ORIGINAL RESEARCH PAPER

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THE POSSIBLE CURE OF ULCERATIVE COLITIS BASED ON THE HYPOTHESIS THAT SOME CANCERS ARE RICH SOURCE OF NON-APOPTOTIC AND FUNCTIONAL TREG CELLS

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ABSTRACT

Many of the Autoimmune diseases, if not all, arise because either the levels of regulatory T cells (Tregs) have reduced in the milieu of organ affected or the Tregs in the milieu of organ affected have impaired. When the Tregs undergo either of these two fates, the conventional T cells wreck havoc on the healthy cells of the body, killing them and causing chronic inflammation. Such a state in the colon and rectum together is mostly the disease called Ulcerative Colitis (UC). It has been hypothesized that the impaired functioning of Tregs cause UC. Hence if the milieu of colon and rectum in the UC patients is populated with non-apoptotic fully functional Tregs, they can perhaps be cured. But from where to get such Tregs ? From the studies of Immunotherapies in Cancers I hypothesize that some cancers including the colitis-associated cancer can be the source of such Tregs. Based on these ideas I propose in this paper two possible curative therapies for UC which I call the CAR-Treg therapy and the E-Treg therapy. CAR-Treg therapy is based on the theory of multispecific Chimeric Antigen Receptors, and E-Treg therapy is based on the theory of cell encapsulation.

Introduction

Ulcerative Colitis (UC) is an inflammatory autoimmune disease of the colon and rectum, the main cause being the impairment in the normal functioning of regulatory T cells (Treg) in the colonic milieu and not the change in the levels of Foxp3+ Treg cells.[1] One of the functions of Treg cells is that they protect the healthy cells of human body from immune attack by a variety of mechanisms one of which could even be the direct killing of immune cells.[2-12] Foxp3 is the most important transcription factor expressed in activated Treg cells or in the Treg cells being developed; mutations in Foxp3 gene have been linked with some autoimmune disorders in both human and mice.[13]

There has been no cure of UC so far.[14] The treatment strategy adopted in UC is to lessen its symptoms. The latest in UC treatment is to use Tumor Necrosis Factor-alpha (TNF-α) blockers or inhibitors in different forms.[15,16] Elevated levels of pro-inflammatory cytokine TNF-α has been observed in UC.[17] But it has been documented that in high doses against UC TNF-α blockers lead to Colon Cancer.[18] The cancer arising this way is called Colitis-Associated Cancer (CAC).[19] Previous knowledge on the genesis of UC was that it arises when the competition of dominance between the two helper T cells T_h1 and T_h2 is won by T_h1.[20] But the paper so latest as that published in 2018[21] claims that in UC the competition of dominance between T_h1 and Th2 is won by T_h2. If we consider this claim to be correct, TNF-α being a Th1 cytokine one cannot explain the elevated level of TNF-α in UC. Possibly, we have yet to stumble upon the correct genesis of UC. But this is not the focus of this paper. In this paper, building a certain hypothesis from the success of Immunotherapy (IT) in cancer, I propose a possible cure for UC.

Immune Checkpoint Inhibition and Cancer

Treg cells are not the sole regulators of immune tolerance to self-antigens; the Programmed Cell Death-1 (PD-1)/Programmed Death Ligand-1 (PD-L1) pair is also the key regulator of immune tolerance to self-antigens. PD-1 is the receptor (on most of the immune cells including activated T cells) to the ligand PD-L1 (expressed upon inflammation in some immune cells and some epithelial cells).[22] PD-L1 has also been found to be highly expressed on tumors in a variety of malignancies.[22] Ever since the discovery of PD-1[23], the most studied PD-1/PD-L1 combination is one wherein PD-1 is expressed on effector T cells (Teff) and PD-L1 is expressed on tumor. When PD-L1 on tumor binds to the PD-1 on Teff the process of the suppression of immune response (of Teff against tumor) begins by the dysfunction or the exhaustion of Teff cells, or by the repression of Teff cytokine production.[22] Hence because of their roles in keeping a check on immune response, PD-1 and PD-L1 are called immune checkpoint molecules.

