"A novel feasible cure for demyelinating diseases?": not yet but here is the concept

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Abstract

Will the people with demyelinating diseases ever be able to use CAR Treg cells to be cured, if it proves effective in the future? Probably not, considering its gigantic price. Researchers should focus on developing an affordable safe Treg-specific viral vector to produce CAR Treg's in-vivo.

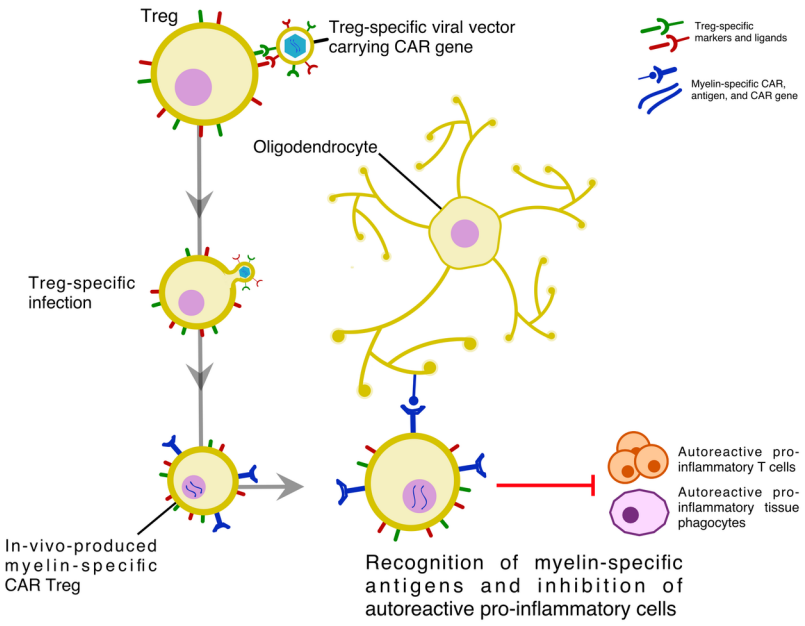

Figure 1: Schematic figure of in-vivo CAR Treg cell therapy for curing demyelinating diseases

Regulatory T (Treg) are a line of immune cells, which regulate the immune responses. In simple words, if a Treg recognizes a cell, that cell will be immune to immunity (the immune system will not harm it). Naturally occurring human Treg's are identified as CD3+ CD4+ CD25(high) CD127(low) FOXP3+ cells in flow cytometry (1). They are either developed in thymus (tTreg's) or induced in peripheral lymph tissue (iTreg's).

The Treg's are thought to function with three main arms, after major histocompatibility complex (MHC)-dependent recognition of self-antigens: 1) The cytokines interleukin-10 (IL-10) and tumor growth factor-ß (TGF-ß), both of which inhibit the pro-inflammatory responses of effector T cells, macrophages, and dendritic cells; 2) The CTLA-4 molecule, which by blocking the B7 molecules on surface of antigen-presenting cells (APCs), competitively inhibits the CD28 costimulatory signal required for effector T cell activation; and 3) Proliferating by consumption of IL-2, which leaves less IL-2 to be used as a pro-inflammatory signal for other cells.

Daclizumab is a monoclonal antibody against the CD25 molecule (or the IL-2 receptor alpha chain), previously approved for treating multiple sclerosis (MS). It worked by binding to the CD25 molecule, blocking the high- and low-affinity IL2 receptors, therefore, blocking the IL2 signaling in immune system. It first proved pretty promising however, it was later retracted. Apparently, it also bound to the CD25 on Treg's and led to their depletion, resulting in autoimmunity, as there were no heroes left to rescue self-antigens from the immune system (2). As a Persian metaphor says, "it wanted to fix an eyebrow, but blinded the eye".

