T Cell Therapy: Underlying Mechanisms and Current Advancements

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Abstract

As the second leading cause of death in 2016, cancer is one of the most serious diseases facing the world today. T cell therapy is a current area of research attempting to address the disease with two primary division: CAR-T and TCR-T cell therapy. The immune system naturally produces T lymphocytes to aid in the recognition and removal of cells infected with viruses or transformed into cancer. Under normal circumstances, T lymphocytes identify and destroy cancerous cells; however, some cancerous cell types can evade this system. With the use of genetic editing technology, T lymphocytes can gain the ability to recognize these evasive cancers. The editing process is known as T cell therapy.

T Cell Therapy: Underlying Mechanisms and Current Advancements

Discussion

Overview

Cancer is one of the most serious diseases facing the United States today (Siegel et al., 2016). In 2016, it was the second leading cause of death behind heart disease with an average of 4,600 new diagnoses daily. With the severity of the disease, new research is continually being conducted to better comprehend its molecular mechanisms. Great strides have been made in the reduction of cancer deaths; however, improvements can still be made in many cancer types. One avenue currently being evaluated for this is T cell therapy. To better understand how this treatment interacts with cancerous cells, a base understanding of cancer will be set first.

Hallmarks of Cancer

In their discussion on cancer, Hanahan & Weinberg (2011) set out six distinctive hallmarks of cells that make up these growths. Essentially, cancerous cells begin as normal functioning cells; however, as they mutate, they gradually gain each of these six hallmarks. Arguably, at their most base level, cancerous cells "sustain chronic proliferation" or the tendency to grow uncontrollably without accountability (Hanahan & Weinberg, 2011, p. 646). If these cells live, they will continue to divide without check, spreading well beyond their intending plane of living. They accomplish this by "evading growth suppressors," another hallmark of the transformation (Hanahan & Weinberg, 2011, p. 648). Under normal conditions, cells exhibiting uncontrolled growth would be rendered inert and killed by various cellular mechanisms regulating tumor growth such as the RB protein. This protein "integrates signals from diverse extracellular and intracellular sources and, in response, decides whether or not a cell should proceed through its growth-and-division cycle," (Hanahan & Weinberg, 2011, p. 648).

Additionally, cancerous cells possess the ability to bypass the replicative limitations imposed on normally functioning, non-stem cells. While most normally replicating cells can only replicate for a few generations before dying, cancerous cells have bypassed this inhibition to replicate indefinitely. They also induce angiogenesis to create extra blood vessels to support the growing mass of cells. Going further, cancerous cells generate the ability to invade neighboring cell types and spread to the rest of the body, inducing metastasis. Finally, these cells also resist apoptosis. Through both extrinsic and intrinsic signaling pathways, programmed cell death can be induced in damaged cells. T lymphocytes naturally survey the environment for altered-self or damaged cells requiring the induction of this process for the maintenance of homeostasis. Unfortunately, evasive cancer types avoid the mechanism to further promote survival. By altering the T lymphocytes' natural recognition and killing ability, T cell therapy attempts to address the problem of evasive cancerous cells.

T lymphocytes

One of the functions of T lymphocytes is to meditate the killing of damaged cells via the humoral and cellular immune responses (Chaplin, 2010). There are a variety of subsets that facilitate this function. The two main varieties are cytotoxic (T_c) and helper (T_h) T lymphocytes, the first serving to scan for damaged or altered-self cells and the second as controller cells granting permission for activation of cytotoxic and B lymphocytes. This is accomplished through interactions between the lymphocytes' T cell receptor (TCR) and surface markers on surrounding cells. To determine if a peripheral cell is damaged or altered, the TCR interacts with cell-surface glycoproteins known as major histocompatibility molecules (MHCs). MHCs present peptide antigens processed from both internal and external pathways, known as endogenous and exogenous respectively. For a TCR to interact with an MHC and its presented antigen, it must

also possess surface proteins known as clusters of differentiation (CDs). A wide variety of CDs are observed across the body that serve a variety of functions; however, central to T cell therapy are two: CD4 and CD8.

T_c and T_h cells are differentiated mainly on whether they are $CD4^+$ or $CD8^+$ as they are central to function (Chaplin, 2010). Cytotoxic cells express CD8 while helper cells express CD4. CD4 and CD8 determine T lymphocyte function by limiting interactions to specific MHCs and activating different signaling pathways. There are two main classes of MHC molecules: I and II. MHC class I presents antigens processed endogenously to $CD8^+$ T cells while class II presents exogenously processed antigens to CD4. By interacting with antigens processed extracellularly, T_h cells work to modulate the humoral and cellular response to potentially pathogenic organisms while also alerting T_c cells to potential threats. Contrarily, T_c cells function by killing damaged or infected cells as they primarily interact with antigens originating from intracellular products. Through the natural endogenous processing system, intracellular products are broken down into small peptide fragments for presentation on MHC class 1 molecules to $CD8^+$ T lymphocytes.

