IMMUNOTHERAPY

T_H9 cells tackle advanced tumours

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Therapeutic approaches using adoptive cell therapy (ACT) with tumour-specific T cells have shown some promising results in cancer patients, but they are rarely curative. Now, reporting in *Cancer Cell*, Qing Yi and colleagues show that ACT with $CD4^+$ T helper 9 (T_H9) cells can completely eradicate large established tumours in mouse models of melanoma.

Currently, most anticancer ACT protocols use $CD8^+$ cytotoxic T lymphocytes (CTLs), but are hampered by the side effects of IL-2, which needs to be administered systemically to ensure CTL survival. $CD4^+$ T cells, which don't require IL-2, may also hold promise for ACT, and studies so far have focused on tumour-specific T_H1 and T_H17 cells. T_H1 cells can be potently cytolytic, but ex vivo generated tumour-specific T_H1 cells display an exhausted phenotype and low persistence after transfer. T_H17 cells, which have an early memory and/or stem cell phenotype, are less cytolytic than T_H1 cells, but outperform them owing to much higher persistence in vivo. However, there is concern that some T_H17 cells may convert into regulatory T cells.

The authors had previously shown that T_H9 cells have anticancer properties and can promote CTL-mediated anticancer activity. Now they compare the suitability of tumour-specific T_H1, T_H17 and T_H9 cells for ACT in mouse models of melanoma (the B16-OVA and the less immunogenic B16 model, using T_H cells derived from naive OVA-specific or tyrosine-related protein 1-specific $CD4^+$ cells, respectively). Similar to ACT protocols in the clinic, mice were treated with cyclophosphamide before transfer to induce temporary lymphopaenia and also received tumour antigen-loaded dendritic cells (DCs) to boost antitumour responses.

Strikingly, in both melanoma models, only T_H9 cells induced significant regression of large established tumours and allowed for long-term survival. They also protected mice against re-challenge with tumour cells. In contrast, ACT with T_H1 and T_H17 cells only led to temporary tumour regression, followed by aggressive regrowth. The antitumour response in mice treated with T_H9 cells did not depend on CTLs, as antitumour responses were only mildly impaired in $CD8^+$ T cell-deficient mice, and did not require IL-9 or IFNγ expression, as shown with *Il9*$^{-/-}$ and *Ifng*$^{-/-}$ T_H9 cells. They did, however, require the co-administration of DCs.

To investigate the molecular underpinnings of the superior performance of the T_H9 cells, the transferred cells were retrieved 12 days after ACT and subjected to genetic and molecular analysis. T_H9 cells did not share the 'exhaustion gene signature' of T_H1 cells and expressed higher levels of co-stimulatory molecules and much lower levels of inhibitory receptors (such as PD1, LAG3, KLRG1 and CD244) than T_H1 cells. Their gene signature also suggested that T_H9 cells are mature effector T cells, but not terminally differentiated like T_H1 cells.

Interestingly, T_H9 cells expressed high levels of eomesodermin (EOMES), a transcription factor that indicates effector cell development and is also known as a master controller of granzyme expression. Compared with T_H1 and T_H17 cells, T_H9 cells expressed the highest levels of granzymes and showed the most potent tumour-specific cytolytic activity — which depended on the presence of EOMES and granzyme B.

Transferred T_H9 cells showed extraordinary persistence in vivo, equal to or better than T_H17 cells. The persistence of T_H17 cells is attributed to their early memory or stem cell-like features and enhanced resistance to apoptosis. In contrast, T_H9 cells appeared to persist owing to hyperproliferation.

Further analysis revealed that the hyperproliferation was driven by late-phase nuclear factor-κB (NF-κB) hyperactivation, activated by high levels of the ubiquitin ligase TRAF6. The high expression of TRAF6 appeared to be due to epigenetic changes in the *Traf6* promoter, opening it up to transcription factors such as PU.1. The authors speculate that accumulated TRAF6 serves as a crucial adaptor that links to the MALT1–CARMA1–BCL-10 complex downstream of the T cell receptor and upstream of NF-κB.

Overall, the study shows that T_H9 cells display an ideal combination of T_H1-like cytolytic and T_H17-like persistence characteristics, which may render them particularly suitable for ACT therapies.

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