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Interaction Between Immune and Cancer Cells and Promising CAR T-Cell Therapy

Hyeonjee Rho[1] and Haley Halasz[#]

[1]Maine Central Institute, Pittsfield, ME, USA
[#]Advisor

ABSTRACT

$CD8^+$cytotoxic T cells, $CD4^+$helper T cells, and regulatory T cells interact differently with cancer. $CD8^+$cytotoxic T cells recognize the antigens presented by cancer cells and directly attack cancer cells. $CD4^+$helper T cells recruit other immune cells and promote them to destroy cancer cells. On the other hand, regulatory T cells suppress T cell proliferation and the immune response of both $CD8^+$cytotoxic T cells and $CD4^+$helper T cells. This suppressive mechanism often leads to the progression of cancer. The T cell-cancer cell interactions can often render conventional cancer treatments ineffective, leaving a need for new and improved therapies. Chimeric antigen receptor (CAR) T-cell therapy has been an emerging immunotherapy especially against B cell cancers. While CAR T-cell therapy has shown some successful cases, there are still limitations in CAR T-cell therapy that need to be overcome.

Introduction

Cancer is one of the leading causes of deaths throughout the world. In 2020, nearly 10 million people died of cancer (World Health Organization, 2022). By 2040, it is projected that cancer will affect 29.5 million people and will account for deaths of 16.4 million people annually (National Cancer Institute, 2020). Thus, new promising treatments for cancer are very needed to save lives of those.

Cancer results from uncontrolled growth of abnormal or damaged cells in the human body. They can begin growing in any part of the human body and spread to other tissues, called metastasis. Typically, genetic programs control the formation of new cells in the human body and their growth and division. However, errors during cell division or direct damage to DNA by outside environmental factors can cause mutations in proto-oncogenes which are supposed to promote normal cell growth-and-division cycle. The errors can also lead to mutations resulting in inactivation of tumor suppressor genes whose role is to prevent cell division or to cause apoptosis. Cancer cells are usually driven by these mutations. Mutated proto-oncogenes are called oncogenes which allows the cell to divide excessively without any regulation. Inactivation of tumor suppressor genes can also lead to the uncontrolled cell growth (National Cancer Institute, 2021).

Abnormal, uncontrollable cancer cells are often recognized and suppressed by immune cells called T cells. Typically, when pathogens enter into the human body, the immune system tries to recognize and attack them. Each pathogen expresses antigens on its surface, and T cells use their receptors to recognize and bind to the antigens. When T cell receptors bind to antigens, T cells secrete signaling molecules such as interferon (IFN)- γ or cytotoxins to aid in the destruction of pathogens. Cancer cells also present antigens. T cells can recognize and bind to these antigens in order to kill cancer cells (American Cancer Society, 2022). However, because of genetic mutations over time and constant interaction with T cells, cancer cells have been able to decrease their antigen expression to avoid T cells and to attract regulatory T cells as a protection against suppression by other types of immune T cells (Vinay et al., 2015). This paper summarizes different types of T cells

and their interaction with cancer and discusses how T cell biology is now being used to develop new, promising immunotherapies to treat cancer.

T cells and Cancer

There are three major types of T cells: cytotoxic T cells, helper T cells, and regulatory T cells. Cytotoxic T cells and helper T cells are lumped together in the next section. Cytotoxic T cells directly attack foreign cells while helper T cells activate cytotoxic T cells, macrophages, and B cells to produce an immune response against foreign cells. Cytotoxic T cells and helper T cells have different roles but have the same goal which is getting rid of foreign cells in the body (Alberts et al., 2002).

