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Rewiring regulatory T cells for tumour killing

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Abstract

In mouse models of cancer, the inhibition of a set of regulatory proteins improves checkpoint-blockade therapy by causing regulatory T cells to produce the cytokine interferon-γ.

Immune surveillance is the first line of the body's defence against the uncontrolled proliferation of tumour cells, as leukocytes such as natural killer (NK) cells and cytotoxic $CD8^+$ T lymphocytes (CTL) efficiently kill neoplastic cells. But tumour cells can explore immunosuppressive networks and evade anti-tumour immunity[1]. As an endogenous control mechanism over autoimmunity that can nevertheless also result in impaired anti-tumour immunity, forkhead-box P3 $(FOXP3)^+$ $CD25^+$ regulatory T (T_{REG}) cells suppress the effector function of NK cells, CTLs and antigen-presenting cells (APCs) through multiple pathways[2] (Fig. 1). In light of their immune suppressor activities[2–4], and given that high numbers of T_{REG} cells in the tumour microenvironment are associated with poor prognosis, T_{REG} cells are an attractive target for anticancer therapy.

In humans, T_{REG}-cell deficiency caused by mutations in the T_{REG}-cell master transcription regulator FOXP3 results in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome — a severe and systemic autoimmune disorder. The dramatic effects of systemic T_{REG}-cell deficiency suggest that the complete removal of T_{REG} cells is not a feasible approach for cancer immunotherapy. In a study published in *Nature*, Thorsten Mempel and colleagues now report that the disruption of the T-cell receptor (TCR) signalosome complex CARMA1–BCL10–MALT1 (CBM) in T_{REG} cells causes a reversion in the cell's phenotype, from immune suppression to immune activation, leading to the production of interferon (IFN)-γ in the tumour microenvironment and enhancing local anti-tumour activity[5].

Mempel and colleagues identified a role for the CBM signalosome in T_{REG} cells by crossing *Foxp3*$^{YFP–Cre}$ mice to *Carma1*$^{flox/flox}$ mice, which produced offspring with T_{REG}-cell-specific *Carma1* deficiency. In these mice, genetic deletion of *Carma1* in T_{REG} cells did not affect the expression of FOXP3 or other markers of T_{REG} cells, but triggered the expression of the immune-effector transcription factors T-box transcription factor TBX21 (T-bet) and RAR-related orphan receptor-γt (ROR-γt), as well as the production of the pro-inflammatory cytokines tumour necrosis factor (TNF), interleukin (IL)-17 and IFN-γ. Importantly, the monoallelic deletion of *Carma1* in T_{REG} cells also resulted in tumour

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control while avoiding systemic autoimmunity in mice bearing either poorly immunogenic D4M.3A melanoma or immunogenic MC38 adenocarcinoma. In these models, IFN-γ production by T_{REG} cells was critical for controlling tumour progression in a process restricted to the tumour microenvironment.

To explore the mechanism underlying the anti-tumour effect of *Carma1*$^{-/-}$ T_{REG} cells, Mempel and colleagues explored the effects of the acute deletion of *Carma1* in the tamoxifen-induced *Foxp3*$^{Cre–ERT2}$ *Carma1*$^{flox/flox}$ model. In agreement with prior data, tumour growth was arrested in these animals only after tamoxifen administration. Notably, macrophages from *Foxp3*$^{Cre–ERT2}$ *Carma1*$^{flox/flox}$ tumour-bearing animals expressed higher levels of major histocompatibility complex (MHC) class II when compared with *Foxp3*$^{Cre–ERT2}$ *Carma1*$^{+/+}$ control mice. Concurrently, tumour cells expressed higher levels of MHC-I, making them more sensitive to CTL-dependent killing. However, tumour cells also expressed higher levels of programmed death-ligand 1 (PD-L1, also known as B7-H1), which may have contained the anti-tumour immune response. This was found to be the case as, while *Foxp3*$^{Cre–ERT2}$ *Carma1*$^{+/+}$ animals responded moderately to PD-1 blockade, *Foxp3*$^{Cre–ERT2}$*Carma1*$^{flox/flox}$ mice manifested significantly enhanced tumour regression in response to the checkpoint inhibitor.

The lack of pharmacological inhibitors for CARMA1 prevents the non-genetic interrogation of the function of this factor in T_{REG} cells, but inhibitors exist for MALT1, an enzymatic component of the CBM complex. Administration of two MALT1 inhibitors (mepazine and MI-2) in the D4M.3A tumour model triggered a phenotype that approximates that of conditional deletion of *Carma1* in T_{REG} cells, including an increase in the frequency of T cells producing IFN-γ and TNF, and the elevated expression of MHC-I and PD-L1 on cancer cells. Furthermore, when D4M.3A melanoma-bearing male mice were treated with mepazine and anti-PD-1, the combination therapy yielded synergistic anti-tumour activity. Hence, the authors conclude that the inhibition of the CARMA1–BCL10–MALT1 signalosome induces an inflammatory phenotype in T_{REG} cells and generates potent anti-tumour immunity (Fig. 2).