T_c Function

Tc lymphocytes play an important role in the immune system by responding to endogenous antigens (Chaplin, 2010). Nearly every cell in the body expresses MHC class I molecules for the expression of endogenous antigens. The intracellular antigens expressed on these MHCs originate primarily from expired cellular products. Through the processes of negative selection and tolerance, T_c cells are unable to bind self-antigens, thus any cell expressing them is passed over preventing the immune system from attacking normally functioning cells. Due to this system of endogenous antigen presentation, T_c lymphocytes are also able to catch viral or altered-self products of these cells. Upon identifying such non-self

products, apoptosis can be induced in the target cell through cytokine signaling or ligand binding. This is a spontaneous and natural process constantly occurring in the body as T lymphocytes pass tissues through normal bodily function.

Negative Selection and Tolerance

To properly discriminate between self and non-self antigens, T lymphocytes undergo a thorough selection process during development followed by tolerance programming in peripheral tissues (Passos et al., 2017). After initial differentiation in the bone marrow, T lymphocytes are sent to the thymus to undergo development. Here, they are selected based on their antigen recognition. Generally, if they recognize a self antigen, they are killed; however, if they recognize a non-self antigen, they are allowed to survive. The process occurs in two steps: positive and negative selection. During negative selection, specialized medullary thymic epithelial cells (mTECs) expressing the autoimmune regulator (*Aire*) gene present self-antigens to naïve T lymphocytes. The *Aire* gene allows mTECs to display antigens from tissues found throughout the body. These cells effectively serve as a trial run for naïve T lymphocytes. If the T lymphocytes were to react with the repertoire of self antigens displayed on these cells, an apoptotic signal would be induced, promoting cell death in a process known as clonal deletion. Functionally, this serves the body well, because if numerous self-reactive T cells were allowed to mature, an autoimmune reaction would ensue.

Unfortunately, negative selection is not a perfect process as not all self antigens are presented (Redmond et al., 2008). This allows some self-reactive T cells to escape. To atone for these errors, self antigens are again presented to lymphocytes in secondary lymphoid tissues via APC cross-presentation and regulatory T cells (T_{reg}) (Beissert et al., 2006). If T cells interact with these antigens, peripheral tolerance mechanisms are activated that serve to either induce

anergy or apoptosis in the self-reactive cells. For example, through interactions with APCs via cytotoxic T lym-

phocyte-associated antigen – 4 (CTLA-4), T_{reg} cells can impede APC production of tryptophan, a proliferative stimulus for T_c cells, to inhibit T_c cell activation. This process presents a roadblock to a T lymphocyte's ability to fight evasive cancer types.

T_c Cell Limitations and Cancer

Largely, $CD8^+$ T_c cells function to destroy self cells that have been infected or altered so that they no longer serve their original bodily function. They screen for such cells by examining antigens presented on MHC class 1 molecules. Non-self antigens presented on these cells' MHCs mark them for death based on the programming of central and peripheral tolerance. Through this system, many diseases are fought by T_c cells; unfortunately, diseases in which the damaged cell continues presenting normal antigens are not as easily detected. Many proliferative diseases that produce tumors such as cancer fall under this category as they suppress the expression of tumor markers on MHC class 1 molecules. Therefore, normally reproducing T_c cells are largely unable to destroy cancerous growths.

Additionally, these tissues are often surrounded by an abundant number of immunosuppressive cells such as regulatory T lymphocytes and M2 macrophages in a dense fibrous matrix (Zhao & Cao, 2019). The dense matrix impedes the ability of the lymphocytes to access the rampantly dividing cells. Meanwhile, immunosuppressive cells can inhibit T lymphocytes by binding to negative regulatory ligands found on their surface such as cytotoxic T lymphocyte antigen-4 (CTLA-4) (Smith-Garvin et al., 2009). CTLA-4 is an important inhibitory receptor found on the surface of T lymphocytes important in self-tolerance. An additional inhibitory receptor found on T lymphocytes is the programmed death-1 (PD-1) receptor. Both

these receptors are targeted by the immunosuppressive matrix surrounding dense tumors to limit the expansion and activation of T lymphocytes. Also, suppressive cytokines are released to further limit the activity of these cells such as IL-10. Finally, the dense matrix has been found to induce lymphocyte exhaustion due to a lack of essential amino acids, low oxygen concentration, and high acidity (Zhao & Cao, 2019). Taken together, the environment surrounding solid tumors specifically targets the proliferative ability and activation of T lymphocytes through cytokine secretion, inhibitor binding, and nutrient deprivation.

