Teens In Health

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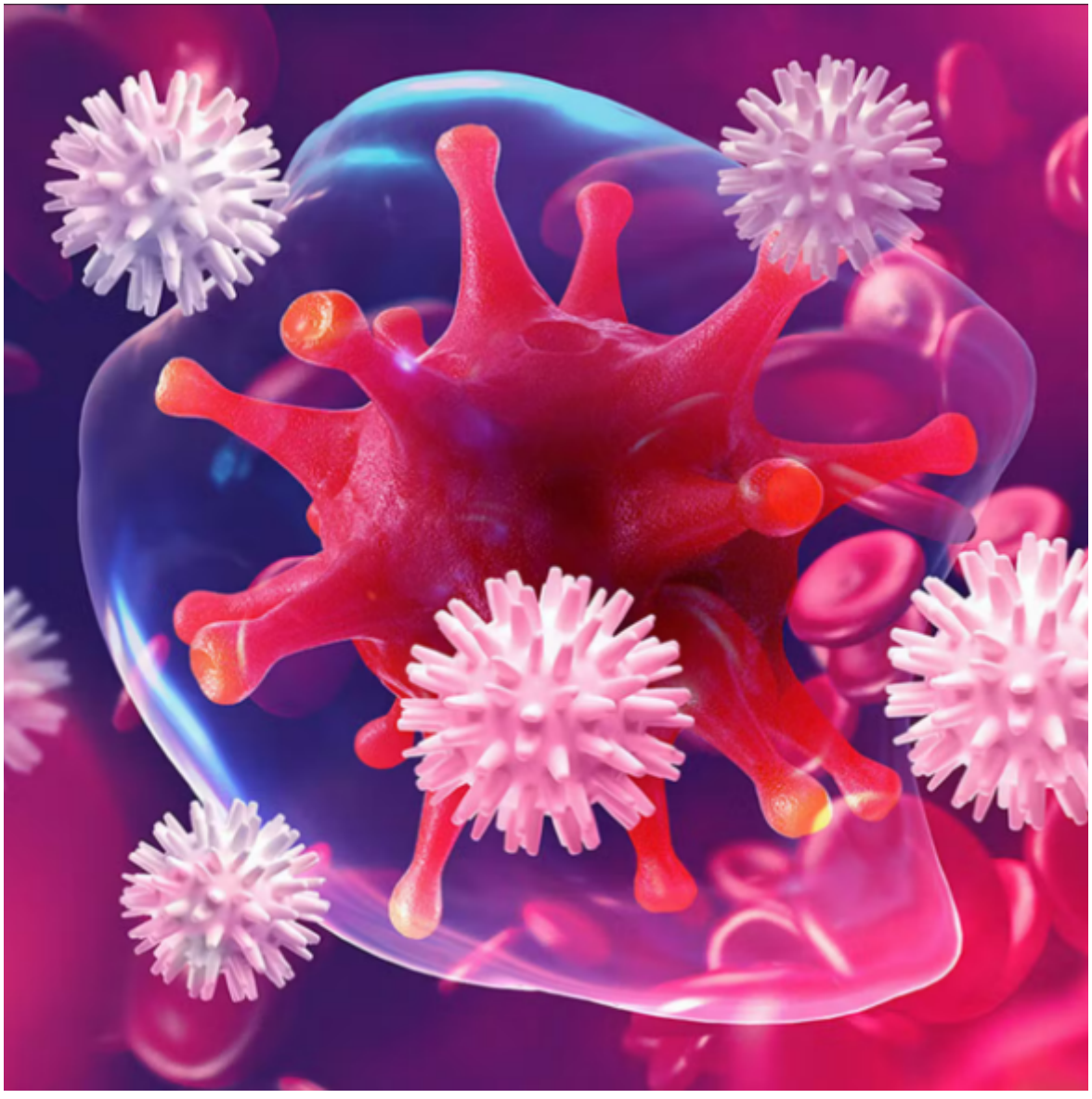
Immune Disorders Article Writing
Summer 2023 Journal



Teens in Health: Immune Disorders Article Writing Summer 2023 Journal

Teens in Health is a teen-led organization that provides students with opportunities to learn about various topics related to biological sciences and medicine and apply concepts in their own research articles or literary reviews. This four-week program is one of the eight various programs offered during Teens in Health's Summer 2023, focusing on exploring the fundamental basics of immunology and disease, enhancing student understanding of various disease pathways, and developing hypothetical experiments with treatment options through weekly lectures and writing workshops.

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Literary Review

Antibody Cancer Therapy: Advancements, Challenges, and Future Perspectives.

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Keywords: Monoclonal antibodies, bispecific monoclonal antibodies, antibody-drug conjugates, immune checkpoint inhibitors, immunosuppressive tumor microenvironment, combination therapies, personalized medicine, bispecific T-cell engagers, biomarkers, genomics, artificial intelligence, antibody therapies

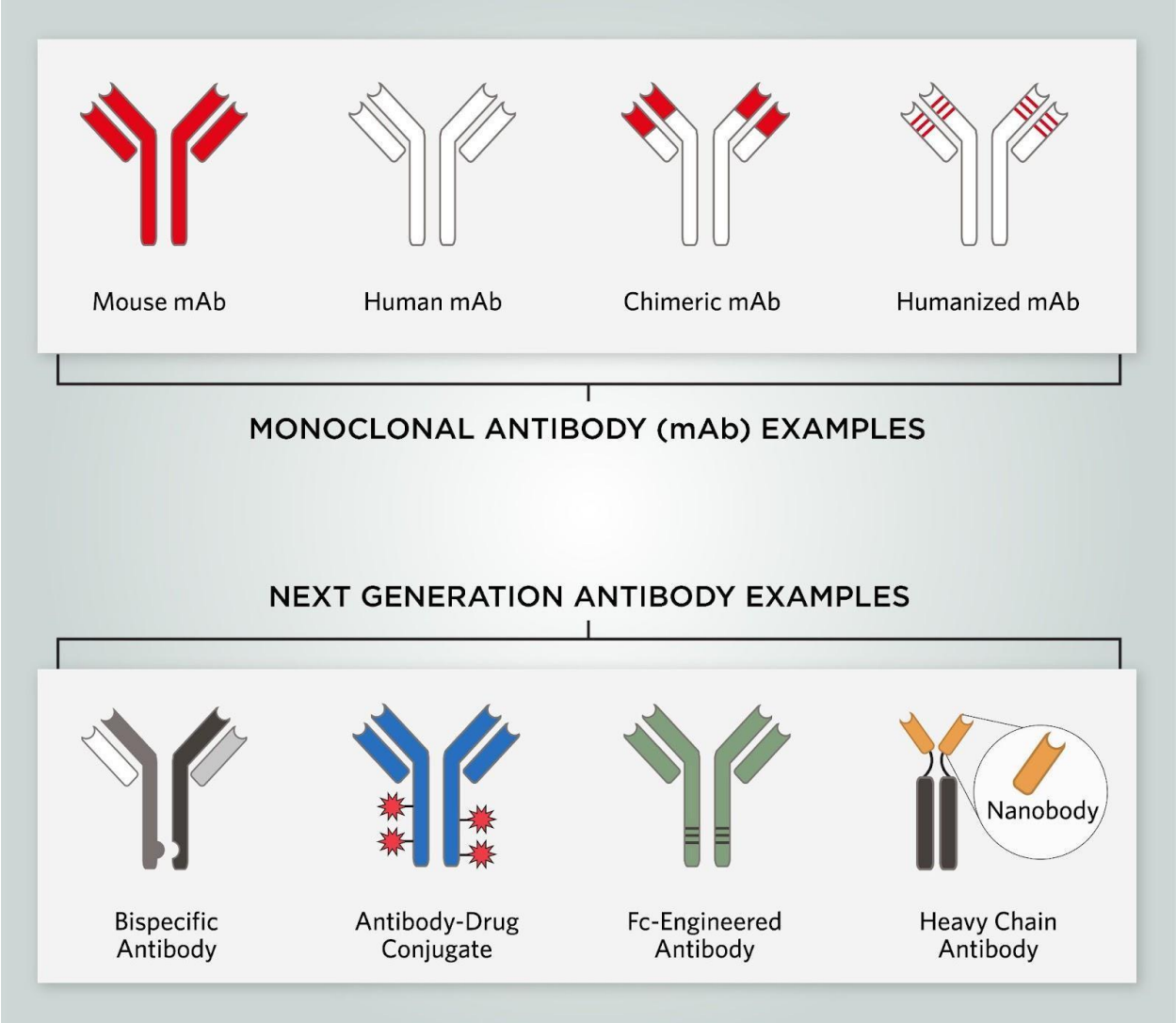
Abstract

Antibody cancer therapy has emerged as a promising strategy for treating various malignancies. This literary review examines the advancements, challenges, and future perspectives in antibody-based cancer therapy. It explores the mechanism of action and types of antibodies used, including monoclonal antibodies, bispecific monoclonal antibodies, and antibody-drug conjugates. The paper discusses the significant advancements in antibody cancer therapy, such as immune checkpoint inhibitors and antibody-drug conjugates, which have improved treatment outcomes. It also has challenges like resistance to antibody treatment and the immunosuppressive tumor microenvironment. Strategies to overcome these challenges, including combination therapies and personalized medicine, are explored. The paper concludes with future perspectives, including next-generation antibody formats like bispecific T-cell engagers and incorporating biomarkers, genomics, and artificial intelligence in optimizing antibody therapies. Overall, antibody cancer therapy holds great potential, and ongoing advancements offer new possibilities in the fight against cancer.

Introduction

Monoclonal antibodies (mAbs)

To understand the impact of antibody cancer therapy, it is essential to delve into its mechanism of action and the different types of antibodies utilized. Monoclonal antibodies (mAbs) have been at the forefront of antibody-based cancer therapy, designed to specifically recognize and bind to antigens expressed on cancer cells. These antibodies can evoke diverse therapeutic effects, such as blocking signaling pathways, enhancing immune-mediated cytotoxicity, or delivering cytotoxic payloads directly to cancer cells (Basel, 2005).



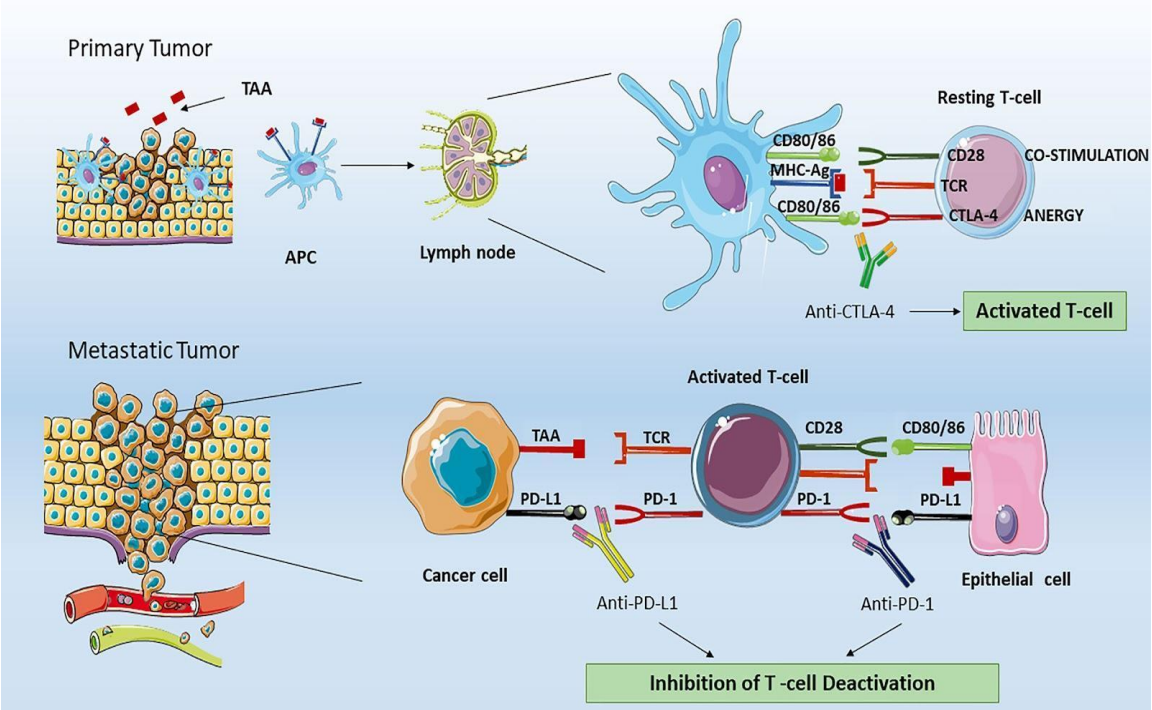
Credit: The Scientist

In recent years, bispecific monoclonal antibodies (bsAbs) have gained prominence. These engineered antibodies possess the ability to simultaneously bind to two distinct antigens, facilitating the engagement of immune cells and boosting anti-tumor immune responses.

Moreover, the development of antibody-drug conjugates (ADCs) represents a significant advancement in the field. ADCs combine the targeting specificity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs, enabling the selective delivery of powerful therapeutic agents to cancer cells (Nguyen, 2023). This specialized approach minimizes systemic toxicity and enhances therapeutic effectiveness.

The notable advancements in antibody cancer therapy have yielded significant improvements in treatment outcomes. Immune checkpoint inhibitors, a class of antibodies that disrupt the inhibitory signals between cancer cells and immune cells, have demonstrated remarkable success in various malignancies. These

inhibitors, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors, have shown durable responses and improved survival rates in patients (Seidel, 2018).



Immunosuppressive Tumor Microenvironment

Despite the remarkable achievements in antibody cancer therapy, challenges persist in achieving optimal treatment outcomes. One significant hurdle is the immunosuppressive tumor microenvironment (TME). The TME consists of various cellular and noncellular components, such as immune cells, fibroblasts, endothelial cells, and extracellular matrix, which interact with cancer cells to create a hostile environment that promotes tumor growth and immune evasion (Baghban, 2023). The immunosuppressive TME is characterized by the presence of immunosuppressive cells such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), as well as the release of immunosuppressive factors, including transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10) (Lindau, 2023). Together these factors create a state of immune tolerance, preventing the effective recognition and elimination of cancer cells by the immune system. To overcome the immunosuppressive TME, researchers are exploring combination therapies that target multiple components of the tumor microenvironment simultaneously (Ucken, 2021). By combining monoclonal antibodies with other therapeutic agents, such as immune checkpoint inhibitors, chemotherapeutic drugs, or targeted therapies, it is possible to enhance the anti-tumor immune response while simultaneously

addressing other tumor-promoting factors. This multifaceted approach aims to disrupt the immunosuppressive signals, activate immune cells, and restore immune surveillance against cancer cells within the TME.

Personalized Medicine and Biomarkers

Another crucial aspect in advancing antibody cancer therapy is the collage of personalized medicine. Personalized medicine recognizes that each patient's tumor is unique and requires specialized treatment strategies based on individual characteristics. Biomarkers play a pivotal role in this study by providing valuable information about the tumor's biology, immune response, and potential treatment response. Biomarkers can include genetic mutations, protein expression levels, immune cell infiltration, or circulating tumor DNA (ctDNA) (Sanjay, 2015). By analyzing these biomarkers, clinicians can select the most appropriate antibody therapy for each patient, maximizing the chances of a favorable treatment response.

Genomics and Artificial Intelligence

Advancements in genomics have also contributed significantly to the field of antibody cancer therapy. Genomic profiling allows the identification of genetic alterations within tumors, such as driver mutations or gene amplifications, which can guide the selection of targeted therapies or immunotherapies (Berger, 2018). Additionally, the emergence of artificial intelligence (AI) has revolutionized data analysis and interpretation. AI algorithms can analyze large datasets, identify patterns, and generate predictive models, thereby assisting in the identification of potential therapeutic targets, prediction of treatment response, and optimization of treatment regimens (Schork, 2019). The combination of genomics and AI holds great promise in enhancing the precision and efficacy of antibody therapies.

Bispecific T-Cell Engagers

While monoclonal antibodies have proven effective in many cases, "novel" antibody formats are continuously being developed to further improve treatment outcomes. Bispecific T-cell engagers (BiTEs) are one such innovation. BiTEs are antibodies engineered to simultaneously bind to a tumor-associated antigen and CD3, a molecule expressed on the surface of T cells. By bridging cancer cells and T cells, BiTEs facilitate the formation of an immunological synapse, leading to the activation and directed killing of cancer cells by T cells (Coupet, 2014). This approach harnesses the power of the patient's immune system to specifically target and eliminate tumor cells, offering a potential breakthrough in antibody cancer therapy.

Summarization and Importance of Antibody Cancer Therapy and Its Beneficiaries

Overall, Antibody Cancer Therapy has emerged as a crucial and promising strategy for the treatment of various cancers. This research paper uncovers the advancements, challenges, and future perspectives in this field, shedding light on the importance of antibody-based cancer therapy and who can benefit from it.

Firstly, antibody cancer therapy is important because it offers a targeted approach to treating cancer. Monoclonal antibodies (mAbs) specifically recognize and bind to antigens shown from cancer cells, allowing for precise targeting of tumor cells while sparing healthy tissues, as previously mentioned. For the most part, this targeted approach minimizes off-target effects and reduces systemic toxicity, leading to improved patient outcomes.

Moreover, the advancements in antibody cancer therapy have introduced innovative treatment modalities. Bispecific monoclonal antibodies (bsAbs) and antibody-drug conjugates (ADCs) have expanded the therapeutic possibilities. BsAbs can simultaneously engage immune cells and cancer cells, enhancing the anti-tumor immune response. ADCs combine the specificity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs, enabling the selective delivery of powerful therapeutic agents to cancerous cells. These advancements have resulted in improved treatment outcomes and increased treatment options for patients. One of the significant beneficiaries of antibody cancer therapy is patients with advanced or metastatic cancer who may have limited treatment options. Immune checkpoint inhibitors, a class of antibodies that disrupt the inhibitory signals between cancer cells and immune cells, have demonstrated remarkable success in various cancers.

All together, antibody cancer therapy is of paramount importance in the fight against cancer. Through targeted approaches, personalized medicine, and innovative advancements, antibody therapies offer new possibilities and improved treatment outcomes. By addressing the challenges of tumor heterogeneity and the immunosuppressive TME, antibody cancer therapy benefits patients with limited treatment options, providing hope for enhanced survival and quality of life.

Discussion

Antibody cancer therapy has witnessed significant advancements in recent years, revolutionizing the treatment landscape for numerous cancers. Monoclonal antibodies have been at the front of this progress. These antibodies are designed and specialized to recognize and bind to antigens expressed on cancer cells, leading to diverse therapeutic effects. For instance, mAbs can block signaling pathways essential for cancer cell growth, enhance immune-mediated cytotoxicity, or deliver cytotoxic payloads directly to cancer cells (Zahavi, 2020). The specificity of monoclonal antibodies minimizes off-target effects, reducing systemic toxicity and improving patient outcomes. In addition to monoclonal antibodies, bispecific monoclonal antibodies (bsAbs) have emerged as a promising advancement in antibody cancer therapy. BsAbs are engineered antibodies that can simultaneously bind to two distinct antigens. This unique property allows for the engagement of immune cells and the amplification of anti-tumor immune responses. BsAbs can redirect immune cells, such as T cells, to recognize and eliminate cancer cells more effectively. This approach harnesses the power of the immune system and provides an innovative strategy

for targeted cancer therapy. Another significant advancement is the development of antibody-drug conjugates (ADCs). ADCs combine the targeting specificity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs. The antibodies deliver the attached cytotoxic payloads directly to cancer cells, enabling selective and localized delivery of potent therapeutic agents (Dean, 2021). This targeted approach minimizes systemic toxicity, improves drug efficacy, and expands the therapeutic options available for patients.

Impact of Immune Checkpoint Inhibitors

Immune checkpoint inhibitors represent a groundbreaking class of antibodies in cancer therapy. These inhibitors disrupt the inhibitory signals between cancer cells and immune cells, thereby unleashing the immune system to recognize and eliminate cancer cells effectively. In particular, antibodies targeting programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have shown remarkable success in various malignancies. Clinical trials and real-world evidence have demonstrated durable responses and improved survival rates in patients receiving immune checkpoint inhibitors.