Human T_{REG} cells can express inflammatory and effector cytokines, including IL-8, IL-17 and IFN-γ in the tumour microenvironment and in inflammatory conditions[6–8]. Thus, that Mempel and colleagues observed IFN-γ^{+} T_{REG} cells in the mouse tumour microenvironment is not surprising. However, that CARMA1 can dramatically alter the functional properties of FOXP3^{+} cells to enable IFN-γ expression in T_{REG} cells is an important observation. Although the specific deletion of neuropilin-1 results in IFN-γ expression in T_{REG} cells[9], whether the CBM signalosome and neuropilin-1 are connected in regulating the T_{REG} phenotype is unknown.

As T_{REG} cells are regulated by the strength of the TCR signal[10], the CBM signalosome may quantitatively and qualitatively alter TCR signalling in T_{REG} cells. This potential mechanistic relationship would partially explain why T_{REG} cells express IFN-γ in the tumour and not in lymph nodes. In addition, it is known that the CBM signalosome can activate several downstream pathways, including nuclear factor-κB (NF-κB), activator protein 1 and mammalian target of rapamycin (mTOR). Mempel and co-authors provided

evidence that NF-κB has no role in their models. Given that the integrity of T_{REG} cells is particularly regulated by the balance of mTORC1 and mTORC2 (ref.[11]), that the phenotypic plasticity of T_{REG} cells can be regulated by the crosstalk of metabolic pathways, and that IFN-γ production is linked to the metabolic state of T cells[12], it is sound to investigate the mechanistic association between CBM signalosome components, mTOR and IFN-γ expression in T_{REG} cells. The authors show that CARMA1 deficiency induces the expression of T-bet and ROR-γt; it is therefore tempting to speculate whether CARMA1–CBM has a role in the interplay between the three key T-cell transcription factors FOXP3, T-bet and ROR-γt in T_{REG} cells, and whether this interplay may determine the functional fate of T_{REG} cells.

The observation of an increase in MHC-I expression in macrophages, and of PD-L1 expression on tumour cells under stimulation by IFN-γ^+ T_{REG} cells, offers a mechanism for the therapeutic efficacy of the phenotypically reverted T_{REG} cells and would explain why PD-1 blockade concomitant with *Carma1*-deletion leads to the strong rejection of tumours. However, it is unclear whether IFN-γ from IFN-γ^+ T_{REG} cells also led to the induction of PD-L1 expression on macrophages or myeloid dendritic cells (mDCs). PD-$L1^+$ antigen-presenting cells are the primary targets of PD-L1 and PD-1 blockade therapy[13–15], and several studies have documented that PD-L1 is expressed on macrophages and mDCs in human tumour-draining lymph nodes and in the tumour microenvironments. Ultimately, multiple environmental, genetic and metabolic determinants may shape the outcome of PD-1 and PD-L1 blockade therapy in different experimental and clinical settings[16]. A clear understanding of the IFN-γ^+ T_{REG} cell's interacting partners may help to develop new mechanistically informed therapeutic strategies for cancer patients.

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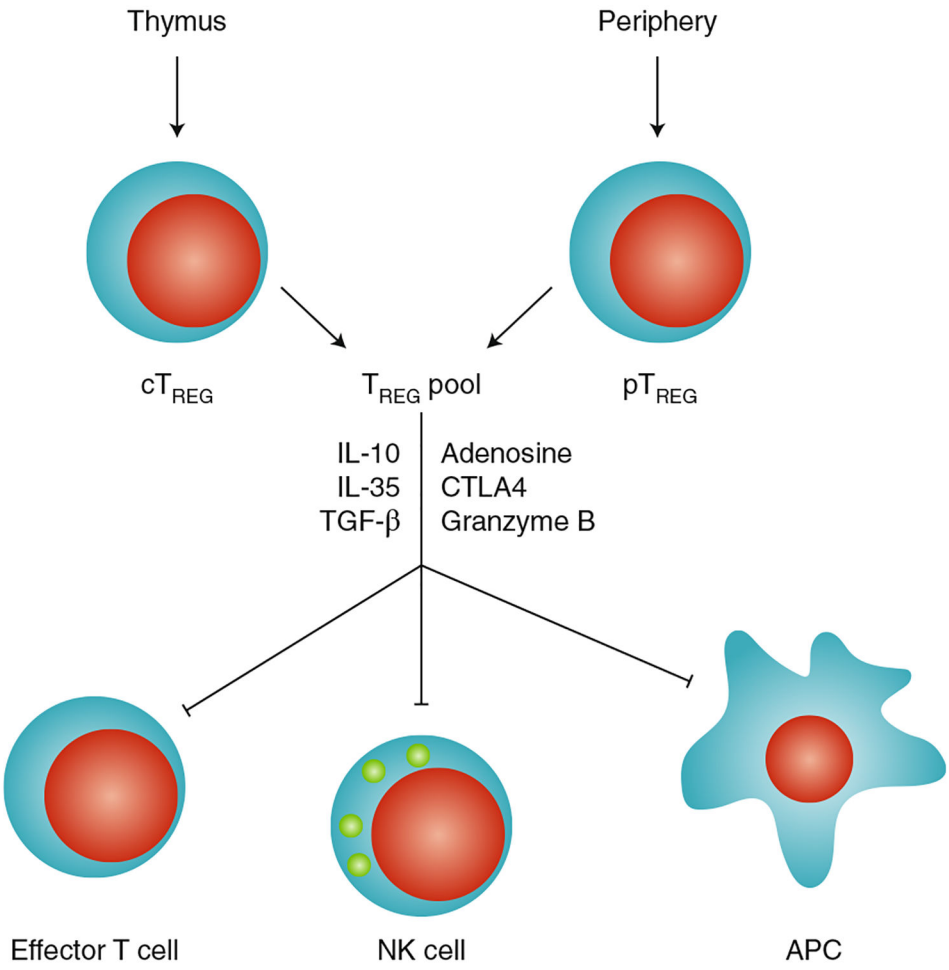

