TGT	CGG	GTC	TCC	ATC	AGG	GGC	AAC		
62	I	L	W	G	V	K	K	S	D	G	G	V	Y	Q	Ⓢ	R	V	S	I	R	G	N		
363	CCA	GCC	TCG	GAG	TCC	TGC	GGC	ACC	TAC	CTC	AGG	GTT	CGA	G	<i>gtgagt...cctag</i>		AG	CCA	ATA	CCT	AGA			
84	P	A	S	E	S	Ⓢ	G	T	Y	L	R	V	R		<i>II (967)</i>		E	P	I	P	R			
417	CCC	TTC	CTG	GAC	ATG	GGA	GAG	GCC	ACC	AAG	AAC	AGG	ATT	ATC	ACT	GCA	GAG	GGA	ATC	ATT	CTG	CTT		
102	P	F	L	D	M	G	E	A	T	K	N	R	I	I	T	A	E	G	I	I	L	L		
483	TTC	TGC	GCT	GTC	GTC	CCG	GGG	ACC	CTC	CTG	CTC	TTC	AGA	<i>gtaaga...ctgtag</i>		AAG	CGG	TGG	CAG	AAT				
124	F	C	A	V	V	V	G	T	L	L	L	F	R		<i>III (552)</i>		K	R	W	Q	N			
537	GAG	AAA	TAC	GCT	GTG	GAC	CAC	CTG	GAC	TAC	TAT	GAT	GAT	GAG	AAC	CTG	TAT	GAG	<i>gtgaga...ctccag</i>					
142	E	K	Y	A	V	D	H	L	D	Y	Y	D	D	E	N	L	Y	E		<i>IV (140)</i>				
591	GGC	CTT	AAC	CTC	GAT	GAC	TGT	GCC	ATG	TAT	GAG	GAC	ATC	TCC	CGG	GGC	TTG	CAG	GGG	ACC	TAC	CAG		
160	G	L	N	L	D	D	C	A	M	Y	E	D	I	S	R	G	L	Q	G	T	Y	Q		
657	GAT	GCC	ACC	ACC	CTC	AGG	GTG	GGG	GAT	GTC	CAG	CTG	GAG	AAG	CCG	TGA								
182	D	A	T	T	L	R	V	G	D	V	Q	L	E	K	P	*								
705	GGGCCCTGCGTGAAGTCTCTAGCTTCGACCTGCCTTAACCTGCCCGCTTtaggtcttctgacttgctccgctctccgtctcctcggg																							
792	ACCAGATGGTTTCCAAGGTCTCTCCAGCTCTGATGTTCCATGTTTCTAAATCTGTCCCTTTGCCCTCTCCTCCCTCCCTTCTCTG																							
879	GCCGTTACTTCTAACTTGCTCCCCGTAATCCAGGAGATGCTGAAGCAGGATCCCCCAAGGCCCAAGCTTTGTTCATGGCCAC																							
966	TAATGATCTAATAAATGTGGGGCCGTTGGGAAGAAGGGGCTCCATTGCGCAGCAAAAAAAAAAAAAAAAAAAAAA																							
(B)	1	AGGCAAAGACAGCTCTCCTTTTCCACAGCACGATCTCAGCTAGTTGGGGACCCTTTGGGG																						
62	ATG	ATG	TCC	AGC	CTC	ATT	CCG	CCC	TTG	GTC	CTG	CTC	CTC	ATC	TCT	GTT	CTG	AAC	CCT	GGC	TTC	CTC		
-23	M	M	S	S	L	I	P	P	L	V	L	L	L	I	S	V	L	N	P	G	F	L		
128	ACC	TGG	GGT	CTG	AGA	GTG	GAG	CCT	GGC	CAG	CCC	TCC	GTG	ACT	GTG	CCT	GTG	GGG	GGC	ACG	GCC	CTG		
-1	T	▲	W	G	L	R	V	E	P	G	Q	P	S	V	T	V	P	V	G	G	T	A	L	