Challenges

While antibody cancer therapy has shown tremendous promise, several challenges need to be addressed to optimize treatment outcomes. One significant hurdle is the immunosuppressive tumor microenvironment (TME). The TME consists of various cellular and noncellular components that interact with cancer cells, creating a hostile environment that promotes tumor growth and immune evasion. Immunosuppressive cells, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), along with immunosuppressive factors like transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10), contribute to immune tolerance within the TME. To overcome the immunosuppressive TME, combination therapies targeting multiple components of the tumor microenvironment simultaneously have shown promise. By combining monoclonal antibodies with other therapeutic agents such as immune checkpoint inhibitors, chemotherapeutic drugs, or targeted therapies, it is possible to enhance the anti-tumor immune response while simultaneously addressing other tumor-promoting factors (Vanneman, 2012). Preclinical and clinical studies exploring combination approaches have demonstrated synergistic effects, suggesting the potential for improved treatment outcomes in patients with challenging tumors.

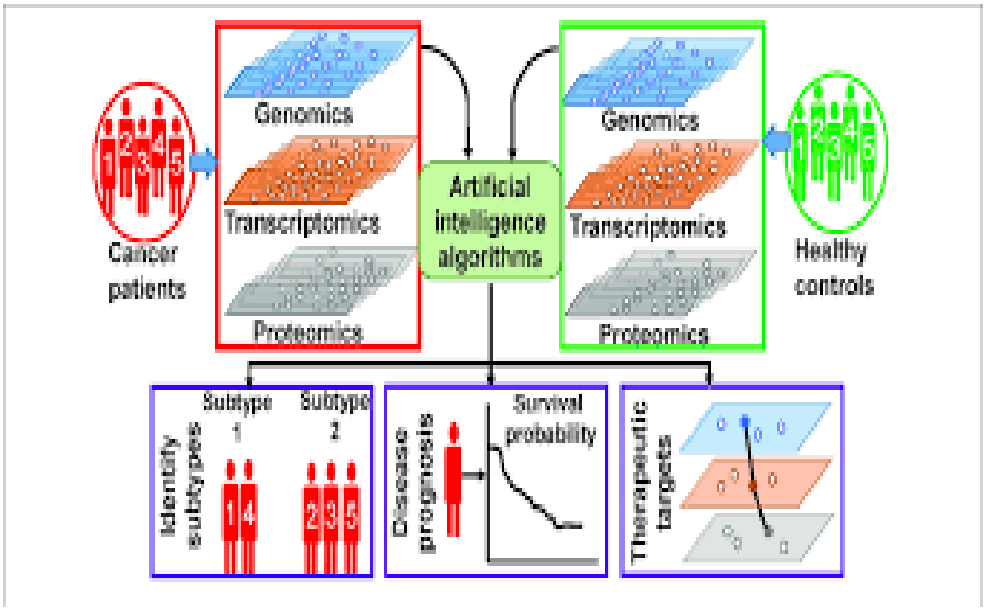
Personalized Medicine and Biomarkers

Personalized medicine plays a critical role in advancing antibody cancer therapy. Biomarkers serve as invaluable tools for tailoring treatment strategies to individual patients. Genetic mutations, protein expression levels, immune cell infiltration, and circulating tumor DNA (ctDNA) are examples of biomarkers that provide insights into tumor biology, immune response, and treatment response. Analyzing

these biomarkers enables clinicians to select the most appropriate antibody therapy, maximizing the chances of a favorable treatment response.

Integration of Genomics and Artificial Intelligence (AI)

The field of genomics has significantly contributed to antibody cancer therapy. Genomic profiling allows the identification of genetic alterations within tumors, such as driver mutations or gene amplifications, which can guide the selection of targeted therapies or immunotherapies. Moreover, the emergence of artificial intelligence (AI) has revolutionized data analysis and interpretation in cancer research. AI algorithms can analyze large datasets, identify patterns, and generate predictive models, thereby assisting in the identification of potential therapeutic targets, prediction of treatment response, and optimization of treatment regimens (Johnson, 2020). The integration of genomics and AI holds great promise in enhancing the precision and efficacy of antibody therapies.



Future Perspectives

Looking ahead, the future of antibody cancer therapy appears promising. Ongoing research and development efforts are focused on advancing novel antibody formats, such as bispecific T-cell engagers (BiTEs). BiTEs are engineered antibodies designed to simultaneously bind to a tumor-associated antigen and CD3, a molecule expressed on the surface of T cells. By bridging cancer cells and T cells, BiTEs facilitate the formation of an immunological synapse, leading to the activation and directed killing of cancer cells by T cells. The potential of BiTEs to harness the patient's immune system represents a breakthrough in antibody cancer therapy.

Conclusion

Antibody cancer therapy has emerged as a crucial and promising strategy for the treatment of various malignancies. This literary review has explored the advancements, challenges, and future perspectives in antibody-based cancer therapy, shedding light on its significance and potential benefits.

The development of monoclonal antibodies (mAbs) has revolutionized the field by providing a targeted approach to cancer treatment. These antibodies specifically recognize and bind to antigens expressed on cancer cells, allowing for precise targeting while sparing healthy tissues. The use of mAbs has demonstrated diverse therapeutic effects, including blocking signaling pathways, enhancing immune-mediated cytotoxicity, and delivering cytotoxic payloads directly to cancer cells. This targeted approach minimizes off-target effects, reduces systemic toxicity, and improves patient outcomes.

In addition to mAbs, the advancements in antibody cancer therapy have introduced innovative treatment modalities such as bispecific monoclonal antibodies (bsAbs) and antibody-drug conjugates (ADCs). BsAbs have the unique ability to simultaneously engage immune cells and cancer cells, enhancing the anti-tumor immune response. ADCs combine the specificity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs, enabling the selective delivery of powerful therapeutic agents to cancerous cells. These advancements have resulted in improved treatment outcomes and increased treatment options for patients, particularly those with advanced or metastatic cancer who may have limited treatment options.

One of the significant beneficiaries of antibody cancer therapy is patients with limited treatment options. Immune checkpoint inhibitors, a class of antibodies that disrupt the inhibitory signals between cancer cells and immune cells, have demonstrated remarkable success in various cancers. Clinical trials and real-world evidence have shown durable responses and improved survival rates in patients receiving immune checkpoint inhibitors.

Despite these advancements, challenges persist in achieving optimal treatment outcomes. The immunosuppressive tumor microenvironment (TME) poses a significant hurdle, creating a hostile environment that promotes tumor growth and immune evasion. Strategies such as combination therapies targeting multiple components of the TME simultaneously have shown promise in overcoming this challenge. By combining monoclonal antibodies with other therapeutic agents, it is possible to enhance the anti-tumor immune response while addressing other tumor-promoting factors.

Personalized medicine and the use of biomarkers play a crucial role in advancing antibody cancer therapy. Biomarkers provide valuable information about the tumor's biology, immune response, and potential treatment response. Analyzing these biomarkers enables clinicians to select the most appropriate antibody therapy for each patient, maximizing the chances of a favorable treatment response.

Furthermore, advancements in genomics and artificial intelligence (AI) have contributed significantly to the field. Genomic profiling allows the identification of genetic alterations within tumors, guiding the

selection of targeted therapies or immunotherapies. AI algorithms assist in data analysis and interpretation, aiding in the identification of potential therapeutic targets, prediction of treatment response, and optimization of treatment regimens. The integration of genomics and AI holds great promise in enhancing the precision and efficacy of antibody therapies.

Looking to the future, ongoing research and development efforts are focused on advancing novel antibody formats such as bispecific T-cell engagers (BiTEs). BiTEs have the potential to harness the patient's immune system by bridging cancer cells and T cells, leading to the activation and direct killing of cancer cells. These innovations offer new possibilities and improved treatment outcomes in the fight against cancer.

In conclusion, antibody cancer therapy holds great potential in the fight against cancer. Through targeted approaches, personalized medicine, and innovative advancements, antibody therapies offer new possibilities and improved treatment outcomes. By addressing the challenges of tumor heterogeneity and the immunosuppressive tumor microenvironment, antibody cancer therapy benefits patients with limited treatment options, providing hope for enhanced survival and improved quality of life. Ongoing research and future advancements in antibody-based cancer therapy offer exciting prospects for the future.

Works Cited

"Advances in targeting the Ras/Raf/MEK/ERK pathway in melanoma and non-small cell lung cancer." (2019). Discovery Medicine, 28(154), 63-78. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5883082/

"Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: Celebrating the 20th anniversary of the discovery of ACE2." (2013). Circulation Research, 131(3), 145-156. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4604043/

"COVID-19 and cancer: Lessons from a pooled meta-analysis." (2021). Journal of Hematology & Oncology, 14(1), 1-3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7551545/

"Emerging role of ACE2 in health and disease: Consistency and inconsistencies of laboratory and clinical findings." (2021). American Journal of Translational Research, 13(5), 1863-1878. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8619951/

"Mitochondrial dynamics and cancer." (2014). Seminars in Cancer Biology, 25, 47-53. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575763/

"Protein kinase inhibitors in cancer therapy: The successes and the shortcomings." (2015). Current Medicinal Chemistry, 22(23), 258-281. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8306582/

"Protein kinase inhibitors: A patent review (2010 - 2013)." (2014). Expert Opinion on Therapeutic Patents, 24(1), 1-18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967236/

"Rationale for targeting the Ras/MAPK pathway in triple-negative breast cancer." (2015). Discovery Medicine, 20(111), 359-369. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445461/

"Targeting the MAPK signaling pathway in cancer: Promising preclinical activity with the novel selective ERK1/2 inhibitor BVD-523 (ulixertinib)." (2020). Molecular Cancer Therapeutics, 19(1), 1-13. https://biosignaling.biomedcentral.com/articles/10.1186/s12964-020-0530-4

"The renin-angiotensin system and the ACE-2/Ang1-7/Mas cascade: Studies of angiotensin-converting enzyme 2 inhibition in myocardial infarction and heart failure." (2015). Critical Care, 19(1), 1-3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7877825/

"Therapeutic implications of targeting the PI3K/AKT/mTOR signaling module in melanoma therapy." (2015). American Journal of Cancer Research, 5(3), 897-908. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8122391/

"Translational implications of tumor heterogeneity." (2019). Clinical Cancer Research, 25(6), 2066-2074. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6658089/

"Understanding the mechanisms of action and resistance of inhibitors of the MAPK pathway in cancer treatment." (2015). Cancers, 7(2), 871-888. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8300931/v

"Update on the therapeutic significance of estrogen receptor β in malignant gliomas." (2020). ASN Neuro, 12, 1-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7580505/

"Zebrafish as a model for the study of solid human tumors." (2016). Methods in Molecular Biology, 1451, 121-137.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9913659/#:~:text=improve%20ADC%20tolerability.-,Abstract,cytotoxic%20agents%20to%20malignant%20cells

Research Article

Chimeric Antigen Receptor (CAR)- T cell Therapy for Progressive Multifocal Leukoencephalopathy (PML)

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Keywords: PML, polyomavirus, JCV, HIV, T-cell, antiretroviral therapy, RRMS, CAR-T, MRI, PCR, CSF, TCR

Abstract

This paper examines the use of chimeric antigen receptor T- cell therapy as a novel treatment against the John Cunningham virus in efforts to fight the disease, Progressive multifocal leukoencephalopathy. Although no research has been conducted on CAR-T therapy for PML specifically, anti-HIV CAR-T therapy has shown promising results in clinical trials. Through the engineering of the host's own T-cells, they will be able to recognize and attack any cells infected with the John Cunningham virus. The anticipated results include decreased viral presence, improved neuroimaging findings, and increased functional capacity in most patients. However, the limitations and challenges associated with CAR-T cell therapy, such as the potential for disease progression and cytokine toxicity, need to be addressed through further research and refinement of the therapy. Additionally, efforts are needed to make CAR-T therapy more accessible and cost-effective. In conclusion, CAR-T cell therapy holds promise as a potential treatment option for PML, offering specificity and the potential to reverse disease progression in patients

Introduction

Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a disease caused by the viral infection called Human polyomavirus II, more commonly recognized as John Cunningham Virus (JCV). PML affects the central nervous system in a debilitating and often fatal manner. PML primarily presents in individuals with suppressed immune systems, thus has become increasingly common due to the outbreak of HIV/AIDS. Additionally, PML is associated with the use of the drug Natalizumab, which is used to treat RRMS (Iannnetta et. al). In a host with active PML, the JCV infects oligodendrocytes, which causes demyelination of axons and disrupts the white matter of the brain. The host may experience enhanced demyelination due to a disrupted immune system and develop PML- immune reconstitution inflammatory syndrome, or PML-IRIS, thus requiring corticosteroids (Pavlovic et al). Comparatively, in HIV+ patients,

a low CD4+ T-cell count puts them at a higher risk for developing PML (Engsig et al). In essence, PML is often a result of the inactivity of the immune system against JCV, yet if the immune system goes too far, one can develop IRIS. Thus, treatment of PML becomes a delicate balance for the immune system. The major treatment path for PML is immune reconstitution, which aims to restore the body's defense against the JCV and possible IRIS. (Pavlovic et al). A somewhat successful model for treatment has been combination antiretroviral therapy (cART). In a study using a five-drug combination, HIV+ PML patients showed promising survival rates in the first months following a PML diagnosis. However, the individual survival of the patients depended heavily on the CD8+ T Lymphocyte responses, which are dependent on the JCV-specific CD4+ T lymphocytes. In the five-drug cART study conducted by Gasnault and others, patients who died displayed lower baseline naive and effector CD8+ T-cell counts, as well as lower central memory CD4+ T-cell numbers. While the five-drug cART showed a significant increase in CD4+ T cells, a total decrease in CD8+ T cells was witnessed. (Gasnault et. al). Due to the significant risk of developing IRIS associated with altering T-cell count with drugs, a new and prevalent immunotherapy presents itself as a possible treatment.

Chimeric Antigen Receptor T cell therapy

Chimeric Antigen Receptor (CAR)-T cell therapy has taken the immunotherapy world by storm. The complex idea of developing programmed killer cells in a lab has been successful in its trials against cancer. The approved brands Kymriah ® and Yescarta ® primarily treat leukemia and lymphoma, respectively. CAR cells are developed by collecting existing T cells from a patient and producing proteins on their surfaces, CARs, which bind to specific antigens. The Chimeric Antigen Receptors are synthetically produced to match the pathogen in the patient's body. After the lab expansion of these cells, the CAR-T cells are infused into the patient's body, where they are able to multiply and kill any cells with the target antigen presented (National Cancer Institute).

Contrary to cancer, the antigen presented by a virus, such as JCV, will remain the same patient to patient; while researched treatment methods of PML can reduce the effects of the disease, CAR-T therapy presents the opportunity to eliminate the JCV reservoir. Pathogen specific T-cells exist in small quantities and may be ineffective to viral escape mechanisms, like MHC. CAR-T allows for the creation of more pathogen specific T-cells with the ability to bypass any escape mechanisms and protection from their own infection. As of now, no research has been conducted on the application of CAR-T for PML or JCV at all. However, anti- HIV CAR-T therapy has reached clinical trials and has shown advancement (Seif et. al). As HIV+ patients are the largest population of PML hosts, this development will likely decrease the prevalence of PML (Pavlovic et. al).

While CAR-T therapy uses the host's own T cells to generate pathogen specific T cells, similar tactics use third party donor cells to treat infectious diseases. Virus specific T cells (VSTs) were used to treat BKV

and showed promising results of preventing disease progression. Almost all patients with BKV associated hemorrhagic cystitis saw a decrease in symptoms; there was a complete resolution of gross hematuria (Tzannou et. al). While this study may seem unrelated to PML, BKV is a polyomavirus incredibly similar to JCV. The two viruses are highly homologous at the DNA and proMassimotein levels (Barbanti-Brodano et. al). Thus, the success rates of VST to a similar virus displays a potential success for the application of modified T cells to JCV. To support this idea, researchers were able to successfully create “anti-JCV designer T cells", which successfully secreted cytokines in a TCR based experiment involving chimeric immune receptors. (Yang et. al).

Materials and Methods

It is important to note that this experiment is hypothetical, and that the results may not be accurate since the experiment was not performed.

The objective of this experiment is to create a CAR-T cell that performs the destruction of the JCV and is able to replicate and retain memory in the host’s body, in order to stop PML. This would prevent the use of steroids and the need for multiple antiretroviral drugs. In this experiment, the CAR cells will be second generation to allow for better killing and control over the viral replication (Seif et. al).

Patient Characteristics

This study contains fifty adults between the ages of eighteen and sixty. These patients will have diagnosed PML and the confirmed presence of JCV in the cerebral spinal fluid (CSF). These patients may be HIV+, but none have MS nor have been treated with natalizumab. In addition, none of the patients take corticosteroids. None of the patients may have cancer or have been treated with immunotherapies, such as CAR-T therapy due to correlation between its use in oncology and the presence of PML (Ahrendsen et. al).

Engineering CAR-T cells

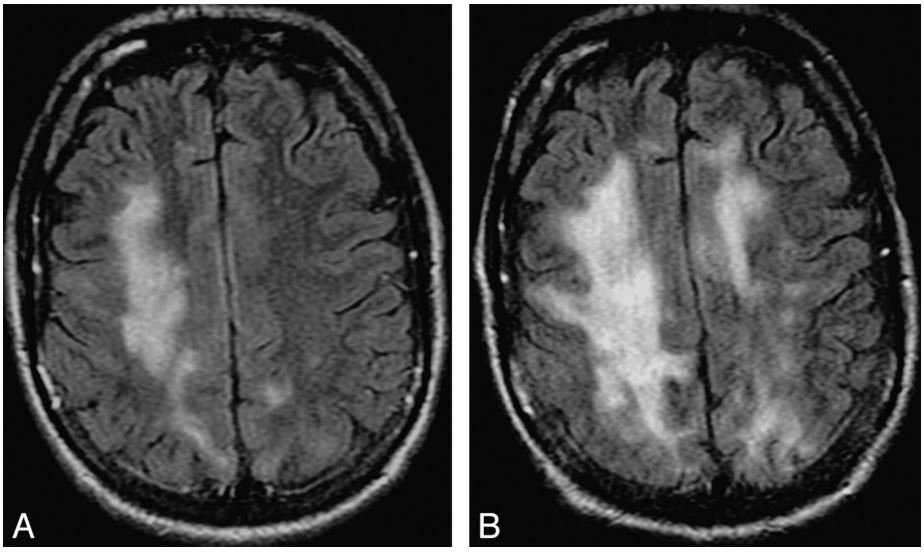
The first step to engineering the appropriate CAR-T cells would be to identify the target antigens on JCV-infected cells. For this experiment, p36 and p100 CTL epitopes derived from JC virus will be used (Yang et. al). The CAR consists of an extracellular antigen recognition domain, a transmembrane domain, a costimulation doman, and an activation domain. The activation domain will be CD3ζ and the costimulation domain will be 4-1BB in order to better expand memory CD8+ T cells (Zhang et. al).

In order to apply this, leukapheresis will be performed on each patient, in order to harvest leukocytes. The T-cells grow in perfusion bioreactor in the presence of IL-2 and aAPCs. The T-cells are incubated with a third generation lentivirus vector which encodes the CAR construct. The RNA from the vector “is then transcribed and translated by the patient cells, and the CAR is expressed on the cell surface” (Levine et. al). Using flow cytometry and cytotoxicity assays, the cells will be tested for specificity and cytotoxicity

against JCV cells. These cells will then be infused back into the patient's body cells through a central line. The CAR-T cells will optimally recognize and eliminate JCV-infected cells, potentially reducing the viral load and associated symptoms (Levine et. al). The experimental results will be based on the first six months following the infusion.

Testing for Efficacy

Included in the engineering of the CAR-T cells, they are tested for cytokine release and antigen recognition. At this point in the testing, there is more interest in the overall effects on the JCV and PML symptom progression. During the six month testing period, these tests will be performed at checkpoints once a month for the first three months, the bimonthly for the remaining three months. The first test will be a regular MRI scan to monitor the changes in the white matter lesions. If treatment is effective, the lesions will improve or stop progression and stabilize.