T Cell Therapy

Due to the body's inability to fight some transformed cells, numerous treatments have been devised to address the disease. To do so, the treatments must first identify and then exploit physiological differences between normal and cancerous cells. Some of the most common such as chemotherapy and radiation therapy are devised to target cancer's hallmark of chronic proliferation. The majority of normally functioning cells in the body do not proliferate continually; therefore, these chemicals can be used to destroy cancer while leaving most healthy cells unaffected. Unfortunately, cells that multiply quickly under normal circumstances are also killed. Subsequently, these therapies have a high toll on the body, killing cells such as hair follicles and many activating immune cells limiting clonal expansion. To fully eradicate the disease, surgery is often used to remove the weakened growths.

While this strategy is effective for some cancers, many types remain unaffected by it. Additionally, the effects it has on the body are destructive. Therefore, new treatments are continually being developed to target other hallmarks. T cell therapy is one such treatment as it attempts to address the roadblocks keeping T lymphocytes from destroying cancerous cells (Chi et al., 2016). First, through the genetic altering technology of CRISPR/Cas9 or retroviral vectors,

TCR genes can be edited to code for a binding pocket that recognizes specific cancerous antigens, programming the T lymphocytes to target these cells (Milone & O'Doherty, 2018). This binding pocket is composed of two polypeptide chains, which both contribute to the specificity of the antigen binding domain (Roth, 2014). The chains responsible for the binding domain are encoded by exons composed of variable (V), diversity (D), and joining (J) regions. These are normally assembled through the process of V(D)J recombination. During development in the thymus, these regions are rearranged by ligating random regions sequentially from V to D to J. This generates variability in the antigen binding domain. Further variability is also added by random addition and deletion of the nucleotides in the junctions between regions. The result of this variability is that each antigen binding domain binds one specific antigen. In T cell therapy, this process is altered by addition of specific genes encoding for a specific binding pocket.

Finally, these altered cells are aided in accessing the diseased tissue by editing additional genes that promote survival and cell penetration (Zhao & Cao, 2019). If fully developed, the technique could attack cancer while leaving the rest of the body untouched. Improvements must be made; however, strides are routinely being made to further progress the treatment.

CRISPR/Cas9

The origins of the CRISPR/Cas9 system are found in bacteria as a defense mechanism from foreign, invading genetic material such as viral DNA and plasmids (Ren & Zhao, 2017). Viruses routinely attempt to take over bacterial, host machinery to replicate progeny by inserting their genetic material for insertion into the host genome. Undefended, the host cell would be destroyed. The CRISPR/Cas9 system is used to fight this attack by recognizing foreign genetic information and marking it for degradation. Essentially, the system is divided into two separate components forming a complex: a CRISPR DNA library and a Cas9 endonuclease. If the cell

recognizes foreign, genetic material, it is destroyed while a copy is created and inserted into a DNA library. After addition to the library, CRISPR RNA (crRNA) is transcribed from the stored DNA sequence to bind the genetic material if infected again. If the bacterium is again targeted, the crRNA serves as a guide RNA for Cas9. Upon recognition, the crRNA binds the invading genetic material, complexing with and activating Cas9. This triggers the endonuclease to cleave the viral genetic material.

This defense system can be utilized to induce exact edits to genetic information (Chi et al., 2016). By leading Cas9 to specific sites, researchers have been able to induce DSBs at precise locations in targeted DNA. To begin, guide RNAs engineered to pair with specific DNA sequences are produced to bypass the use of crRNA. This guide RNA complexes with the Cas9 endonuclease to create double-stranded breaks (DSBs). Meanwhile, a significant amount of donor sequence is introduced to the break site (Zhan et al., 2018). The donor sequence possesses blunt ends matching that of the DSB to encourage cell-mediated homology-directed repair (HDR). The process ends with the permanent insertion of the new sequence into the host genome to direct downstream transcription. While effective, this method of gene editing is not commonly utilized in T cell therapy.

Retroviral Vectors

An alternate avenue of insertion of donor DNA can be achieved using retroviral vectors (Milone & O'Doherty, 2018). These vectors are created from genetically altered retroviruses. Normally these viruses survive by integrating their genetic information into the host genome. This is accomplished through viral integration. Under normal conditions, the mature retrovirus begins its life cycle after infecting the host. At this stage, viral proteins and genetic material are unloaded into the host cell. The viral proteins proceed to form an integration complex with a

primary component known as viral integrase (IN). IN prepares the donor information via 3 strand cleavage with the genetic information to form the reverse transcription complex (RTC). This complex converts the RNA into viral DNA in the process of reverse transcription. Additionally, it aids in the process of inserting the viral DNA into the host genome. Upon insertion, the host machinery transcribes the viral information into viral particles to produce viral progeny.