194	CTG	CAC	TGC	GAA	CAC	GAT	GGG	AGC	TCC	GAT	GCC	AAC	GTG	ACT	TGG	TGG	CGA	GTC	AAC	ATG	GAT	AGC		
22	L	H	Ⓢ	E	H	D	G	S	S	D	A	<u>N</u>	<u>V</u>	<u>T</u>	W	W	R	V	N	M	D	S		
260	TTC	ACA	TGG	CCC	AGG	AAA	AAG	GTG	GAG	AAA	TTC	ATT	CAT	CCC	AAT	GGG	ACA	CTG	GTC	ATC	TTG	GCA		
44	F	T	W	P	R	K	K	V	E	K	F	I	H	P	<u>N</u>	<u>G</u>	<u>T</u>	L	V	I	L	A		
326	GCA	AAC	AAG	AGC	GAC	GGA	GGC	ATG	TAC	CTA	TGT	CAA	GTT	TCT	GTC	GAG	AAT	GAT	CAA	GCC	TCA	GAG		
66	A	<u>N</u>	<u>K</u>	<u>S</u>	D	G	G	M	Y	L	Ⓢ	Q	V	S	V	E	N	D	Q	A	S	E		
392	TCC	TGT	GGC	ACC	TAC	CTC	AGG	GTT	CGT	GAG	CCA	ATA	CCC	AGA	CCC	TTC	TTG	GAC	ATG	GGC	GAA	GGC		
88	S	Ⓢ	G	T	Y	L	R	V	R	E	P	I	P	R	P	F	L	D	M	G	E	G		
458	ACA	AAA	AAC	AGA	ATT	ATC	ACC	GCA	GAG	GGA	ATC	ATC	CTG	CTC	TTC	TGT	GCA	GTC	GTC	CCT	GGC	ACA		
110	T	K	N	R	I	I	T	A	E	G	I	I	L	L	F	C	A	V	V	P	G	T		
524	CTG	CTG	CTC	TTT	AGG	AAG	CGT	TGG	CAG	AAT	GAG	AGA	TAT	GCT	GTG	GAT	CAC	TTG	GAC	TAT	TAT	GAT		
132	L	L	L	F	R	K	R	W	Q	N	E	R	Y	A	V	D	H	L	D	Y	Y	D		
590	GAT	GAG	AAC	CTA	TAT	GAG	GGT	CTT	AAC	CTC	GAT	GAC	TGC	GCC	ATG	TAT	GAG	GAC	ATC	TCC	CGG	GGT		
154	D	E	N	L	Y	E	G	L	N	L	D	D	C	A	M	Y	E	D	I	S	R	G		
656	CTG	CAG	GGG	ACC	TAC	CAG	GAT	GCC	ACT	ACC	CTC	AGG	GTG	GGA	GAT	GTC	CAG	CTA	GAG	AAG	CCA	TGA		
176	L	Q	G	T	Y	Q	D	A	T	T	L	R	V	G	D	V	Q	L	E	K	P	*		
722	TGGCCCTTCTTGCCCTTGCTCAAAGCAGCCTCCCTCCCTTAAACTGCCCGCTTTCAGGTCTTTCTGACTGTCCCACTCTCAGTTTCCT																							
809	CAGGAGTCAGTTTGACTAGATGGTATCTAAGGCCCTCCAGCCCTGACATCCCTGTTCGAAGGCCCTCCAGCCTTTGATATTC																							
896	CTGTTCCAAGATCCCTCCAGCTCTGATATTTTCATGTTCTGTTTCCACGTTTGACATGTTTCTCTCTCCCATGATATGCCCTT																							
983	CTCTATCCCTCATGCTTGACTCCTGATCCCTTTTAACTTTTCCCTATAACTCTTTCCTATAATGCAGGAGGTGCTTGAAGC																							
1070	AGGATCCCTCCCTAAGTTTGTGTATAGCTACTAATGATAATAAATCTGTGGCCCATGGGGAAGGAAAGGGTTCATTCTCCATTG																							
1157	GGCGGGATGGTAAAAAAAAAAAAAAAAAAAAAAAAA																							