Cytotoxic T cells and Helper T cells

Each type of T cells has specific proteins on the cell surface that differentiates it from other types of T cells. Cytotoxic T cells express the surface protein known as CD8 and can be referred to as $CD8^+$cytotoxic T cells. The main role of $CD8^+$cytotoxic T cells is to recognize and directly destroy foreign cells such as virus-infected cells or cancer cells. $CD8^+$cytotoxic T cells produce an anticancer immune response and suppress the growth of cancer. One study shows that a number of $CD8^+$cytotoxic T cells have been found to infiltrate the environment surrounding the cancer (Kawai et al., 2008). $CD8^+$cytotoxic T cells recognize fragments of antigens presented by class I major histocompatibility complexes (MHC) of cancer cells. When a $CD8^+$cytotoxic T cell receptor binds to these antigens, biomechanical signals are secreted and trigger the activation of the $CD8^+$cytotoxic T cell receptor complex. Then, an additional receptor on $CD8^+$cytotoxic T cells, CD28, binds to costimulatory signal CD80 or CD86 presented by the cancer cells for the full activation of $CD8^+$cytotoxic T cells. CD80 and CD86 are important signals for full activation of T cells, sufficient cytokine production, and sensitive recognition of cancer cells. After that, phosphorylation of intracellular tyrosine residues attracts phosphatidylinositol 3-kinase (PI3K) which has a role of promoting T cell survival and normal cell growth-and-division cycle. PI3K triggers the activation of protein kinase B and nuclear factor-κB which, in turn, increases the expression of Bcl-xL. High expression of Bcl-xL on $CD8^+$ cytotoxic T cells promotes the anti-apoptosis of them, which allows them to survive longer. As a result, $CD8^+$cytotoxic T cells are ready to attack foreign cells (Raskov et al., 2021).

In addition to cytotoxic T cells, helper T cells have an important role in attacking the cancer cells. Helper T cells present surface protein CD4, which allows them to be referred to as $CD4^+$helper T cells. Their receptor binds to the fragments of antigens presented by class II MHC of cancer cells for activation of themselves. Unlike $CD8^+$cytotoxic T cells, $CD4^+$helper T cells help to gather other types of immune cells such as B cells and macrophages and trigger their immune responses to help attack cancer cells (Reiner, 2007).

While $CD4^+$ helper T cells have an indirect effect on cancer cells, $CD8^+$ cytotoxic T cells interact directly with cancer cells via two different mechanisms. The first mechanism is known as granule exocytosis, where cytoplasmic granules are released from $CD8^+$ cytotoxic T cells to tumor cells. The cytoplasmic granules mostly consist of a pore forming protein perforin 1 (PRF1) and granzyme B. PRF1 helps granzyme B infiltrate into the cytoplasm of tumor cells as it can form pores on the surface of cancer cells allowing the granzyme B to enter. When granzyme B successfully gets into the cancer cells, it breaks down intracellular substrates that control cell survivals leading to cancer cell death. Granzyme B can also sometimes destroy the cancer cells by causing cell lysis, which means rupturing the membrane of cancer cells. The second mechanism by which these immune cells work to destroy cancer cells is through the death ligand and death receptor system. $CD8^+$ cytotoxic

T cells dominantly express three death ligands: tumor necrosis factor (TNF)-α, tumor necrosis-related apoptosis-inducing ligand, and Fas ligand. These death ligands belong to the TNF family. TNF is a cytokine responsible for cell survival, growth, and death. When the death ligands bind to death receptors on the cancer cells, an extracellular apoptotic signal is released into the inside of the cancer cells, inducing apoptosis of cancer cells (Martínez-Lostao et al., 2015).

Regulatory T cells

CD4⁺ regulatory T cells

$CD4^+$regulatory T cells are a subset of $CD4^+$T cells but have different functions from $CD4^+$helper T cells (Saleh & Elkord, 2020). There are two types of regulatory T cells: natural regulatory T cells (nTregs) and induced regulatory T cells (iTregs). nTregs are developed from the thymus. They also refer to as $CD4^+CD25^+$ T cells since CD25 is commonly found on nTregs as a cell surface marker. iTregs are derived from conventional $CD4^+$ T cells by stimulation of cytokines IL-10 and TGF-β which are known to have suppressive functions on other immune cells. nTregs and iTregs with immunosuppressive functions express the transcription factor known as forkhead box protein 3 (FOXP3). FOXP3 is an important regulator of development, function, and homeostasis of $CD4^+$regulatory T cells (Workman et al., 2009). FOXP3 expression is induced when regulatory T cells bind to antigens presented by class II MHC of antigen presenting cells. Besides FOXP3, CD3, CD4, CD25, cytotoxic T-lymphocyte-associated protein 4, and CD103 are main cell surface markers of $CD4^+$regulatory T cells (Togashi & Nishikawa, 2017). Generally, $CD4^+$regulatory T cells restrain the immune response by directly interacting with $CD8^+$cytotoxic T cells and $CD4^+$helper T cells or by promoting apoptosis of these T cells. Most $CD4^+$regulatory T cells bind to targeting T cells directly for immune suppression. Sometimes, they produce PRF1 and granzyme B as one of their mechanisms to attack $CD8^+$cytotoxic T cells and $CD4^+$helper T cells (Wang, 2008).

