Addendum

Highly diverse TCR α chain repertoire of pre-immune CD8⁺ T cells reveals new insights in gene recombination

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The TCR $\alpha\delta$ locus undergoes TCR δ gene recombination on DN thymocytes. Because TRDV segments are scattered through the TCR $\alpha\delta$ locus, and can coincide with TRAV segments, this can result in variable truncations of the locus (Addendum Figure 1A, Sleckman *et al*, 1998, Krangel, 2009). While most (twelve) of the TRDV and TRAV/DV genes are located in the 3' portion of the locus, some (four) are located upstream (i.e. TRAV14D-3/DV8 (68), TRAV6D/DV11 (70), TRAV15D-2/DV6D-2 (79) and TRAV15D-1/DV6D-1 (87)) (<http://www.imgt.org/IMGTrepertoire/LocusGenes/>). Therefore, if secondary TRAV–TRAJ gene recombination on DP thymocytes proceeded in a coordinated sequential manner, their 'starting points' would be different, which might affect the conclusion reached in our recent article (Genolet *et al*, 2012). To examine this, we re-analyzed our data.

An implication of such TRAV locus truncations is that distal TRAV segments would recombine more frequently than proximal ones. Plotting the TRAV–TRAJ recombination frequency against functional TRAV genes indicated that several proximal TRAV exhibited low recombination frequencies (Addendum Figure 1B). This is consistent with the said TRAV locus truncations; however, there were also low recombination frequencies of TRAV segments located upstream i.e. past $\frac{1}{4}$ of TRDV segments (e.g. TRAV6-6 (30), TRAV7-4 (35), TRAV14N-1, TRAV14D-2 (80) and TRAV11D (92)). Moreover, a large part of the TRAV locus (TRAV14-1 (25) to TRAV7N-4 (67)) harbors no TRDV segments and hence rearrangements of these 42 TRAV should exhibit a bias, if they were coordinate. The recombination frequencies of these TRAV genes depended dramatically on TRAV usage (Figure 2C in Genolet *et al*, 2012). To compensate for this, we normalized the recombination frequencies, such that their sum is 100% for each TRAV segment. For these TRAV segments, the resulting 2D plot revealed remarkably similar distributions of recombination frequencies and prominent variations in TRAJ usage, i.e. some TRAJ segments were overrepresented and others underrepresented (Addendum Figure 1C). This pattern is inconsistent with secondary TRAV–TRAJ gene recombination proceeding in a coordinated fashion, which predicts that TRAJ gene usage is skewed from proximal to distal TRAJ for progressively more distal TRAV segments. Moreover, it predicts that the proximal TRAV14-3 (6) preferentially rearranges with proximal TRAJ segments. However, our sequencing data indicated preferential recombination with distal TRAJ segments (Addendum Figure 1D). Related divergences were observed for other TRAV genes, e.g. TRAV14D-3 (68) or TRAV6D-6 (93) (Figure 2C in Genolet *et al*, 2012). Collectively, these additional analyses better support a model according to which DNA looping in the TRAV locus provides equal opportunities for all TRAV genes to recombine (Chaumeil and Skok, 2012).

Addendum Figure 1 Impact of TCR δ recombination on TRAV-TRAJ rearrangements. **(A)** Cartoon of truncations of the TRAV/TRDV locus by TRDV-TRDD rearrangements on DN thymocytes. **(B)** The frequencies of all TRAV-TRAJ recombinants (y-axis; % of total) are plotted against TRAV gene segments, listed in order of their chromosomal location (x-axis). For TRAV numbering (from proximal to distal). The 10 TRAV segments that are also used for TRDV recombination are marked in red and highlighted with an asterisk. **(C)** The frequency of recombination of TRAV14-1 (25) to TRAV7N-4 (67) with TRAJ genes are represented in a 2D graph, in which functional TRAV and TRAJ segments are plotted on the x- and y-axis, respectively. The recombination frequencies are normalized such that for each TRAV segment the sum of its recombination is 100% and are ranked by the colour code as defined on the bar. **(D)** The frequencies of recombination of TRAV14-3 (6) (y-axis; % of total) are plotted against the TRAJ genes listed on the x-axis in order of their chromosomal location.

References

- Chaumeil J, Skok JA (2012) Equal opportunity for all. *EMBO J* **31**: 1627–1629
- Genolet R, Stevenson BJ, Farinelli L, Østerås M, Luescher IF (2012) Highly diverse TCR α chain repertoire of pre-immune CD8 $^{+}$ T cells reveals new insights in gene recombination. *EMBO J* **31**: 1666–1678
- Krangel MS (2009) Mechanics of T cell receptor gene rearrangement. *Curr Opin Immunol* **21**: 133–139
- Sleckman BP, Khor B, Monroe R, Alt FW (1998) Assembly of productive T cell receptor delta variable region genes exhibits allelic inclusion. *J Exp Med* **188**: 1465–1471