Fig. 1. The nucleotide sequence (numbered at the top) and deduced amino acid sequence (one-letter abbreviation code, numbered below in bold) for CD79a in the opossum (A) and the tammar wallaby (B). The location of the introns in the opossum sequence are shown in italics (gt/ag sites in italics and underlined; intron number in roman numerals and length of intron in parentheses). For intron 1 the length is designated unk for unknown). For both sequences, the predicted mature polypeptide begins at position +1 indicated with a filled arrowhead. Extracellular cysteine residues are circled. Potential N-linked glycosylation sites are underlined and the polyadenylation signal is double underlined.

The opossum *CD79b* gene spanned 13 kb and was much larger than its *CD79b* homologs in humans, rats and chickens, which are 3.8, 3.1 and 4.3 kb in length, respectively (Hashimoto et al., 1994a; Katsukura et al., 2001; Nakazato et al., 1998). The vast size of the opossum *CD79b* gene was due to larger intronic lengths, which totalled 12.3 kb compared to only 2.3 kb in human *CD79b*. In contrast, the length of the six exons in the human and opossum *CD79b* genes were well conserved, with the exception that exon 2 in opossum *CD79b* is 3 nucleotides longer than its human homolog. This conservation of exon structure also extended to the sequences and locations of the exon/intron boundaries, resulting in an identical structural domain arrangement in the predicted opossum polypeptide and the human *CD79b* protein (Fig. 3B). Thus, exon 1 encoded the first 22 amino acids of the leader peptide. Exon 2 encoded the rest of the leader peptide (7 amino

Fig. 2. The nucleotide sequence (numbered at the top) and deduced amino acid sequence (one-letter abbreviation code, numbered below in bold) for CD79b in the opossum (A) and the tammar wallaby (B). The location of introns in the opossum sequence is shown in italics (gt/ag sites in italics and underlined; intron number in roman numerals and length of intron in brackets). The predicted mature polypeptide begins at position +1 indicated with a filled arrowhead. Extracellular cysteine residues are circled. Potential N-linked glycosylation sites are underlined and the polyadenylation signal is double underlined.

(B)

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1  AAGGAACACAGCCACGGAGGTCAAGGCCTGGGTCAAGGACAGAATCAATTTGACC

57  ATG GCT GGG CTG GGG CTG CCC ACC ATC TCC AAA GAA GGA CTG AGA GTG CTG CTC CTG ATA CTA TCA
-34  M  A  G  L  G  L  P  T  I  S  K  E  G  L  R  V  L  L  L  I  L  S

123  GGT GAG ATG ATC CTG AAA GTC CAA ACA GCG AAT GGT GAT GTT GTC CAG GCT TCT AGA GCA AAT TCA
-12  G  E  M  I  L  K  V  Q  T  A  N  G  D  V  V  Q  A  S  R  A  N  S

189  TGC AGC AAG TTA TGG CAA CGT CTA CGT TTT GTG GCC AAA AAG AGG GAC AGC TCC TTC GAA GTG ACC
11  C  S  K  L  W  Q  R  L  R  F  V  A  K  K  R  D  S  S  F  E  V  T

255  TGC CAC GTA GAA CAT CCC AGG GAG GTG ACT TGG CTG GTG AAG CGG GAG TCA GAC CTT GTA CCC CAG
33  C  H  V  E  H  P  R  E  V  T  W  L  V  K  R  E  S  D  L  V  P  Q

321  GCC CTT CCA CCC GAT AAT GGA CGT CTA AGG CGG GTG ACA AAC AAC AAC ACC GCC ACT CTC ATC ATC
55  A  L  P  P  D  N  G  R  L  R  R  V  T  N  N  N  T  A  T  L  I  I

387  ACA AAG ATA CAA TTT GAG GAC AAT GGC ATC TAT TAT TGT GAA GAA AAG TGT ATG GAA GGT CCT AGA
77  T  K  I  Q  F  E  D  N  G  I  Y  Y  C  E  E  K  C  M  E  G  P  R