(American Journal of Neuroradiology)
The scan shows the progression of lesions in a patient with PML over a four month period. In order to characterize successful treatment, the results would be reversed, or would stay stable at the "A" lesions.

Secondly, the patient's CSF will be tested for the presence and quantity of the JCV using PCR. In addition to the biological testing, symptoms and neurological progression will be tested. This will occur through the tracking of changes in symptoms, such as headaches, clumsiness, weakness, and visual and speech changes. Neurological progression will be determined through Karnofsky's performance-status (Gasnault et. al).

Ethical Considerations

In order to engineer CAR-T cells, the patients' blood must be drawn. To perform this, the patients will sign consent forms. In addition, the patients must consent to a central line to reinfuse the modified T-cells into them. Consent forms will also cover the possibilities of worsened lesions, moderate to severe

disability, or even death. Each patient that enters the clinical trial must sign said consent forms in order to ethically perform experimental treatment.

Results

Once again, this experiment is hypothetical, thus any results are formed using knowledge and hypothesis. After the six month period, the last MRI scans will display that patients have seen either a decrease in lesions in the brain, or the stabilization of the lesions previously formed. In addition to this, a significant number of patients will show a decrease in the presence of JCV in the CSF. On the Karnofsky performance-status scale, most patients will see an increase in functional capacity. Optimistically, all patients will be in the 50-100 range, which is above the disabled mark. However, not all patients will have optimal outcomes. The CAR-T cells may not retain memory and function in all patients. Patients may see an increase in the presence of JCV as well as additional lesions and physical symptoms; they may lose functional capacity and enter the 40's and below on the Karnofsky Performance Status scale, signaling disability and rapidly progressing disease.

Discussion

The results above display a new chapter of medicine, in which PML is manageable or even treatable. As observed, the engineered CAR-T cells were successfully able to recognize the antigen of JCV. Along with recognition, the CAR-T cells effectively killed the JCV infected cells. This is seen by the decrease in JCV presence in the CSF. With the decrease of viral presence, the demyelination risk decreases significantly, thus preventing lesions in the brain. While CAR-T cell therapy has proven effective against PML, it carries significant limitations. Firstly, CAR-T cell therapy for cancer has seen to be a trigger for PML (Ahrendsen et. al). The application of this therapy to patients with active PML may pose significant risk of the rapid progression of the disease. However, an advantage to this therapy is the specificity it has to the JCV, potentially countering this risk of further progression. In addition to this, CAR-T cell therapy poses the associated risk of toxic levels of cytokine release. Due to the popular research of CAR-T however, many scientists and researchers are undergoing the process of refining the use of the therapy to prevent issues such as cytokine toxicity. In terms of accessibility, CAR-T cell therapies are priced at several thousands of dollars per patient (Potnis). While this seems cost ineffective, current treatment methods of PML, specifically antiretroviral therapies, expand a wide range of prices. In the ten thousands, these drug prices often fluctuate and remain heavily dependent on dosage. With the effectiveness of cART increasing with more drugs, these prices continue to rise while effectiveness is not guaranteed. Thus, the implementation of CAR-T therapy would allow for a single and stable treatment, not variable to drug companies and dosages. In order to better this treatment option, more research needs to be conducted on

CAR-T cell therapy on viral infections. In addition, more JCV specific research needs to be conducted in order to ensure the most effective antigens for the CAR. Research also needs to continue to decrease the side effects associated with CAR-T therapies and finding cost effective engineering strategies to create an accessible and safe treatment option.

Conclusion

PML is a devastating disease that affects the central nervous system through the JCV. The application of CAR-T cell therapy introduces specificity in the attacks against the viral infected cells and can potentially reverse lesions in the brain. This occurs by the modification of the host's own T- cells to be able to attack and withstand against the JCV. CAR- T cell therapy provides hope for this degenerative disease and could revolutionize its treatment.

References

Ahrendsen, Jared T, et al. “Progressive Multifocal Leukoencephalopathy after Chimeric Antigen Receptor T-Cell Therapy for Recurrent Non-Hodgkin Lymphoma.” *Journal of Hematology* , vol. 10, no. 5, 1 Oct. 2021, pp. 212–216, www.ncbi.nlm.nih.gov/pmc/articles/PMC8577592/, https://doi.org/10.14740/jh903. Accessed 23 July 2023.

American Journal of Neuroradiology. “Progression of CPML. A and B, These FLAIR Images, 4 Months Apart, from an HIV-Positive Patient with CPML Demonstrate Typical Progression of the Supratentorial White Matter FLAIR Hyperintensity.,” *American Journal of Neuroradiology*, Oct. 2010, www.ajnr.org/content/ajnr/31/9/1564/F4.large.jpg. Accessed 22 July 2023.

Barbanti-Brodano, Giuseppe, et al. “BK Virus, JC Virus and Simian Virus 40 Infection in Humans, and Association with Human Tumors.” *Polyomaviruses and Human Diseases*, 24 June 2007, pp. 319–341, https://doi.org/10.1007/0-387-32957-9_23. Accessed 23 July 2023.

Gasnault, Jacques, et al. “Improved Survival of HIV-1-Infected Patients with Progressive Multifocal Leukoencephalopathy Receiving Early 5-Drug Combination Antiretroviral Therapy.” *PloS One*, vol. 6, no. 6, 2011, p. e20967, www.ncbi.nlm.nih.gov/pmc/articles/PMC3127950/, https://doi.org/10.1371/journal.pone.0020967. Accessed 22 July 2023.

Iannetta, Marco, et al. “Natalizumab Affects T-Cell Phenotype in Multiple Sclerosis: Implications for JCV Reactivation.” *Plos One*, vol. 11, no. 8, 3 Aug. 2016, pp. e0160277–e0160277, https://doi.org/10.1371/journal.pone.0160277. Accessed 15 July 2023.

Levine, Bruce L., et al. “Global Manufacturing of CAR T Cell Therapy.” *Molecular Therapy - Methods & Clinical Development*, vol. 4, Mar. 2017, pp. 92–101, https://doi.org/10.1016/j.omtm.2016.12.006.

National Cancer Institute. “CAR T Cells: Engineering Immune Cells to Treat Cancer.” *National Cancer Institute*, Cancer.gov, 10 Mar. 2022, www.cancer.gov/about-cancer/treatment/research/car-t-cells.

Pavlovic, Dejan, et al. “Progressive Multifocal Leukoencephalopathy: Current Treatment Options and Future Perspectives.” *Therapeutic Advances in Neurological Disorders*, vol. 8, no. 6, 7 Sept. 2015, pp. 255–273, www.ncbi.nlm.nih.gov/pmc/articles/PMC4643867/, https://doi.org/10.1177/1756285615602832.

Potnis, Kunal C, et al. “Cost-Effectiveness of Chimeric Antigen Receptor T-Cell Therapy in Adults with Relapsed or Refractory Follicular Lymphoma.” *Blood Advances*, vol. 7, no. 5, 3 Mar. 2023, pp. 801–810, ashpublications.org/bloodadvances/article/7/5/801/487027/Cost-effectiveness-of-chimeric-antigen-receptor-T, https://doi.org/10.1182/bloodadvances.2022008097. Accessed 15 July 2023.

Seif, Michelle, et al. “CAR T Cells beyond Cancer: Hope for Immunomodulatory Therapy of Infectious Diseases.” *Frontiers in Immunology*, vol. 10, 21 Nov. 2019, www.ncbi.nlm.nih.gov/pmc/articles/PMC6881243/, https://doi.org/10.3389/fimmu.2019.02711. Accessed 22 July 2023.

Shaw, Maggie L. “What Is the True Cost of the High Price of ART?” *AJMC*, 3 Feb. 2020, www.ajmc.com/view/what-is-the-true-cost-of-the-high-price-of-art.

Tzannou, Ifigeneia, et al. “Off-The-Shelf Virus-Specific T Cells to Treat BK Virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections after Allogeneic Hematopoietic Stem-Cell Transplantation.” *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, vol. 35, no. 31, 1 Nov. 2017, pp. 3547–3557, www.ncbi.nlm.nih.gov/pubmed/28783452, https://doi.org/10.1200/JCO.2017.73.0655. Accessed 14 July 2023.

Yang, Wanli, et al. “Chimeric Immune Receptors (CIRs) Specific to JC Virus for Immunotherapy in Progressive Multifocal Leukoencephalopathy (PML).” *International Immunology*, vol. 19, no. 9, 28 July 2007, pp. 1083–1093, academic.oup.com/intimm/article/19/9/1083/665240, https://doi.org/10.1093/intimm/dxm076. Accessed 15 July 2023.

Zhang, Hua, et al. “4-1BB Is Superior to CD28 Costimulation for Generating CD8+Cytotoxic Lymphocytes for Adoptive Immunotherapy.” *The Journal of Immunology*, vol. 179, no. 7, 18 Sept. 2007, pp. 4910–4918, https://doi.org/10.4049/jimmunol.179.7.4910. Accessed 14 July 2023.

Literary Review

Using Mesenchymal Stem Cells in Rheumatoid Arthritis Treatment

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Keywords: Mesenchymal Stem Cells (MSCs), T-cells, rheumatoid arthritis

Abstract

Arthritis is an autoimmune and inflammatory disease that causes the immune system to attack body tissues within the joints, causing joint pain and stiffness. Arthritis has become more common among the population, making it the lead cause of disability in the United States. Since cartilage is avascular and lacks blood vessels, damage from arthritis cannot be repaired. This has led to new scientific experiments being conducted in order to find more effective treatments that alleviate severe symptoms. Recent studies have found that the infusion of mesenchymal stem cells (MSCs) have potential benefits in reducing arthritis symptoms. These studies involve animal testing with DBA/1 mice that are infused with collagen-induced arthritis, and different sources of MSCs to test its effects on experimental arthritis symptoms. The results of these experiments are determined by measuring the amount of T cells and the amount of inflammation, and harmful antibodies found in the joints. This review will focus on three different research experiments related to the efficacy of using mesenchymal stem cells in arthritis treatment.

Introduction

At a cellular level, in rheumatoid arthritis, T cells enter the synovial membrane, where macrophages are held to detect bacteria and harmful organisms and synovial fibroblasts that repair tissues (National Library of Medicine, 2000). T-cell dysfunction then transforms them into effector cells that destroy tissues within the body. This has led to research studies aimed at targeting molecular and cellular pathways to reduce symptoms, avoid further joint damage, and repair tissues. Mesenchymal stem cells (MSCs) are found in the bone marrow and can be used to reconstruct skeletal tissues, including cartilage, bone, and the fat found in the bone marrow. They consist of different cell types, such as osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells), and fat cells that allow them to increase the adiposity of people's bones (Mayo Clinic, n.d.). Through previous research studies, MSCs have been proven to deplete T-cell responses and reduce the severity of arthritis symptoms. Infusing MSCs can induce the development of anti-inflammatory lymph nodes and joints. Additionally,

it can decrease the production of inflammatory responses, ultimately suppressing self-reactive T effector responses.

Discussion

Treatment of Experimental Arthritis by Inducing Immune Tolerance with Human Adipose-derived Mesenchymal Stem Cells

A research team from the American College of Rheumatology published a research article in the National Library of Medicine regarding the infusion of MSCs in arthritis treatment. The researchers used DBA/1 mice with collagen-induced arthritis to test the effectiveness of adding human mesenchymal stem cells on their autoimmune and inflammatory response. After they were treated, they observed the different levels of inflammation in their joints to measure the inflammatory response to the treatment. The Th-1 mediated autoreactive response, which indicates a reaction to intracellular pathogens, was calculated by finding the proliferative response, which can be used to assess the biological reaction to the treatment tested. They also measured the cytokine profile of the draining lymph node cells (structures that filter foreign substances) that were stimulated with the autoantigen introduced. The frequency of Treg cells are lower in many autoimmune diseases, including rheumatoid arthritis. They help control immune responses to antigens and avoid autoimmune diseases. The amount of Treg cells were measured to determine the treatment's effects on arthritis symptoms (González, 2009).

As a result of the experiment, the systemic infusion of human AD-MSCs (adipose derived mesenchymal stem cells) significantly reduced the severity of experimental arthritis symptoms. The Th-1 autoimmune and inflammatory responses were regulated and the production of inflammatory cytokines and chemokines were lowered, thus reducing the amount of inflammation in the lymph nodes and joints. The treatment also resulted in the development of more antigen-specific Treg cells with the ability to control T effector responses, which led to the improvement of systemic arthritis symptoms. The results of this experiment show that MSCs are key regulators of immune tolerance in arthritis treatment by generating and activating more Treg cells, which control how the immune system reacts and limit the dysregulation of regulatory T cells.

Mesenchymal Stem cells Alleviate Experimental Rheumatoid Arthritis through MicroRNA-regulated IκB Expression

Another research team published a research study under the Scientific Reports Journal in 2019, testing the effects of using MSCs to alleviate arthritis symptoms. In this study, collagen-induced arthritis was applied to test rodents to imitate rheumatoid arthritis and its effects on human joints. This led to the development of B- and T- lymphocyte responses, which produce antibodies and destroy harmful cells in

the body. This also resulted in the production of anti-collagen type II antibodies and collagen-specific T-cells, increasing the severity of the experimental disease.

Nuclear factor-κB (NF-κB) can be used to regulate inflammation from rheumatoid arthritis (RA). However, in recent studies it has been associated with the development of T helper 1 responses and more RA-associated synovial cells that increase the severity of RA symptoms. In addition, it can lead to abnormal cell death (aberrant apoptosis) that is related to developmental abnormalities and the reproduction of RA-associated synovial cells found in the joints.

The results of this experiment concluded that MSC transplantation inhibited NF-κB activity, lessening the severity of systemic arthritis symptoms. It also caused growth in β receptor signaling, preventing arthritis symptoms that are induced by type II collagen antibodies. Their data suggests that MSC transplantation in joint tissue could alleviate experimental RA and shows its potential benefits in RA treatment.

Comparison of Therapeutic Effects of Different Mesenchymal Stem Cells on Rheumatoid Arthritis in Mice

In addition, a group of researchers under the National Institute of Health conducted another experiment in 2019, related to the use of MSCs in arthritis therapy. The results were determined by measuring the effects of three different sources of MSCs on collagen-induced arthritis in test mice. This includes bone marrow derived MSCs (BMSCs), umbilical cord derived MSCs (UCs), and MSCs from human exfoliated deciduous teeth (SHED). In this research experiment, type II collagen was infused in 24 DBA/1 mice to module experimental arthritis. To determine the effects of each MSC source, bone erosion and joint damage were measured using micro-computed tomographic analysis (Micro-CT), hematoxylin staining, and eosin staining.

The results of this experiment further demonstrated how systemic delivery of MSCs could alleviate symptoms related to experimental arthritis. The Micro-CT concluded that its infusion reduced bone erosions. It was also discovered that treatment with MSCs reduced synovitis, joint inflammation, and cartilage destruction. UCs were shown to be more effective in alleviating RA symptoms, ultimately concluding that it could be the most useful source to infuse MSCs in RA treatment.

The Purpose of Using Mesenchymal Stem Cells

MSCs have multiple characteristics that can be beneficial in cell-based therapy. Its infusion creates cartilage formation, vascularization (an increase in muscle size and density) and immunoregulation (National Institutes of Health, 2010). It also stimulates anti-inflammation, which can lessen the severity of arthritis symptoms. These cells are found in bone marrow, trabecular bone, the synovial brain, and many other tissues. So, they have the potential to regenerate chondrocytes, induce cartilage formation, and differentiate into cartilage. MSCs can reduce joint damage, ultimately reducing pain and increasing

mobility. Additionally, many clinical trials have proven the potential benefits of MSC infusion in RA treatment. Although stem cell therapy can't be considered as a potential cure for arthritis, it shows significant benefits in reducing its symptoms and alleviating pain.

Challenges Caused by Arthritis

Severe arthritis can cause many symptoms that challenge normal daily activities and movements. It can lead to chronic pain, impair physical functioning, and cause permanent joint damage that can't be repaired. These symptoms can cause tiredness and damage a person's overall health and well-being. This autoimmune disease has become a significant cause of disability in the United States. It is also associated with depression due to its negative impact on people's bodily function (Arthritis Foundation, (n.d.). Arthritis is linked to osteoporosis, a condition that weakens bones, increasing the chances of bone fractures under any form of pressure. The severity of arthritis symptoms can make people more susceptible to mortality risks. For example, the CDC has found that people with RA are more susceptible to having other diseases, such as heart disease and diabetes through systemic inflammation. Recent research has shown that the risk of having arthritis is increased with age, body mass, inactivity, and poor mental health (CDC). These factors have become more prevalent issues and have introduced many health risks on a global scale. There has been a significant increase in autoimmune disease rates and a greater need for more effective treatments that can alleviate their symptoms.

Conclusion

In conclusion, multiple research studies have demonstrated the potential benefits of MSC infusion in cell therapy. With the rise in arthritis cases, we need to find a more effective way to alleviate symptoms and avoid other potential health risks that are linked with the autoimmune disease. Although it isn't considered as a potential cure, it has been proven to have many benefits that can regulate the immune response and reduce inflammation in the joints. This treatment can improve bodily function, improving people's quality of life, and reducing the chances of joint/bone damage. Stem cell therapy isn't a standard form of treatment, but studies have introduced its potential benefits. The need for more effective arthritis treatment has led to the rise of cell therapy research, which has demonstrated the potential efficacy of repairing joints and relieving arthritis pain. MSCs have many characteristics that have been proven to reduce inflammation and inhibit harmful T-helper responses that cause tissue damage, ultimately improving arthritis conditions and avoiding any further damage.

Works Cited

Chandra, Abhishek. “Mesenchymal Stem Cell Biology.” Mayo Clinic, 19 Feb. 2020, www.mayo.edu/research/labs/bone-injury-repair/research/mesenchymal-stem-cell-biology#:~:text=Mesenchymal%20stem%20cells%20(MSCs)%20are%20multipotent%20stem%20cells%20found%20in,into%20lipid%2Daccumulating%20fat%20cells.

Gonzalo-Gil, Elena, and María Galindo-Izquierdo. “Role of Transforming Growth Factor-Beta (TGF) Beta in the Physiopathology of Rheumatoid Arthritis: Reumatología Clínica.” *Reumatología Clínica (English Edition)*, 1 May 2014, www.reumatologiaclinica.org/en-role-transforming-growth-factor-beta-tgf--articulo-resumen-S217357431400077X#:~:text=TGF%2Dbeta%20type%20I%20receptor,by%20type%20II%20collagen%20antibody.

González MA;Gonzalez-Rey E;Rico L;Büscher D;Delgado M; “Treatment of Experimental Arthritis by Inducing Immune Tolerance with Human Adipose-Derived Mesenchymal Stem Cells.” *Arthritis and Rheumatism*, 2009, pubmed.ncbi.nlm.nih.gov/19333946/.

González MA;Gonzalez-Rey E;Rico L;Büscher D;Delgado M;, Manuel, et al. “Treatment of Experimental Arthritis by Inducing Immune Tolerance with Human Adipose-Derived Mesenchymal Stem Cells.” *Arthritis and Rheumatism*, 2009, pubmed.ncbi.nlm.nih.gov/19333946/.

JJ;, Weyand CM;Bryl E;Goronzy. “The Role of T Cells in Rheumatoid Arthritis.” *Archivum Immunologiae et Therapiae Experimentalis*, 2000, pubmed.ncbi.nlm.nih.gov/11140470/.

“Types of Arthritis Pain: Arthritis Foundation.” Types of Arthritis Pain | Arthritis Foundation, www.arthritis.org/health-wellness/healthy-living/managing-pain/understanding-pain/sources-of-arthritis-pain. Accessed 23 July 2023.

Yan, Xin, et al. “Mesenchymal Stem Cells Alleviate Experimental Rheumatoid Arthritis through Microrna-Regulated IKB Expression.” *Nature News*, 29 June 2016, www.nature.com/articles/srep28915.

Zhang1, Qing, et al. “Comparison of Therapeutic Effects of Different Mesenchymal Stem Cells on Rheumatoid Arthritis in Mice.” *PeerJ*, 3 June 2019, peerj.com/articles/7023/.

