Preprint Digest Form

Title

PD-1 controls differentiation, survival, and TCR affinity evolution of stem-like CD8+ T cells

Keywords

- Stem-like T cells
- PD-1 regulation
- Tumour immunotherapy

Main Findings

In the face of tumour and viral challenge, stem-like TCF-1^{+}PD-1^{+} cells (T_{SL}) represent a critical CD8^{+} subset capable of self-renewal and replenishing intratumoural cytotoxic effector T cells. Crucially, T_{SL} are reported to expand upon immune checkpoint blockade, thus potentiating therapeutic recovery of anti-tumour T cell responses. In a recent pre-print, Hor et al. deeply interrogate stem-like T cell niches in tumour-draining lymph nodes (tDLNs), defining a SLAMF6$^{+}T_{SL}$ subset with high TCR affinity. Maintenance of this subset is governed by regulatory PD-1 signalling and prolonged cDC1 association.

The authors leveraged 3D multiplex immunofluorescence imaging workflows in optically cleared tDLNs of mice bearing subcutaneous KP-OVA tumours (Kras$^{G12D/+}$/Trp53$^{-/-}$ derived lung adenocarcinoma), enabling spatial and functional profiling of antigen-targeting CD8^{+} T cells. The use of an XCR1-Venus host model allowed additional visualisation of XCR1^{+} cDC1 populations.

In tumour-inoculated mice adoptively transferred with naïve OT-1 cells, distinct clusters of cDC1-interacting SLAMF6^{+}TCF-1^{+}PD-1^{+} T_{SL} subsets were observed resident in T cell zones of tDLNs. These populations exhibited elevated TCR signalling, signified by nuclear localisation of NFAT. The essential role for cDC1 in maintaining SLAMF6$^{+}T_{SL}$ was highlighted by their collapse upon selective cDC1 depletion, using diptheria toxin treatment in tumour-inoculated XCR1-DTR mice.

Persistent late-stage (post-priming) antigen presentation resulting from cDC1 exposure was argued to drive elevated TCR affinity within SLAMF6$^{+}T_{SL}$ pools. Despite their chronic engagement in cDC1 tDLN niches, SLAMF6$^{+}T_{SL}$ cells maintained quiescent profiles, attributed by the authors to their heightened PD-1 signalling. PD-1 activation was inferred by PD-1 surface clustering and T cell-cDC1 PD-1/PD-L1 co-localisation. Consistently, inhibition of this axis in the KP-OVA model by αPD-L1/L2 or αPD-1 antibody administration induced dramatic loss of high affinity SLAMF6$^{+}T_{SL}$ subsets, and instead triggered proliferative expansion of heterogenous SLAMF6^{-}TCF-1^{+} populations and TCF-1^{-} effector cells. To address potential experimental variability, Bayesian linear modelling was performed which validated

PD-1 signalling as the critical driver of SLAMF6^{+}T$_{SL}$ abundance and TCR affinity in this system. Moreover, CD4^{+} depletion experiments highlighted that this effect of PD-1 blockade on T$_{SL}$ phenotype was independent of PD-1-expressing CD4^{+} compartments (e.g. regulatory T cells). PD-L1 inhibition alone also did not result in loss of SLAMF6^{+}T$_{SL}$ populations.

In summary, the current study challenges the prevailing dogma that chronic antigen stimulation and TCR engagement triggers terminal differentiation by default. Instead, PD-1-mediated TCR regulation enables the persistence of high affinity SLAMF6^{+}T$_{SL}$ clones in tDLNs, which evolve upon association with antigen-enriched cDC1 hubs. This enables continued replenishment of high affinity tumour-targeting effector progeny. The work here advocates for further investigation of nuanced PD-1-targeting treatment regimes, that balance anti-tumour efficacy with the preservation of high affinity T$_{SL}$ populations, calling for future translational research into the clinical impact of these subsets.

Limitations

A key question remains concerning the therapeutic value of the SLAMF6^{+} T cell compartment. Although their high affinity for tumour antigens suggests a positive role in anti-tumour responses, explicitly demonstrating their direct impact on tumour control would strengthen the findings. Future work could focus on specifically depleting these populations and assessing tumour growth, or phenotyping effector progeny to evaluate enhanced effector functions.

Significance/Novelty

This study reveals the pivotal role of PD-1 signalling in sustaining high affinity stem-like T cells during chronic antigen stimulation. This may encourage the re-evaluation of current strategies for PD-1 checkpoint blockade, suggesting the implementation of dosing regimes which allow for the preservation of high-affinity stem-like T cell populations.

By leveraging a unique, high-dimensional imaging workflow, the authors were able to provide detailed understanding of the spatial distribution, as well as the functional profile of stem-like T cells, and their relation to cDC1 populations (previously undefined).

This builds on foundational work by Tyler Jacks' group, indicating that therapeutic recovery of migratory DCs restores SLAMF6^{+} stem-like T cell populations during tumour progression (Schenkel et al., 2021, https://doi.org/10.1016/j.immuni.2021.08.026).

Preprint rating

- 5
- 5
- 4

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Credit

Reviewed by **Matthew Jackson** and **Eileen Parkes** as part of a cross-institutional journal club between the Icahn School of Medicine at Mount Sinai, the University of Oxford, the Karolinska Institute and the University of Toronto.

The author declares no conflict of interests in relation to their involvement in the review.