

## Preprint watch

# Distinct human T follicular regulatory cell subsets with helper and regulatory lineage origins

Germinal centre (GC) responses, during which CD4<sup>+</sup> CXCR5<sup>hi</sup>PD-1<sup>hi</sup> T follicular helper (Tfh) cells provide help to B cells, are crucial for the development of affinity-matured antibodies and memory B cells, but they require careful regulation to limit autoantibody generation. Regulatory cells in/around GCs include a subset of CD4<sup>+</sup> Foxp3<sup>+</sup>CD25<sup>hi</sup> regulatory T (Treg) cells termed follicular regulatory T (Tfr) cells whose access to B cell follicles is facilitated by CXCR5 upregulation. Intriguingly, Tfr cells can suppress and/or enhance humoral responses.

Treg cells can be thymically-derived (tTreg) cells or develop from mature T cells that acquire stable Foxp3 expression in the periphery. Tfr cells were initially assumed to differentiate from tTreg cells. However, they express the Tfh lineage-defining transcriptional repressor BCL-6 and Tfh markers CXCR5, PD-1, and ICOS, and T cell receptor (TCR) analysis indicates some clonal overlap between Tfr and Tfh cells in mice, suggesting alternative pathways for Tfr generation.

In this preprint (not peer-reviewed), Le Coz and colleagues identified a human CD4<sup>+</sup> Tfr subset originating from Tfh-lineage cells, providing compelling evidence that Tfr cells can develop from both Tfh and Treg lineages. They exploited single-cell transcript profiling of CD25<sup>-</sup> Tfh, CD25<sup>hi</sup> Tfh, CD25<sup>hi</sup>CXCR5<sup>+</sup>PD-1<sup>int</sup> Tfr, and CD25<sup>hi</sup>CXCR5<sup>-</sup>PD-1<sup>-</sup> Treg cells sorted from paediatric tonsils, coupled with analysis of Treg and Tfh populations in a tonsil organoid immunisation model, to identify two distinct Tfr development pathways: a Treg-to-Tfr route generating “natural” nTfr cells, and a Tfh-lineage differentiation axis involving induction via CD25<sup>hi</sup> Tfh intermediates of a Foxp3-expressing iTfr subset. Clonal overlap between nTfr and Treg (but not Tfh) and between iTfr and Tfh (but not Treg) cells further supported seeding of the Tfr pool by both Treg and Tfh-lineage cells.

Transcript profiling and CITE-seq analyses identified CD38 as an iTfr-distinguishing cell-surface marker. CD38<sup>-</sup> nTfr cells exhibited T cell-inhibitory activity exceeding that of Treg cells, whilst CD38<sup>+</sup> iTfr cells acquired T cell-inhibitory activity comparable to that of Treg cells but were also able to help co-cultured GC B cells produce antibodies, partly via IL-21/10 production. Notably, multiplexed imaging and CODEX analysis located Tfh-like CD38<sup>+</sup> iTfr cells in GCs while Treg-like CD38<sup>-</sup> nTfr cells were in follicular mantles.

In summary, Tfh lineage-derived CD38<sup>+</sup> iTfr cells acquire suppressive function while retaining B cell help capacity, whereas Treg-derived CD38<sup>-</sup> nTfr cells mediate elite T-cell suppressor activity outside GCs. The mechanisms via and extent to which iTfr and nTfr cells contribute to shaping GC outputs at different stages of an immune response remain unclear. Dissecting these should inform design of next-generation vaccination strategies and precision immunotherapies.

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**Original article:** Le Coz, C. et al. Human T follicular helper clones seed the germinal center-resident regulatory pool. *bioRxiv. Journal* <https://doi.org/10.1101/2022.10.26.513910> (2022)

**Related articles:** Nguyen, Q. N. N. Preprint Journal Club. *PreprintClub* <https://www.preprintclub.com/2023-jan-Le-Coz> (2023)

Quote: “Interventions differentially targeting Tfr subsets may provide therapeutic opportunities to boost immunity or more precisely treat autoimmune diseases”