Literary Review

A Review of Chronic Granulomatous Disease and Viable Treatments

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Keywords: Chronic Granulomatous Disease, NADPH oxidase, allograft, interferon gamma, lentiviral vector

Abstract

Chronic Granulomatous Disease is an immune deficiency that leaves those affected with an inability to fight bacterial and fungal infections. As a result, patients diagnosed with this deficiency are likely to experience frequent, severe infections that often require prolonged hospitalization and antibiotic treatments. Though research in treating this disease has advanced greatly in recent years, the benefits of treatment vary between patients and are not consistent. A greater understanding of the pathophysiology and previous treatment results of Chronic Granulomatous Disease is required to further improve the quality of life for those diagnosed. This literary review will focus on three different experiments focusing on treatment of Chronic Granulomatous Disease with hematopoietic allograft transplantation, Interferon Gamma, and CRISPR-mediated genotypic and phenotypic alteration.

Introduction

Chronic Granulomatous Disease is a genetic condition which affects the ability of the white blood cells known as neutrophils to kill bacterial and fungal pathogens. Mutations in one of five genes can cause defects in the NADPH Oxidase enzyme, weakening the immune system (“Chronic”). This enzyme contributes to the formation of hydrogen peroxide (H_2O_2), which can later be transformed into more reactive and antimicrobial metabolites such as hypochlorous acid (HOCl) (Yu *et. al.*). NADPH Oxidase is essential in forming Neutrophil Extracellular Traps (NETs), which are comprised of modified chromatin decorated with bactericidal proteins from granules and cytoplasm (Vorobjeva and Chernyak). Neutrophil samples from patients diagnosed with CGD are deficient in NETosis (the process by which neutrophils release NETs), thus leading to their inability to trap and kill pathogens (Yu *et. al*). The main symptom of CGD is frequent bacterial and fungal infections, which often require a prolonged hospital stay with extensive antibiotic treatments. This condition can be lethal, especially to older patients, and has required extensive research and clinical trials to attempt to treat it.

Discussion

NADPH oxidase

NADPH oxidase enzymes are a class of enzymes responsible for the generation of reactive oxygen species (ROS) from superoxide anions. NOX2 is the specific enzyme in this family required for the proper function of neutrophils and macrophages and is the central cause for Chronic Granulomatous Disease when mutated (Taylor and Tse, vol. 48). The NADPH oxidase enzyme consists of a membrane-bound heterodimer and three cytosolic subunits (Rider *et. al.,* vol. 7). Upon activation, the cytosolic members translocate to the membrane-bound portion and form a 5-component oxidase complex. It also forms an electron-transport chain that can generate microbial killing agents using NADPH oxidase as an electron donor. Different genetic mutations inhibit the proper assembly of the various subunits that make up this protein, with the most common mutation being the CYBB mutation.

Treatment of Chronic Granulomatous Disease With Nonmyeloablative Conditioning and a T-Cell Depleted Hematopoietic Allograft

A study from New England Journal of Medicine detailed a clinical trial in which a group of ten patients diagnosed with Chronic Granulomatous Disease, five adults and five children, underwent a transplantation of peripheral blood stem cells from a Human Leukocyte Antigen (HLA) identical sibling (Horwitz *et. al*., vol. 344, no. 12, pp. 881–888). Prior to the actual transplantation, the patients underwent a non-myeloablative regimen consisting of treatment with cyclophosphamide, fludarabine, and antithymocyte globulin in order to prepare their bodies for the actual transplant. Also, all prophylactic Interferon Gamma treatment ceased two to four weeks before the treatment began. To reduce the risk of graft-versus-host disease, the allograft was depleted of T-Cells before being inserted into the recipient's body. During the allograft transplantation, patients were infused with a minimum of 5.0×10^6 CD34+ cells per kilogram and 1.0×10^5 CD3+ T cells per kilogram. Post-transplantation, donor-recipient chimerism, or the presences of both donor and recipient T cells and myeloid cells in the recipient's body, was measured on a weekly basis. If donor T cells made up less the 60 percent of the patients CD3+ cells by 30 days post-transplantation, each patient received an infusion of 2.0×10^6 CD3+ cells per kilogram of body weight. They received a second infusion of 1.0×10^7 CD3+ cells per kilogram if donor T cells made up less than 60 percent of the patients circulating CD3+ cells by day 60. Three Donor-lymphocyte infusions containing 1.0×10^7 of CD3+ cells per kilogram were given at 90 day intervals after the ceasement of cyclosporine treatment, for a varying period that lasted as long as the patient had less than 60 percent of the donor's cells. The results of this trial were promising, with hematopoietic recovery being relatively rapid in all patients. In four of the five adult patients, all of the circulating myeloid cells were of donor origin by day 150. In two of the child patients, half of the circulating myeloid cells were of donor origin by day 100, but this proportion increased after cyclosporine treatment ended and more infusions were given. One patient never showed any signs of donor cell engraftment, and another experienced allograft rejection at eight months post-transplant. At the 17 month follow-up, eight patients had NADPH

oxidase-positive neutrophils in their blood at levels that could be expected to mount a normal defense for the host, with the median level being 100 and the range being from 33-100 percent. By the conclusion of the trial, three adult patients, ranging from ages 18-36, died of graft-versus-host disease or another infection.

The most glaring weakness of this trial is its limited patient amount. With a total of only ten patients, both the success and failures of this trial cannot be universally applied to the pool of CGD patients. Also, the effectiveness of this treatment varied greatly amongst the age range of the patients, with younger children generally seeing more success than the adult patients. To further explore this variable, current and future studies may want to consider closely comparing the effects of this treatment on a larger pool of child and adult patients. Also, this treatment requires a donor from an HLA-identical match, which limits the ability for this treatment to be used on a widespread scale because of the fact that an HLA match may not be available for all CGD patients. Despite these limitations, this trial provides substantial evidence that transplantation of HLA-identical match blood stem cells paired with a non-myeloablative regimen may be a successful method if restoring effective, NADPH Oxidase positive, neutrophils into the bloodstream.

A Controlled Trial of Interferon Gamma to Prevent Infection in Chronic Granulomatous Disease

Another study in the New England Journal of Medicine investigated the treatment of CGD using recombinant human interferon gamma. A group of 128 patients spanning four countries were randomly assigned to receive a subcutaneous treatment using the interferon gamma or a placebo three times a week for up to one year unless unacceptable toxicity was observed (Gallin *et. al*, vol. 324, no. 8, pp. 509–516). A total of 63 patients received the interferon gamma, and 65 received the placebo. By the end of the treatment, 57 patients had completed the trial with interferon gamma, while 54 had completed the placebo trial. Phagocyte-function assays such as a Neutrophil Nitroblue Tetrazolium Test and a Phagocyte Superoxide-Production Assay were conducted pre-treatment and during treatment for comparative purposes. Phagocytic killing of *Staphylococcus aureus* was measured in patients based on colony counts and bacteria levels in cell-growth-control preparations before treatment and on 4, 90, 180, 270, and 360 days after treatment. No serious toxic effects could be directly attributed to the administration of interferon gamma, but common side effects included fever, chills, headache, and erythema at injection site. Symptoms were seen twice as much in patients above the age of ten compared to those under ten. The end of this experiment for each patient was marked by the first serious infection requiring hospitalization and antibiotic treatment. Forty-six percent of the 65 placebo patients had at least one serious infection, while only twenty-two percent of the 63 interferon gamma patients had at least one serious infection. At the end of this trial, no patients had died as a result of the treatment. Data from this study provides substantial evidence that interferon gamma has the ability to reduce infection more efficiently in children under the age of ten. This treatment was effective for all types of genetic CGD.

A weakness of this experiment lies in its results. While the overall trial was successful, there was no statistically significant difference between the interferon gamma and placebo groups in superoxide production or bacterial killing. Despite the decrease in serious infection witnessed in the interferon gamma patient group, it cannot be attributed to improved phagocytic function. Being that, this trial cements interferon gamma as a viable and successful prophylactic treatment, that when paired with another treatment that does improve phagocytic function, would likely produce favorable results.

CRISPR-Mediated Genotypic and Phenotypic Correction of Chronic Granulomatous Disease Mutation in Human iPS Cells

A study conducted in June of 2015 and published by Elsevier Inc. describes the use of regularly interspaced short palindromic repeat CRISPR-Cas9 site-specific nuclease systems to encourage repair of the endogenous gene by enhancing the levels of homologous recombination (Flynn *et. al*, vol. 43, no. 10). In order to restore oxidative burst function, a mutation in the Cytochrome B β (CYBB) gene of an induced pluripotent stem (iPS) cell derived from a patient with Chronic Granulomatous Disease was corrected. After culturing the cells, the researchers constructed a lentiviral vector to deliver the CRISPR-Cas9 system and the WT copy of the gene into the iPS cells. This also included a guide RNA (gRNA) to ensure that the Cas9 nuclease would bind to the region of the genome containing the CYBB mutation in the iPS cell. Genetic information for the expression of blue fluorescent protein (BFP) that differs from enhanced green fluorescent protein (eGFP) by two amino acid substitutions within the chromophore. The BFP was used to signify the initial uptake of the vector, while the eGFP was used to affirm the correction of the CYBB. The lentiviral vector was transduced into the iPS cells and the eGFP expression was measured using flow cytometry. After the gene editing was carried out, individual iPS cells were isolated on separate culture plates allowing them to multiply creating corrected clones through the process of single-cell cloning. This experiment achieved above a 10% rate of homologous recombination in both HEK293 cells and iPS cells. In doing so, this restored the 10% of circulating neutrophils and monocytes with a functional NADPH oxidase complex required to relieve the symptoms of CGD patients.

Despite the abundant successes of this experiment, one of its weaknesses lies in the fact that this solution exclusively applies to Chronic Granulomatous Disease caused by the CYBB genetic mutation. There are four other mutations that can cause this disease, each with several possible mutations, so different gRNA strands must be used in different patients to ensure that this alteration affects the proper segment of DNA. Though the use of this process to treat CGD on a larger, clinical scale could be limited by its need for detailed personalization for each patient, it still has an immense potential for effectively treating patients. Unlike the more common methods of treatment for CGD, this has the unique ability to resolve the

condition on a genetic level. This study provides ample proof of the viability and effectiveness for treating Chronic Granulomatous Disease using CRISPR-Cas9 to correct the mutation(s) responsible.

Treatment Comparison

All of the aforementioned treatments had concrete successes that should guide the future of treatments for Chronic Granulomatous Disease. The allograft and interferon gamma treatments work to address the effects of CGD and sought to improve immune function without altering genetic material. Contrary to these treatments, the CRISPR mediated treatment directly altered the genetic material of human induced pluripotent stem cells to introduce immune cells with a corrected CYBB gene. The allograft treatment saw three patient deaths, while the other two did not see any deaths by their conclusion. The interferon gamma treatment was unable to produce results pointing towards increased neutrophil functions, while neutrophil function was directly improved in the other trials.

Neutrophils

Being the most abundant immune cell in the human body, the neutrophil is an essential component of the innate immune system. Neutrophils have been observed to possess functional and phenotypic plasticity, further cementing their importance and versatility as an immune cell (Silvestre-Roig *et. al,* vol. 40, no. 7, pp. 565–583). A disruption of its activity causing an inability to carry out its defensive processes can bring about disaster for the human immune system. Upon an initial injury or infection, neutrophils will exit the bloodstream, where they typically reside, and enter the affected tissue (Burn *et. al*, vol. 54, no. 7, pp. 1377–1391). They act as the primary line of immunological defense aside from physical barriers. When they come into contact and recognize a foreign pathogen, they employ a variety of proteins and molecules to neutralize them. The most pertinent of these molecules being the reactive oxygen species (ROS), which are generated by the crucial NADPH oxidase. They are also crucial to the immune response because of their use of neutrophil extracellular traps (NETs). NETs are composed of decondensed chromatin and occupy three to five times the space of condensed chromatin (Delgado-Rizo *et. al*, vol. 8). Granules and histones bind to these NETs, some of which have antimicrobial properties. Those diagnosed with CGD have been found to have lower levels of NETs when compared to those without CGD.

Genetic Basis of CGD

There are five genes whose potential mutation can cause a defect in the subunit of the NADPH oxidase enzyme that it codes for. A mutation in the CYBB gene is the cause for a majority of the cases of CGD and is found on the X-chromosome. The NCF1 mutation follows this mutation in frequency and is found on the seventh chromosome (Merling *et. al*, vol. 23, no. 1, pp. 147–157). Using CRISPR-Cas9 techniques similar to those used in the study conducted by Flynn et. al., it may be possible to target this mutation in human stem cells and correct it.

CGD Patients: Life Quality and Expectancy

Patients diagnosed with Chronic Granulomatous Disease have a median age of death between 30 and 40, with their quality of life decreasing with age (Arnold and Heimall *et. al*, pp. 2543–2557). They also experience inflammation and infections in entire organs, leading to further complications. These patients most often have to endure a life of strong antibiotic prophylaxis, being that they have little to no ability to combat fungal and bacterial infection. With antibiotic resistance on the rise, the viability of antibiotic prophylaxis may see a decline. At this time, consistently curative treatments are sparse, experimental, and nonwidespread, creating a need to develop a treatment that can see consistent success across a large body of patients.

Infections and Inflammation

CGD patients most often experience infection from catalase-positive organisms such as *Staphylococcus aureus*, *Burkholderia cepacia*, *Salmonella,* etc. These patients are at an incredibly high risk when infected with these organisms, due to the fact that their already defected neutrophils cannot influence the microbes' hydrogen peroxide production. Fungal infections contribute to the mortality of patients with CGD greatly. With a variety of rare fungal species that are difficult to treat causing infections, an instance of these infections should be an indicator to doctors that the patient may have CGD.

Besides experiencing regular infections, CGD patients may face irregular inflammation. This inflammation often manifests in Gastrointestinal (GI) conditions that may even recede the CGD diagnosis. Other organs that may develop inflammatory conditions include the liver, eyes, lungs, and urogenital tract (Arnold and Heimall *et. al,* pp. 2543–2557). As CGD patients enter adulthood, the likelihood of experiencing autoinflammatory conditions increases and may determine their morbidity (Rieber *et. al*).

Conclusion

As shown above, there have been several teams of researchers who have investigated treatment options for Chronic Granulomatous Disease. With each of the trials having undeniable successes, more research and development should be allocated towards the treatment of CGD, using these studies and those that are similar to guide future trials. It is crucial that CGD gains more awareness in both the general and scientific community and becomes an immune disorder that more people are familiar with. With more people being educated on this topic, diagnosis and treatment will hopefully start as early as possible, seeing that both the hematopoietic allograft and interferon gamma treatments had more success and less side effects on young children. With more awareness and research into Chronic Granulomatous Disease and those diagnosed with it, there will hopefully be more treatments that could be implemented on a large clinical scale instead of being confined to the few participants in a clinical trial. Overall, there has already been an extensive amount of research done in the areas treating Chronic Granulomatous Disease via

hematopoietic allograft transplant, interferon gamma, and gene therapy that has produced promising results. With even more focus and dedication to developing and refining such treatments, more patients with CGD may live a longer, healthier life and may hopefully be cured.

Works Cited

Arnold, D.E., Heimall, J.R. A Review of Chronic Granulomatous Disease. *Adv Ther* 34, 2543–2557 (2017). https://doi.org/10.1007/s12325-017-0636-2

Burn, Garth Lawrence, et al. "The Neutrophil." *Immunity*, vol. 54, no. 7, 2021, pp. 1377–1391, https://doi.org/10.1016/j.immuni.2021.06.006.

"Chronic Granulomatous Disease (CGD)." *National Institute of Allergy and Infectious Diseases*, www.niaid.nih.gov/diseases-conditions/chronic-granulomatous-disease-cgd#:~:text=Chronic%20granulomatous%20disease%20(CGD)%20is,threatening%20bacterial%20and%20fungal%20infections. Accessed 7 July 2023.

Delgado-Rizo, Vidal, et al. "Neutrophil Extracellular Traps and Its Implications in Inflammation: An Overview." *Frontiers in Immunology*, vol. 8, 2017, https://doi.org/10.3389/fimmu.2017.00081.

Flynn, Rowan, et al. "CRISPR-Mediated Genotypic and Phenotypic Correction of a Chronic Granulomatous Disease Mutation in Human IPS Cells." *Experimental Hematology*, vol. 43, no. 10, 2015, https://doi.org/10.1016/j.exphem.2015.06.002.

Gallin et al."A Controlled Trial of Interferon Gamma to Prevent Infection in Chronic Granulomatous Disease." *New England Journal of Medicine*, vol. 324, no. 8, 1991, pp. 509–516, https://doi.org/10.1056/nejm199102213240801.

Horwitz, Mitchell E., et al. "Treatment of Chronic Granulomatous Disease with Nonmyeloablative Conditioning and a T-Cell–Depleted Hematopoietic Allograft." *New England Journal of Medicine*, vol. 344, no. 12, 2001, pp. 881–888, https://doi.org/10.1056/nejm200103223441203.

Merling, Randall K, et al. "An AAVS1-Targeted Minigene Platform for Correction of Ipscs from All Five Types of Chronic Granulomatous Disease." *Molecular Therapy*, vol. 23, no. 1, 2015, pp. 147–157, https://doi.org/10.1038/mt.2014.195.

Rider, N L, et al. "Chronic Granulomatous Disease: Epidemiology, Pathophysiology, and Genetic Basis of Disease." *Journal of the Pediatric Infectious Diseases Society*, vol. 7, no. suppl_1, 2018, https://doi.org/10.1093/jpids/piy008.

Rieber, Nikolaus, et al. "Current Concepts of Hyperinflammation in Chronic Granulomatous Disease." *Clinical and Developmental Immunology*, vol. 2012, 2012, pp. 1–6, https://doi.org/10.1155/2012/252460.

Silvestre-Roig, Carlos, et al. “Neutrophil Diversity in Health and Disease.” *Trends in Immunology*, vol. 40, no. 7, 2019, pp. 565–583, https://doi.org/10.1016/j.it.2019.04.012.

Taylor, Jared P., and Hubert M. Tse. “The Role of NADPH Oxidases in Infectious and Inflammatory Diseases.” *Redox Biology*, vol. 48, 2021, p. 102159, https://doi.org/10.1016/j.redox.2021.102159.

Vorobjeva, N V, and B V Chernyak. “Netosis: Molecular Mechanisms, Role in Physiology and Pathology.” *Biochemistry. Biokhimiia*, Oct. 2020, www.ncbi.nlm.nih.gov/pmc/articles/PMC7590568/.

Yu, Hsin-Hui, et al. “Chronic Granulomatous Disease: A Comprehensive Review.” *SpringerReference*, 2020, https://doi.org/10.1007/springerreference_172807.

Research Article

Nano Scaffolds in Nanomedicine for Enhancing Rheumatoid Arthritis Treatment

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Keywords: Rheumatoid Arthritis (RA), Immune System, Cells, Inflammation, Nano Carriers, Nano Scaffolds

Abstract

Rheumatoid Arthritis (RA) is classified as an inflammatory and autoimmune disease where the immune system mistakenly identifies healthy cells as foreign invaders that threaten and attack the body, leading to inflammation. The inflammation usually affects the major joints of the body, such as through the lining of the joint, causing joint tissue damage. Nano scaffolds are three-dimensional structures assembled of polymer fibers at the nanoscale level. The nano scaffold's 3D system allows for it to be used as nanocarriers to improve drug delivery effectiveness for RA treatment. These scaffolds are used as a framework for the nanoparticles to promote tissue restoration in the affected joints, allowing researchers to ease the regeneration of damaged tissues for RA. Currently, there is no cure for RA, but utilizing nano scaffolds would provide a favorable environment for developing, attaching, and differentiating cells involved in joint repair and regeneration. The new treatment approaches for rheumatoid arthritis offer optimistic expectations for enhancing patients' overall well-being and quality of life.