453  GAG AAC CTC CGG GGC TGT GGC ACT GAG CTG CGT GTC ATG GGC TTC AGC ACT ATG GCC CAG CTG AAG
99  E  N  L  R  G  C  G  T  E  L  R  V  M  G  F  S  T  M  A  Q  L  K

519  CGG AGG AAC ACT CTG AAA GAT GCC ATT ATT ATG GTC CAG ACC CTA CTC ATC ATT GTC TTC ATC ACC
121  R  R  N  T  L  K  D  A  I  I  M  V  Q  T  L  L  I  I  V  F  I  T

585  GTC CCT ATT TTC CTG TTG TTA GAT AAG GAT GAG AGC AAG GTG GCA TTG GAG GAT GAT CAC ACC TAT
143  V  P  I  F  L  L  L  D  K  D  E  S  K  V  A  L  E  D  D  H  T  Y

651  GAG GGT CTG GAA ATT GAC CAG ACG GCC ACC TAT GAG GAC ATC GTG ACA CTT CGG ACA GGA GAA GTA
165  E  G  L  E  I  D  Q  T  A  T  Y  E  D  I  V  T  L  R  T  G  E  V

717  AAA TGG TCA GTG GGT GAG CAC CCG GGC CAG GAG TGA
187  K  W  S  V  G  E  H  P  G  Q  E  *

753  AGGGTTCCTGACCTGGCAATCCTTCCTACGCTCCCCAGCCCTCCATCAACTTCCTGGAGGAGCTCATCTAGCAAATCAGCTTCAT
840  GGACCTGCTCTGGTTTCCATCCTATTACTCCTTTATCATAACTGTCCTGCCTCCAGGCATACTGACCCAGCTCCGCCTTCCTATTTTC
927  CAGTCCCTCGAGACTCACAACCATCTCCAGAGGGATGTAGGCCTGCAGTGCTTAACAGAGCACAGGTTGGGGAAGTTGTCTCTCTGA
1014  GTTCTTTGGAGTTGGCCATGTCCATGATGGCTGGGGGAAGGAGACAGAGTATAGAGGGAGACTTAGACTTAGTGGGGAGAAGGGAGAT
1101  AAGACAAAGGAAACAGATTTGGAGTCTGGTGGGGAAAAAATGGAAGGAACCTGTAAATCTGACTCAGACTGATCTATGAGACCAGA
1188  CCATACTTGGACCTTTACAGCATCATGCCTCCAGTTTTGTATGCAAGCAAGGTCAGGATAGACTCTAAACAGTTGGCCAGCCAACCTG
1275  ACTCAATCCAAATTAGGGTTGTGGAAATATATCCCTTCTCCATCTCCAGGTCCTATTGGGCCCTTCTGGTGTCTGTGTCAT
1362  AGCTCCTGATGTAAATAAAGCTGTGTGTTTGAATAAAAAAAAAAAAAAAAAAAAAAAAAA

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Fig. 2. (Continued).

acids) and the first 12 amino acids of the Ig domain whilst exon 3 coded for most of the Ig domain (104 amino acids). The remainder of the Ig domain (16 amino acids), the entire transmembrane region (22 amino acids) and the first 2 amino acids of the cytoplasmic tail are encoded on exon 4 and the rest of the cytoplasmic tail is divided between exons 5 and 6 (14 amino acids and 32 amino acids, respectively). Analysis of the opossum *CD79b* gene introns revealed agreement with those of other *CD79b* genes including human (Hashimoto et al., 1994a), mouse (Hermanson et al., 1988), rat (Nakazato et al., 1998) and

chicken (Katsukura et al., 2001) whereby introns 1–3 contain the type I (also known as phase I) splice motif and introns 4 and 5 contain the type 0 (phase 0) motif.