Fig. 1 |. Effect of T_{REG} cells on immune cells.

T_{REG} cells are developed in the thymus (central T_{REG} cells; cT_{REG}) or generated and expanded outside the thymus (peripheral T_{REG} cells; pT_{REG}); can directly target antigen-presenting cells, NK cells and effector T cells; and mediate immunosuppression via distinct molecular mechanisms.

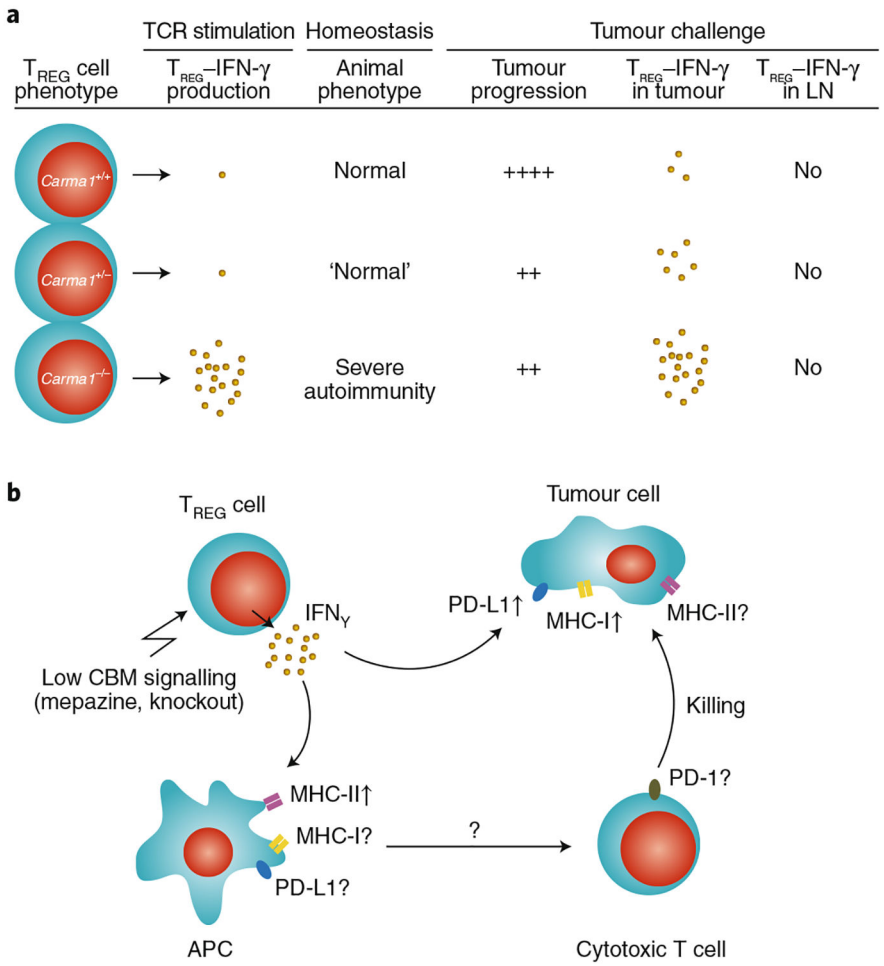

Fig. 2 |. Effect of the cBm signalosome on the phenotype of T_{REG} cells.

a, Expression levels of the CBM-complex scaffold protein CARMA1 are associated with different levels of IFN-γ production in T_{REG} cells following in vitro stimulation and in vivo tumour challenge. LN, lymph node. **b**, The genetic or pharmacological loss of CBM signalling results in the increased production of IFN-γ in tumour-associated T_{REG} cells. IFN-γ production by T_{REG} cells may affect MHC-I, MHC-II and PD-L1 on tumour cells and on antigen-presenting cells. ↑, increased expression; ?, unknown effects.