This process can be harnessed to engineer the genetic information of T lymphocytes by inserting donor DNA encoding either an edited receptor or additional binding components into the cell's genome (Milone & O'Doherty, 2018). To begin, the subclass of retrovirus is selected as each possesses different insertion preferences. Unfortunately, the exact point of insertion is difficult to control. Each vector possesses different affinities for insertion sites; however, the points of insertion are still variable (Ren & Zhao, 2017). A way to increase site specificity is by incorporating the CRISPR system as a guide for the viral machinery. This technique allows the viral genetic information to gain access to the cell through retrovirus machinery while also allowing site specificity through the CRSIPR-Cas9 system.

In addition, steps are taken to decrease the virulence ability of the retrovirus (Ren & Zhao, 2017). Viral vectors are crippled to remove their ability to replicate on their own. If the vector were to retain its replicative ability, it would hijack the cell for creation of progeny. To do this, specific virulence genes are removed while additional viral genes are separated into separate plasmids. The donor sequences are then packaged and added to the retrovirus. Once accomplished, the viral vector is no more than a delivery agent incapable of self-replication. From this point, the targeted cell is transformed with the vector to accomplish genetic editing.

CAR-T and TCR-T Cell Therapy

T cell therapy researchers use genetic editing technology to genetically alter the binding and activation components of T lymphocytes (Duong et al., 2019). They do this in one of two ways. Either the TCR is altered to create a binding pocket specific to cancerous antigens or an additional binding component known as a chimeric antigen receptor (CAR) is added to the binding repertoire. Both techniques alter the binding capability of the cell; however, they do so in varying ways.

TCR-T Cell Therapy

The first editing technique, TCR-T cell therapy, originated from an experiment by Bluthmann et al. (1988) in which TCR genes were transfected from one T cell to another giving them identical binding capabilities. This technique was adapted to alter TCR genes to bind specific antigens presented by cancerous cells (Zhao & Cao, 2019). The first step is to identify antigens presented specifically by the cancerous growth being targeted. According to Rath & Arber, four criteria are required for successful antigen selection (2020). First, the antigen must be selectively expressed on tumor cells. It must also have a low or negligible expression on healthy cells. After this, it must possess sufficient immunogenicity to generate an immune response against it. Finally, the tumor's survival must rely on the antigen's presentation. Once an antigen fitting these criteria is discovered, its structure is analyzed to determine the structure of the required TCR binding pocket necessary for high-affinity binding to both MHC and antigen (Zhao & Cao, 2019). The complementarity determining regions (CDRs) of the variable amino acid chains in the TCR are altered to bind and generate an activation signal in the T lymphocyte (Rath & Arber, 2020). To accomplish this, live, naïve T lymphocytes are removed *ex vivo* for genetic editing (Zhao & Cao, 2019). While gene editing could be accomplished *in vivo,* extreme

risks accompany such a technique. Such risks include generating T lymphocytes with autoimmune tendencies. For example, through improper editing, the effects of negative selection and peripheral tolerance could be overridden. As previously stated, T lymphocytes with high affinity for self antigens are destroyed to decrease the risk for immune targeting of healthy bodily cells. If TCR therapy results in lymphocytes with affinity for healthy cells, the targeting of evasive cancer species would not only fail but cause potentially lethal side effects. Therefore, the cells are removed for editing outside the body. Once removed, genetic loci are targeted in the host genome by genetic editing mechanisms. Insertion of the DNA donor sequence coding for the new binding pocket follows. The engineered T lymphocytes are tested for successful conversion then reinstituted into the body near the site of cancerous activity to induce cellular death in the diseased cells.

Clinical Manifestations

Successful applications of this technique have been shown in numerous different settings. One example is found in a study conducted by Rapaport et al. in which TCR engineered T lymphocytes were utilized to target multiple myeloma (MM) tumor cells (2015). The lymphocytes were set to recognize the NY-ESO-1 and LAGE-1 antigens. Through past experimentation, it was discovered that approximately 60% of advanced MM cells express the NY-ESO-1 protein antigen, an immunogenic cancer-testis antigen, on their MHC class 1 molecules. This antigen has previously been shown to produce spontaneous and vaccine-induced immunity making it a good target for this therapy. Therefore, Rapaport et al. hypothesized that a TCR engineered to recognize these antigens could induce cell death in the targeted cells.

To generate T lymphocytes capable of binding these targeted antigens, the immune cells were injected with an HIV-1 derived lentiviral vector (Rapaport et al., 2015). The vector was

produced by addition of a transfer vector expressed on four plasmids and other components responsible for the insertion of the donor sequence into the host genome. Following this, the T lymphocytes were edited by transformation with the generated lentiviral vector and re-inserted *in vivo*. The lymphocytes were allowed to interact with host tissues and analyzed incrementally after implantation. To study the effect of the TCR lymphocytes, samples were taken from bone marrow and peripheral blood tissues. For example, after running tests such as Q-PCR analysis and TCR clonotype analysis, it was determined that a statistically significant number of T lymphocytes expressed the desired engineered TCR. In conclusion, the research team found that NY-ESOc259 TCR-engineered T-cells generated ex-vivo did not experience rejection from host tissues or stimulate significant negative side effects. Further, they were found to expand in vivo, traffic well to the tumor site, and show persistent, on-target anti-tumor activity. This study provides significant proof of the potential effectiveness of this type of treatment model.

