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Mini review

Immunogenicity in CAR T cell immunotherapy

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Abstract

Currently, the most accessible forms of cancer treatment include surgery, chemotherapy, and radiation. However, these forms of treatment may damage or destroy healthy tissue as well as cancerous cells, resulting in side effects such as fatigue, hair loss, diarrhea, etc. Immunotherapy, an alternative form of cancer treatment, is a growing treatment method of interest that uses bodily substances made by the body or in a laboratory to boost the immune system's activity against tumor cells. One type of immunotherapy is CAR T cell therapy, in which a patient's T cells are genetically modified in a lab to express Chimeric Antigen Receptors (CARs) that help T cells identify and destroy their target. However, because CARs are constructed in the lab and currently consist of non-self components, genetically engineered CAR T cells have the potential to induce anti-CAR immune responses. The following paper will explore the causes of anti-CAR immunity, its possible solutions, and the potential implications of these discoveries.

Abbreviations

CAR: Chimeric Antigen Receptor; scFv: single chain variable Fragment; V_H: Variable Heavy; V_L: Variable Light; MHC: Major Histocompatibility Complex; APC: Antigen-Presenting Cell; TCR: T Cell Receptor; HLA: Human Leukocyte Antigen; NK cells: Natural Killer cells; CDR: Complementarity-Determining Region; FR: Framework Region

Introduction

Chimeric Antigen Receptors (CARs) are recombinant receptors that are expressed on genetically modified CAR T cells to promote T cell recognition and destruction of tumorous cells. CARs are made of two parts: an antigen-binding single chain Variable Fragment (scFv) and an intracellular signaling molecule. The scFv is typically made of the Variable Heavy (V_H) and Variable Light (V_L) chains of an antibody. The intracellular portion consists of a signaling chain that couples antigen recognition to intracellular signal-transduction pathways and optional co-stimulation domains [1]. Different CARs are recombined to recognize specific antigens presented on tumorous cells that the body's normal T cells may not identify as foreign [2].

CAR T cell immunotherapy results in immunogenicity

Because current methods of artificially constructing CARs use non-self fragments, immunogenicity is a critical contributor to the lower efficacy and success rates of administered CAR T cells. The body may induce anti-CAR immune responses to non-self components of the CAR constructs or to residual proteins from the inherently immunogenic gene-transfer vectors that are used in the process of genetically engineering CAR T cells [3,4].

The body's natural immune response may reject administered CAR T cells through both cellular and humoral anti-CAR responses. Cellular immunity likely arises from the cross-presentation of foreign sequences in the CAR molecule by a Major Histocompatibility Complex (MHC). When CAR T cells naturally die through apoptosis, foreign mice-derived

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scFv fragments may be displayed through MHC molecules by Antigen-Presenting Cells (APCs) and used to prime T cell responses, thereby turning the body's T cells against the CAR molecule. Humoral immunity is also primed through CAR proteins, but the foreign CAR construct fragments are instead presented by follicular dendritic cells to B cells. CAR-specific B cells can then undergo plasma cell differentiation and class switching, in which B cells change the type of antibodies they produce, thereby enabling the immune system to manufacture anti-CAR antibodies that induce the death of CAR T cells [5].

Although initial clinical administrations of CAR T cells have high response rates, studies have noted an increase in the amount of detected anti-CAR antibodies following infusion [6] and disease relapse after the first round of CAR T cell treatment [7]. These results suggest that anti-CAR immunity is a prevalent issue that significantly impacts the efficacy of CAR T cell immunotherapy. Fortunately, there are a few, promising methods that have the potential to combat anti-CAR immune responses (Figure 1).

Lymphodepletion

One method to minimize anti-CAR immune responses is lymphodepletion, in which the number of immune cells that have the potential to attack CAR T cells is decreased. The primary method of lymphodepletion is through chemotherapy; fludarabine and cyclophosphamide are recognized as the most promising chemotherapy medications so far [9]. Fludarabine interferes with ribonucleotide reductase and DNA polymerase to inhibit DNA synthesis [10] and cyclophosphamide forms cross-linkages within and between DNA strands at the guanine N-7 position, resulting in permanent modifications that lead to programmed cell death [11]. The combined use of fludarabine and cyclophosphamide is currently the most commonly used combination for inducing immune cell death before CAR T cell treatment therapy. Some patients, though, fail to develop a favorable immune environment that is inhibited by lymphodepletion even with the best lymphodepletion regime, suggesting that the effectiveness of lymphodepletion chemotherapy also depends on the host biological response [9].