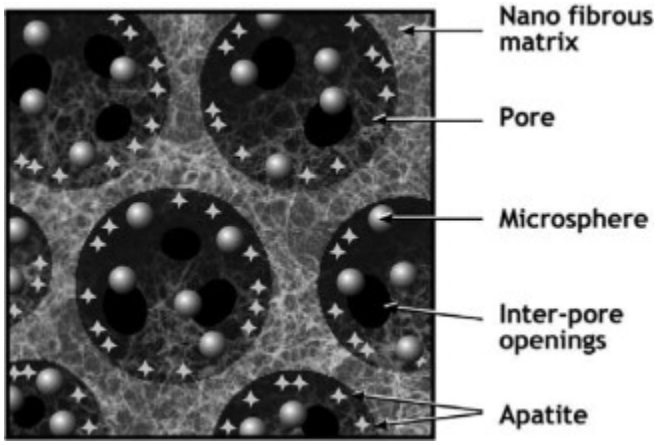
Introduction

Rheumatoid Arthritis

Rheumatoid arthritis (RA), an inflammatory and autoimmune disease primarily affects the joints and is associated with autoantibodies that target numerous molecules, including modified self-epitopes (Nature Journal). Although RA usually affects small joints of the feet and hand, which include the knuckles, wrists, and toes, it can act on multiple joints in the body, and the severity of the condition may vary significantly between individuals. The joint involvement would be dissimilar, with some suffering lesser symptoms and others experiencing more severe symptoms, such as permanent damage to the joint, which would need joint replacement surgery (arthroplasty). When the inflammation spreads and worsens, RA can act on the cartilage, which acts as a "shock absorber" in our joints, and over time, the damaged cartilage leads to joint deformity (Cleveland Clinic).

Nano Scaffolds

Nano scaffolds are scaffolds at the nanoscale level that are constructed from polymeric biocompatible materials. Nano scaffolds serve an essential purpose in tissue engineering, as when a nano scaffold is implanted or injected inside the body, the surrounding tissue reacts and releases damaged cells that would attach to the scaffold's surface to restore and regenerate the damaged tissues in the body. As the cells attach to the scaffold, they begin to multiply and invade it.



The scaffold with a structure of a cylinder composition would have a diameter of 200 nanometers and a length of 300 micrometers, loaded with PLGA polymer. The PLGA polymer would be engineered to be porous for the movement of the drug to the inflamed tissues. In addition to the pores in PLGA, the scaffold's structure would also be porous to imitate the characteristics of the natural tissues, such as function and porosity, to allow for the transportation of the Methotrexate through its open and interconnected network of pores to the inflammation sites. When Methotrexate is encapsulated within the scaffold, it travels through pathways the pores create for effective drug delivery through the channels, allowing controlled release of the Methotrexate to enable efficient and localized drug delivery, lessening potential side effects and enhancing therapeutic efficacy. The network of pores would create a mechanical interlock between the surrounding tissue and the scaffold.

Extracellular Matrix (ECM)

The extracellular matrix (ECM) consists of a complex network of macromolecules structured in a tissue-specific manner that plays a role in growth, tissue healing, and cell signaling (PMC). The ECM elements work together to construct a secure composite that provides structural integrity to the tissues. This creates a suitable environment for attachment and communication with nearby cells, allowing for cell growth, movement, and other functions (PMC). It provides the cellular support and microenvironment necessary for cell growth, migration, and response to signals, and its composition contributes to the mechanical qualities of the tissue by providing stiffness and elasticity adapted to certain tissue functions.

ECM with a core microsome composed of 300 proteins, including glycoproteins, collagens, and proteoglycan are used to support tissue function, allowing them to continue regulating cell behavior and providing adhesion sites to the cells.

Furthermore, ECM serves as a site for bioactive regulators like cytokines and growth factors that help stimulate cellular responses such as proliferation, differentiation, and migration for tissue growth and development (PMC). These ECM regulators allow them to have specific impacts on nearby cells. The ECM would be regulated during pathological processes like inflammation to allow tissue repair and regeneration. Matrix metalloproteinases (MMPs) are enzymes that are activated for the degradation and movement of ECM components. In turn, this would authorize damaged ECM to be removed, and the new ECM proteins would be deployed to help with tissue healing. The newly synthesized ECM provides mechanical support and signaling cues that guide cell migration and tissue reconstruction.

ECM Mimicking

ECM mimicking is a technique used for the nano scaffolds to mimic the ECM's natural microenvironment. This process would involve modifying the ECM's porous structure through degradation and cross-linking processes. The ECM cross-linking can occur through the action of lysyl oxidase, which allows for the collagen and elastin to cross-link, forming covalent cross-links that would allow for the structure (Loh and Cloong 33). Using various ways, such as incorporating matrix metalloproteinases (MMPs) and attaching the Arg-Gly-Asp peptide for cell attachment, tissue engineering can stimulate specific signaling pathways and cellular responses essential for tissue development, regeneration, and functionality. MMPs role in ECM for nano scaffolds allows it to promote degradation and remodeling necessary to break down and repair ECM, which is necessary for assisting in joint tissue repair. They are known to serve in ECM by breaking down and remodeling parts in the ECM, which is crucial for repairing joint tissues.

The ECM’s natural structure promotes regulatory immune cells such as anti-inflammatory macrophages or T cells. Similarly, when the nano scaffold copies the natural environment of the ECM by carrying the special ECM proteins, it can perform the actions of transmitting immune cells that assist at sites of inflammation. Additionally, the scaffold could be integrated with bioactive molecules and growth factors from the ECM network for tissue repair and development.

Materials and Methods

This experiment uses nano scaffolds that serve as nanocarriers to provide a foundation for the assembly of drug delivery systems, which would deliver therapeutic agents to the inflammation site and assist with tissue repair. The engineered nano scaffolds work as nanocarriers to help assemble drug-loaded nanoparticles. These nanoparticles would then be cell membrane-coated through biomimetic drug

delivery systems to improve the efficiency of the therapeutic agents to be delivered to the site of inflammation. The nano scaffolds' structure would be engineered to encase the nanoparticles loaded with therapeutic drugs to successfully provide the therapeutic drugs at the inflammation site and encourage the repair of the damaged tissues.

Materials

- PLGA nanoparticles
- Methotrexate therapeutic agent
- Neutrophil cell membrane
- Polyvinyl alcohol
- Dichloromethane (DCM)

Methods

Poly (Lactic-co-glycolic acid) (PLGA) nanoparticles are a synthetic polymer that can serve as nano scaffolds to encapsulate the therapeutic agents in its polymer matrix. The matrix would allow the drugs to be encapsulated and protected, away from any factors that might restrict their efficiency. The matrix would consist of both the hydrophobic core and the hydrophilic shell, further safeguarding the encapsulation of the drugs released at the appropriate site. During the manufacture of the PLGA nanoparticles, the drug can be loaded into the core by being integrated at the front of the nanoparticle formulation or using a polymer solution to break down the drugs.

Methotrexate is a therapeutic medicine that can be used to fight the inflammation in the joints in RA. The Methotrexate would be encapsulated by the nanoparticles to be delivered at the site.

Neutrophils are white blood cells that assist at the site of the inflammation by starting an immune response when pathogens invade the body. The neutrophil cell membrane is the neutrophil's outer membrane, which contains a complicated array of adhesion molecules and receptors. These adhesion molecules and receptors are compatible with binding to specific ligands in the inflamed tissues. Coating the nanoparticles with a neutrophil cell membrane would grant the nanoparticles with the unique properties of the neutrophil membrane to specially target the inflammation site. The nanoparticles possess adhesion molecules and receptors similar to those found on neutrophils, allowing them to mimic the natural ability of neutrophils.

Experimental Procedures

Weigh 100 - 500 mg of PLGA polymer and break it down in a dichloromethane (DCM) solvent to create a one-phase solution. In a separate water-immiscible solvent, dissolve the therapeutic agent Methotrexate with a particle size of 20-30 μm to be added to the solution of PLGA polymer to spread the particles within the solvent. The PLGA polymer and Methotrexate would be stirred with a large water volume in an oil-in-water emulsion, where polyvinyl alcohol, an emulsifier, would be added to stabilize the mixture.

Continue to emulsify the mixture for a minute under the mixing conditions. Remove the DCM solvent from the emulsion to harden the scattered droplets (containing PLGA and Methotrexate) into solid particles for suitable drug encapsulation. Next, extract the cell membrane from Neutrophil cells to coat the PLGA nanoparticles through membrane fusion, allowing for the integration of the two bilayers. To support cell-material interactions, incorporate ECM-mimicking proteins and peptides, including fibronectin, hyaluronic acid laminin, and collagen (NIH). Replicating the natural ECM's structural components will allow the PLGA nanoparticles to interact with the damaged tissues. The presence of the ECM-mimicking components would allow the promotion of cellular adhesion, proliferation, and tissue integration for the interaction with the damaged tissues. Wash the final nanoparticles to remove any unwanted and harmful chemicals. Lastly, resuspend the nanoparticles into an injectable solution such as phosphate-buffered saline (PBS), as it is biocompatible and has the potential for buffering capacity and ionic balance.

Clinical Trials

The in vivo experimental study involves lab mice and uses mouse-specific collagen-induced arthritis (CIA) that serves as an autoimmune model of human rheumatoid arthritis.

Select a compatible mice strain, such as the DBA/1J mice, as they are vaccinated with type II collagen and trigger the development of severe polyarthritis (inflammation in multiple joints).

Male or female of the selected mice strain between the ages of 8-12 weeks, where they would be considered young adults, ensuring consistency in their developmental stage and susceptibility to arthritis development.

Mice experiencing arthritis symptoms such as joint inflammation, swelling, erythema, and reduced mobility without any pre-existing health conditions are selected. The selection of mice with arthritis symptoms is essential as it allows the therapeutic effects of the Methotrexate-loaded PLGA nanoparticles to be evaluated in a related disease context.

Begin the initiation of CIA by immunizing the mice with an emulsion of type II collagen and Freund's adjuvant to promote arthritis. This can be done by injecting the base of the tail or near a rigid limb. The Freund's adjuvant would consist of heat-killed Mycobacterium tuberculosis in mineral oils at the 4 mg concentration, which would be mixed equally. Using a 26 gauge x 12 needle, inject 50-100 µl of volume into the mice. Administer the dosage and create booster immunizations using an emulsion of type II collagen in incomplete Freund's adjuvant without the heat-killed M. tuberculosis. Administer the booster or secondary immunization 14 or 21 days after the initial immunization.

The study will include three test groups aimed at assessing different outcomes.

Group 1 includes mice induced with CIA that are not treated for their arthritis symptoms. This specific mice group serves as a baseline for the experiment to allow researchers to monitor and track the progress

of the arthritis symptoms without any disturbance. It allows researchers to perceive how severe the symptoms are to allow researchers to perceive the severity of the symptoms in the absence of treatment. It provides a basis for evaluating the effectiveness of the Methotrexate-loaded PLGA nanoparticles in treating the severity of arthritis symptoms in the treated groups.

Group 2 includes mice that receive placebo treatment which involves inactive substances. In the case of human clinical trials, the placebo treatment is designed to look like the medicine that is being tested, to allow the patients to believe that they are receiving treatment that leads to improvements in arthritis symptoms, even if the treatment itself has no therapeutic effect. Despite not having a therapeutic effect, the placebo treatment would be aimed toward the patient's psychological well-being, enhancing their expectations and perceptions of the treatment. This approach ensures the unbiasedness of the patients and enables a more explicit evaluation of the actual effects of the active treatment.

Group 3 trials mice that receive the actual treatment, the Methotrexate-loaded PLGA nanoparticles with neutrophil cell membrane for collagen-induced arthritis to be monitored for the effectiveness of the treatment using nanoparticles. The trial is designed to track the treatment's impact on reducing arthritis symptoms and inflammation.

Severity score	Degree of inflammation
0	No evidence of erythema and swelling
1	Erythema and mild swelling confined to the tarsals or ankle joint
2	Erythema and mild swelling extending from the ankle to the tarsals
3	Erythema and moderate swelling extending from the ankle to metatarsal joints
4	Erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb

The mice of each of the three groups would be examined 2 to 3 times a week, where each of the mice's paws would be evaluated and scored on a scale of 0 - 4, where 4 indicates the most severe inflammation (Nature Protocols).

Hypothetical Results

The focus of the hypothetical results lies within the clinical phases, explicitly evaluating the efficiency of the Methotrexate-loaded PLGA nanoparticles for treating RA.

Group 1, consisting of individuals with untreated induced CIA symptoms, displayed signs of erythema and mild swelling extending from the ankle to the tarsals, as shown in the severity score. The severity of these symptoms is assessed using a scoring system, where higher scores indicate more severe inflammation and swelling. The severity scores for mice in Group 1 range from 2 to 3 on the scale, indicating moderate to moderately severe arthritis symptoms. However, without any treatment, the inflammatory response in the joints may continue to increase, leading to worse symptoms and a higher severity score, such as a 4.

Similar to Group 1, Group 2, which received placebo treatment for the arthritis symptoms, also exhibited signs of erythema and mild swelling in the ankle to the tarsal region. The severity scores for mice in Group 2 ranged from 1 to 2 on the scale, indicating mild to moderate arthritis symptoms. Unlike the results in Group 1, Group 2 symptoms would be slightly less severe as the placebo treatment may have a minimal effect, such as less stress and a more robust immune response. Similarly to Group 1, Group 2 degree of inflammation will increase due to improper treatment, leading to severity scores of 3 or 4.

In Group 3, the mice showed signs of erythema and moderate swelling extending from the ankle to the metatarsal joints. After the active treatment, the mice displayed a significant decrease in erythema and swelling in the joints, indicating a significant improvement in the degree of inflammation. The treated mice demonstrated enhanced mobility and reduced joint stiffness compared to their initial condition. These results strongly indicate the potential effectiveness of Methotrexate-loaded PLGA nanoparticles in targeting the inflammatory processes associated with arthritis. The result is a reduction in severity scores, ranging from 1 to 2.

Discussion

Disadvantages of Methotrexate-loaded PLGA nanoparticles

While using Methotrexate-loaded PLGA nanoparticles with a neutrophil is a potential treatment for RA, there are setbacks to this method. While the nanoparticles are deployed to target and deliver the methotrexate therapeutic agent to the inflamed areas in the joint, the drug could be delivered to unwanted areas in the body, increasing the risk of side effects in the system and potential toxicity in non-target tissues and areas. Furthermore, the immune responses to the nanoparticles themselves can serve as a threat. Since the nanoparticles enter the body as foreign substances, the body may recognize it as a threat and produce an inflammatory response. This immune response may target the nanoparticles and affect the surrounding tissues, worsening the inflammation and potentially causing tissue damage. Also, the release of the nanoparticles may be unregulated, delivering the drugs inconsistently. There may not be enough of the drug at one site, and there could be excessive drug concentrations at another site. Poor drug release

kinetics can cause negative lead to insignificant therapeutic effects, as the medicine may need to reach the desired concentration to treat joint inflammation adequately.

Comparing Methotrexate-loaded PLGA nanoparticles with Immunotherapy

Immunotherapy is a type of treatment that uses substances made from living organisms to treat diseases (NIH). It uses the body's own immune system to activate or suppress the immune system and is commonly used in fighting cancers and infections through the use of biological agents, such as tumor necrosis factor (TNF) inhibitors, interleukin (IL) inhibitors, or B-cell modulators. Unlike how the nanoparticles deliver therapeutic drugs directly to the inflammation site, Immunotherapy uses systemic delivery of immune-modulating drugs to attain disease control throughout the body. Regarding time efficiency, the nanoparticles serve as a faster route due to their direct delivery at the inflammation site. On the other hand, Immunotherapy may require several extended treatment cycles and periods until the proper control of RA is achieved.

Nano Scaffolds Instead of Regular Scaffolds

Nano Scaffolds are scaffolds at the nanoscale level (10 times smaller than the human hair) and have a higher surface area and porosity than regular scaffolds. This allows higher concentrations of Methotrexate and other factors to be integrated into the scaffold. Also, the nano scaffold’s larger surface area per unit volume allows for more adhesion sites for more interactions between the cells and the scaffold. The larger surface area per unit volume would lead to increased pores for improved delivery of the therapeutic agents. Nano scaffolds also have more control over release kinetics than regular scaffolds due to the more effective diffusion of the therapeutic agents. Overall, nano scaffolds have a small size but a higher surface area per unit volume that have more benefits than regular scaffolds.

Conclusion

In conclusion, Methotrexate-loaded PLGA nanoparticles as scaffolds can potentially treat and prevent the spread of RA by delivering the therapeutic agent methotrexate directly at the site of inflammation. The delivery of the agent allows for a more concentrated and efficient treatment method, limiting potential adverse effects and systemic exposure. PLGA nanoparticles as scaffolds provide a three-dimensional structure of polymer fibers to encourage tissue restoration and regeneration in the affected joint to repair damaged tissues. Furthermore, incorporating a neutrophil cell membrane onto the nanoparticles allows for enhanced targeting and interaction with the inflamed joint tissues. While the concept of methotrexate-loaded PLGA nanoparticles is relatively new, the research for it can be expanded to manifest it as a possible cure.

Works Cited

Boehler, R. M., Graham, J. R., & Anseth, K. S. (2011). Tissue engineering tools for modulation of the immune response. BioTechniques, 51(4), 239–254. https://doi.org/10.2144/000113754

Brand, D. D., Latham, K. A., & Rosloniec, E. F. (2007). Collagen-induced arthritis. Nature Protocols, 2(5), 1269–1275. https://doi.org/10.1038/nprot.2007.173

Bullock, J., Rizvi, S. S., Saleh, A. M., Ahmed, S., P, D., DO, Ansari, R. A., & Ahmed, J. (2018). Rheumatoid arthritis: A brief overview of the treatment. Medical Principles and Practice, 27(6), 501–507. https://doi.org/10.1159/000493390

Gupta, K. C., Haider, A., Choi, Y., & Kang, I. (2014). Nanofibrous scaffolds in biomedical applications. Biomaterials Research, 18(1). https://doi.org/10.1186/2055-7124-18-5

He, X., & Xu, C. (2020). Immune checkpoint signaling and cancer immunotherapy. Cell Research, 30(8), 660–669. https://doi.org/10.1038/s41422-020-0343-4

Huang, S., Wu, T., & Yu, H. (2013). Biomimetics Using Nanotechnology/Nanoparticles in Dental Tissue Regeneration. In Nanofibrous Scaffold. https://doi.org/10.1016/b978-1-4557-3127-5.00020-9

Loh, Q. L., & Choong, C. (2013). Three-Dimensional scaffolds for tissue engineering applications: role of porosity and pore size. Tissue Engineering Part B-reviews, 19(6), 485–502. https://doi.org/10.1089/ten.teb.2012.0437

Naskar, S., Das, S., Sharma, S., & Kuotsu, K. (2021). A Review on Designing Poly (Lactic-co-glycolic Acid) Nanoparticles as Drug Delivery Systems. Pharmaceutical Nanotechnology, 9(1), 36–50. https://doi.org/10.2174/2211738508666201214103010

NCI Dictionary of Cancer Terms. (n.d.). National Cancer Institute. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/ecm

Pham, C. T. (2011). Nanotherapeutic approaches for the treatment of rheumatoid arthritis. Wiley Interdisciplinary Reviews-nanomedicine and Nanobiotechnology, 3(6), 607–619. https://doi.org/10.1002/wnan.157

PLGA NanoParticles | Luna NanoTech. (n.d.). Luna Nanotech. https://www.lunanano.com/product-page/plga-nanoparticles?utm_term=&utm_campaign=&utm_source=adwords&utm_medium=ppc&hsa_acc=5536580572&hsa_cam=17142624182&hsa_grp=137964352764&hsa_ad=596006214472&hsa_src=g&hsa_tgt=dsa-1654967648858&hsa_kw=&hsa_mt=&hsa_net=adwords&hsa_ver=3&gad=1&gclid=Cj0KCQjwwISlBhD6ARIsAESAmp7J7RIuDH-adRPee2p0eF5YToMJUQYZh9v9oEBX55rFmy0ZGgoDPL4aArpOEALw_wcB

Rheumatoid arthritis (RA) | Arthritis | CDC. (n.d.). https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html#:~:text=Rheumatoid%20arthritis%2C%20or%20RA%2C%20is,usually%20many%20joints%20at%20once.