Natural function of effector T cells depends on two signals: 1) The MHC-dependent antigen-binding signal (via T cell receptors); and 2) The costimulatory activating signal (via B7-CD28 interactions). The artificially-engineered chimeric antigen receptors (CARs) integrate both of these signals in one receptor, while also eliminating the dependency to MHC. The effector T cells, genetically engineered to express CARs, are called the CAR T cells, and are used to target specific antigens in immunotherapy. CAR T cell therapy has proved to be one of the most effective methods of treating (or somehow, curing) therapy-resistant cancers. Similar to effector T cells, Treg's can also be genetically engineered to express CARs. Considering the immuno-modulatory effects of Treg's, CAR Treg cell-therapy has been proposed for treatment of autoimmune conditions, such as multiple sclerosis and type-I diabetes mellitus. However, transferring Treg's to patients may be a little bit risky, as they may change to pro-inflammatory T cells in-vivo (3), an issue pointed to as "T cell plasticity".

CAR Treg cell-therapy with myelin-specific CARs has shown promising in curing experimental demyelination models i.e., experimental autoimmune encephalomyelitis (EAE), in murine experiments (4, 5). Simply, the CAR Treg's recognize the myelin-specific antigens (MHC-independently) and keep them safe from the immune system with their mentioned arms. CAR Treg cell-therapies are currently being further tested for curing demyelinating diseases e.g., MS. However, as mentioned, they may be risky, and even if prove effective and safe, they cannot be considered feasible for people with demyelinating diseases (pwDD), as they are super expensive. To produce the CAR Treg cells, first, host cells should be extracted, then the CAR gene should be delivered to host Treg's ex-vivo via a vector e.g., a lentiviral vector. After being cultured and ex-vivo confirmation of safety and capability, the CAR Treg's will be infused back to the patient, eventually resulting complete remission of the disease. This process requires large amounts of time, expertise, precision, equipment, and therefore, money. The currently-approved CAR T cell therapies (for some types of leukemia/lymphoma) are estimated to cost more than 350,000 dollars (6), which honestly, is a big number for insurance companies and foundations, let alone the patients themselves.

The HIV is a lentivirus (ironically, it is oval in shape unlike other elliptical lenti "lens-shaped" viruses), primarily affecting the CD4+ immune cells. Its entry to cells, involves interactions with the CD4 molecule via the glycoprotein 120 (gp120). However, to be able to enter the cells it also requires co-interaction with chemokine co-receptors, like the CCR5 (7).

The lentiviral envelop proteins (including HIV's) are interchangeable, through a widely-practiced process called "pseudotyping". Along with other factors e.g. infecting non-dividing cells etc., this has made the lentiviruses the perfect viral vectors for the CAR gene, in CAR T cell therapy. With some genetic modifications, eliminating their pathogenicity, transmissibility, and limiting their power of replication, the third-generation lentiviral self-inactivating vectors are also deemed to be safe.

Lentiviral envelope proteins e.g. gp120 in HIV, can even be pseudotyped with artificially-engineered proteins. However, the exact requirements for an efficient pseudotype are not completely clear yet (8). In the currently-

practiced CAR T cell therapy, the lentiviral envelope is often pseudotyped with VSV-G protein of the vesicular stomatitis virus, to be able to infect a wide variety of cell types.

The COVID-19 pandemic was a great opportunity to test the novel ways to develop human vaccines. Among which, have been the viral vector vaccines. They work by delivering SARS-CoV-2-specific antigens to host cells. The host cells will then express SARS-CoV-2-specific antigens, which leads to immunization to the actual virus. Viral vector COVID-19 vaccines mostly use pseudotyped adenoviral vectors, and cost around 5-10 dollars a dose.