Going further, significant progress has also been seen in the treatment of other solid tumor types. For example, the MART-1 antigen has been targeted in the treatment of metastatic melanoma. In a study of 20 people, the average person experienced a 30% objective antitumor response. Even more impressive, researchers studying this disease and synovial cell carcinoma attempted treatment through targeting the NY-ESO-1 antigen. In this study of 17 people, two saw complete remission while one saw partial remission. Additional studies have also revealed promise in targeting leukemia and lymphoma. By generating proprietary antibody-TCR (AbTCR) T cells, these hematological malignancies have begun to be addressed.

Advantages and Challenges

TCR-T cell therapy has several advantages over traditional cancer therapy techniques. For example, any antigen presented on MHC molecules can induce an antitumor response in the

engineered T lymphocytes. This provides a wide range of targets for this therapy technique in comparison to the hallmark of proliferation frequently targeted by traditional methods. Additionally, because the lymphocytes maintain all the TCR auxiliary molecules of signal transduction, they are capable of full activation by antigen recognition. This retention of function allows engineered T lymphocytes to generate potent anti-tumor effects surpassing that of conventional techniques.

Unfortunately, TCR-T cell therapy also has a certain number of challenges that limit the application of this technique to cancer treatment. For example, because it is using a TCR to recognize cancerous antigens, the antigens must be processed endogenously and presented on MHC class 1 molecules. This excludes any antigen presented either without an MHC or on an MHC class 2 molecule on the target cell's membrane This is known as MHC restriction as antigen recognition is restricted only to those capable of presentation on the correct MHCs. This poses a great obstacle to the application of the disease as the number of presented cancerous antigens is limited. Additionally, there is a risk of host vs. graft disease. Essentially, the grafted tissue, in this case reintroduced T cells, can attack the normal host tissue. By altering the affinity of TCRs towards self-MHC molecules, dangerous cross-reactivity can occur (Rath & Arber, 2020). Instead of generating a killing response against the targeted antigen, physiologically normal antigens are targeted and activate the altered T lymphocyte. This can lead to lethal reactions in the patient. Finally, the infiltrating TCR-T cells must overcome the tumor microenvironment (TME). While altered to recognize cancerous antigens, the TCR lymphocytes retain the limitations to the TME as unaltered T lymphocytes. Each TCR lymphocyte encounters the dense, physical barrier of tumor-associated cells surrounding the area obstructing invasion. Anergy is also encouraged by cytokines such as IL-10 and inhibitory receptor binding such as

CTLA-4 and PD-1. Additionally, the tumor lacks homing factors specific to T lymphocytes, which leads to a lack of directed movement to the tumor and a subsequent lack of tumor antigen detection. To further inhibit T lymphocytes, the TME also recruits additional immunosuppressive cells such as tumor-associated macrophages and myeloid derived suppressor cells.

CAR-T Cell Therapy

CAR-T cell therapy addresses the problem of cancer similarly to TCR-T cell therapy; however, significant differences exist in the recognition and activation of the T lymphocyte (Wang et al., 2017). The main difference lies in the core of the CAR-T cell: the chimeric antigen receptor. This receptor complex allows the T lymphocyte to recognize any antigenic protein located on the surface of the plasma membrane of the target cell without MHC restriction. Essentially, the CAR bypasses the need for the use of the TCR complex. From here, a CAR-T cell is placed into one of three classes (or generations) based on its components (Barrett et al., 2014). For the latest generation, it is routinely comprised of the single-chain variable fragment domain (scFv) of the targeted antibody for antibody recognition and intracellular signaling domains including that of CD3z for signaling and T lymphocyte activation (Wang et al., 2017). The CAR is also complexed with several costimulatory CDs such as CD28, CD 137, or CD134 to help achieve activation. By including these components, the CAR acts to replace the TCR, excluding the need for the normally required MHC/TCR stimulatory interaction. With this in mind, to develop a CAR-T cell, an antigen on the surface of the targeted cell must be chosen for CAR development. Once picked, the CAR complex is converted into its corresponding genetic code and inserted into the T lymphocyte genome *ex vivo* via genetic editing technology. After

testing for successful transfection and safety, like TCR-T cells, they are transferred back to the body for immunogenic activity.