Schematic of a ‘biomimetic nano scaffold’. The scaffold combines the. . . (n.d.). ResearchGate. https://www.researchgate.net/figure/Schematic-of-a-biomimetic-nano-scaffold-The-scaffold-combines-the-novel-nano-fibrous_fig7_248555655

Seely, A. J., Pascual, J. L., & Christou, N. V. (2003). Science review: Cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance. Critical Care, 7(4), 291. https://doi.org/10.1186/cc1853

Singh, R., & Lillard, J. W. (2009). Nanoparticle-based targeted drug delivery. Experimental and Molecular Pathology, 86(3), 215–223. https://doi.org/10.1016/j.yexmp.2008.12.004

Smith, I., Liu, X., Smith, L. C., & X, P., MA. (2009). Nanostructured polymer scaffolds for tissue engineering and regenerative medicine. Wiley Interdisciplinary Reviews-nanomedicine and Nanobiotechnology, 1(2), 226–236. https://doi.org/10.1002/wnan.26

Smolen, J. S., Aletaha, D., Warren, R. B., Burmester, G. R., Emery, P., Firestein, G. S., Kavanaugh, A., McInnes, I. B., Solomon, D. H., Strand, V., & Yamamoto, K. (2018). Rheumatoid arthritis. Nature Reviews Disease Primers, 4(1). https://doi.org/10.1038/nrdp.2018.1

Sokolova, V., Kostka, K., Shalumon, K., Prymak, O., Chen, J., & Epple, M. (2020). Synthesis and characterization of PLGA/HAP scaffolds with DNA-functionalised calcium phosphate nanoparticles for bone tissue engineering. Journal of Materials Science: Materials in Medicine, 31(11). https://doi.org/10.1007/s10856-020-06442-1

Wang, A., Qin, X., Fang, J., & Sun, X. (2021). Nanomedicines for the treatment of rheumatoid arthritis: State of art and potential therapeutic strategies. Acta Pharmaceutica Sinica B, 11(5), 1158–1174. https://doi.org/10.1016/j.apsb.2021.03.013

Wikipedia contributors. (2023). Nano-Scaffold. Wikipedia. https://en.wikipedia.org/wiki/Nano-scaffold
X, P., MA. (2004). Scaffolds for tissue fabrication. Materials Today, 7(5), 30–40. https://doi.org/10.1016/s1369-7021(04)00233-0

Yi, H., Rehman, F. U., Zhao, C., Liu, B., & He, N. (2016). Recent advances in nano scaffolds for bone repair. Bone Research, 4(1). https://doi.org/10.1038/boneres.2016.50

“000670 - DBA1 Strain Details.” *The Jackson Laboratory*, www.jax.org/strain/000670. Accessed 30 July 2023.

Zhu, L., Luo, D., & Liu, Y. (2020). Effect of the nano/microscale structure of biomaterial scaffolds on bone regeneration. International Journal of Oral Science, 12(1). [Original source: https://studycrumb.com/alphabetizer]

Literary Review

Hashimoto's Disease: The Importance of Nutrition and Lifestyle

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Keywords: Hashimoto's thyroiditis, lifestyle, diet, nutrition, thyroid hormones

Abstract

Hashimoto's disease is an autoimmune disorder affecting the thyroid gland, in which the thyroid is unable to create thyroid hormones for the body, which can affect the entire immune system. Despite having thyroid hormone medicine, many individuals with Hashimoto's disease continue to experience symptoms and impaired quality of life. However, new approaches in research have found that nutrition and lifestyle have come to play a big role in regulating a patient's symptoms. By eating the right nutrition/foods, exercising, and creating a good daily way of living, patients who have Hashimoto's disease have significantly improved their health by improving their routines. In this article, 3 experiments have differently shown ways in which Hashimoto's thyroiditis patients could change up their lifestyles to have a healthy thyroid.

Introduction

Hashimoto's disease, also known as chronic lymphocytic thyroiditis, is thyroid inflammation or hypothyroidism. The thyroid is the neck gland that makes hormones that help regulate the body and control metabolism. Hashimoto's disease happens when the thyroid is inflamed and can't make thyroid hormones, which the immune system automatically responds to by attacking the body's tissues and organs. White blood cells or lymphocytes (essential to the immune system) make antibodies or proteins that attack thyroid cells. Thyroid hormones are necessary to support many functions: regulating body temperature, heart rate, menstrual cycle, growth, and development (Mincer *et al*. 2021). Some of the common symptoms of Hashimoto's can range from fatigue, weight gain, constipation, heavy or irregular menstrual periods, etc. For some, it may lead to complicated life-threatening health issues such as high blood pressure or heart disease. While there are medicines such as levothyroxine that help restore thyroid hormone levels, there isn't an actual cure for the disease. Many patients are heavily impacted by symptoms, ruining their quality of life. However, dietary and changing lifestyles of patients have been seen as a possible way to improve symptoms and health and adjust daily routines to treat Hashimoto's.

Discussion

Thyroid hormone functions

To establish a better understanding of Hashimoto's disease, the thyroid and thyroid hormones are the initial target that the disease attacks. The job of the thyroid is to produce thyroid hormones, which are responsible for controlling metabolism, growth, etc. There are two main hormones, triiodothyronine (T3) and thyroxine (T4). The hormones are controlled by the pituitary and thyroid glands along with the hypothalamus. These two hormones affect every cell and all the organs in the body, by regulating the rate at which the body uses calories, weight loss or weight gain, slowing down or speeding up heart rate, body temperature, speed of digestive tract, brain development, etc (Shahid *et al*, 2021). When hypothyroidism happens, the thyroid doesn't make or release enough thyroid hormone into the bloodstream, causing metabolism to slow down. Thyroid hormones are crucial to one's body in order to maintain a healthy thyroid that helps with metabolism and body functioning.

Effects of powdered black cumin on thyroid function

A researcher at the Tabriz University of Medical Science wanted to experiment with black cumin or *Nigella sativa* seed powder on oxidative stress (possibly related to the development/pathogenesis of Hashimoto's disease). Oxidative stress is a condition where lifestyle and other factors could affect one's body, creating antioxidants that damage cells and tissues. In the experiment, 40 patients with Hashimoto's thyroiditis, ages 22 to 50, were randomly assigned to two groups, one receiving black cumin daily and the control group, for 8 weeks. The usage of cumin seeds was deemed effective for their antioxidant, anti-inflammatory, and regulating immune system properties. As a result, the researchers saw a significant reduction in weight and body mass index, observed in patients treated with black cumin seeds. Serum concentrations of thyroid-stimulating hormone (TSH) and anti-thyroid peroxidase (anti-TPO) antibodies decreased while serum T3 concentrations increased in the *Nigella sativa*-treated group after 8 weeks. None of these changes had been observed in the control group. The experiments conclude on black cumin powder's effect on thyroid status and anthropometric variables in patients with Hashimoto's thyroiditis. Powdered *Nigella sativa* can help improve thyroid status, as it helps with the antibodies that develop Hashimoto's disease. Considering the effect of the seeds in treating the disease's severity, it can be a therapeutic approach to managing Hashimoto's thyroiditis.

A weakness of this experiment is the need to be aware of potential side effects. Although the article itself explained in depth the benefits of black cumin seeds as therapy/treatment for Hashimoto's disease, there was a diagram that provided data on the experimental group: 23 participants received the powdered black cumin, but 3 participants were lost to the follow up due to nausea and itching. With this in mind, black cumin seeds may not help with reducing symptoms because there is still uncertainty with the powder

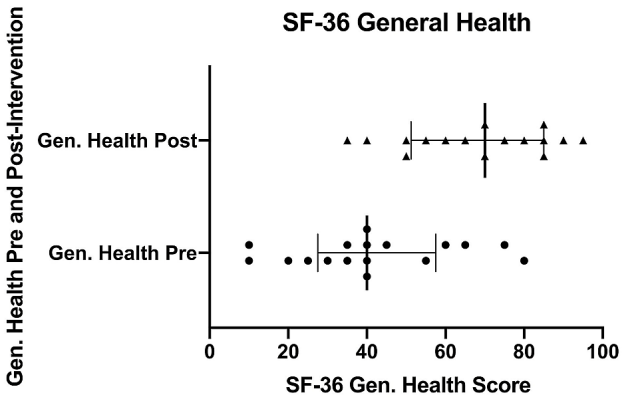
consumed by every Hashimoto's thyroiditis patient, it can't be assumed that there was a sign of allergy or reaction from the immune system.

Autoimmune Protocol Diet/Supported Lifestyle Intervention

Another approach to improving Hashimoto's symptoms was done by researchers Robert D. Abbott, Adam Sadowski, and Angela G. Alt on lifestyle intervention at the Helfgott Research Institute. In their study, they wanted to determine the efficacy of a 10-week diet and lifestyle intervention implemented by nutritional therapy practitioners, health coaches, and physicians. Before the initial experiment, selected Hashimoto's thyroiditis patients completed surveys regarding their health (36-Item Short Form Health Survey), quality of life, and medical symptoms (Medical Symptoms Questionnaire) along with their blood tests and demographic information. Once all prerequisites were completed, 17 participants began an online dietary and health program for 10 weeks of lifestyle intervention. This involved six weeks of food elimination, adding nutrient-dense foods, and modifying lifestyles, followed by a four-week maintenance period. Foods eliminated consisted of all grains, dairy, eggs, legumes, coffee, alcohol, seeds, nuts, refined/processed sugars, oils, and food additives. Eliminating foods has become a crucial treatment because these foods can trigger thyroid inflammation. For replacement, anti-inflammation foods that are added to the patient's diet include foods rich in mono and polyunsaturated fatty acids, bone broth, seafood, fermented foods, and organ meats. Lifestyle modifications included sleep hygiene, stress management, exercise, promotion of support systems, and increased time spent outdoors. Thyroid hormones help control weight and metabolism, making physical activity a necessity for good thyroid health. All health coaches and nutritional therapy practitioners provided help virtually through email and private Facebook group chats. They encouraged when participants faced challenges, answered questions, and troubleshot participants who experienced difficulty with the process. For the analysis of the experiment, the researchers compared the data from surveys, before the start of the experiment and during the 10-week lifestyle/diet adjustments. The Medical Symptoms Questionnaire scores over four weeks decreased substantially from an average of 92 (Standard Deviation 25) before the program to 29 (SD 20) after the program. Along with general health, the data suggested drastic improvements. The overall experiment evaluated the use of online surveys by nutrition practitioners and health coaches to improve the quality of life of Hashimoto's disease patients and the severity of their symptoms (along with thyroid health).

There was one resolved problem about this experiment, where there were errors in calculating data from the 36-Item Short Form Health Survey, as individual subscales failed the Shapiro-Wilk test for normality, and so the data sets could not be assumed to be normally distributed. Because this experiment was based on surveying participants and using those surveys to record results, it could lead to issues such as response and questionnaire bias. To correct the error when performing statistical analyses for multiple

hypotheses, the main researchers utilized a false discovery rate control adjustment and they found median values with the subscales.



This figure shows a scatter plot of SF-36 general health scores pre and post-intervention: SF-36 (36-Item Short Form Health Survey), Gen. Health (general health), Pre (pre-intervention), and Post (post-intervention). Individual pre-intervention scores are depicted with circles and individual post-intervention scores are depicted with triangles. Comparing the pre-intervention points to the post-intervention points, there is an increase in general health scores. As for the error, the researchers created median error bars, the side vertical lines as the interquartile range (the middle half of the data set), and the bolded vertical lines within the interquartile indicate the median.

Why is managing a diet necessary for Hashimoto's thyroiditis?

Thyroid hormones have a crucial role in the regulation of metabolism, with decreasing thyroid function, Hashimoto's thyroiditis patients have decreased resting metabolic rates (which leads to a possible increase in body weight resulting from excessive energy consumption). At the University of Life Sciences in Warsaw, Poland, a study was conducted regarding the importance of nutrition and dietary management. An analysis of the diet of Polish patients with Hashimoto's disease showed that the average energy value of over 80% of menus checked daily was 1,600 kcal (±600 kcal), which was below the assumed value (Ihnatowicz *et al*. 2020). Among these patients, there were similar numbers of women with normal body weight, overweight, and obesity. This implies that regardless of body weight, energy intake in this group could be insufficient. Hashimoto's disease can be managed by eating a diet that promotes the immune system's function in regulating inflammatory processes through the composition and preparation of meals. In addition, it requires the elimination of problematic food antigens. Among patients suffering from autoimmune thyroid disease, there is a deficiency of minerals such as iodine, iron, zinc, copper, magnesium, potassium, and vitamins A, C, D, and B group vitamins. Iodine is a key nutrient that supports thyroid hormone production, being that T3 and T4 thyroid hormones contain iodine. Many of these minerals and vitamins help with the functioning of the thyroid. The main issue of Hashimoto's disease is

the condition of the thyroid, so keeping the thyroid in good condition is essential. Cooperating with the idea of switching diets is a good focus to build up thyroid hormones so that the immune system can operate properly.

Conclusion

In conclusion, research has shown that the balance of a healthy lifestyle will benefit the function of the thyroid of Hashimoto's thyroiditis patients. The current treatments involve finding ways to help manage the thyroid and the creation of thyroid hormones. More natural ways such as consuming black cumin powder to produce more hormones, mentoring patients in their lifestyles, and incorporating vitamins and minerals in diets are critical elements that can help with Hashimoto's disease. The thyroid gland is an important part of the body, with its ability to grow and keep up metabolism and the immune system, affecting every other function of the entire body. Lots of research is still being done, with new treatments such as harnessing stem cells to regulate the immune system, the growth of understanding Hashimoto's disease still continues to develop and hopefully find possible cures.

Works Cited

Abbott, Robert D, et al. “Efficacy of the Autoimmune Protocol Diet as Part of a Multi-disciplinary, Supported Lifestyle Intervention for Hashimoto’s Thyroiditis” *Cureus*, 27 Nov. 2019, https://www.cureus.com/articles/18455-efficacy-of-the-autoimmune-protocol-diet-as-part-of-a-multi-disciplinary-supported-lifestyle-intervention-for-hashimotos-thyroiditis#!/

Farhangi, Mahdieh Abbasalizad, et al. “The effects of Nigella sativa on thyroid function, serum Vascular Endothelial Growth Factor (VEGF) - 1, Nesfatin-1 and anthropometric features in patients with Hashimoto's thyroiditis: a randomized controlled trial” *Pubmed*, U.S. National Library of Medicine, 16 Nov. 2016, https://pubmed.ncbi.nlm.nih.gov/27852303/

Ihnatowicz, Paulina, et al. “The importance of nutritional factors and dietary management of Hashimoto’s thyroiditis” *Annals of Agricultural and Environmental Medicine*, 2020, https://www.aaem.pl/The-importance-of-nutritional-factors-and-dietary-management-of-Hashimoto-s-thyroiditis,112331,0,2.html

Mincer, Dana L, et al. “Hashimoto's Disease” *National Institutes of Health*, 2021, https://www.niddk.nih.gov/health-information/endocrine-diseases/hashimotos-disease#:~:text=Hashimoto's%20disease%20is%20an%20autoimmune,the%20front%20of%20your%20neck.

Shahid MA, et al. “Thyroid Hormone” *Cleveland Clinic*, 12 May. 2021, https://my.clevelandclinic.org/health/articles/22391-thyroid-hormone

Literary Review

Cell-targeted Immunotherapy in Systemic Lupus Erythematosus (SLE)

Gabriela Dominguez (author), Alan Wang (advisor), Christina Thomas (advisor)

Keywords: Systemic lupus erythematosus (SLE), immunosuppressive drugs, biologics, B cells, clinical trials, targeted therapies.

Abstract

The most prevalent form of lupus, known as systemic lupus erythematosus (SLE), affects over 5 million individuals worldwide. SLE may develop at any age, although it more commonly affects women who are of reproductive age, with a 9:1 female-to-male ratio. Studies have indicated that ethnic minorities have a greater prevalence of the disease and a higher risk of developing it. The mortality rate of SLE has progressively lessened in recent years, given the increased understanding of the disease and considerable advancements in treatments. As the field of immunotherapy continues to rapidly evolve, the potential to revolutionize the SLE treatment landscape continues to be promising, enhancing patient outcomes and quality of life. This review will highlight the most recent advances and clinical trials in cell-targeted immunotherapy for SLE, particularly those targeting B-cells.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease whereby the immune system attacks its own tissues. Clinical manifestations can range depending on the disease's different presentations. However, common clinical manifestations include joint pain, swelling, and fatigue (CDC). There are severe forms of the disease, such as lupus nephritis which many patients with SLE may develop during their disease course. LN involves the inflammation of the kidneys, along with other much more severe symptoms. Due to its diverse clinical presentations, SLE remains challenging for specialists and healthcare professionals to diagnose. However, the American College of Rheumatology has made specific criteria for patients to be easily diagnosed. While the exact cause of SLE remains uncertain, many scientists suggest that the disease occurs in response to a combination of environmental, genetic, and hormonal factors. Therapy utilized in treating the disease has relied on immunosuppressants, agents that decrease the body's immune responses, such as corticosteroids, azathioprine, and methotrexate ("Treating Lupus with Immunosuppressive Medications"). In recent years, however, there have been noteworthy advances in the treatments for SLE, leading to substantial improvements in patient outcomes.

Discussion

B lymphocytes in SLE pathogenesis

It has been widely investigated how B cells, also referred to as B lymphocytes play a role in autoimmune illnesses like systemic lupus erythematosus (SLE). Understanding the role of B cells in SLE is critical for developing tailored treatment approaches. In autoimmune diseases, B cells become dysregulated, contributing to the pathogenesis of SLE through the production of pathogenic antibodies thar target the body's cells and tissues (Fregoso, *et al.*). In addition, B cells act as antigen-presenting cells, activating T-cells and pro-inflammatory cytokines, further exacerbating the inflammatory response and contributing to tissue damage.

Targeted Biologic Agents

One emerging area of research in the field of immunology is the development of targeted biological agents. B-cell therapies are a form of treatment that has escalated during the past decade that directly targets and modulates the activity of B cells in autoimmune diseases like systemic lupus erythematosus (SLE). B-cell depletion has already been employed successfully to manage autoimmune diseases, such as rheumatoid arthritis. The multifaceted nature of B cells in SLE has prompted further research to be conducted into the development of therapies that target these cells directly, eliminating abnormalities and restoring immunological balance. B cell targeting in SLE has been explored utilizing a range of approaches including depletion and inhibition of B cell survival and maturation (Lee, Amengual).

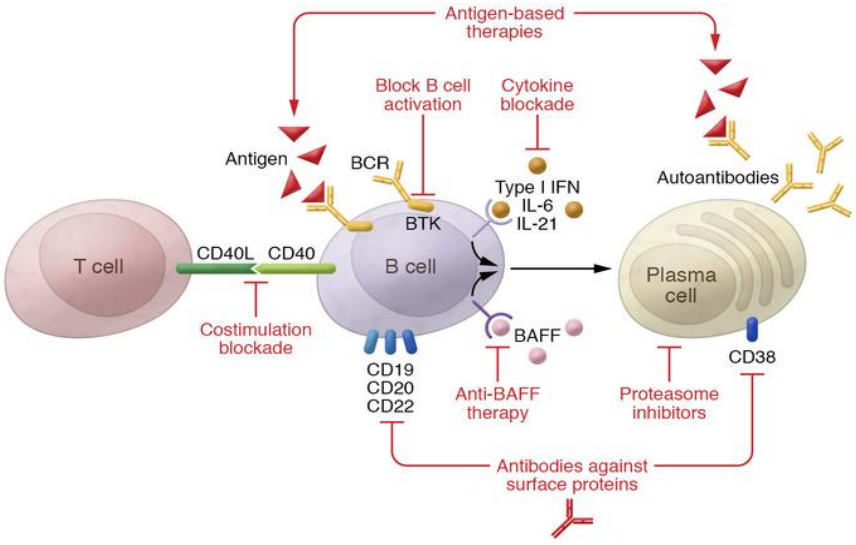


Figure 1. This figure outlines antigen-based therapies for SLE to modulate the immune response. The process begins with the contact of self-antigens by antigen-presenting cells, activating T cells in draining lymph nodes. The stimulation of plasma cell development in B cells is thus assisted by these activated T cells. Autoantibodies produced by these plasma cells result in the formation of immunological complexes.

These immune complexes can accumulate in numerous tissues, such as the kidneys, leading to organ damage.