CD8⁺ regulatory T cells

$CD8^+$ regulatory T cells are another population of T cells with capabilities of immune suppression. They develop in the tumor microenvironment or in a cytokine-rich environment that favors the production of regulatory T cells by antigen stimulation. There are five subsets of $CD8^+$ regulatory T cells: Qa-1-specific $CD8^+$ regulatory cells, alloantigen-specific $CD8^+CD28^-$ regulatory T cells, $CD8^+CD25^+$ regulatory T cells, γδ- TCR regulatory T cells, and natural killer T regulatory T cells. Qa-1-specific $CD8^+$ regulatory T cells recognize Qa-1 molecules on $CD4^+$ helper T cells and prevent natural immune response of $CD4^+$ helper T cells. Alloantigen-specific $CD8^+CD28^-$ regulatory T cells directly interact with dendritic cells and other antigen presenting cells that have an ability to suppress $CD4^+$ helper T cells. Alloantigen-specific $CD8^+CD28^-$ regulatory T cells increase expression of inhibitory receptors on antigen presenting cells so that they can effectively hinder the activity of $CD4^+$ helper T cells. $CD8^+CD25^+$ regulatory T cells are also activated by antigen presenting cells and have a crucial role in inhibiting growth and function of $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. Activated $CD8^+CD25^+$ regulatory T cells release IL-10 which is a cytokine that suppresses immune response of T cells. γδ- TCR regulatory cells suppress development of dendritic cells and T cell proliferation by releasing IL-10 and TGF-β. TGF-β is a cytokine that functions as a controller of immune response of T cells similar to IL-10. Natural killer T regulatory T cells also show immunosuppressive traits on T cells (Wang, 2008).

Interaction Between Regulatory T cells and Cancer cells

In the tumor microenvironment, a number of highly immune-suppressive regulatory T cells are found along with other immune cells, blood vessels, extracellular matrix, and other types of cells such as fibroblast (Murfin,

2021; Togashi et al., 2019). Cancer cells produce regulatory T cell-recruiting chemokines CCL22 and CCL1. These chemokines recognize and bind to specific receptors of regulatory T cells CCR4 and CCR8 respectively and recruit regulatory T cells into the tumor microenvironment (Togashi et al., 2019). These regulatory T cells are activated by actively growing and dying cancer cells that present a number of antigens. Since regulatory T cells have a higher T cell receptor affinity than the other T cells, regulatory T cells are better at recognizing antigens presented by the cancer cells and expand themselves in the tumor microenvironment (Tanaka & Sakaguchi, 2017; Togashi & Nishikawa, 2017).

The immunosuppressive characteristics of regulatory T cells have detrimental effects on treating cancers. One study shows that every doubling of $CD4^+$ regulatory T cells drops the survival rate from prostate cancer by 12% (Davidsson et al., 2013). Regulatory T cells can recognize antigens presented by cancer cells and suppress the antitumor immune response which results in growth and development of cancer cells. One study conducted in a murine fibrosarcoma model observed the effects of depleting regulatory T cells. A late stage of murine fibrosarcoma was infiltrated by abundant regulatory T cells. These regulatory T cells secrete inhibitory cytokines IL-10 and TGF- β to suppress the anti-tumor response of $CD4^+$ helper T cells. When levels of these inhibitory cytokines were significantly reduced, immune response of $CD4^+$ helper T cells on cancer cells was mostly restored. In addition, when the number of regulatory T cells were decreased, it was observed that the cancer was destroyed and that long-term anti-tumor memory was created. Regulatory T cells have a dominant influence on growth of cancer and that, even at the late stage of cancer, removal of the suppression of regulatory T cells can be effective to treat cancer (Beyer & Schultze, 2006).