3.4. Promoter analysis of the marsupial *CD79a* and *CD79b* genes

The 5' flanking region upstream of the opossum and tammar *CD79a* and *CD79b* genes (Fig. 4) was analysed for known transcription factor-binding sites (Faisst and Meyer, 1992). An alignment of this region revealed a high level

Fig. 3. Genomic organisation of the opossum *CD79a* (A) and *CD79b* (B) genes. Not drawn to scale. Exons are boxed and numbered in roman numerals whilst introns are designated by solid lines. The length of each exon and intron are indicated except for intron 1 in opossum *CD79a* where the length is unknown. The structural arrangement of the protein domains in relation to coding exons (shaded areas) is also shown. L, leader; Ig, extracellular Ig domain; TM, transmembrane; Cyt, cytoplasmic tail.

Fig. 4. Alignment of the putative promoter region in the opossum and tammar *CD79a* (A) and *CD79b* (B). Consensus recognition sequences of potential factor-binding sites in the promoter region are shown in bold and named above the opossum and below the tammar sequence. The ATG translation start site is shown in bold and italics.

of sequence conservation, showing 60% identity between the marsupial *CD79a* sequences and 79% identity between the marsupial *CD79b* sequences. As with the human and murine *mb-1* (*CD79a*) and *B29* (*CD79b*) genes (Ha et al., 1994; Hermanson et al., 1989; Kashiwamura et al., 1990; Thompson et al., 1996), both marsupial genes lacked a TATA box. TATA-less eukaryotic promoters commonly have multiple initiation sites for transcription. Several possible transcription initiator (Inr) consensus sequence motifs (Smale and Baltimore, 1989) were found in the marsupial sequences. For the marsupial *CD79a* gene, the putative Inr shown for opossum (Fig. 4A) was located in the same position as in human and mouse *mb-1* (*CD79a*) (Ha et al., 1994; Kashiwamura et al., 1990), where it overlaps two of the major transcription start sites. In marsupial *CD79b*, the putative Inr shown for opossum and tammar (Fig. 4B) was located just upstream of the major transcription start sites in human and mouse *B29* (*CD79b*) (Hermanson et al., 1989; Thompson et al., 1996).

Several putative transcription factor-binding sites were identified in the *CD79a* promoter region in both marsupials (Fig. 4A) including Ets (Bassuk and Leiden, 1997), Lyf-1 (Lo et al., 1991) and Sp1 (Wierstra, 2008). However, other motifs found in the human (Ha et al., 1994), murine (Kashiwamura et al., 1990) and bovine (Youn et al., 1997) *mb-1* (*CD79a*) promoter regions, including EBF/BlyF (Feldhaus et al., 1992), MUF-2 (Travis et al., 1991) and NF- κ B (Lenardo and Baltimore, 1989), were absent from the marsupial *CD79a* promoter regions. In the mouse, the expression of *mb-1* (*CD79a*) is developmentally regulated in the B cell lineage, appearing in very early B cells and mature B cells but not in plasma cells (Sakaguchi et al., 1988). It is perhaps not unexpected that the critical control element of the *mb-1* (*CD79a*) gene in humans and

mice, EBF (early B cell factor, also referred to as BlyF), has been identified in pre-B and B cells but not in plasma cells. This may be one reason for the developmentally regulated expression of the *mb-1* (*CD79a*) gene during the early, but not the late, stages of B cell differentiation (Hagman et al., 1991). The absence of a binding site for EBF in the putative promoter region of the *CD79a* gene in both marsupials was therefore surprising. Analysis of the Ensembl *Monodelphis* genome revealed the opossum *EBF* gene on chromosome 1, upstream of the gene *RNF145* as is the mouse *EBF* gene on chromosome 11. Based on this data, it is possible that an EBF binding site for the marsupial *CD79a* gene occurs in a different location to that observed in human and mouse. However, it is currently unknown if the *EBF* gene is expressed in marsupials and further studies are required to establish the function of EBF in the regulation of the *CD79a* gene in marsupials. If the *CD79a* gene is developmentally regulated in the marsupials as it is in human and mouse it is likely that another regulatory mechanism may be operating.