Clinical Manifestations

Large potential for CAR-T cell therapy has been witnessed by researchers in a wide variety of cancer treatments. One of the greatest advancements has been revealed in the treatment of B cell malignancies. For example, one study by Ying et al. (2019) delved into the possibility of treating refractory B cell lymphoma. It has been previously shown that B cells express CD19 throughout development and are present on the majority of cancerous B cells. Therefore, anti-CD19 CAR-T cells (CART-19) targeting the CD19 immunoglobulin domain are highly utilized in B lymphocyte cancer immunotherapy. Specifically, the study by Ying et al. chose to target B cell lymphoma by generating a new species of CART-19 lymphocytes known as CD19-BBz(86). This species was adapted from the previous CD19-BBz species which possessed an scFv from the FMC63 antigen, several costimulatory domains including CD3 signaling domains, and CD8α transmembrane and linking domains. To improve its safety, the CD8α domains were genetically altered resulting in the CD19-BBz(86) variant. This CART-19 species was then packaged into a lentiviral vector for transfection of T lymphocytes. These CART-19 cells were found to produce cytokines at decreased levels, express anti-apoptotic molecules at higher levels, and proliferate slower than the prototype CD19-BBz CAR T cells while retaining potent cytolytic activity.

After this discovery, the newly engineered CART-19 cells were infused to 25 patients exhibiting refractory B cell lymphoma. Of the 25 patients, six received a low dose, eight received a medium dose, and eleven received a high dose. Three of the patients receiving a low dose manifested a treatment response with two attaining partial remission and one complete

remission. In addition, three of the medium dose patients attained partial remission. Remarkably, complete remission was seen in six of the high dose patients while two achieved partial remission. The results of the study revealed an incredible new avenue for the treatment of this disease.

Another disease seeing marvelous improvement through CAR-T cell therapy is acute lymphoblastic leukemia (ALL) (Maude et al., 2014). Similar to B cell lymphoma, the researchers studying ALL engineered T lymphocytes to recognize $CD19^{+}$ cancerous B cells. By generating a CD19-BBz variant capable of recognizing ALL cells, the researchers were able to synthesize CART-19 cells capable of fighting the disease. Again, like the study by Ying et al., the cells were engineered via a lentiviral vector and returned to the host for immunogenic activity. Incredibly, one month after infusion twenty-seven of the thirty participants were in complete remission. Unfortunately, only half remained in full remission at six months after treatment. While CAR-T cell therapy has shown incredible potential for cancer treatment, advances are required for remission to be achieved more safely and to be applied to a greater number of people.

Barriers

One of the greatest advantages to CAR-T cell therapy is its ability to target non-HLA presented peptide antigens (Zhao & Cao, 2019). Often, cancerous cells downregulate MHC molecules to reduce the presentation of cancerous antigens to T lymphocytes. CAR-T cells are successfully able to circumvent this strategy. A subsequent advantage is also found in that any antigen found on the cell's surface is viable for targeting. This presents a large range of targetable antigens for use in CAR-T cell therapy.

Unfortunately, while this treatment shows great promise for the targeting of cancerous cells, several barriers stand in the way of further application in the field. One of the largest barriers is found in cytokine release storms (CRSs) as it is the most widely reported adverse side effect of CAR-T cell therapy (Zhao & Cao, 2019). Essentially, CRS is the release of a significant amount of inflammatory cytokines from the interaction of CAR-T cells with their environment. However, the cytokines do not originate solely from the engineered lymphocytes. Myeloid cells such as monocytes, macrophages, and dendritic cells also synthesize and release cytokines, which add to those caused by the engineered T lymphocytes. Ultimately, CRS can result in life-threatening complications from the accumulation of dangerous symptoms such as long term fever, hypotension, dyspnea, and organ problems. Additionally, patients undergoing CAR-T cell therapy risk neurotoxicity. Overall, 40% of those treated experience this dangerous complication. Symptoms include decreased consciousness, confusion, seizures, and brain edema. While it can be paired with CRS, this side effect can be found alone.

Another significant challenge associated with CAR-T cell treatment is on-target/off-tumor toxicity (Zhao & Cao, 2019). When selecting antigens for CAR recognition, the ideal would be to only select those expressed on the surface of cancerous cells; unfortunately, the majority of cancerous antigens are not specific to the diseased cells. Due to this lack of specificity, there is a high difficulty associated with identifying antigens exclusively expressed on cancerous cells. Subsequently, most CAR-T treatments possess at least small amounts of on-target antigen recognition on off-target cells. This aspect of the treatment leads to most of the side effects seen in CAR-T cell therapy.

