

NEWS AND COMMENTARY

Grafting chimeric antigen receptors to redirect T cells

Carving the CAR

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Chimeric antigen receptors (CARs) are major histocompatibility complex (MHC)-independent one-poly-peptide chain receptor molecules that, when expressed in T cells, can redirect specificity towards tumor or viral antigens. The antigen-binding domain of the CAR is derived from a single-chain antibody fragment and the transmembrane and signalling domains from the T-cell receptor CD3 ζ chain, and optionally includes a costimulatory molecule such as CD28. CARs therefore combine the antigen specificity of an antibody and the property to activate a cytotoxic T cell in a single fusion molecule. A spacer is required between the extracellular antigen-binding domain and the transmembrane moiety. Most commonly, the constant immunoglobulin G (IgG)1 hinge-CH2-CH3 Fc domain is used as a spacer domain, resulting in dimerization and thereby increasing CAR expression on the cell surface.

In this issue, Hombach *et al.*¹ reveal that the IgG1 spacer used in most CARs may also bind to surface IgG Fc gamma receptors expressed on macrophages and natural killer cells. This binding activates both the engineered T cells and the innate immune cells independent of the specificity of the CAR binding domain. Activation of CAR-positive T cells and Fc receptor-bearing cells through Fc γ R binding led to an unwanted, 'off-target' pro-inflammatory immune response. Hombach *et al.*¹ provide a solution to this problem by modifying the sequences required for Fc binding in the constant immunoreceptor Fc domain, which eliminated interaction with Fc-positive cells but preserved stable expression of CARs on the surface of T cells and specific antigen recognition.

In recent years, a variety of CARs with different scFv-binding domains have been designed.² These CARs possess binding specificities for

tumor-associated antigens, such as carcinoembryonic antigen and melanoma-associated antigens, CD30 and CD19, or for virus-infected cells, such as HIV or hepatitis B virus.³ Clinical trials that use CAR-modified T cells for adoptive immunotherapy of malignant diseases have been implemented and additional trials are planned. Owing to its one-poly-peptide chain design and modular composition, the use of CARs substantially simplifies redirecting T cells with defined specificity. Upon antigen engagement, the intracellular signalling domain initiates T-cell activation, resulting in T-cell proliferation, secretion of pro-inflammatory cytokines and cytolysis of the antigen-positive target cell. There are major differences between the natural T cell receptor and a CAR, which confer potential advantages and disadvantages. CAR recognition is independent from antigen presentation on MHC, and allows broad clinical application for patients of any HLA haplotype. The lack of MHC restriction may also allow effective therapy of tumors that escape MHC-restricted T cells by downregulating MHC expression or through defects in antigen processing. However, early versions of CARs often provided weak activation of engineered T cells, mainly because CAR expression was unstable and costimulatory molecules were not, or only inadequately, engaged. The modular nature of CARs has enabled researchers to modify the CAR structure to achieve better activity. In the early research on CARs, it became obvious that inserting a 'spacer' domain, the IgG1 Fc being most commonly used, between the antigen-binding and transmembrane moieties improved expression and binding.⁴ Later on, research focussed on improving the signalling capacity by fusing costimulatory domains, such as CD28, OX40, 4-1BB or the src family kinase lck,

to the CD3 ζ signalling domain.^{5–8} T cells carrying CARs with CD3 ζ and CD28 signalling domains proved to be superior *in vivo* and were able to reject established tumors in mice.⁹ Using T cells with natural specificity for Epstein–Barr virus is another strategy to provide appropriate costimulation through intermittent T-cell receptor ligation, and improved the persistence and probably also the activity of CAR-grafted T cells in tumor patients.¹⁰

Severe side effects reported from recent clinical trials of adoptive immunotherapy with CAR-redirection T cells are a poignant reminder that much remains to be learned to realize the therapeutic promise of this approach. In one case, reactivity of the CAR antibody-binding domain with normal antigen-expressing cells was implicated as the cause of a cytokine storm and organ toxicity,¹¹ and an unexplained sepsis-like syndrome occurred in a second case.¹²

The work of Hombach *et al.*¹ illustrates the potential for 'off-target' activation of engineered T cells via FcR binding to induce unwanted cytokine release and tissue damage—and, importantly, provides an improvement in CAR design that may help to avoid these side effects. It is anticipated that further improvements in CAR design will focus on increasing the specificity of the CAR-redirection T-cell response and minimizing the inflammatory response that is elicited. This should improve the safety and efficacy of adoptive immunotherapy with CAR-engineered T cells.

Conflict of interest

The authors declare no conflict of interest. ■

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