The putative *CD79b* promoter region in the tammar and opossum (Fig. 4B) contained motifs for Oct (Matthias, 1998), Ets and Lyf-1. These elements were highly conserved in sequence and position to all known mammalian *B29* (*CD79b*) promoter regions, including human (Thompson et al., 1996), mouse (Omori and Wall, 1993) and rat (Nakazato et al., 1998). Unlike the developmentally regulated expression of the *mb-1* (*CD79a*) gene, the *B29* (*CD79b*) gene is expressed at all stages of B cell differentiation including plasma cells of mice and humans. The high conservation of regulatory elements in the *B29* (*CD79b*) promoter region across mammals including the marsupials suggests that the marsupial *CD79b* gene may also be expressed at all stages of cell differentiation in the B cell lineage.

Fig. 5. Alignment of the CD79a (A) and CD79b (B) amino acid sequence of opossum with other vertebrates (including tammar) showing the level of sequence identity (Id%) with the opossum sequences. The alignment was generated using ClustalW (BioManager) and identical residues (>60%) are shaded. Dashes indicate gaps introduced into the sequence to optimise the alignment. The leader sequence, Ig domain, transmembrane region and cytoplasmic tail boundaries indicated above the sequences were predicted using Interproscan with the exception that the CD79b leader sequence length was based on the human and mouse CD79b sequence due to discrepancies in the length of the SignalP predicted marsupial CD79b leader sequences. The extracellular cysteines are indicated with an asterisk whilst the potential N-linked glycosylation sites in the extracellular domain are shown in bold. Features of note in the cytoplasmic tail are the ITAM consensus motifs that are underlined, the peptide sequence of the anti-human CD79a antibody and the peptide sequence of the anti-human CD79b antibody that are shown in bold. GenBank accession numbers are as follows: Human (CD79a, M74721; CD79b, S52229), Mouse (CD79a, X13450; CD79b, J03857), Cow (CD79a, D16412) and Pig (CD79a, AM915272), Norway rat (CD79b, AB004831) and Chicken (CD79b, AB062512).

3.5. Comparison of the marsupial CD79 deduced protein sequences with their homologs in other species

A multiple alignment of the marsupial CD79a and CD79b deduced amino acid sequences with other known CD79 sequences is shown in Fig. 5, and illustrates the level of sequence identity with the opossum CD79a (Fig. 5A) and CD79b (Fig. 5B) sequences. Highest overall identity was observed between the marsupial sequences, showing identities of 76% (CD79a) and 72% (CD79b) whilst identity with their eutherian homologs was 60–61% (CD79a) and 58–59% (CD79b). For the structural domains, the signal

peptide and extracellular domain of the CD79a and CD79b sequences were poorly conserved across species examined. This was particularly evident for the leader sequence of the CD79a sequences, which revealed 52% identity between the marsupial sequences whilst identity with the eutherian CD79a leader sequences ranged from 42% (mouse) to 29% (pig).

Despite the low level of sequence conservation in the extracellular domains of CD79a (48–66%) and CD79b (48–60%) amongst mammals, the structural features of this domain were conserved in the opossum and tammar. The extracellular domain of opossum and tammar CD79a

revealed the presence of three conserved cysteine residues. The two cysteines (C-50, C-101 in mouse) capable of forming an intrachain bond in the Ig fold of this domain were conserved, as was the membrane proximal cysteine (C-113 in mouse) capable of intermolecular dimer formation between the CD79a and CD79b subunits of the BCR, as seen in human and mouse CD79a (Sakaguchi et al., 1988; Yu and Chang, 1992). The number of N-linked glycosylation motifs (NX[T/S], where X is any residue except proline) in eutherian CD79a sequences varies considerably. There are six motifs present in human CD79a and two predicted sites in the mouse whilst the cow and pig both have four predicted N-linked glycosylation sites. There were three potential N-linked glycosylation sites in both the opossum and the tammar CD79a sequences that were each conserved in their positioning in relation to their mammalian counterparts (see Fig. 5A).

