Author/s:
Call, MJ;Davey, AS

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Hello Possums!

Strapline: Introducing the "mu"-γ T cell receptor

Melissa J Call and Ashleigh S Davey

Corresponding author: mjcall@wehi.edu.au

Structural Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia, Department of Medical Biology, The University of Melbourne, Parkville VIC 3052, Australia.

While thymic development in most eutherian mammals is initiated prenatally and offspring are born relatively immuno-competent, marsupials are comparatively less mature at birth with an undifferentiated thymus and few or no circulating lymphocytes. Both cell-mediated and humoral immune responses are consequently absent in marsupials until at least 2 weeks postpartum, with offspring relying significantly on maternal immune protection for the first few weeks of life. The differences in immune development pre- and post-partum and significant time since marsupials and eutherians shared a common ancestor (170–180 million years ago) has sparked the question of whether marsupial immunity may have evolved to possess unique differences from eutherians?

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In 2007, Miller and colleagues discovered that marsupials indeed had a rather unusual T cell receptor (TCR) chain in addition to the well-described TCR α, β, δ and γ chains that heterodimerise within αβ and γδ T cells of all mammals[1]. This chain termed TCRμ had a constant (Cμ) and variable (Vμ) domain like other TCR chains, but sandwiched between these domains was a second variable-like domain that was already rearranged in the germline (Vμj) (**Figure 1a**). In a recent *Science* paper[2], Miller joins forces with Rossjohn and Le Nours to report that TCRμ pairs with TCRγ to create a heterodimeric γμTCR. Crystal structures of two examples from the gray short-tailed opossum show that Vγ and $V\mu_j$ create a scaffold that supports the Vμ domain at the membrane distal region of the receptor. This novel research sets the scene to understand what roles these T cells have in the marsupial immune system, identify what type of ligands they recognise and discover why other mammals have lost this third type of TCR. In a world in which we are only just beginning to extend our own immune repertoire by co-opting other species immune recognition systems (e.g. camelid antibodies) and through de novo receptor design (e.g. chimeric antigen receptors), the marsuipial γμTCR provides a new source of nanobodies and its structure may inform receptor engineering designs in the future.

To define the architecture of a TCR containing TCRμ, Morrissey & Wegrecki *et al.* [2] set about determining which TCR chain pairs with TCRμ with a single cell transcriptome RNA sequencing campaign. After finding no TCRμ transcripts in peripheral blood T cells, they moved on to the spleen where 37% of cells contained TCRμ. The authors measured transcripts in these cells for the presence of TCRα, β, γ and δ chain transcripts. While the odd $TCR\mu^+$ T cell had transcripts of rearranged TCRβ and δ, all contained functional TCRγ transcripts confirming a pairing between TCRμ and TCRγ that was hypothesised because of TCRμ's similarity to TCRδ.

Further mining of the single cell dataset enabled the authors to look for co-receptor expression and TCR signalling chains (summarised in **Figure 1b**). γμ T cells are mostly $CD8\alpha\alpha^+$, with around a quarter having neither CD4 or CD8 transcripts, and γμ T cells contain the TCR receptor machinery required for signalling (CD3 ε, γ, δ, and ζ). The μ chain indeed contains the canonical basic residues required for productive CDδε and ζζ pairing[3], which strongly suggests the isolated γμ T cells are able to respond upon ligand recognition. That they are $CD8\alpha\alpha^+$ suggests that they may have innate characteristics. CD8αα is a poor coreceptor for MHC class I compared to

CD8αβ indicating that like γδ T cells they may recognise antigen directly rather than in combination with MHC class I[4]. It will be interesting to see further analysis of the transcriptome data as it no doubt provides a wealth of information on the activation state of these cells, and what cytokines and chemokines they produce or respond to.

The power of single cell transcriptomics also provides a wealth of information on the nature of V (variable), D (diversity), J (joining) recombination, and which splicing events are more heavily represented in both chains of the γμ T cells, as well as providing CDR3 (complementary determining region 3) loop analysis to provide clues as to what type of antigen these receptors might respond to. From this analysis, antigen engagement is likely restricted to the N-terminal variable domain (Vμ) of the TCRμ chain as the Vμj domain is germline encoded and thus invariant, and the TCRγ chains that pairs with TCRμ also seems to display less diversity than those paired with TCRδ in γδ T cells. In contrast, the CDR3 loop of Vμ is unusually long and most often incorporates three D segments (as opposed to the single D segments that are typical in αβ and γδ TCRs), providing a larger surface area for the direct engagement of antigen.

There is restricted usage of V(D)J segments in both the TCRμ and TCRγ chains found in γμ T cells, suggesting that these T cells are selected on antigen or that there are other factors at play that limit diversity. While the bulk of γμ T cells had TCRγ chains splicing Vγ2 and Jγ3, γδ T cells of the spleen mostly bore TCR with Vγ3.2 and Jγ1 splicing events indicating that γδ and γμ T cells likely have distinct biological functions. The TCRμ gene locus contains 5 clusters in which V, D, J, & C segments can be spliced; however, interrogation of ~30 γμ T cells only found evidence that clusters 3, 5 and 7 were used, with 57% using cluster 5, again hinting that these T cells recognise a restricted diversity of antigen. Care must be taken in extending these findings to all γμ T cells as only splenic γμ T cells were interrogated. Indeed γδ T cells tend to localise to different tissues depending on Vγ specificity[5] and γμ T cells if found in other tissues may do the same.

