Does selecting ligand shape γδ-TCR repertoire?

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In a recent publication entitled "Role of a selecting ligand in shaping the murine γδ-TCR repertoire," Fahl et al. (1) report that the expression of nonclassic MHC T10 and its related molecule T22 influences the T10/T22 specific γδ-T cell receptor (TCR) repertoire. We maintain that caveats in their experimental approaches and analysis significantly limit what can be inferred from these data with regards to the role of ligand expression in shaping γδ-TCR repertoire.

The study (1) claims that "Reactivity of the γδ-TCR with . . . H2-T10/22, is critically dependent upon the EGYEL motif in the complementarity determining region 3 (CDR3) region of TCRδ. In the absence of H2-T10/T22 ligand, the commitment of H2-T10/22 reactive γδ-T cells . . . exhibit a profound alteration in the γδ-TCR repertoire, including depletion of the EGYEL motif and reductions in both CDR3δ length and charge." However, the TCRδ motif for T10/T22-specific γδ-TCRs is W...EGYEL (2), not EGYEL. The W...EGYEL motif was assembled by gene rearrangement using the V_δ segment (W) or the $D_\delta1$ segment (W) together with the $D_\delta2$ segment (EGYE) and P nucleotides (L). Importantly, the Trp (W) residue in the motif is critical for T10/T22 binding (3).

Despite asserting that T10/T22-specific γδ-TCRs have the EGYEL motif, Fahl et al. (1) also report: "It is important to note that the majority of T22-reactive, committed, and mature γδ-T cells developing in H2T-expressing mice express a TCRδ subunit that lacks the EGYEL motif, suggesting that additional motifs are capable of supporting T22 reactivity." If this is the case, what are the T10/T22-specific γδ-TCR sequences in mice with and without T10/T22 expression? What is the rationale for comparing the frequencies of the EGYEL motif in T10/T22-specific γδ-TCRs in wild-type and mutant mice? It should be pointed out that, while TCRs with the TCRδ W...EGYEL motif can bind T10/T22 (3), not all T10/T22-specific γδ-TCRs have this motif (2).

Fahl et al. (1) use cDNA-based bulk sequencing to determine T10/T22-specific γδ-TCR sequences. This approach does not adequately estimate the abundance of T cells with a given TCR, because the frequencies of cDNA encoding a given TCR sequence do not correlate with the abundance of the T cells alone. Rather, it also reflects the abundance of TCR mRNA within the T cells. It is well established that TCR mRNA levels in individual T cells are variable. Single-cell TCR determination or bulk sequencing using genomic DNA as templates (to better determine the abundance) have been in use for some time, and yet these techniques were not applied in this study. Moreover, there is no report of how many T10/T22-specific γδ-T cells were sorted for sequencing, the purity of the cell population, sequencing depth, or the numbers of biological repeats. It is also unclear how many TCR sequences were obtained and how many were analyzed. No TCR sequences are reported in either Fahl et al.'s main text or supplemental results. TCR sequences made available on the public website show only the primary DNA sequences without annotation.

1 Fahl SP, et al. (2018) Role of a selecting ligand in shaping the murine γδ-TCR repertoire. *Proc Natl Acad Sci USA* 115:1889–1894.
2 Shin S, et al. (2005) Antigen recognition determinants of gammadelta T cell receptors. *Science* 308:252–255.
3 Adams EJ, Strop P, Shin S, Chien YH, Garcia KC (2008) An autonomous CDR3delta is sufficient for recognition of the nonclassical MHC class I molecules T10 and T22 by gammadelta T cells. *Nat Immunol* 9:777–784.

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