Understanding the mechanisms that determine the ability of CD8⁺ T cells to infiltrate tumours is paramount to improving responses to cancer immunotherapy. Jansen et al. show in patients with cancer that intratumoural stem-like CD8⁺ T cells contribute to the immune response by giving rise to terminally differentiated T cells and reside in antigen-presenting cell (APC) niches – the lack of which in patients is associated with progressive disease.

The authors started out by analysing the composition of populations of tumour-infiltrating CD8⁺ T cells in patients with kidney cancer, using flow cytometry. The authors identified two distinct populations of CD8⁺ T cells based on expression levels of checkpoint molecules and key transcription factors. One population of CD8⁺ T cells had high expression levels of the checkpoint molecules TIM3, PD1, CTLA4 and TIGIT (PD1⁺TIM3⁺ cells), similar to the exhausted T cell phenotype, whereas another population had low expression levels of checkpoint molecules but high expression levels of co-stimulatory CD28 and the transcription factor TCF1, likely representing previously described stem-like T cells (TCF1⁺TIM3⁺CD28⁺ cells). In vitro analysis of the proliferative potential of these cell populations showed that TCF1⁺TIM3⁺CD28⁺ cells proliferated upon stimulation, whereas PD1⁺TIM3⁺ cells did not. Moreover, TCF1⁺TIM3⁺CD28⁺ cells, upon proliferation, upregulated checkpoint molecule expression, indicating that upon proliferation, they acquire a more terminally

differentiated phenotype. Also, there was significant overlap of T cell receptor in stem-like and terminally differentiated T cell populations from distant sites within tumours, indicating that stem-like T cells give rise to terminally differentiated T cells in the tumour.

Overall, the CD8⁺ T cell infiltration in tumours relative to the total number of cells in the tumour ranged from 0.002% to 20% in the study's patient cohort. In tumours with high CD8⁺ T cell infiltration, PD1⁺TIM3⁺ cells were consistently present, but not in tumours with poor CD8⁺ T cell infiltration, across patients with kidney, prostate or bladder cancer. By contrast, TCF1⁺TIM3⁺CD28⁺ cells were consistently detected in tumours with poor infiltration, albeit at very low numbers, overall supporting a model in which stem-like T cells give rise to terminally differentiated T cells in tumours and thereby support the T cell response.

RNA sequencing analysis of the identified T cell populations showed that terminally differentiated T cells expressed higher levels of granzymes and perforin than stem-like T cells, which expressed higher levels of survival genes (*IL7R* and *IL2RA*). Of note, the gene expression profiles were similar to those of previously described stem-like and terminally differentiated exhausted T cells from mice with chronic lymphocytic choriomeningitis virus (LCMV) infection. Moreover, whole-genome DNA methylation analysis revealed demethylation events in key genes including those encoding TCF1 and PD1 during transition from stem-like to differentiated T cells.

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Next, the authors explored how stem-like T cells, which are known to reside in lymphoid tissue in LCMV-infected mice, are able to survive in the microenvironment of kidney, prostate or bladder tumours. TCF1⁺CD8⁺ T cells correlated in number with the presence of dendritic cell populations across those tumours and preferentially resided in zones of tumours that were APC dense, based on expression of major histocompatibility complex II (MHCII). There were multiple of these MHCII dense regions across larger tumour sections. TCF1⁺CD8⁺ T cells however were distributed across the tumour tissue, without preference for MHCII dense regions. In addition to correlating with the number of CD8⁺ T cells in the tumour, these APC niches were located inside the stromal barrier of the tumour. This is in contrast to tertiary lymphoid structures (TLS), which were found in 5 out of 33 patients, usually outside the tumour border, and the presence of which did not correlate with CD8⁺ T cell infiltration. Also, APC niches more closely resembled extra-follicular regions of lymphoid tissue where T cells reside, whereas TLS more closely resembled B cell follicles, as shown by immunofluorescence.

Was the presence of intratumoural APC niches correlated with disease progression? In patients with kidney cancer that had undergone surgery, APC niches with resident TCF1⁺CD8⁺ T cells were identified in all patients, yet the number of these MHCII dense zones was significantly higher in patients with controlled disease than in patients with progressive disease, and the levels of MHCII density could be used to stratify patients with progressive disease.

This study offers valuable insight into the phenotype of intratumoural stem-like CD8⁺ T cells and its relationship to the exhausted CD8⁺ T cell phenotype in human cancer. Yet, the origin of intratumoural stem-like CD8⁺ T cells and how APC niches are maintained remain unclear.

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