Another considerable challenge facing CAR-T cell therapy is that of solid tumor treatment (Watanabe & Nishikawa, 2021). Incredible potential has been seen in the treatment of

hematological tumors; however, solid tumors have shown less promise. Clinical trials often result in unsatisfactory results compared to blood related malignancies. This is due to several factors including the type of antigens found on solid tumors and the accumulation of immunosuppressive cells. Unfortunately, while the antigens presented on hematological tumors are tissue specific, that is not the case with solid tumors. Often, these antigens are also expressed on normally functioning tissues making it hard to find antigens for targeting by the CARs. Going further, even after targeting specific solid tumor antigens, as in TCR-T cell therapy, the TME of the solid tumor actively counteracts the effects of the T lymphocytes.

Finally, CAR-T cell therapy is incredibly expensive (Zhao & Cao, 2019). In 2019, only two therapy options were available for patients willing to test the experimental treatment. The more costly option equated to an average of $510,963 per patient while the less expensive cost an average of $402,647. If no effect was seen after one month, the fees were waived; however, if an effect was seen, the price was high. Unfortunately, after nearly three years of research, the price still hovers around $400,000 excluding inpatient hospitalization costs and any treatment costs resulting from complications (Bhaskar et al., 2021). Going further, not only is the treatment costly, but it also requires a significant amount of time (Zhao & Cao, 2019). It takes anywhere from half a month to one month to generate specific CAR-T cells for any one patient. This large amount of time carries the ability to prevent patients from receiving treatment in a time-efficient manner. Considerable challenges face the development and applicability of this treatment.

TCR-T vs CAR-T Cell Therapy

Both treatment options possess associated pros and cons, some shared while others specific (Zhao & Cao, 2019). For example, TCR-T cells can target not only cell-surface antigens but also intracellular derived antigens. Through this capability, antigens such as NY-ESO-1 are

utilized by TCR-T cells to target cancerous cells in a way CAR-T cells cannot. Unfortunately, the antigens must be presented by MHC molecules targetable by the TCR. This largely limits the number of targetable antigens, especially if the cancerous cell downregulates or mutates its MHC molecules. A plateau has been seen in recent years in TCR-T cell therapy due to the limiting nature of MHC restriction associated with this technique. Until additional intracellular cancer antigens are identified, this treatment method will remain limited in its scope of treatment.

Contrarily, while the CAR component of CAR-T cells cannot target intracellularly derived antigens, it can target any cell surface antigen (Zhao & Cao, 2019). As previously stated, the CAR is not inhibited by MHC restriction. This adds an ability to the CAR repertoire that the TCR does not possess. Additionally, since TCR-T cell therapy relies on MHC presentation, it is also restricted to recognizing peptide antigens. This is another restriction bypassed by the CAR model. Any antigen, regardless of molecular composition, can be targeted by the CAR-T cell. Because of these properties, the CAR possesses a much broader range of targetable antigens. However, while virtually any cell can be targeted, antigens must be identified that are expressed solely on the cancerous cell. Without selective cancerous antigenic expression, the CAR-T cell would stimulate cell death in normal host tissue, causing more harm than good. Additionally, the dosage required for CAR-T cell therapy is significantly less than that required for TCR-T cell therapy due to the CAR's high specificity, clear target, and lack of MHC restriction.

Continuing, both CAR-T and TCR-T cell therapy experience similar setbacks in their respective treatment models (Zhao & Cao, 2019). One of the largest examples is the barrier associated with the TME in solid tumors (Watanabe & Nishikawa, 2021). Both models have shown promise in the treatment of hematological tumors, with great success shown in CAR-T models specifically; unfortunately, the immunosuppressive nature of the TME found in solid

tumors has yielded challenges in the function, infiltration, and survival of T lymphocytes. While both CAR-T and TCR-T cells possess the ability to target specific antigens associated with the cancerous tissue, the lymphocytes often encounter resistance discouraging proliferation and activation. From this action of the TME, both therapy techniques have encountered significant resistance in the treatment of solid tumors. Both have shown great promise in the treatment of hematological malignancies; however, strides need to be made in this area for either treatment to successfully address solid tumors.

Future Directions

CRS

Both CAR-T and TCR-T cell therapy models have revealed promise in their respective treatments; however, to broaden their scope, improvements must be made (Zhao & Cao, 2019). For example, by addressing the problem of CRS, CAR-T cell therapy could be advanced. As previously stated, the impact of CAR-T cells on the body often initiates the release of pro-inflammatory cytokines, primarily IL-6, which result in potentially life-threatening symptoms. If CRS was reduced or negated, the safety of CAR-T cell therapy would be greatly improved. Currently, treatment options for those threatened with CRS are limited to “intensive medical care, including the use of ventilators, drugs to increase blood pressure, and antiepilepsy drugs,” (Zhao & Cao, 2019, p. 6). Additionally, an IL-6 inhibitor drug has been approved by the FDA known as tocilizumab. This drug has begun to be used widely in the effort to suppress the inflammatory immune response and has been shown repeatedly to reduce CRS (Brudno & Kochenderfer, 2019). Further, the use of corticosteroids in tandem with tocilizumab has resulted in even greater immune suppression. Unfortunately, corticosteroids can also have the effect of T lymphocyte impairment, so their use is reserved for high grade CRS. Additionally, the use of

these drugs does not always abolish CRS, only serving to lessen the severity of symptoms. The development of next generation CAR-T cells could address this problem at its source. By generating lymphocytes capable of secreting anti-inflammatory cytokines or possessing additional co-stimulatory domains, the cells inducing the inflammatory response could be trained to avoid this complication.