The extracellular domain of opossum and tammar CD79b contained five conserved cysteine residues. In these sequences, there are two potential sites for disulphide linkage between the first four cysteines (C-43, C-65, C-120, C-124 in mouse) that would involve a canonical disulphide linkage of the Ig fold between cysteine residues C-65 and C-120 (in mouse) and an additional covalent bond between cysteine residues C-43 and C-124 (in mouse). Moreover, the membrane proximal cysteine (C-135 in mouse) capable of dimerisation between the two subunits was conserved as in human and mouse CD79b (Hashimoto et al., 1993; Hermanson et al., 1988; Siegers et al., 2006). For N-linked glycosylation sites, the human and mouse CD79b sequences each have three potential sites, there are two possible sites in the rat whilst the chicken has 5 potential N-linked glycosylation sites. In the opossum and tammar CD79b sequences a single N-glycosylation site was observed in both marsupial sequences at one of the sites common to all known CD79b sequences in vertebrates (see Fig. 5B).

The transmembrane region has remained highly conserved during evolution, with identities as high as 100% (CD79a) and 90% (CD79b) between the marsupials and ranging from 95–100% (CD79a) and 77–81% (CD79b) between the marsupials and eutherians. This high sequence conservation is not surprising since the transmembrane region of the CD79 subunits are known to be critical for the formation of heterodimers during the assembly of the BCR complex (Dylke et al., 2007). Of note are two polar residues that are conserved across all known CD79 molecules, namely glutamic acid in CD79a (E-148 in human) and glutamine in CD79b (Q-165 in human), that potentially form strong ionic and hydrogen bonds thus stabilising the heterodimer (Dylke et al., 2007).

The high conservation of the cytoplasmic tail amongst mammals (CD79a, 75–96%; CD79b, 87–93%) reflects the presence of a conserved regulatory motif in this region, the ITAM (D/E(x)₇D/ExxYxxL(x)₇YxxI/L) (Reth, 1989). This motif is conserved in the marsupial CD79a and CD79b sequences. In mammals, phosphorylation of the ITAM tyrosine residues on the CD79a and CD79b subunits promotes the recruitment and activation of the Syk tyrosine kinase that binds through its tandem SH2 domains to the paired phospho-tyrosines in the ITAM (Dal Porto et al., 2004; Gold,

2002). In addition to the ITAM tyrosines, there are two additional tyrosine residues (Y-176, Y-204 in mouse) in mammalian CD79a including the marsupials. Phosphorylation of the residue at position 204, recruits the B cell linker protein BLNK that binds through its SH2 domain thus forming a platform for downstream signaling pathways (Engels et al., 2001; Kabak et al., 2002).

Despite the clear structural homology between the CD79a and CD79b subunits, they are not closely related to each other, with amino acid identities being as low as 21% (human), 18% (opossum, tammar) and 17% (mouse). This is also evident in the phylogenetic tree based on the amino acid alignment (Fig. 6) where the marsupial CD79a and CD79b sequences are each more closely related to their eutherian homologs than they are to each other and, as such, form two distinct clusters. Within the CD79a and CD79b clusters, the marsupial sequences form a monophyletic clade as a sister group to the eutherians.

3.6. Tissue-specific distribution of CD79a and CD79b in adult and pouch young marsupials

The tissue-specific profile of CD79a and CD79b in the tissues of adult and pouch young marsupials has previously been analysed using anti-human CD79a and anti-human CD79b antibodies by immunohistochemistry (Hemsley et al., 1995; Old and Deane, 2003; Old et al., 2004). These antibodies have shown wide cross-reactivity in mammals including the mouse, rat, pig and cow as well as chicken (Jones et al., 1993) but the results in marsupials have been variable. Despite the fact that the anti-human CD79b antibody showed good staining in the tissues of a broad range of marsupials, the anti-human CD79a antibody showed no reactivity in most marsupials including the tammar, opossum, koala, ringtail possum and eastern gray kangaroo (Hemsley et al., 1995; Jones et al., 1993; Old and Deane, 2001). This lack of reactivity can be explained by the sequence data presented in this study. The marsupial CD79b sequences are 100% identical to the anti-human CD79b peptide sequence (15 residues) whilst both the marsupial CD79a sequences have a mismatch of 5 residues at positions 6, 7, 8, 10 and 11 to the peptide sequence (14 residues) of anti-human CD79a (Fig. 5).