CAR T-Cell Therapy

One of the conventional treatments for cancer is immunotherapy. It enables the immune system of the patients to better recognize and fight off cancer cells. This conventional immunotherapy can not always be effective since cancer often develops the mechanisms to resist against the treatment. Therefore, there is a large need to develop new treatments for cancer.

Currently, chimeric antigen receptor (CAR) T-cell therapy is perhaps one of the most promising immunotherapies against malignant cancers, especially hematological malignancies. CAR T-cell therapy uses a patient's own T cells and genetically modifies them to express CAR. CAR is a man-made receptor added onto a T cell designed to recognize one of the antigens specific to cancer (Han et al., 2021). Currently, most CARs are designed to target CD19. CD19 is an antigen selectively expressed by cancer cells, not normal cells, which makes CD19 an optimal target for CARs (Raskov et al., 2021). Furthermore, CAR T-cells have a fully functional MHC that helps easily bind to the antigens expressed by cancer cells. When CAR T-cells bind to antigens on cancer cells, they release cytotoxic agents previously discussed, PRF1 and granzyme B. These proteins promote apoptosis of cancer cells by inducing the increase of apoptosis signaling pathways (Han et al., 2021).

CAR Structure

CAR is composed of three domains: the ectodomain, the transmembrane domain, and the endodomain. The ectodomain is the region of recognition of cancer cell antigens. It is made up of a linker, a single-chain fragment variable (scFv), and a hinge region. The scFv is the component that identifies antigens and activates the T cell receptors (Cho et al., 2018). The transmembrane domain links the ectodomain to the endodomain and secures itself to the cell membrane for overall stability of the receptor. In addition, it typically helps the signal to be transmitted from the extracellular domain to the intracellular domain (Huang et al., 2020). Endodomain is composed of stimulatory molecules such as CD3ζ and costimulatory molecule(s) such as CD8, CD28, or CD137. A stimulatory molecule is a main signal that activates CAR T-cells to produce an immune response against cancer cells. Costimulatory molecules are for amplifying the main signal to help with full activation of CAR T-cells. Also, most importantly, costimulatory signals can increase a release of IL-2 which is a cytokine that

promotes the proliferation of CAR T-cells. Overall, costimulatory molecules are responsible for persistence of CAR T-cells (Sterner & Sterner, 2021). Endodomain also contains three immunoreceptor tyrosine-based activation motifs (ITAMs) which help to transduce signals to T cells (Zhang et al., 2017).

FDA Approved CAR T-cell Therapies

Currently, the U.S. Food and Drug Administration (FDA) approved three CAR T-cell therapies: Tisagenlecleucel, Axicabtagene ciloleucel, and Brexucabtagene autoleucel. All of them are to treat relapsed or refractory B cell cancers and target CD19 on the surface of cancer cells.

Tisagenlecleucel

Tisagenlecleucel is FDA approved CAR T-cell therapy to treat pediatric and young adults under 25 years old with relapsed or refractory B cell acute lymphoblastic leukemia (ALL) according to "KYMRIAH (tisagenlecleucel)" written by FDA in 2021. In the phase II clinical trials conducted as multi studies at 25 different sites around the world, 75 patients who are between 3 and 21 years of age with relapsed or refractory B cell ALL participated in the clinical trial with 3 months of follow up. They received tisagenlecleucel and showed promising results proving the effectiveness of the treatment. Among the patients who were followed up for 3 months, 60% of patients showed complete remission with complete hematologic recovery. 21% of patients had complete remission without complete hematologic recovery (Maude et al., 2018).

Axicabtagene ciloleucel

According to "YESCARTA (axicabtagene ciloleucel)" written by FDA in 2022, Axicabtagene ciloleucel is another FDA approved CAR T-cell therapy. This is for patients who have relapsed or refractory large B cell lymphoma with unsuccessful results from conventional chemoimmunotherapy. Axicabtagene ciloleucel is targeting CD19 expressed on cancer cells. In the phase II clinical trial, 54% of patients showed the complete response rate after the treatment. Even after 15.4 months, 40% of patients still showed the complete response rate (Neelapu et al., 2017).

