IMMUNOTHERAPY

Remote control CARs

Therapies using T cells that are engineered to express chimeric antigen receptors (CARs) are promising for the treatment of diseases such as cancer and autoimmune disease. But these approaches are blighted by life-threatening side effects associated with the potent immune activity of CAR-expressing T cells. Now, Lim, Onuffer and colleagues have found a way to control the activity of the infused T cells by developing a CAR that is switched on only in the presence of both of its cognate antigen and a small-molecule drug. This reversible switch allows for precise control over the amount, timing and location of CAR T cell activity.

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Conventional CAR constructs combine an antigen recognition domain (single-chain variable fragment (scFv)) with signalling motifs from the T cell receptor CD3ζ chain and co-stimulatory molecules, and they are activated upon binding to the cognate antigen. To better regulate their activity, Wu *et al.* designed different constructs in which the extracellular antigen-binding and intracellular signalling components were split and included heterodimerizing domains that assemble only in the presence of a small molecule (such as a modified form of rapamycin, referred to as 'rapalog'). The authors identified one construct that induced strong cytokine production by Jurkat T cells, comparable to that stimulated by a conventional single-component CAR, only when exposed to both antigen and rapalog. Single-molecule imaging confirmed that the two components of the 'ON-switch' CAR assemble only in the presence of the small-molecule dimerizer.

Next, they tested the ability of the construct to induce cytokine secretion, proliferation and cytotoxicity by primary human T cells. The ability to regulate each of these effector functions is vital to limit off-target toxicities. Dual stimulation with antigen and increasing amounts of rapalog led to a dose-dependent increase in cytokine secretion by ON-switch CAR-expressing T cells. Similarly, increasing amounts of rapalog increased the fraction of proliferating cells. To test for target cell killing, the authors used fluorescently labelled target cells expressing either the cognate antigen or a non-cognate antigen and incubated them with primary human T cells expressing the ON-switch CAR construct. When rapalog was added to the cultures, efficient killing of cognate but not non-cognate target cells was observed. This response was titratable by changing the concentration of rapalog and could be turned on and off and on again according to the presence of rapalog.

Mouse in the House/Alamy

Finally, the authors tested the ON-switch CAR construct *in vivo*, by injecting the target cells into the peritoneum of a mouse, followed by the CAR-expressing T cells and several doses of rapalog (or vehicle control). Only in mice that received the T cells and rapalog did the authors observe a selective reduction in the number of cognate antigen-expressing target cells.

So, this new class of synthetic CARs allows for effective control of specific T cell activity by small-molecule drugs. This opens up opportunities to delay the activation of CAR T cells to avoid off-target toxicity that can occur immediately after T cell infusion or to target activation to the diseased tissue only, using local delivery of the small molecule.

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Nature Reviews Immunology

This article is modified from the original in *Nat.Rev. Immunol.* (http://dx.doi.org/10.1038/nri3938)

ORIGINAL RESEARCH PAPER Wu, C.-Y. *et al.* Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. *Science* http://dx.doi.org/10.1126/science.aab4077 (2015)