Clinical Trials

Rituximab is one such agent used in B-cell depletion, classified as a chimeric monoclonal antibody against the protein CD20. It is currently a viable treatment option for managing refractory SLE manifestations, including nephritis and neuropsychiatric SLE. Rituximab has gone through extensive trials to ensure its efficacy. Most notably through randomized controlled trials: EXPLORER and LUNAR. In the EXPLORER trial, 257 patients from the ages of 16-75 with moderate to severe non-renal SLE were randomized to obtain Rituximab or placebo. Exclusion criteria for this trial consisted of patients who had previously taken cyclophosphamide or calcineurin inhibitors, presented a severe central nervous system or life-threatening form of lupus, or any conditions requiring steroid usage. To assess the activity of the disease, researchers utilized a system called the British Isles Lupus Assessment Group (BILAG) organ system, which assigned scores based on the need to either adjust or increase the treatment for different organs. Severe disease was indicated by numerous high-scoring organs, whilst moderate disease was indicated by at least two moderate-scoring organs. The primary and secondary efficacy endpoints, and the BILAG-defined response, failed to propose any substantial variations between the placebo and Rituximab groups. However, the treatment group particularly showed promising results among Hispanic and African American populations. While the EXPLORER trial assessed Rituximab in non-renal lupus, the LUNAR trial assessed the efficacy of Rituximab in lupus nephritis (LN). In this particular study, patients were randomized 1:1 to receive either a placebo or Rituximab. The evaluation of the renal response status at week 52 served as the primary endpoint of the study (Reddy, Isenberg). The trial's findings revealed that Rituximab failed to significantly improve renal response compared to the placebo group. Therefore, primary endpoints were not achieved in either of the trials. Furthermore, there has been further research into alternate approaches prompted by the trial's findings, including the use of Rituximab as a stand-alone treatment or in combination with other medications to target different pathways involved in SLE pathogenesis.

Successes with other targeted biological agents, such as Belimumab, which has been developed to specifically target the immune system are showing promise in treating active SLE. In more than 50 years since the use of corticosteroids and immunosuppressive therapy, Belimumab, an anti-B cell activating factor (BAFF) became the first biological drug to be approved by the US Food and Drug Administration (FDA) for the treatment of adult patients with active SLE. Belimumab is a monoclonal antibody that suppresses the action of B-cell activating factor (BAFF), a cytokine essential for B-cell survival and proliferation that is thought to have a role in the development of SLE (Singh, Shah, *et al.*). Belimumab has proved to be successful in many large clinical trials and is currently approved in several countries for

its use in treating SLE and other severe manifestations of it such as lupus nephritis. Two phase III trials, BLISS-52 and BLISS-76, studied the efficacy of intravenous Belimumab on patients with active SLE (Ramos-Casals, *et al.*). These trials showcased the effectiveness of Belimumab in reducing disease activity, thus improving symptoms in patients. In both trials, patients receiving Belimumab in addition to standard therapy experienced a substantial decline in disease activity compared to those who received placebo plus standard therapy. At week 52, more patients in both studies treated with Belimumab (10 mg/kg) with SOC than those treated with SOC (standard-of-care) alone saw a decline in disease activity. The BLISS-76 study examined the effectiveness of Belimumab over a longer time period of 76 weeks in a larger group of patients. Overall, both studies demonstrated that Belimumab was efficient in diminishing disease activity and enhancing outcomes in SLE patients when added to SOC medication as the primary goals of both clinical trials were achieved, as evidenced by the treatment groups' superior results over the placebo groups. As per the SRI-4 (Systemic Lupus Erythematosus Responder Index) response, improvement in general health was one notable outcome from these trials. At week 52, the treatment groups in each of the studies had a statistically significant improvement in SRI-4 response compared to the placebo groups. The success of both these trials implies Belimumab is a promising treatment in the management of SLE (Wise, Stohl). As of recent, Belimumab has shown favorable outcomes in different patient populations, as evidenced by approval for its use in the treatment of pediatric SLE in 2019. A year later, in 2020, Belimumab also received approval for treatment in adult lupus nephritis. Ongoing clinical studies continue to assess Belimumab’s effectiveness in various indications beyond SLE.

Emerging Therapies for SLE and future prospects

Despite the fact chimeric antigen receptor (CAR) T-cell therapy was first developed for the treatment of cancer, its ability to eliminate pathologic cells extends beyond just cancer and is currently being seen as a promising hope for patients with autoimmune diseases. In fact, CAR-T cell therapy offers a novel therapeutic approach for patients suffering with SLE. CAR-T cell therapy involves removing T cells from the immune system, which are responsible for recognizing and targeting specific pathogens. These removed cells are then genetically modified and infused back into the patients’ bloodstream (“CAR T-cell Therapy and Its Side Effects”). In their *Nature Journal* publication, researchers performed a study on 5 patients presenting severe forms of SLE between the ages of 18-25 (Xuexiao Jin, *et al*.). The findings of this study produced groundbreaking results, as the patients achieved a state of sustained drug-free remission. A decrease in the number of B cells and their inability in producing lupus-specific autoantibodies was another significant observation in this study, highlighting the effectiveness of CAR-T therapy in modulating abnormal immune responses. While successful results have been demonstrated in this study, CAR-T is still an area of ongoing research requiring further studies to determine its efficacy, and safety as a possible and viable treatment option for SLE patients.

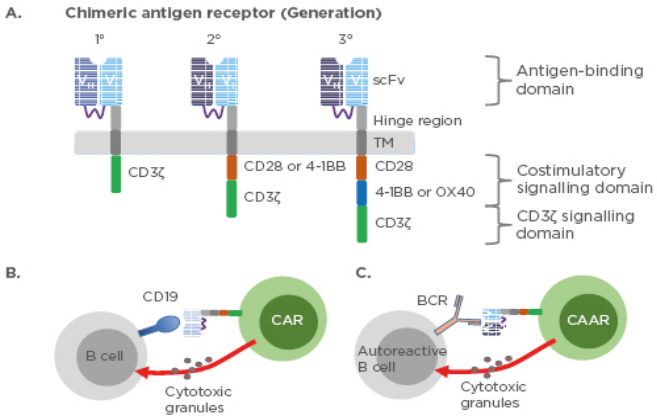


Figure 2. Illustrates chimeric antigen receptor (CAR). In the context of immunotherapy, the chimeric antigen receptors (CARs) are novel and complex molecular constructions that combine antigen binding and signaling domains in order to enable a receptor-driven response.

Conclusion

In conclusion, SLE remains a challenging disease due to its complexity and varied manifestations, necessitating continued research and collaborative efforts between researchers and clinicians. A greater understanding of SLE pathogenesis, advances in cell-targeted immunotherapy and immunosuppressive medications provide promising avenues to improve the progression and outcomes of the disease in patients. While considerable progress has been already made, as evidenced by novel approaches like CAR-T cell therapy, it is crucial for prospective studies and large-scale clinical investigations to continue evaluating the intricate mechanisms underlying SLE.

Works Cited

Atisha-Fregoso, Yemil, et al. “Meant to B: B Cells as a Therapeutic Target in Systemic Lupus Erythematosus.” *The Journal of Clinical Investigation*, 15 June 2021, www.jci.org/articles/view/149095.

“Car T-Cell Therapy and Its Side Effects.” *American Cancer Society*, 1 March 2022, www.cancer.org/cancer/managing-cancer/treatment-types/immunotherapy/car-t-cell1.html.

“Cochrane Database of Systematic Reviews: All Issues: Cochrane Library.” *Cochrane Database of Systematic Reviews: All Issues | Cochrane Library*, 25 February 2021, www.cochranelibrary.com/cdsr/table-of-contents.

Jin, Xuexiao, et al. “Car-T Cell Therapy: New Hope for Systemic Lupus Erythematosus Patients.” *Nature News*, 4 Nov. 2021, www.nature.com/articles/s41423-021-00787-5#citeas.

Li, Wen Shi, and Olga Amengual. “Full Article: B Cells Targeting Therapy in the Management of Systemic ...” *B Cells Targeting Therapy in the Management of Systemic Lupus Erythematosus*, 9 Dec. 2019, www.tandfonline.com/doi/full/10.1080/25785826.2019.1698929.

Patel, Shreya, et al. “Expanding the Role of CAR-T Cell Therapy to Systemic Lupus Erythematosus.” *European Medical Journal*, 24 Nov. 2021, www.emjreviews.com/hematology/article/expanding-the-role-of-car-t-cell-therapy-to-systemic-lupus-erythematosus/.

Ramos-Casals, Manuel, et al. “B-Cell-Depleting Therapy in Systemic Lupus Erythematosus.” The American Journal of Medicine, Apr. 2012, www.ncbi.nlm.nih.gov/pmc/articles/PMC3925418/.

Reddy, Venkat, et al. “B-Cell Depletion in SLE: Clinical and Trial Experience with Rituximab and Ocrelizumaband Implications for Study Design - Arthritis Research & Therapy.” *BioMed Central*, 11 Feb. 2013, https://pubmed.ncbi.nlm.nih.gov/23566295/.

"Systemic Lupus Erythematosus (SLE)." *Centers for Disease Control and Prevention*, 5 July 2022, www.cdc.gov/lupus/facts/detailed.html#:~:text=Systemic%20lupus%20erythematosus%20(SLE)%2C,damage%20in%20the%20affected%20organs.

Wise, Leanna M., and William Stohl. "Belimumab and Rituximab in Systemic Lupus Erythematosus: A Tale of Two b Cell-Targeting Agents." *Frontiers*, 27 May 2020, www.frontiersin.org/articles/10.3389/fmed.2020.00303/full.

Multiple Sclerosis and the use of Stem Cell Therapy

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Keywords: Multiple Sclerosis, Clinical Trials, Mesenchymal Stem Cells, Stem Cell therapy

Abstract

Multiple Sclerosis is an autoimmune condition that affects the nervous system. There is currently no cure for it, but there are a lot of different treatments to help control and minimize the symptoms. Currently, there are clinical trials and research being done surrounding the use of Stem Cells, more specifically Mesenchymal Stem Cells to stop the progression of the disease itself. The findings so far have yielded some hopeful results and improvements in a large number of patients. Although this presents very optimistic effects, there is still more research and testing that needs to be done before it can be available for all Multiple Sclerosis patients. This paper is going to be analyzing the uses and effectiveness of stem cell therapy in both treating and preventing the effects of multiple sclerosis.

Introduction

Multiple Sclerosis (MS) is a prevalent topic of discussion due to its aggressive nature and widespread reach, as one in every 333 people in America has it. MS is an autoimmune condition where your immune system mistakes your myelin for an antigen, misleading the T cells in their body to attack the myelin, leading to nerve damage. It is thought to be caused by a variety of factors both genetic and environmental, but there has been no concrete evidence pointing to a single cause (Ascherio and Munger). This autoimmune condition has an array of effects, including losses of mobility and cognitive functions, fatigue, pain and more. While there is currently no cure available, there are lots of medications available to help treat the symptoms, as well as ongoing research for a cure and better symptom management (Doshi and Chataway). Most of the current medications treat and manage the symptoms, but they can't prevent any damage, or stop the progression of MS. To add to that, the symptoms and severity of MS varies for every patient, making it more difficult to find a solution that will work for everyone. Currently, the most promising research being done surrounds blocking the attacks from the immune system, or giving the patient a "new" immune system. This won't just treat symptoms, but is expected to help slow and stop the progression of the disease itself, possibly even reverse the effects through regeneration.

Discussion

Hadassah University Hospital Study

Currently there are a lot of ongoing trials for new drugs and methods of helping to prevent the progression of Multiple Sclerosis. A lot of them include the use of stem cells, more specifically Mesenchymal Stem Cells, which are stem cells that typically originate from bone marrow. These cells have the ability to repair skeletal tissue. One instance where this was used was in a 3 group randomized experiment (Petrou et al.). There were 48 total patients with varying types of Multiple Sclerosis. They prepared the experiment by retrieving the Mesenchymal Stem Cells (MSC) from each of the patients themselves, known as autologous MSC because they are the patients' cells. After they acquired the stem cells and had cultured them, they diluted them with saline and put them into syringes for administration. The first group was given the MSC through the spine canal (Intrathecal, known as IT), and then given a round of Saline through an IV. The second group was given saline through an IT and then the MSC through an IV. Finally the third group was given 2 rounds of Saline. The researchers then followed up with second treatments where everyone (including group 3 patients) received the MSC, but the method of receiving it through the spinal canal or through an IV varied. The researchers then tested the patients with a variety of cognitive, medical and coordination tests, including an MRI, 9-peg hole test, etc. The treatment itself didn't cause any side effects, but the administration caused mild effects, usually just headaches and back pain. When testing for the effectiveness of the treatment, they found that within the sham treatment group (group 3, given only saline), 76.4% had a deterioration in a minimum of one of the functional scorings, while in the treatment group it was drastically lower at 31 and 27.6%. But, after the sham group received treatment, there were signs of improvement through the EDSS scoring.

Overall there were positive effects that the stem cell therapy had on the MS patients. But there were a few things that this treatment did not address, including the inflammation of the central nervous system as well as remyelination. MS attacks the myelin, but this treatment doesn't help with the remyelination (regeneration of the myelin) which is crucial to preventing further nerve damage. Being able to not only counter the current damage, but prevent future damage could be the difference between a treatment and a cure. In this experiment, it seems that they eventually did give the sham treatment group the MSC treatment. This seems contradicting because then the only thing you can really test for is the effectiveness of more of the MSC compared to less, because everyone still received it. But from the paper, they were trying to see the effectiveness of the treatment, which would have been easier to determine if one group was only given saline during the duration of the trial. This would have given a more clear answer of the full extent of the effectiveness of the treatment itself as well as the value of the different administration sites. By mixing it up for each MSC session, there was no clear answer as to why there were improvements, or why there weren't. They would only be able to test the general improvement of the symptoms and disease based on the scores from before the treatment. By keeping their groups separated they could have determined if the site played a role, as well as the full effect that the treatment had.

Clinical Trial at Jordan University Hospital

Another similar experiment conducted showed similar results. It was a phase 2 study (Dahbour et al.), aimed to evaluate the effectiveness of stem cell therapy both through radiology as well as physical symptoms. They tested this using Mesenchymal Stem Cells-conditioned media. They accepted patients based on failure from previous treatments as well as being tested for any viruses or other diseases. They ended up with 10 eligible patients (some ended up leaving) for this trial, varying with secondary Progressive MS and Relapse Remitting MS. They had their MSC-CM obtained through bone marrow aspiration, and they were injected Intrathecally immediately after. During the tri-monthly check ins, they performed both cognitive and coordination tests on all of the patients. They also took MRIs to check the imaging for any effects, positive or negative. There weren't any complications from the treatment, but there were some mild symptoms from the administration of the stem cells, such as fatigue, bruising, swelling, etc. To test the effectiveness of this treatment, they mainly used the EDSS, which stands for Extended Disability Status Scale, where the higher the number, the more loss of mobility, and the lower the number, the more mobility. Only 20% of the patients had an improvement based on the EDSS scaling, but they did find an increase in brain white matter lesions as well as spinal cord white matter lesions. But these results were not able to lead to definite conclusions about the complete effects that stem cell therapy has on Multiple Sclerosis. There is still more research and testing on the limits and effectiveness of stem cell therapy for Multiple Sclerosis that needs to be done.

Compared to the first experiment mentioned in the paper, both of these experiments have some improvements in their various scaling methods and tests from the stem cell therapy treatment but the increase in the lesions in this particular experiment was intriguing. There is no definite explanation whether or not there were increased lesions because of the treatment itself, or because of the disease progression. It could be that the treatment didn't affect the lesions at all, letting the condition get worse and spread. It seems unlikely compared to the other positive results that the stem cell therapy has had that it was the treatment that caused more lesions, but there is no sure answer yet. One limitation that these results have is that they didn't have a large number of patients in these trials. Testing them again in a larger group, around 50-80 people, would yield more accurate and reliable results. Keeping the groups down to 15 and 10 makes sense for a first run, but after completing that it is important to do it multiple times. This way you can compare the data of the different groups, and check for any discrepancies or new information. Doing multiple runs, even with different trial groups, will be able to provide much more useful data, especially to figuring out why there was an increase in the lesions, both in the brain and spinal cord.

Study from Hadassah Medical Organization

Another experiment on the use of Stem Cell therapy was conducted, and it was a Phase 1/2 trial (Karussis et al.). Their main goal was to evaluate the safety of using Stem Cells for Multiple Sclerosis. The parameters to be a part of this study included being in the age range of 25-65, having MS for at least 5 years, and to have a failed response to at least one available treatment .There were also some ALS (Amyotrophic Lateral Sclerosis) who participated in this trial as well. To get the stem cells, they performed bone marrow aspiration on each of the patients and then cultured them for around 40-60 days. After this they were checked to make sure that they were sterile and of quality and then added to saline. They administered the treatment in 2 parts. They injected ⅔ of it intrathecally, and the other ⅓ intravenously. The patients had MRI's taken hours after the administration as well as 1, 3, and 6 months after. As a result of the administration, some of the patients suffered from a slight fever, as well as headaches. The EDSS scores either declined or stayed the same after the treatment which shows signs of improvement. From the brain scans there were no new lesions, which seems promising. The treatment also seemed to be improving the immune effects that MS had, not only that but the positive effects it had seemed to be greater than the current medications that are typically used to help with the immune system. They were able to confirm that MSC had the ability to regenerate nervous tissue as well as prevent cell deaths. These results helped open up the doors to more research on the use of MSC, as well as hope for the prevention of the progression of MS, due to its regenerative properties.

This experiment shows some of the earlier phases of clinical testing on the use of MSC. It's important to go over phase one results like this because it shows us the first things that were learned and observed about the treatment. One thing that seemed interesting was the fact that the stem cell therapy seemed to have almost immediate results, improving the immune system only around 4 hours after the administration. This is something that wasn't mentioned in the other articles, and this discrepancy could be due to numerous variables. But it seems like the main variable at play is the method of administration. Some of the experiments used a mixture of both intrathecal and intravenous injections, and the 2nd detailed experiment was only intravenously. This experiment had both, but they were both MSC, while the first was half MSC half saline. Having the MSC administered to both of the areas where the most damage is present could speed up the effects that this treatment has. Only administering it to one area could force the treatment to have to travel to the other locations, taking up more time to heal and regenerate the nervous system.

Conclusion

Overall, these clinical trials and tests using Mesenchymal Stem Cells to treat Multiple Sclerosis have had a seemingly positive effect on most areas that the disease affects. They have had a lot of success in helping heal tissue, as well as improving the symptoms caused by MS. But there are some negative

effects, such as a possibility of an increase in lesions. There is a lot that is still unknown about the usage of MSC, and it will require more research before it can be considered safe and effective. MSC is not the only new method that is being tested to treat MS, there are also wellness interventions that are being researched. This entails things such as changing lifestyle habits, eating healthier, exercise, but it is a more passive approach to dealing with MS. Stem cell therapy would be the preferred option, because it can tackle MS head on with a faster improvement compared to wellness interventions, which would most likely take months to years to see any kind of results. There are a lot of different ongoing types of research for Multiple Sclerosis and it is imperative that this research be continued. Multiple Sclerosis is such a widely spread disease, and with the continuation of research like this there is hope to find a cure.

Works Cited

Ascherio, Alberto, and Kassandra L. Munger. "Environmental Risk Factors for Multiple Sclerosis. Part I: The Role of Infection." *Annals of Neurology*, vol. 61, no. 4, Apr. 2007, pp. 288–99.

Cona, Louis A. *Multiple Sclerosis Cure: Breakthroughs & Promising Treatments (2023)*. 12 May 2023, https://www.dvcstem.com/post/multiple-sclerosis-cure.

Croxford, Andrew L., et al. "Mouse Models for Multiple Sclerosis: Historical Facts and Future Implications." *Biochimica et Biophysica Acta*, vol. 1812, no. 2, Feb. 2011, pp. 177–83.

Dahbour, Said, et al. "Mesenchymal Stem Cells and Conditioned Media in the Treatment of Multiple Sclerosis Patients: Clinical, Ophthalmological and Radiological Assessments of Safety and Efficacy." *CNS Neuroscience & Therapeutics*, vol. 23, no. 11, Nov. 2017, pp. 866–74.