Current methods of lymphodepletion chemotherapy come with harmful side effects, however, including pancytopenia and prolonged immune suppression. Pancytopenia is a condition characterized by low levels of red blood cells, white blood cells, and platelets. This consequently leads to greater chances of anemia, infection, and excessive bruising or bleeding [12]. Prolonged immune suppression may be caused by the lingering effects of fludarabine and cyclophosphamide after lymphodepletion is no longer necessary, thereby increasing the risk of infections. Additionally, fludarabine can induce neurotoxicity and fever, while cyclophosphamide can induce hemorrhagic cystitis and pericarditis, and both may increase the risk of secondary malignancies [9]. Although lymphodepletion has the potential to increase the efficiency of CAR T cell therapy, the side effects of current treatment methods warrant further research into the mechanism of immune suppression by chemotherapy medication.

Elimination of MHC molecules on CAR T cells

Another proposed method to combat anti-CAR T cell immune responses takes advantage of programmable nucleases that use the CRISPR-Cas9 genome editing system. Researchers can use gene editing to eliminate cell-surface MHC expression on CAR T

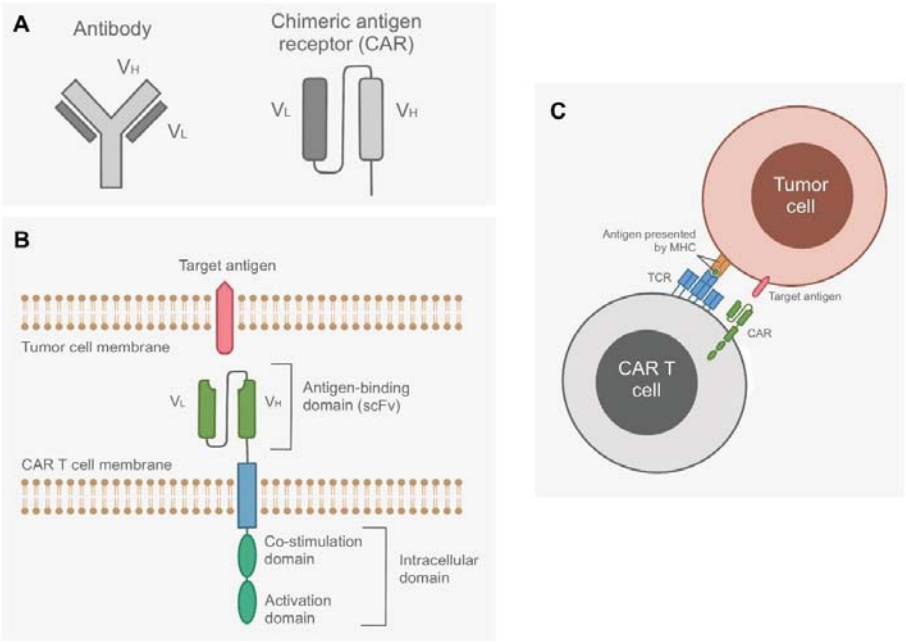

Figure 1: (A) The V_H and V_L chain components of the CAR construct are derived from an antibody. (B) The CAR construct consists of an antigen-binding domain and an intracellular domain. The antigen-binding domain, also known as scFv, is made of the antibody-derived V_H and V_L chains. The intracellular domain includes an activation domain and optional co-stimulation domains. (C) The antigen-binding domain can be reconstructed to target specific antigens expressed on tumor cells. The purpose of the CAR construct is to recognize surface antigens unique to cancer cells that the T Cell Receptor (TCR) cannot.

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cells and thereby prevent the detection and elimination of CAR T cells by T cell-mediated anti-CAR immunity. Researchers first tried to remove MHC I from the surface of resting CAR T cells, as MHC I molecules are expressed on all nucleated cells and play a crucial role in alerting the immune system to infected cells [13]. Eliminating surface MHC I expression indeed prevents alloimmune reactions, but it requires the genetic editing of the human leukocyte antigen (HLA) locus, which are genes in MHCs that help code for proteins that differentiate between self and non-self [14]. The eradication of HLA expression would consequently increase natural killer (NK) cell-mediated cytotoxicity. A potential way to combat this issue is to prevent the expression of HLA-A and HLA-B while allowing the expression of HLA-C, an MHC component that is assumed to act as a ligand for killer immunoglobulin receptors expressed on NK cells and may minimize NK cell-mediated cytotoxicity [15].

MHCs may also be eliminated from the cell surface by disrupting the functional expression of CIITA, which encodes the master transcriptional regulator of MHC II [16]. The inhibition of CIITA expression would remove MHC II from the cell surface without provoking alloimmune responses to activated CAR T cells. However, this method again introduces the issue of NK cell-mediated cytotoxicity [5]. No matter what approach is used to eliminate MHC surface-expression levels, it is evident that the identification of foreign cells by the immune system via MHCs is still an ambiguous area of study that, if clarified, could increase the efficacy of CAR T cell therapy (Figure 2).

Development of CAR constructs with humanized scFv

The most critical issue facing current CAR T cell therapy methods, however, is the development of CARs from scFv fragments that are derived from mice. When CAR T cells die through apoptosis, the foreign fragments in CAR constructs are displayed by MHC molecules expressed on APCs, leading to an anti-CAR T cell immune response.

