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IMMUNOTHERAPY

# Throwing $T_{reg}$ cells off balance

> intratumoural $T_{reg}$ cells lose their immunosuppressive function

In the tumour microenvironment (TME), infiltrating effector T ($T_{eff}$) cells function to control tumour growth whilst infiltrating regulatory T ($T_{reg}$) cells act to suppress the immune responses elicited by $T_{eff}$ cells, therein promoting tumour progression. As a consequence the maximal antitumour activity of $T_{eff}$ cells will likely be limited even under immune checkpoint blockade. Hence, one approach to improve patient outcomes to immune checkpoint therapies could be to target tumour-reactive $T_{reg}$ cells. However, complete $T_{reg}$ depletion would result in systemic autoimmunity. To avoid this obstacle, Di Pilato et al. selectively modulated the function of $T_{reg}$ cells in tumours by targeting the CARMA1–BCL10–MALT1 (CBM) signalosome complex in only a fraction of $T_{reg}$ cells and found that this was sufficient to impede tumour growth and prime the TME for immune checkpoint blockade.

In searching for T cell receptor signalling components that could be disrupted to weaken intratumoural $T_{reg}$ cells, the authors focused their attention on the CBM complex, given its key role in controlling lymphocyte activation, proliferation and function. They observed that conditionally deleting one or both copies of the gene encoding the scaffold protein CARMA1 in mature FOXP3$^+$ $T_{reg}$ cells in mice proportionately decreased levels of CARMA1 in CD4$^+$FOXP3$^+$ $T_{reg}$ cells in the lymph nodes (LNs). As expected, complete loss of CARMA1 in $T_{reg}$ cells was fatal, in line with the essential role played by $T_{reg}$ cells in sustaining immune tolerance. However, up to a 50% loss in the expression of CARMA1 could be tolerated.

Next, to investigate how CARMA1-deficient $T_{reg}$ cells might influence tumour development, D4M.3A mouse melanoma or MC38 mouse colon adenocarcinoma cells were subcutaneously implanted into mice with partially or fully CARMA1-depleted $T_{reg}$ cells. This resulted in a pronounced reduction in tumour growth compared with mice carrying CARMA1-sufficient $T_{reg}$ cells. Interestingly the CARMA1-deficient $T_{reg}$ cells present in the tumour tissue secreted the inflammatory cytokines interferon-γ (IFNγ) and tumour necrosis factor (TNF), indicative of a gain of effector function in situ. Strikingly, this reprogramming of $T_{reg}$ cells was specific to the TME as CARMA1-deficient $T_{reg}$ cells in tumour draining LNs (tdLNs) or non-lymphoid tissues did not secrete effector cytokines.

Furthermore, intravenous injection of CARMA1-depleted $T_{reg}$ cells but not CARMA1-sufficient $T_{reg}$ cells into D4M.3A tumour-bearing wild-type or *Ifng*$^{-/-}$ mice could attenuate tumour growth in both hosts. Yet, the inhibition of tumour growth in *Ifng*$^{-/-}$ mice could be reversed upon neutralization of IFNγ with an IFNγ antibody, confirming the requirement for this cytokine in the antitumour activity of the converted intratumoural $T_{reg}$ cells. Even if tumours were already established and further $T_{reg}$ cell egress from tdLNs was blocked prior to CARMA1 deletion in $T_{reg}$ cells, subsequent tumour growth was impeded. Additionally, the antitumour phenotype was associated with increased major histocompatibility complex class II (MHC II) expression on macrophages as well as increased MHC I and programmed cell death 1 ligand 1 (PDL1) expression on tumour cells.

To assess whether this strategy had potential therapeutic applicability, the authors chose to pharmacologically inhibit the CBM complex. As CARMA1 inhibitors do not currently exist, the activity of two MALT1 protease inhibitors, mepazine and MI-2, were instead tested on D4M.3A allografts. Both inhibitors attenuated melanoma growth to the same degree as that following genetic targeting of CARMA1 in $T_{reg}$ cells. As MALT1 inhibitors would be expected to block lymphocyte effector functions, this outcome is likely to be specific to the weakened $T_{reg}$ cells — this is reinforced by the finding that decreases in tumour growth were not observed in RAG1-deficient mice, which lack lymphocytes. Mepazine treatment also induced expression of MHC I and PDL1 on tumour cells, expression of *Ifng* and IFNγ-associated genes in tumour tissue, and, importantly, increased infiltration of cytotoxic T lymphocytes and natural killer cells into tumours.

As T helper 1 ($T_H1$)-type inflammation and adaptive immune resistance simultaneously ensued following targeting of the CBM complex, this suggested tumours could be sensitized to subsequent programmed cell death protein 1 (PD1) blockade. Indeed, combining a PD1 antibody with CARMA1 deletion or MALT1 inhibition in $T_{reg}$ cells produced much more effective, synergistic control of the otherwise poorly immunogenic D4M.3A tumours than with mepazine alone. This outcome was also reproduced by treating mice bearing MC38 tumours with the combination; in this case, most mice were free from relapse for more than 12 months after treatment had stopped.

An interesting question that arises from this study is why only intratumoural $T_{reg}$ cells lose their immunosuppressive function when the CBM complex is disrupted. Once answered, there is promise that the reprogramming of $T_{reg}$ cells into IFNγ-secreting inflammatory effector cells could be successfully translated to the clinic.

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