Immune checkpoint inhibitors (ICIs) against PD-1 and PD-L1 have been attractive ITs in a number of malignancies.[24] By lowering the levels of PD-1 and or PD-L1 in patients of some cancers the immune response against tumor is restored. But in this IT there is a risk of autoimmunuty related adverse event taking place because immune tolerance is compromised. Let us look at the exact numbers of such adverse events taking place from the work reported in[25]. Little less than 7% (2 out of 29) of cancer pateints receiving Anti–PD-1/PD-L1 monotherapy developed immune related Adverse Events (irAE), the most common irAEs requiring hospitalization were pneumonitis and colitis. In the rest 93% of cancer patients even when it seemed that the immune tolerance was compromised because of therapy, the immune tolerance was not compromised in reality. How ? The possible answer is that the non-apoptotic and fully functional Treg cells were in abundance in these 93% of cancer patients. It is likely that in a majority of these 93% of cancer patients the immune homeostasis between immune attack (on tumor) and immune tolerance (against self-antigens) was reached. There are cases of few cancers (Cervical cancer as reported in[26] and Lung cancer as reported in[27]) in which the Treg cell mediated immunosuppression eventually leads to the evasion of immunity by cancer cells despite the Anti–PD-1/PD-L1 therapy. Hence, in light of these evidences, I hypothesize that some cancers are rich source of non-apoptotic and fully functional Treg cells. CAC is one such type of cancer.[19]

The Possible Cure of UC

The whole idea behind innovating a cure for UC is that the cells obtained in the colonic biopsies from UC patients are turned cancerous, and thereby a pool of non-apoptotic and fully functional Treg cells are obtained which are thereafter introduced into the blood (of UC patients) by certain mechanisms. Following are the steps involved in the curative therapy being proposed for UC

1. Colonoid culture is formed from the cells obtained in colonic biopsy from the concerned UC patient, by the procedure outlined in[28].
2. Mailgnant transformation of the cultured cells. This is accomplished in either of the two ways:

(a) Treating the cultured cells with overdose of anti TNF-alpha drug.

(b) Upregulation of miR-21 has been observed in CAC, and it has been hypothesized that miR-21 supports the genesis of CAC.[29] A technique has been developed to overexpress micro RNAs in cells.[30] Using this technique miR-21 is overexpressed in the cultured cells.

By either of the two ways mentioned above, it is hoped that one generates the CAC cell line (CACcl).

3. By histological examination the formation of CACcl can be confirmed.

4. The CACcl is suspended in the peripheral blood taken out from the UC patient being treated. The immune cells in the peripheral blood will launch immune attack against the CACcl, and it is expected that in response the Treg cells will be generated in enough numbers to evade immune attack. This response is expected because, as stated earlier non-apoptotic and fully functional Treg cells have been found infiltrating the cells of CAC in murine models. The mechanism of this response could be the clonal evolution of CACcl for survival benefit.[31]

5. Next, after few days, the Treg cells could be isolated from the peripheral blood using a technique outlined in[32].

6. One now gets the pool of non-apoptotic and fully functional Treg cells. From here on the further steps could take two routes, which are:

(a) Multispecific Chimeric Antigen Receptors (MCARs) could be introduced into the Treg cell by using a retrovirus or lentivirus, while leaving the rest of the biological machinery of Treg untouched. Active research is going on on MCARs.[33,34] These MCARs for the application being discussed here could be specific for at least any three of the antigens which are the most prominent biomarkers in UC. Some of the prominent biomarkers in UC are TNF-alpha, IL-8, Leukotriene B4, Myeloperoxidase, MCP-1 and MCP-3, ENA-78, macrophage inflammatory proteins 1a and 1b, IFN inducible protein 10, stromal cell derived factor 1, fractalkine etc..[35] Care must be taken that that combination of three antigens be targeted by MCAR which are unique only to the cells of the colon and the rectum affected by UC. Inject the MCAR loaded Treg cells into the blood of the UC patient being treated. I call this therapy CAR-Treg therapy

(b) Encapsulate the Tregs thus prepared (as outlined in step 4) in an outer covering made of pH-sensitive Eudragit P-4135F. Eudragit P-4135F dissolves at pH≥7.4 which is the pH in the milieu of the colon and the rectum affected by UC.[14] Administer the drug thus prepared to the UC patient orally. I call this therapy E-Treg therapy.

DISCUSSION

Before administering either of the two therapies, CAR-Treg or E-Treg, to the UC patient ensure that the patient is not suffering from any type of cancer. There is another matter of concern: these conditioned Tregs (conditioned by CACcl) must maintain themselves in the blood of subjects for many years. The success of CAR-T therapy in the case of a paediatric cancer patient Emily Whitehead[36], raises hope that the Tregs in CAR-Treg therapy must persist for years keeping UC away from the subject. However, in E-Treg therapy same cannot be speculated before conducting clinical trial.

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