The expression profile of CD79a and CD79b transcripts in the tissues of adult opossum and tammar was analysed by RT-PCR and resulted in a similar tissue expression pattern in both marsupials (Fig. 7). CD79a and CD79b transcripts were expressed in the spleen, liver, heart and gut of both marsupials, as well as the blood in the tammar and the lung in the opossum. A low level of expression was detected in the kidneys of the tammar. Transcripts were not observed in the opossum kidney or the brain of either marsupial. In addition, RT-PCR analysis of the expression of CD79a and CD79b transcripts in the tammar pouch young tissues was performed (Fig. 8). CD79a and CD79b transcripts were detected in the bone marrow, cervical thymus and lung at day 10 postpartum. However, although CD79a transcripts were expressed in the spleen and blood at day 10, CD79b transcripts were not detected in these tissues until day 21 postpartum. The gut showed either no expression or a very low level of expression at all ages tested.

Fig. 6. Phylogenetic analysis of vertebrate CD79a and CD79b amino acid sequences showing the marsupial sequences in bold. The complete coding sequences were used in the construction of the tree. Bootstrap values are out of 100. Additional GenBank accession numbers not mentioned elsewhere are as follows: Catfish (CD79a, EF173897; CD79b, EF173898).

By day 120 postpartum, the CD79a and CD79b transcripts were expressed at a high level in the lymphoid organs including the bone marrow, cervical thymus and spleen as well as the blood.

A previous analysis of the distribution of CD79b⁺ cells in the tissues of tammar pouch young by immunohistochemistry using the anti-human CD79b antibody (Old and Deane, 2003) showed CD79b⁺ cells in the spleen at day 21 postpartum and the thymus at day 23 postpartum, but cells were not detected in the bone marrow, gut (except one sample at day 7 postpartum) or lung (CD79b⁺ cells were observed

Fig. 8. RT-PCR analysis of CD79a and CD79b transcripts in the tissues of tammar pouch young at days 10, 21, 60 and 120 postpartum. Tissues analysed were: bone marrow (Bo), cervical thymus (Ce), spleen (Sp), lung (Lu), gut (Gu) and blood (Bl). Expression of G3PDH was included as an internal control. Tissues from two individuals were analysed for each age group with only one result shown.

in the blood vessels of the lung) at any age. With the exception of the bone marrow, these results are consistent with those presented here and suggest that a functional BCR may not be assembled until day 21 postpartum, when the expression of CD79b is evident. As a result, tammar neonates younger than 21 days old may not be capable of mounting an effective adaptive immune response. Further studies are necessary to resolve the apparent discrepancy

Fig. 7. Expression profile of CD79a and CD79b transcripts in adult opossum and the tammar wallaby tissues. Tissues analysed were: spleen (Sp), liver (Li), kidney (Ki), lung (Lu), heart (He), brain (Br), gut (Gu) and blood (Bl). Expression of G3PDH was included as an internal control. Tissues samples were tested in duplicate with only one result shown.

in the mRNA and protein expression of CD79b in the bone marrow.

4. Conclusion

Whilst whole genome sequence data provides a valuable tool for the isolation of homologous genes in divergent species, confirmation of these gene sequences by cDNA or genomic cloning is often necessary to identify the correct exon/intron boundaries and the 5' and 3' ends of genes. Moreover, orientation errors can arise in the assembly of the genome as seen for the opossum *CD79a* gene presented here. In this study, a combination of bioinformatics and laboratory based molecular protocols were used. We initially analysed the opossum genome sequence data to identify the *CD79a* and *CD79b* genes and then used this data to facilitate the isolation of *CD79a* and *CD79b* cDNA transcripts in the opossum and the tammar wallaby. This data provides valuable molecular information on the CD79 subunits in marsupials and will allow further investigation of the role of B cell signaling in the marsupial immune system.

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