TUMOUR IMMUNOLOGY

States of exhaustion

To what extent T cell dysfunction in tumours resembles T cell exhaustion in chronic viral infections, and the mechanisms by which immune checkpoint blockade improves tumour immune surveillance even when T cells are dysfunctional, is poorly understood. In a study published in *Nature Immunology*, Miller, Sen et al. identify a subpopulation of dysfunctional or exhausted CD8+ tumour-infiltrating lymphocytes (TILs) that are polyfunctional and respond to anti-programmed cell death 1 (PD1) therapy. In response to anti-PD1, this subpopulation gives rise to the majority of cytotoxic terminally exhausted TILs.

The authors first compared exhausted CD8+ T cells from mice during chronic infection with lymphocytic choriomeningitis virus (LCMV) with CD8+ T cells isolated from ovalbumin-expressing B16F10 (B16-OVA) mouse melanoma tumours by single-cell expression analysis. Among exhausted CD8+ T cells in LCMV infections, clusters of four subpopulations were found, all of which expressed a T cell exhaustion signature (including *Pd1* and *Tox*). These subpopulations included stem-like or progenitor CD8+ T cells (referred to as progenitor exhausted CD8+ T cells or T_{PE} cells from hereon) and terminally exhausted CD8+ T cells (T_{TE} cells). When analysing TILs, signatures derived from LCMV T_{PE} cells (expressing *Tcf7* (which encodes transcription factor 7 (TCF7; also known as TCF1)) and the gene encoding DNA-binding protein inhibitor ID3) and T_{TE} cells (expressing *Tim3* (which encodes T cell membrane protein 3 (TIM3)) were significantly enriched. For isolation of live T cells and flow cytometry analyses, the authors used the cell surface marker SLAMF6 for T_{PE} cells as it was highly co-expressed with TCF1 in this cell population but not in T_{TE} cells. Gene expression profiles of the corresponding two subpopulations overlapped significantly between TILs and LCMV T cells. However, the two subpopulations were distinct in their transcriptional and phenotypical state and maintained by distinct epigenetic states: T_{PE} cells and T_{TE} cells were distinguishable based on their profiles of chromatin-accessible regions (ChARs), with 13,340 ChARs unique to T_{PE} cells and 8,085 ChARs unique to T_{TE} cells in both tumour and LCMV T cells. These ChARs were associated with genes regulating cytokine production, survival and memory in T_{PE} cells, and cell division, apoptosis and cytotoxicity in T_{TE} cells. The authors then turned their attention to T_{PE} and T_{TE} biology in tumour-bearing mice. In growing tumours, the abundance of T_{TE} relative to T_{PE} cells increased. While the T cell receptor (TCR) repertoire was less diverse in T_{TE} cells than in T_{PE} cells, it overlapped by 50%. In addition, when SLAMF6+TIM3− T_{PE} cells were transferred into tumour-bearing congenic mice carrying the differential *Ptprc*[a] pan-leukocyte marker, SLAMF6+ as well as TIM3+ T cells were recovered 16 days later. TIM3+ T cells were more cytotoxic, meaning they produced more interferon-γ and granzyme B in vitro than SLAMF6+TIM3− T_{PE} cells.

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This suggests that T_{PE} cells differentiate into T_{TE} cells in response to TCR stimulation. Indeed, when SLAMF6+TIM3− T_{PE} cells were transferred into naive mice, in which no antigen would be present and no TCR stimulation occurred, only SLAMF6+TIM3− T cells could be recovered from the spleens 30–40 days later. Also, whereas transferred T_{PE} cells persisted without antigen presence, T_{TE} cells did not.

When naive mice were implanted with B16-OVA tumours 30–40 days after having received T_{PE} cells, T_{PE} cells trafficked into the tumour tissue and proliferated there, as indicated by the increased number of T_{PE} cells recovered from tumour tissue compared with secondary lymphoid organs. Moreover, tumours in mice that received T_{PE} cells as opposed to T_{TE} cells grew slower — likely a sign of the improved ability of T_{PE} cells to proliferate and survive and continuously replenish cytotoxic T_{TE} cells. In response to anti-PD1 treatment in tumour-bearing congenically marked mice, transferred T_{PE} cells expanded significantly, whereas T_{TE} cells did not. T_{PE} cells also converted into the terminally exhausted phenotype at a higher rate than in control tumours.

In patients with melanoma, CD8+ T cell populations expressing TCF1 and PD1, indicative of the T_{PE} cell type, were present in almost all biopsy samples before immune checkpoint blockade therapy. Also, a higher ratio of TCF1+ cells among the total population of PD1+CD8+ T cells positively correlated with prolonged progression-free survival and overall survival on therapy.

T cell exhaustion and the heterogeneity of exhausted T cell populations in tumours are a mirror of the T cell exhaustion and heterogeneity appearing in chronic viral infections. These findings can translate into improved strategies for PD1 blockade, in which the expansion of T_{PE} cells in patients can become a central aim in therapeutic strategies to improve outcomes.

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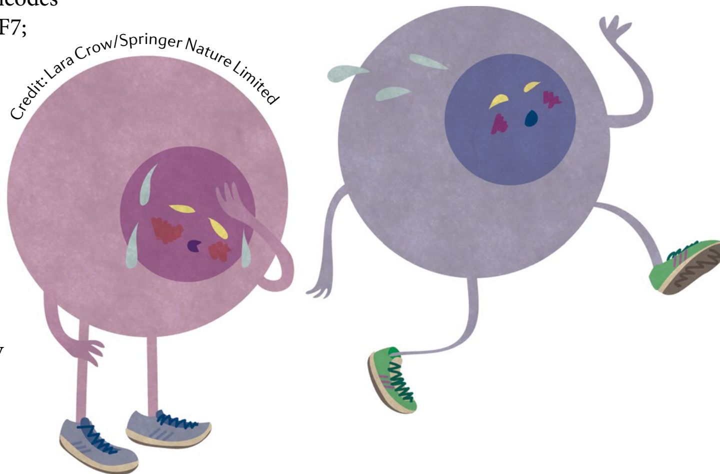
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