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The Other Face of Chimeric Antigen Receptors

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doi:10.1038/mt.2014.58

The concept of chimeric antigen receptor (CAR) was pioneered in 1989 by Eshhar and colleagues with the specific goal of providing an alternative means by which T lymphocytes can engage antigens expressed by target cells.[1] Until recently, the focus of CAR–T cell research has been on achieving effector function in T lymphocytes that can target cancer cells and destroy them. In this issue of *Molecular Therapy*, however, Eshhar's group illuminates another face of CAR-based technology.[2] They engrafted CARs in naturally occurring regulatory T cells (nTregs) to produce "loss of function" of an unwanted T-cell response that causes inflammation, thereby ameliorating an ongoing autoimmune disorder (**Figure 1**).

CARs are chimeric proteins with two main components: the extracellular moiety usually derived from a monoclonal antibody and an intracellular signaling moiety usually derived from the ζ-chain of the CD3–T cell receptor (TCR) complex. By expressing these proteins in T lymphocytes, Eshhar and colleagues in their first discovery overcame two major obstacles limiting the clinical translation of adoptive T-cell therapies.[1] Expression of CARs by polyclonal T cells allows rapid production of antigen-specific effector T cells without having to expand this population from a small number of cell precursors. Moreover, and in contrast to the native αβTCR of T cells, CAR-based antigen recognition does not require antigen processing by the cell for presentation in association with major histocompatibility complex (MHC), and so can be used for all patients, irrespective of their MHC phenotype, even if the targeted tumor cells downregulate their antigen-processing machinery or expression of MHC.

CAR technology was described more than two decades ago, but successful clinical implementation has been much more recent. Although the gradual evolution of effective techniques for producing viral vectors and for transducing and expanding human T lymphocytes certainly contributed to the clinical development of CAR-based T-cell therapies, the key event was the adaptation of the concept of T-cell costimulation to CAR molecules.[3,4] By incorporating a third component within CARs—the intracytoplasmic domains derived from early and late costimulatory molecules—investigators showed that these elements produced sufficient *in vivo* survival and/or expansion of CAR-redirected T cells for the effective control of tumor growth in humans.[5,6]

Although effector T cells that target malignant tissues are of clinical value, an unwanted effector immune response that damages normal tissues, as in autoinflammatory diseases, can be highly destructive. Eshhar and colleagues sought to adapt the CAR–T cell concept so that it could inhibit ongoing, but unwanted, immune responses. Using murine autoimmune colitis as a model, they found that adoptive transfer of nTregs engineered to express a CAR that targets the carcinoembryonic antigen (CEA) can block the inflammatory disease. This model is relevant to human inflammatory colitis (e.g., Crohn's disease), because CEA is expressed in human inflamed intestinal tissues.[7,8] In addition, colitis was observed as a side effect in a clinical trial designed to target CEA expressed by gastrointestinal tumors using T cells redirected with a high-affinity CEA-specific αβTCR, indicating that there is sufficient antigen expression and processing in the intestine to trigger T-cell activation.[9]

Clinical translation of CAR-modified nTregs may be feasible; several approaches have been developed to select and expand functional nTregs *ex vivo* that are sufficiently robust and compliant with good manufacturing practice for clinical use,[10,11] and several clinical studies are investigating their ability to suppress unwanted immune reactivity.[12] If these nTregs could be more specific to antigens expressed by inflamed tissues, then these cells would become activated (and hence fully inhibitory) at the site of autoimmune inflammation, and in humans, as in mice, exert effective and selective immune suppression. Importantly, Eshhar and colleagues point out that even if we do not know the antigens that are the true targets for autoimmune effector cells in human diseases, nTregs can be activated at the site of inflammation if the CAR they express can recognize a known tissue-restricted self-antigen, such as CEA, present at the site of disease.

There remain significant obstacles to the clinical implementation of CAR-modified nTregs. There are, of course substantial differences between mouse and human nTregs. The selection of $CD4^+CD25^+$ T cells in mice allows a significant enrichment of nTregs. In humans, the selection of this scanty T-cell subset does not completely eliminate effector T cells that can express similar phenotypic markers. The addition of rapamycin or its analogs during the *ex vivo* culture of selected human nTregs cells helps to selectively expand nTregs but does not completely eliminate effector T cells.[11,13,14] These residual effector T cells may be of particular concern in an autoimmune disorder because if they are engineered to express a CAR that targets a tissue-restricted self-antigen to the site of autoimmune disease, the consequence may be exacerbation of the disease rather than the intended amelioration. The optimal design of CARs for nTregs also remains unclear. Eshhar and colleagues used a second-generation CAR that encodes the CD28 costimulatory endodomain known to promote costimulation and persistence

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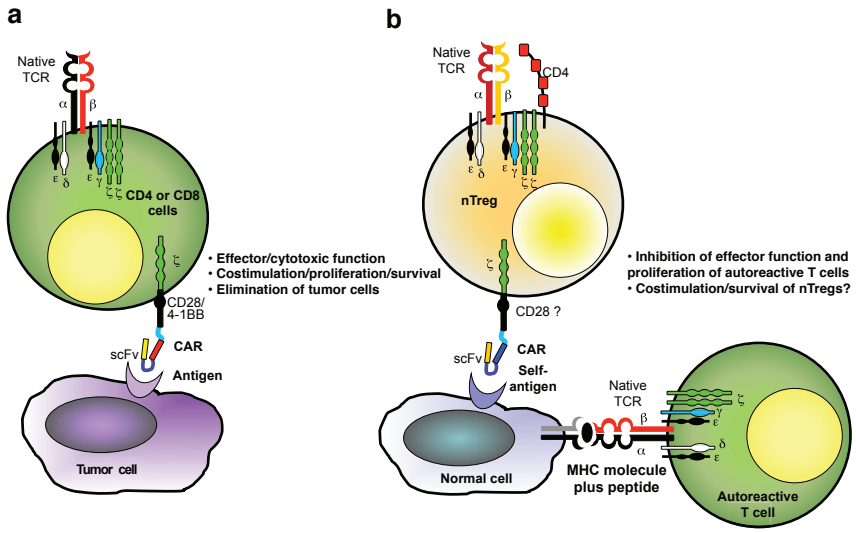

Figure 1 CAR function in (a) effector T cells to eliminate tumor cells and (b) nTregs to control autoimmune diseases. Costimulation in CAR-redirected nTregs remains to be defined. CAR, chimeric antigen receptor; MHC, major histocompatibility complex; nTregs, native T-regulatory cells; scFv, single-chain variable fragment; TCR, T-cell receptor.

of CAR effector T cells in cancer patients.[15] The current study by Eshhar's group was not designed to evaluate the long-term persistence of CAR-modified nTregs. However, because of the usually chronic nature of human autoimmune diseases, including inflammatory bowel diseases, long-term persistence of CAR-redirected nTregs would be desirable. It is unknown whether CD28 costimulation through a CAR will be sufficient for the required long-term persistence of modified nTregs.[16] Indeed, it is unknown whether constitutive costimulation through a CAR will ultimately divert human nTreg cells from their inhibitory function.

Eshhar and colleagues first described CAR-modified effector T cells 25 years ago, and the road to their successful clinical development has turned out to be both long and winding. But we can hope the experience gained on that first journey will enable this new application of CARs to reach its destination more directly and with greater speed.

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