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AUTOIMMUNE DISEASES

CAR-T cells take aim at autoimmunity

> The treatment decreased autoreactive antibody titres, abrogated autoantibody binding and prevented disease manifestation

Current treatments for autoimmune diseases mostly rely on general immunosuppression, leaving patients prone to infections. Approaches that specifically target pathogenic autoimmune cells in autoimmune diseases would be ideal; however, strategies to achieve this have so far been elusive. Now, reporting in *Science*, Ellebrecht *et al.* show that chimeric antigen receptor T cells (CAR-T cells) — which are in clinical trials as novel anticancer agents — can be tweaked to seek out and eliminate self-reactive B cells.

The authors investigated the life-threatening autoimmune blistering disease pemphigus vulgaris (PV), which is caused by autoreactive antibodies targeted at the keratinocyte adhesion protein desmoglein-3 (DSG3). Deletion of B cells with CD20-targeted antibodies can induce short-term remission, but most patients relapse and the treatment leaves them vulnerable to fatal infections. In relapsed patients, the disease-causing DSG3-reactive B cell clones re-emerge, whereas disease remission is associated with the complete disappearance of these clones.

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This observation led the authors to investigate whether CAR-T cells, which have engineered receptors that enable the cells to specifically detect and kill cells bearing a particular surface antigen, could be used to eradicate DSG3-reactive B cell clones. They engineered a CAR using several antigenic epitopes of DSG3 in the extracellular domain, fused to an intracellular signalling domain that has been used in CAR-T cell constructs that have shown clinical efficacy in cancer. The resulting chimeric autoantibody receptor (CAAR) was cloned into T cells, creating DSG3 CAAR-T cells. *In vitro* experiments showed that these CAAR-T cells selectively kill DSG3-reactive B cells while sparing B cells with other specificities.

As PV patients have polyclonal circulating anti-DSG3 antibodies that might either neutralize or stimulate DSG3 CAAR-T cell cytotoxicity, experiments were repeated in the presence of anti-DSG3 antibodies. High-affinity antibodies appeared to mildly decrease CAAR-T cytotoxicity, whereas lower-affinity antibodies had no effect or even increased cytotoxicity, leading the authors to conclude that circulating anti-DSG3 antibodies will not prevent and may even promote CAAR-T cell efficacy.

Next, the DSG3 CAAR-T cells were investigated in a model of PV. This was created by injecting a polyclonal mixture of bioluminescent anti-DSG3 B cell hybridomas into NOD-*scid*-gamma mice, followed by CAAR-T cells 5 days later. The treatment decreased autoreactive antibody titres, abrogated autoantibody binding and prevented disease manifestation. In a PV mouse model created with human $CD19^+$ B cells that expressed anti-DSG3 B cell receptors cloned from PV patients, the CAAR-T cells showed similar efficacy to anti-CD19 CAR-T cells that have been used for clinical studies in B cell malignancies.

Importantly, further *in vitro* and *in vivo* experiments showed that DSG3 CAAR-T cells did not affect keratinocytes; these cells express desmocolins and desmogleins, the physiological binding partners of DSG3. Moreover, in mice that received human skin xenografts, DSG3 CAAR-T cells had no skin toxicity or any other off-target toxicity against other tissues, and did not react against immune cells that might bind to CAAR-bound anti-DSG3 antibodies via their Fc receptors.

Overall, these studies suggest that CAAR-T cells could be developed as a targeted approach to treat autoantibody-mediated immune diseases while avoiding general immunosuppression.

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ORIGINAL RESEARCH PAPER Ellebrecht, C. T. *et al.* Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science* **353**, 179–184 (2016)