How a TCR chain with two variable and one constant domain heterodimerises with a TCR chain containing one variable and one constant domain is revealed by representative crystal structures of two γμ TCRs isolated from splenic γμ T cells. The structures show what looks like a typical

TCR with a tethered Vμ domain extending from the membrane, positioning the CDR loops outwards from the cell in an orientation consistent with its hypothesised role in antigen binding. The elongated CDR3 loop appears flexible, as density for some residues is weak. The two crystal structures orientate the Vμ domain differently atop the VμjVγ domain, but this may not be a physiological difference as each arrangment is likely to be enforced by crystal packing, which is very different between the two structures. The crystal structure that describes the more common Vγ2 γμ TCR heterodimer does show a contact between Vμ and Vγ2 in the form of a hydrogen bond, and a number of key contacts strengthen the interaction between Vμj and Vγ2 domains, perhaps explaining why this pairing is favoured in the majority of γμ T cells. Coupled with the restricted diversity of both the Vμj and Vγ domains, it appears likely that neither domain contributes to antigen binding, but instead provides a scaffold to orient the tethered and variable Vμ domain.

Marsupials and monotremes are not the only species to evolve a small antigen binding domain that is tethered to familiar structures used in antigen recognition. Cartilaginous fishes possess a similar TCR receptor termed γδ-NAR with a very similar architecture[6]. Indeed, a restricted γδ TCR pairing provides the ideal platform for the NAR domain which is spliced into the TCRδ transcript. Antibodies of camelids and sharks also have antigen recognition domains resembling the Vμ domain. Indeed, Vμ and Vμj are more structurally similar to antibody V domains than V domains from TCRδ. In line with Vμ domain being solvent exposed, polar residues in place of hydrophobic residues that would usually be buried in heterodimeric interfaces are present and suggest that the Vμ domain could be released from its hinge as a soluble domain and used as a marsupial-derived nanobody.

Identifying both the pairing partner for TCRμ and the most common splicing arrangement of TCRγ (at least in the spleen) sets the scene to understand the nature of the ligand γμ T cells respond to and what their role might be in the marsupial/monotreme immune system. As TCRα and β chains are rearranged before TCRγ chain in marsupials[7], it does not seem likely that they are examples of foetal T cells produced prior to the establishment of the mature adaptive immune system. One might speculate that γμ T cells play a specialised role in protecting marsupials while they develop in the pouch of their mother, but what of the similar receptor found in cartilaginous

fishes? Curiously, what is the selective pressure for marsupials and monotremes to keep this fifth TCR chain – and are Eutherian mammals, including us, missing out?

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FIGURE CAPTION

Figure 1. A comparison of the marsupial γμ TCR and its αβ and γδ cousins. **(a)** A schematic showing the somatic and germline rearrangements that make up TCRμ, its partner TCRγ and TCRδ. **(b)** A comparison of the TCR markers found in αβ, γδ and γμ splenic T cells. The CD3 chains associated with γδ and γμ TCRs are inferred by the presence of the same motifs that are

well-established to heterodimerise TCRα and β transmembrane domains[8] to provide a platform for CD3 and ζζ recruitment.

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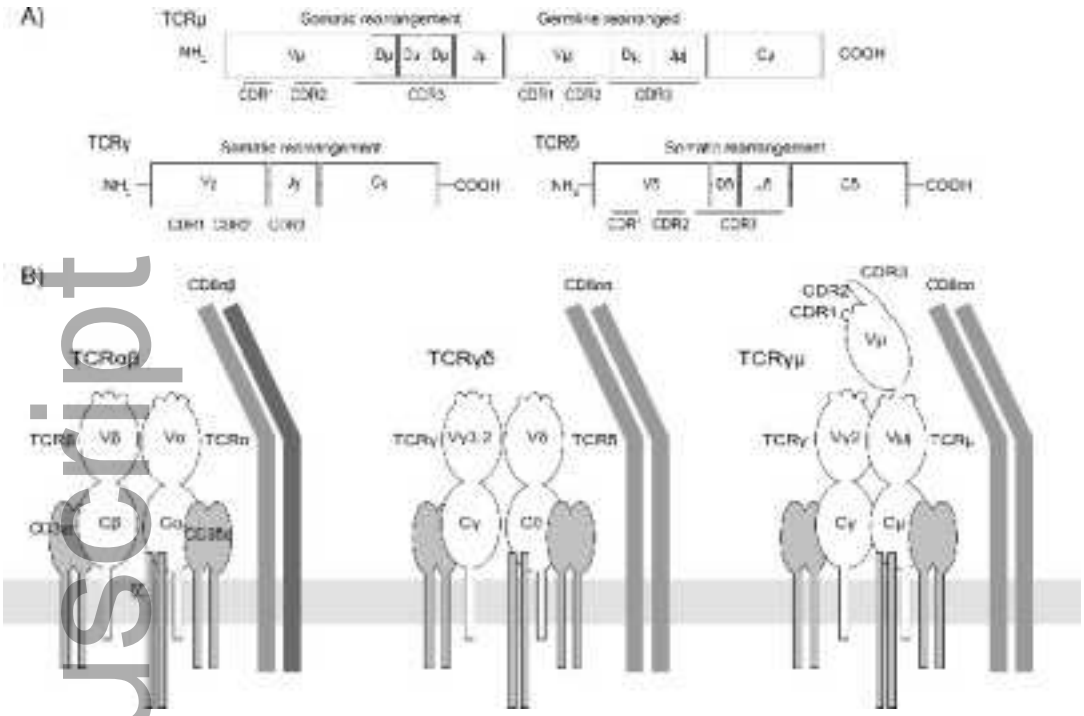

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