CRISPR/Cas9

Another avenue in which improvement can be made is that of the process of insertion of genetic information (Ren & Zhao, 2017). Currently, the process of inserting large DNA sequences encoding CARs requires a viral vector and is preferred in TCR editing. Unfortunately, these vectors, as previously stated, are not site-specific. This limitation can lead to ineffective or disruptive gene insertion especially when the donor information is inserted into essential DNA sequences. To address this, researchers have searched for ways in which to insert genetic information via homology directed repair (HDR). One of the primary methods for doing so is through CRISPR/Cas9. As previously stated, by adding the sgRNA guide sequences and Cas9 endonuclease to the vector, specific sites can be targeted to eliminate the random insertion effect of lentiviral or adenoviral vectors. Unfortunately, advances need to be made in the applicability of this technique. For example, the use of CRISPR/Cas9 requires reagents that can be highly toxic to the T lymphocytes targeted for transformation. In addition, this technique experiences difficulties when inserting large DNA sequences. With these specific avenues addressed, successful on-target DNA insertion could be more easily achieved.

TME

A further direction holding potential for improving T lymphocyte activity is found in addressing the TME (Arina et al., 2016). A significant challenge to both CAR-T and TCR-T cell

therapy is that of the environment surrounding solid tumors. As previously described, it actively inhibits the proliferation and activation of T lymphocytes; therefore, neutralizing its effects could greatly improve the efficacy of both T cell therapy strategies. Although improvements need to be made in each, a variety of methods have been developed to decrease the effects of the TME.

One of these is the generation of tertiary lymphoid structures (TLS) in the proximity of the TME (Arina et al., 2016). Similar in composition to lymph nodes, TLSs commonly develop in the presence of chronic inflammation or cancer to support and promote lymphocyte activity in these regions. The presence of TLSs has been correlated with a stronger immune response to both colorectal and lung cancer. In theory, by promoting the growth of these lymphoid tissues, the persistence of T lymphocytes would be encouraged in these sites. A similar study on LIGHT (TNFSF14), a signaling molecule in the TNF family, revealed results in favor of this theoretical treatment. LIGHT can bind to HVEM on T cells as a costimulatory molecule and lymphotoxin-β receptor (LTβR) on non-lymphoid cells. LTβR results in the organization and maintenance of lymphoid tissues such as lymph nodes through its expression. When LIGHT was overexpressed in cancer patients, T lymphocyte infiltration was greatly improved in their TMEs providing evidence for the validity of the TLS approach. Further research in this area could serve to advance this theoretical treatment option.

Additionally, furthering the understanding of signaling molecules such as chemokines and cytokines contributing to both immunosuppression and stimulation could help decrease the effects of the TME (Arina et al., 2016). For example, through the expression of the CCL2 chemokine tumor-associated macrophages (TAMs) are recruited to the cancerous site to maintain the suppressive nature of the TME. Similarly, the CSF-1 cytokine is an important signaling molecule in the recruitment of immunosuppressive monocytes. If the cytokine and chemokine

milieu surrounding the TME were altered, its immunosuppressive nature could be greatly decreased. One way to accomplish this would be to generate T lymphocytes with the ability to secrete cytokines and chemokines promoting their infiltration and proliferation. One study attempted this by generating CAR-T lymphocytes engineered to produce IL-7 and CCL19 (Watanabe & Nishikawa, 2021). These are commonly seen in the maintenance of T lymphocyte zones in lymph nodes via T-zone fibroblast reticular cells secretion. The modified cells showed an increased anti-tumor effect that could be leveraged for future treatment techniques. Further studies could reveal improved treatment options for both CAR-T and TCR-T cell therapy.

Conclusion

To conclude, T cell therapy is an emerging therapeutic option for patients afflicted by many cancer types. By manipulating the natural defenses of the body, clinicians can target specific cancerous tissues without the undesired effect of killing healthy, bystander cells. Unfortunately, significant challenges such as CRS, the TME, and high cost stand in the way of widespread application of this technique, especially in the treatment of solid tumors. However, in recent years, great progress has been seen. With continued effort, T cell therapy could be applied to larger populations of people in the pursuit of curing cancer.

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