To combat this problem, researchers are looking at the possibility of using humanized CAR constructs, which would be less immunogenic than CARs constructed from mouse-derived scFvs. The humanization of scFvs requires the execution of Complementarity-Determining Region (CDR) grafting with the retention of mouse Framework Region (FR) residues to ensure that the novel humanized constructs maintain the same function and efficacy as the ones previously constructed with mouse scFv fragments. To generate the humanized scFv gene, CDRs of the mouse V_H and V_L regions are grafted onto selected human FRs that show the highest similarity to the amino acid sequence identity of the FRs of mouse V_H and V_L [17].

MHC molecules may be reconstructed using entirely human-derived CAR constructs, or they may have traditional scFvs substituted with immunoglobulin heavy-chain-only recognition domains that lack light chains and potential immunogenic linker sequences. Because linkers are another possible source of immunogenicity, the elimination of linker sequences from CARs would be beneficial. These heavy-chain CAR constructs have shown significant target affinity and efficacy in preclinical models, but constructs that are derived solely from self-human components could still theoretically initiate immune responses in patients [18]. Further head-to-head clinical trials directly comparing the immunogenicity and efficacy of mouse-derived versus humanized CAR constructs are necessary, and if humanized CARs indeed have higher efficacy and lower immunogenicity, they would lead a promising direction for the creation of commercial CAR T cell products. This would make CAR T cell therapy both more convenient and less expensive [19] (Figure 3).

Conclusion

Although immunogenicity in CAR T cell immunotherapy is a pressing issue, there are developing methods that have the potential to combat it. Studying patients' varied responses to fludarabine and cyclophosphamide would allow chemotherapy treatment to eliminate the immune cells that attack administered CAR T cells without downregulating the immune system for an extended period of time. Researching the many caveats of MHC-facilitated immune cell recognition may prevent CAR T cells from being recognized and therefore

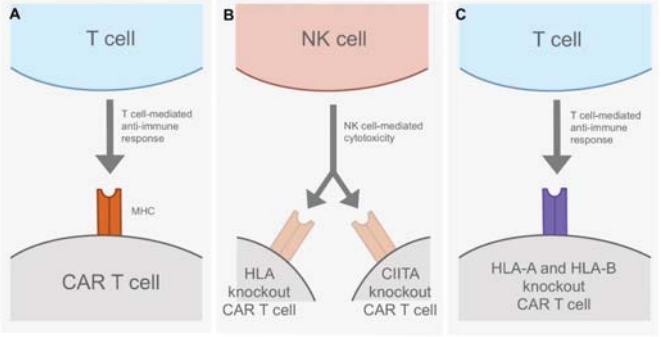

Figure 2: (A) The expression of MHCs on CAR T cells can promote the recognition of foreign CAR constructs and the activation of the anti-CAR immune response by allospecific T cells. (B) Eliminating the expression of HLA or CIITA would remove the surface expression of MHC, thereby minimizing the T cell-mediated anti-immune response. However, this would make the CAR T cells consequently susceptible to NK cell-mediated cytotoxicity. (C) The suppression of HLA-A and HLA-B only, while maintaining the expression of HLA-C, will maintain the surface expression of MHC, although MHC will have a different structure. This minimizes the chance of NK cell-mediated cytotoxicity. But, this reintroduces the issue of the T cell-mediated anti-immune response.

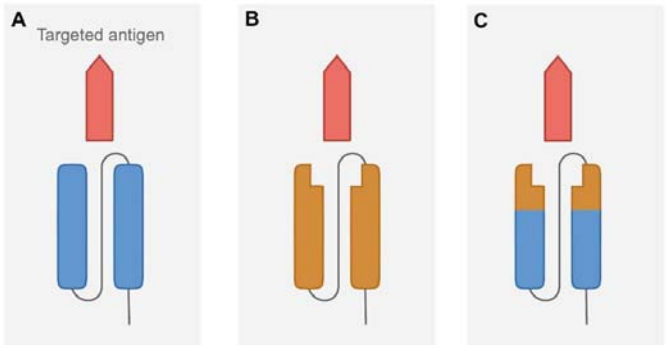

Figure 3: Diagram of (A) a fully human CAR construct, (B) a mouse-derived CAR construct and (C) a humanized CAR construct.

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targeted by the body's immune system. The replacement of mouse-derived scFvs with humanized ones can create CAR constructs that are less likely to stimulate anti-CAR immune responses, but further clinical trials are required to test the constructs' immunogenicity. Finding the solution to immunogenicity in CAR T cell therapy is undoubtedly a difficult process, but it would significantly improve the efficacy of CAR T cell therapy and open the doors of immunotherapy treatment to a plethora of new possibilities.

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