Brexucabtagene autoleucel

Brexucabtagene autoleucel is CD19 directed CAR T-cell therapy for the treatment of relapsed B-cell precursor acute lymphoblastic leukemia. It was approved by the FDA on October 1, 2021. In the clinical trials, 52% of patients showed complete remission within 3 months. The complete remission lasted more than 12 months for majority patients. Drawback of brexucabtagene autoleucel is cytokine release syndrome and neurologic toxicities according to "FDA D.I.S.C.O. Burst Edition: FDA approval of Tecartus (brexucabtagene autoleucel) for adult patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia" written by FDA in 2021.

Current Limitations on CAR T-cell Therapy & Future Directions

CAR T-cell therapy is considered to be a new prospective treatment for cancers. However, there are still limitations on CAR T-cell therapy that are necessary to be overcome for CAR T-cell therapy to be effective, durable, and long-term treatment to treat cancer.

CAR T-cell Associated Toxicities

There are two common adverse effects of CAR T-cell therapy: neurological toxicity and cytokine release syndrome. Neurological toxicity is caused by high levels of cytokines in the cerebrum. It can lead to neurological

damages followed by delirium, expressive aphasia, seizures, and syncope. Currently, neurotoxicity is managed with corticosteroids. Cytokine release syndrome is caused by high levels of cytokines circulating in the body. When CAR binds to the target antigen, CAR T-cells release a large amount of cytokines because of their costimulatory molecules. When excessive cytokines are in the circulatory system, they can cause some symptoms like fever, tachycardia, or hypotension which can ultimately lead to respiratory problems or death. These adverse effects are sometimes used as a sign that CAR T-cells are working properly in the body. Currently, in the clinical trials for CAR T-cell therapy, neurological toxicity and cytokine release syndrome are used to find the right dosage of CAR T-cells for each participant (Han et al., 2021). Improved CAR T-cell therapy with fewer side-effects is needed and is still being researched.

Immunosuppressive tumor microenvironment

An immunosuppressive tumor microenvironment resulting from the metabolism of cancer cells is a barrier that T cells have to overcome. The tumor microenvironment is very acidic, lacking glucose and oxygen. After activation, T cells usually shift from oxidative phosphorylation to glycolysis to boost their expansion. Yet, if there is not a sufficient amount of glucose, which is a reactant of glycolysis, because of the condition of the tumor microenvironment, T cells can not go through glycolysis, thus resulting in reduced levels of proliferation of T cells (Martinez & Moon, 2019). In recent studies, this limitation can be addressed by combining immune checkpoint inhibitors with CAR T-cells. It helps to reduce immunosuppression on CAR T-cells which allows CAR T-cells to proliferate easily even under the immune suppressive condition (Qu et al., 2021).

Lack of successful outcomes in treating solid cancers

CAR T-cell therapy has brought some successful results in treatment of B cell cancers. However, even though a vast amount of research has been conducted to use CAR T-cell therapy for treating solid cancers, there have only been a few successful cases (Martinez & Moon, 2019). It is because of the limited ability of CAR T-cells to locate and penetrate solid cancers growing in immunosuppressive tumor microenvironment. One way to overcome this limitation is to add chemokine receptors on CAR T-cells that can respond to chemokines produced by cancer cells. As chemokines act as chemical attractants to immune cells, CAR T-cells can become better at locating solid cancer. CAR T-cells also have difficulty penetrating a solid cancer because of the stroma which holds the cancer tissue together tightly. This stroma is composed of heparin sulfate proteoglycan. If CAR T-cells can express heparanase which can break down heparin sulfate proteoglycan of stroma, they may be able to better infiltrate into solid cancers (Sterner & Sterner, 2021).

Another reason for having difficulties with tackling solid cancer is that many antigens on solid cancer cells are shared with antigens on healthy normal cells. CAR T-cells are typically engineered to bind to specific antigens expressed on cells and to attack these cells. However, if CAR T-cells are engineered to bind to the antigen that is present on both cancer cells and normal cells, CAR T-cells may bind to cancer cells as well as normal cells and attempt to destroy both types of cells. Attacking normal, healthy cells can ultimately lead to organ dysfunction. Therefore, the target antigen for CAR T-cells has to be specific for solid cancer cells. Since there are not many antigens that are specific to solid cancer cells, it is very difficult to use CAR T-cell therapy for solid cancer (Qu et al., 2021). There has not been a proposed solution to overcome this limitation.