Imagine pseudotyping a lentivirus e.g. HIV, with artificially-engineered ligands, to enter the cells via binding to CD25 and co-interaction with FOXP4, which will lead to specific infection of (tropism for) Treg's (and a small number of other cells, which can be depleted priorly). Now imagine using it as a vector for the myelin-specific CAR gene. It is all coming together isn't it. The result will be host-produced myelin-specific CAR Treg cells, "the guardians of the myelin". This concept, takes the expensive ex-vivo work of producing CAR Treg cells, in vivo, leaving only a cost of mass-producing "genetically-modified pseudotyped HIV's" which carry the myelin-specific CAR genes. Considering the current price of viral vector vaccines, the costs may actually be feasible for pwDD. Following a moderate-intensity anti-CD25 therapy e.g. with Daclizumab (to deplete the small populations of non-Treg CD25+ cells temporarily), administration of these "CAR gene-carrying genetically-modified pseudotyped HIV's with tropism for Treg's" will keep the pwDD in life-long remission without any serious problems.

Issues may rise e.g., the same issue with Daclizumab, issues regarding the transmissibility of the "pseudotyped HIV", the possible mutations/recombinations of the viral vector leading to pathogenicity or a replication-competent lentivirus (RCL), insertion of the viral genome in wrong places causing cancer (insertional mutagenesis), etc. Most of these issues have been previously discussed and thought of, in developing of the lentiviral vectors, currently used ex-vivo in CAR T cell therapy. A major undiscussed issue, is that myelin-specific inhibition of the immune system may increase the risk of development of nerve sheath tumors, due to inhibition of the normally-active immune responses which would have prevented them.

Anyways, there are probably several more fundamental and technical issues in this concept, which I cannot recognize. For example, pseudotyping a lentivirus with synthetic ligands for CD25 and FOXP5, or any other Treg-specific markers, will probably not be that easy and each step of it e.g. protein engineering of the ligands, modeling them, testing them in-vitro etc., will require some time and funding; but with the new AlphaFold and RoseTTAFold tools of protein structure prediction (9, 10), the feasible cure for demyelinating diseases can be considered within reach in a couple of years. Hypothetically, if possible to pseudotype the vector membranes with them, a type of high-affinity fusion trimers needing both CD25 and FOXP3 (or any other Treg-specific pairs like CD25 and CTLA-4) interaction for activation (as in the case of gp120, CD4, and CCR5), can assure successful infection of CD25+FOXP3+ Treg's, while not infecting the CD25+FOXP3- and CD25-FOXP3+ cells. It is unclear however, if the issue with T cell plasticity (3) can be resolved this way, as it is unclear whether the infected FOXP3+ Treg's can turn down the FOXP3 and turn to pro-inflammatory T cells, after getting infected.

Nevertheless, the proposed concept actually combines cell-, gene- and viral vector therapy, all of which being practiced individually before. Apart from its enormous potential for curing demyelinating diseases, it also can be extended to cure other diseases – a lot of them.

References

1. Pitoiset F, Barbié M, Monneret G, Braudeau C, Pochard P, Pellegrin I, et al. A standardized flow cytometry procedure for the monitoring of regulatory T cells in clinical trials. Cytometry Part B: Clinical Cytometry. 2018;94(5):777-82.

2. Loftus P. Biogen and Abbvie Take Multiple Sclerosis Drug Off Market. The Wall Street Journal

2018.

3. Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009;30(5):646-55.

4. De Paula Pohl A, Schmidt A, Zhang AH, Maldonado T, Königs C, Scott DW. Engineered regulatory T cells expressing myelin-specific chimeric antigen receptors suppress EAE progression. Cell Immunol. 2020;358:104222.

5. Fransson M, Piras E, Burman J, Nilsson B, Essand M, Lu B, et al. CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery. J Neuroinflammation. 2012;9:112.

6. Chadwick D. The Price of Hope: Weighing the Cost of CAR-T Cell Therapy in Treating Blood Cancers. CURE. 2020(2).

7. Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. Cold Spring Harb Perspect Med. 2012;2(8).

8. Oleg Tolmachov, Tanya Tolmachova, Al-Allaf FA. Designing Lentiviral Gene Vectors. In: Xu K, editor. Viral Gene Therapy: IntechOpen; 2011.

9. Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, et al. Accurate prediction of protein structures and interactions using a three-track neural network. Science. 2021;373(6557):871.

10. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021.