Tumor Antigen escape

CD19-specific CAR T-cells specifically target a single antigen (CD19) expressed on the cancer cells. Majority of patients with refractory B cell cancers have responded to CD19 CAR T-cell therapy with 50-90% of complete response rate (Labanieh et al., 2018). However, CD19 CAR T-cells become ineffective when cancer cells develop resistance against them through loss of CD19 antigen expression. When CD19 is no longer expressed on the surface of the cancer cells, CAR T-cells are unable to recognize and bind to the cancer cells. In addition, cancer cells can evade CAR T-cell recognition through lineage switching. Lineage switching of the cell is a

case when the phenotype of the cell changes while the genotype of the cell remains the same. Through the lineage switching, cancer cells can hide expression of CD19 on their cell surfaces and escape immunosurveillance of CD19 CAR T-cells (Majzner & Mackall, 2018). In a global phase II clinical trial of Novartis's tisagenlecleucel, 25% of patients who had shown complete remission experienced a CD19 absent relapse in response to antigen escape of cancer cells. In hope of overcoming this limitation, dual-target CAR T cells are being developed. Cancer cells commonly express CD19, CD20, and CD22. Dual-target CAR T-cells are able to target more than one antigen on cancer cells which would decrease the possibility of cancer antigen escape. Currently, there are two models of CAR T-cells that can bind to more than one antigen in clinical trials. One model is referred to as a bicistronic CAR which has two antigen recognition domains with separate scFvs. This reduces the chance of relapse of cancer because of the antigen loss on the cancer. Even though cancer hides its expression of CD19, CAR can still target CD20 on the cancer and produce the anti-tumor response. The other model is referred to as a mono CAR. The mono CAR has two antigen recognition domains such as CD19 and CD22 expressed on the same scFv. It is activated exclusively when cancer both express CD19 and CD22 on its surface. In case of that the cancer may not naturally express both two antigens such as CD19 and CD22, patients get CAR T-cell therapy that contains three different CAR T- cells: CD19 targeted, CD22 targeted CAR T-cells, and mono CD19/CD22 CAR T-cells (National Cancer Institute, n.d.; Sterner & Sterner, 2021; Zhao et al., 2019;).

Axl CAR and Synthetic Notch receptor

Axl CAR and synthetic Notch receptors can be another possible solution for tumor antigen escape as well as for treating solid cancer. The Axl protein is a tyrosine kinase receptor that is highly expressed in many human solid cancers. If CAR T-cells can target Axl instead of CD19, CAR T-cells therapy can be widely used to treat solid cancers besides B cell cancers. Moreover, synthetic Notch receptors have been recently developed. They can be easily engineered by researchers to recognize a specific ligand and to produce a specific response such as release of the cytokines. Because of the high programmability of the synthetic Notch receptors, they have a great potential to be used for cancer therapies. The synthetic Notch receptors can be engineered to target the Axl protein on cancer cells, which have already been created. Researchers expect that if they show clinical benefits from further testing in animal models, the synthetic Notch receptors targeting Axl protein will be worth to be developed as a new cancer therapy (Cho et al., 2018).

Conclusion

Cancer is closely related to different types of T cells. It manipulates itself and its micro tumor environment to avoid immunosurveillance of $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. However, it actively recruits regulatory T cells for its own growth and proliferation. This explains why understanding T cell function is crucial to develop a new cancer treatment. $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells have natural immune surveillance role which is sometimes suppressed by regulatory T cells and cancer cells. Researchers discovered that enhancing the natural defense system of T cells may be able to defeat the cancer cells. CAR T-cell therapy is an example of new, advanced immunotherapies that strengthened the immune surveillance role of T cells by adding CAR on the surface of T cells so that T cells can attack cancer cells better. As CAR T-cell therapy is new, it is followed by several limitations. However, further understanding of the interaction between the immune cells and cancer cells would help to overcome limitations of CAR T-cell therapy and lead to other new promising immunotherapies that can save many lives in the future.

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