Dobson, R., and G. Giovannoni. "Multiple Sclerosis - a Review." *European Journal of Neurology: The Official Journal of the European Federation of Neurological Societies*, vol. 26, no. 1, Jan. 2019, pp. 27–40.

Doshi, Anisha, and Jeremy Chataway. "Multiple Sclerosis, a Treatable Disease." *Clinical Medicine*, vol. 16, no. Suppl 6, Dec. 2016, pp. s53–59.

Harris, Violaine K., et al. "Phase I Trial of Intrathecal Mesenchymal Stem Cell-Derived Neural Progenitors in Progressive Multiple Sclerosis." *EBioMedicine*, vol. 29, Mar. 2018, pp. 23–30.

Hauser, Stephen L., and Bruce A. C. Cree. "Treatment of Multiple Sclerosis: A Review." *The American Journal of Medicine*, vol. 133, no. 12, Dec. 2020, pp. 1380–90.e2.

Karussis, Dimitrios, et al. "Safety and Immunological Effects of Mesenchymal Stem Cell Transplantation in Patients with Multiple Sclerosis and Amyotrophic Lateral Sclerosis." *Archives of Neurology*, vol. 67, no. 10, Oct. 2010, pp. 1187–94.

Multiple Sclerosis (MS). https://www.hopkinsmedicine.org/health/conditions-and-diseases/multiple-sclerosis-ms. Accessed 11 July 2023.

Petrou, Panayiota, et al. “Beneficial Effects of Autologous Mesenchymal Stem Cell Transplantation in Active Progressive Multiple Sclerosis.” *Brain: A Journal of Neurology*, vol. 143, no. 12, Dec. 2020, pp. 3574–88.

Scolding, Neil J., et al. “Cell-Based Therapeutic Strategies for Multiple Sclerosis.” *Brain: A Journal of Neurology*, vol. 140, no. 11, Nov. 2017, pp. 2776–96.

Stuifbergen, Alexa K., et al. “A Randomized Clinical Trial of a Wellness Intervention for Women with Multiple Sclerosis.” *Archives of Physical Medicine and Rehabilitation*, vol. 84, no. 4, Apr. 2003, pp. 467–76.

The Future of Cancer Diagnosis and Treatment: Quantum Dot Nanotechnology

Isabelle Morin (author), Alan Wang (advisor), Christina Thomas (advisor)

Keywords: Oncology, Nanotechnology, Quantum Dots, Cancer, Autoimmune Disease

Abstract

Quantum dots are nanoparticles that have optical and chemical advantages. Quantum dots have been found incredibly useful in the field of oncology. Studies have shown that quantum dot nanotechnology can be used for imaging purposes, making it easier to diagnose cancers. Additionally, the technology can help treat certain conditions as well, including some cancers and autoimmune diseases. However, there are multiple disadvantages to using this technology. The toxicity of QD far outweighs the benefits, and more research needs to be done to create a safer quantum dot bioconjugate that can be used on humans.

Introduction

According to the American Cancer Society, “In the US in 2023, a total of 1.9 million new cancer cases (about 5,370 cases each day) and 609,820 deaths from cancer are expected to occur” (McDowell). Although cancer is a leading cause of death, the mechanisms of carcinogenesis are still unclear and treatment is extremely limited. Malignancies are becoming increasingly common, and while research is always being done, hardly any new drugs have been approved. In 2021, Abemaciclib, a newly approved drug for the treatment of the HR+, HER2-early breast cancer, was the first real advancement in 20 years (WebMD). However, there are some technologies that give hope for the future of the oncology world - such as quantum dots.

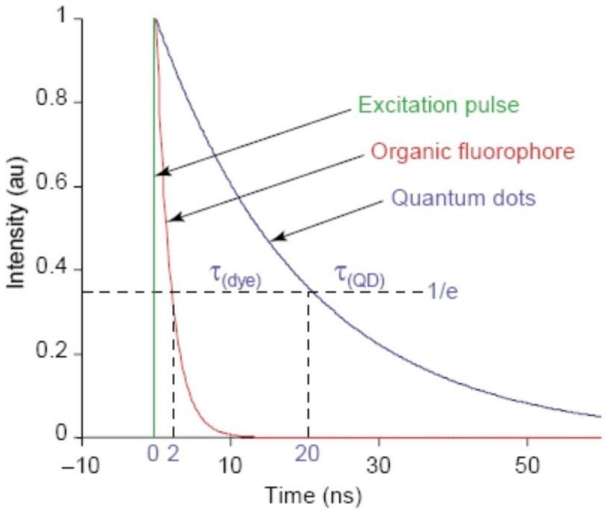
Quantum dots (QD) are man-made nanoparticles that have semiconductive properties (Haynes). Usually when someone thinks of quantum dot technology, they would think of their ability to enhance visuals and colors on modern television screens. However, the optical and chemical advantages of quantum dots have been found to be extremely helpful when studying, diagnosing, and treating cancer.

Discussion

Quantum dots can be beneficial for cancer imaging, both *in vivo* (research done within a living organism) and *in vitro* (research done outside of a living organism) , because of their optical and chemical properties. Essentially, they are nanocrystals that consist of many of the necessary properties for cancer imaging, and can be used in Magnetic Resonance Imaging (MRI), and in other ways as well. Quantum dots have

multiple advantages over traditional organic fluorescent dyes that are usually used. Some of these characteristics include a long lifespan (*see Fig 1*), high fluorescence, and a high resistance to photobleaching. When conjugated with small molecules such as peptides and antibiotics, they can target cancer molecules with high specificity. Min Fang, from the Zhongnan Hospital of Wuhan University, stated that "Several studies have shown that this method is essential for deciphering the molecular mechanism of cancer invasion and is useful for the study of tumor microenvironment" (Fang). Quantum dot fluorescence can be detected in deep tissues. Thus, they are suitable for *in vivo* imaging.

Fig 1: lifespan of quantum dots v. organic fluorophores



In this image, we can see that the time constant of quantum dots is longer than that of traditional organic fluorophores (Okabe). Because of such abilities, these crystalite nanoparticles are preferred over organic fluorophore dyes for imaging cancer cells *in vivo* and *in vitro*, as they are far more superior than the commonly used fluorescent dyes (Okabe).

When conjugated with cancer specific peptides and antibodies, quantum dot imaging has proven to reveal specific details about certain cancers such as prostate, breast, ovarian, gastrointestinal, liver, and pancreatic cancer. A study showed that Doxorubicin (a common chemotherapy drug included in various treatment regimens) conjugated quantum dots can target alveolar macrophages/inflammation (Chakravarthy). Moreover, As Fang et al. stated, "Another group found that QDs impair macrophagic morphology and the ability of phagocytosis by inhibiting Rho-associated kinase signaling, which will contribute to a better understanding of the tumor microenvironment. Hence, QD-based nanotechnology can reveal and detect the role and function of the tumor microenvironment and can be used for novel targeting therapy" (Fang). This proves that quantum dots can help pathologists better understand what is going on in every individual oncology case.

However, there are disadvantages to quantum dots as well. The major problem when working with quantum dots is its toxicity. There is a whole collection of problems that must be addressed before any major biomedical breakthroughs can be made. Their coatings and the cadmium and selenium cores of quantum dots are potentially cytotoxic (meaning they are toxic to cells and can cause significant damage). Shell erosion can cause negative effects *in vivo*, and their composition has been somewhat toxic during *in vitro* studies. In addition, there are many unknowns with quantum dot research. For example, the mechanism of cell death is unknown as well as whether or not the quantum dots ever clear from the body. This can be potentially dangerous, especially considering the possible cytotoxicity (Okabe). There is also not much data on their reproductability and reliability. These threats are not typically a problem with *in vitro* (research done outside of the body, for example; under a microscope) imaging, but they are certainly causing the development to go very slowly, because scientists must find a way to minimize the toxic side effects (Fang). Extensive research must be undertaken before quantum dots can be fully employed for *in vivo* research.

On the contrary, recent research has shown that quantum dots have more potential in oncology treatment than just being used for imaging and the study of carcinogenesis mechanisms. Surprisingly, you can inject humans with quantum dots. Yes, the toxicity is still taken into consideration, but "By injecting a body with these quantum dots, a doctor could see where a tumor or cancer cell was by finding the injected quantum dots, an easy process because of their fluorescence" (Rathhi). Moreover, quantum dots can be used for actual targeted cancer treatment, rather than just to detect cancer. For example, when they are combined with high energy radiation, such as with a laser, quantum dots can emit a thermal field. This phenomena can be used as a form of hyperthermia therapy to destroy malignancies, without damaging surrounding tissues. This could also facilitate live monitoring of the tumor treatment (Scheer). Amit Scheer created a novel bioconjugate nanoparticle that has proven to be effective in many cancer therapies, and has potential in diversifying patient-specific targeting.

Furthermore, the use of quantum dot nanotechnology has been applied to illnesses other than cancer, such as autoimmune diseases. Autoimmune diseases, such as rheumatoid arthritis, are caused when the immune system mistakenly attacks a person's own tissues or organs. Scientists at Scripps University engineered cell-like nanoparticles that only target the immune cells that drive autoimmune reactions, leaving the rest of the immune system healthy. They have delayed and even prevented severe disease in a mouse model of arthritis. The potential advantage of this approach is that it enables safe long-term treatment for autoimmune diseases where the immune system attacks its own tissues or organs without

causing vast immune suppression as most current treatments do (Scripps Research). This advancement shines light on the abilities nanotechnology has for changing the medical field. (Yasss! Loving this)

Conclusion

Because of their proven ability to target tumors and identify metastatic cancer cells (Zhang), research is still being done to create a biomedical conjugate that can effectively kill cancer cells with little to no toxicity. Quantum dots have proven to be a major advance in oncology diagnosis, assessment, and treatment, but the toxicology of quantum dots is a major roadblock in its development. Cancer research has come a long way, but there is still so much to be discovered and developed. Quantum dots have a lot of potential to target cancer cells and help researchers better understand carcinogenesis. Hopefully in the future, a safe quantum dot conjugate that can be used for the treatment of malignancies will be developed.

Works Cited

Chakravarthy, K., et al. “Doxorubicin conjugated quantum dots to target alveolar macrophages/inflammation.” *National Library of Medicine,* 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3363005/.

Fang, M., et al. “Quantum Dots for Cancer Research: Current Status, Remaining Issues, and Future Perspectives.” *National Library of Medicine,* 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3643664/.

Haynes, D. “What is Quantum Dot display technology?” *Samsung,* 2021. https://insights.samsung.com/2021/12/29/what-is-quantum-dot-display-technology/.

McDowell, S. “Incidence Drops for Cervical Cancer Drop But Rises for Prostate Cancer.” *American Cancer Society,* January 12, 2023. https://www.cancer.org/research/acs-research-news/facts-and-figures-2023.html#:~:text=Cancer%20continues%20to%20be%20the,about%201%2C670%20deaths%20a%20day.

Okabe, Y. “Quantum dots for in vivo imaging.” 2007. http://bme240.eng.uci.edu/students/07s/yokabe/main.htm.

Rathhi, R. “Nanotechnology. Technology Revolution of 21st Century.” *S. Chand Publishing,* 2009. https://books.google.com/books?id=agsbEAAAQBAJ&pg=PA179&lpg=PA179&dq=“By+injecting+a+body+with+these+quantum+dots,+a+doctor+could+see+where+a+tumor+or+cancer+cell+was+by+finding+the+injected+quantum+dots,+an+easy+process+because+of+their+fluorescence”&source=bl&ots=sSBf6-WUCf&sig=ACfU3U0P5tlzpFL9qRML2FSXeXB7Oaps2g&hl=en&sa=X&ved=2ahUKEwjzjrP1gOr2AhV9mGoFHfnUBZQQ6AF6BAgDEAM#v=onepage&q=“By%20injecting%20a%20body%20with%20these%20quantum%20dots%2C%20a%20doctor%20could%20see%20where%20a%20tumor%20or%20cancer%20cell%20was%20by%20finding%20the%20injected%20quantum%20dots%2C%20an%20easy%20process%20because%20of%20their%20fluorescence”&f=false.

Scheer, A. “Development of a Novel Quantum Dot-Aptamer Bioconjugate Targeted Cancer Therapy.” *Julian’s Science Fair, 2014.* http://www.projects.juliantrubin.com/science_fair_project/medicine/cancer_2.html.

Scripps Research. “Nanotech strategy shows promise for treating autoimmune disease.” *Scripps Research,* January 1, 2022. https://www.scripps.edu/news-and-events/press-room/2022/20221201-paulson-acs-nano.html#:~:text=disease%20%7C%20Scripps%20Research-,Nanotech%20strategy%20shows%20promise%20for%20treating%20autoimmune%20disease,had%20a%20genetic%20arthritis%20condition.&text=LA%20JOLLA%2C%20CA—Scientists%20at,based%20strategy%20against%20autoimmune%20diseases.

Zhang, H., et al. “Quantum dots for cancer diagnosis and therapy: biological and clinical perspectives.” *Future Medicine,* 2008. https://www.futuremedicine.com/doi/10.2217/17 435889.3.1.83.

WebMD Cancer Center. “New Drug for Early Breast Cancer: First Advance in 20 Years.” *WebMD Cancer Center,* 2021. https://www.webmd.com/breast-cancer/news/20211013/new-drug-for-breast-cancer-approved.

The Correlation Between Chronic/Terminal Illnesses And Mental Health Conditions

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Keywords: Chronic Illness, Terminal Illness, Mental Illness, Inflammation, Neurons

Abstract

Physical and mental conditions have always had a known connection, but it is less understood specifically how chronic and terminal disease is connected to mental health conditions. In order to investigate the exact correlation between chronic and terminal illnesses and mental health conditions, a survey was conducted to gather 107 actual patients' (aged 14-47) perspectives. The survey included a series of questions on patient demographics, diagnoses, personal beliefs on their physical and mental health, and specific experiences that only the patients may encounter. One major finding of this study showed that 98.1% of participating patients feel that having a physical illness has significantly impacted their mental health, which could be a result of long-term inflammation in addition to the emotions faced when being chronically/terminally ill. This study serves to further establish previous knowledge on how substantial the connection between physical and mental health is.

Introduction

Cystic fibrosis, inflammatory bowel disease, diabetes, lupus, colon cancer, rheumatoid arthritis- the list goes on. These are examples of chronic and terminal diseases, which means they cannot be technically cured. Six in ten adults in the United States have at least one chronic or terminal illness (Chronic Diseases in America) Likewise, about one fourth of adults in the United States have at least one mental illness (Mental Health Disorder Statistics) and over fifty percent of people with a mental illness have a chronic condition too (Guerra).

Hormone/neuron imbalances

The claim that is often debated by medical professionals is that chronic/terminal diseases are directly related to mental health conditions through the actual biological qualities they share. However, numerous studies have proven that there is a clear scientific correlation. Physical illnesses can lead to the development of mental illness because most chronic conditions "are associated with abnormal levels of hormones and neurotransmitters that can affect mental health" (McLaughlin). This can be observed in

Parkinson's disease, as it involves dopamine abnormalities that are seen in depression. An additional example is when an individual has a stroke, as the changes that occur to the brain in Parkinson's disease patients and stroke survivors have a direct role in the development of depression (Chronic Illness and Mental Health). Further, chronic pain, which is part of having a chronic/terminal illness, is "associated with imbalances of GABA, serotonin, dopamine, and norepinephrine" (McLaughlin). As aforementioned, these chemical imbalances that occur in physical conditions are also present in psychological conditions, such as depression and anxiety. On the other hand, sometimes people living with a chronic illness actually had a mental illness first, with their physical condition being a result of that. This is due to the way mental illnesses alter hormones and sleep-cycles, and how many medications prescribed for mental illnesses can cause weight gain and arrhythmias. All of these changes can lead to a variety of physical health disorders (The Relationship between Mental Health). However, hormones are not the only direct correlation between physical and mental conditions. Studies at the University of California, Berkeley, have found that the bodies of individuals with chronic illnesses generate less neurons than normal. This was shown to affect the hippocampus, which is the part of the brain that regulates emotions and causes mental disorders (Sanders). This connection shows us how chronic/terminal illnesses have a direct scientific correlation due to their shared biological qualities, such as hormone/chemical imbalances and decreased amounts of neurons.

Stress

Patients diagnosed with a chronic/terminal illness not only have hormone/neuron imbalances, but the stress that they face because of their disease significantly increases their risk for developing a mental condition. About one third of individuals with a serious medical condition also have depression (Chronic Illness and Depression). It makes complete sense that one may face stress while being physically ill as there is constant worrying, grief about your old life, feelings of isolation and like nobody understands what you're going through, financial burdens, and even being invalidated. It is easy to feel misunderstood by family, friends, and overall anyone that has not gone through being sick. Even doctors can make a chronically/terminally ill person feel invalidated. An illness may have you "facing new limits on what you can do and may (make you) feel stressed or concerned about treatment outcomes and the future. It may be hard to adapt to a new reality and to cope with the changes and ongoing treatment that come with the diagnosis" (Chronic Illness and Mental Health). All of this emotional stress, if left untreated, leads to anxiety and depression. Anxiety and depression are not the only common mental illnesses that physically ill patients develop, though. Approximately 35% of people with chronic illnesses develop Post Traumatic Stress Disorder (McLaughlin), which is no doubt a result of the unimaginable medical trauma that is acquired.

Inflammation

It is also important to consider that this mental stress can not only lead to a mental illness, but can result in an individual's physical illness worsening. Stress increases inflammation levels, which "can aggravate the illness, causing a vicious cycle to develop" (Chronic Illness and Depression). Chronic illness flare-ups are when the disease is active again, usually as a result of medications failing to work, but stress is a common cause as well. This is especially prevalent in individuals with an inflammatory chronic illness, such as Crohn's disease, arthritis, or ulcerative colitis. Since stress leads to large-scale body inflammation, and most chronic illnesses involve inflammation, it makes sense that being stressed can cause a flare-up. It is true that it leads to a cycle, because when you are stressed and flare-up, that just makes you more stressed, which makes you feel even worse, and so on. This combination of stress and physical disease can actually be life-threatening. For example, "diabetics who have depressive symptoms have a 46% increased risk for all-cause mortality than diabetics who are not depressed. It's not an exaggeration to say that diabetes and depression together are a deadly combination" (Fernandez). Diabetics with depression have a significantly higher risk for organ failure, which demonstrates how mental illness with physical illness work hand in hand and can be dangerous if not treated well.

The same way that physically ill individuals have an increased risk of developing a mental disorder, patients with mental illnesses face psychological factors that may lead to chronic conditions and just poorer physical health in general. "People living with a serious mental illness are at higher risk of experiencing a wide range of chronic physical conditions" (The Relationship between Mental Health). As aforementioned, this is partly due to the chemical, hormonal, and neuron imbalances that are associated with both physical and mental illness. However, the long-term stress that individuals face while being mentally ill increases their inflammation levels, making them more susceptible for developing an inflammatory disorder or other physical condition. This is because "intense stressors over-activate the immune system, leading to the imbalance of inflammation and anti-inflammation" (Liu). This reinforces the idea that stress is a key factor in the correlation between physical and mental conditions. The following study was created to investigate additional factors that contribute to the correlation between physical and mental health.

Materials and Methods

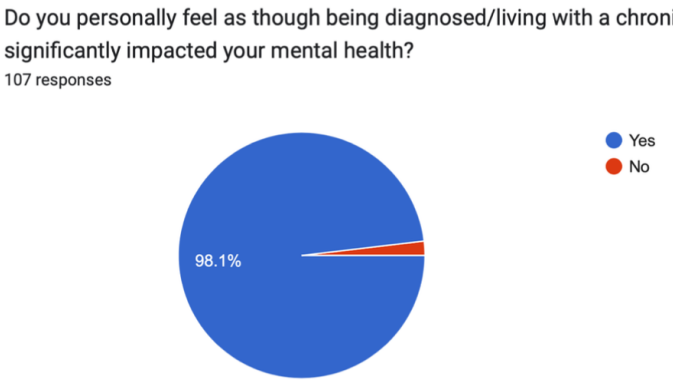
A survey was formed and sent to 107 verified chronicall/terminally ill patients aged 14-47.Consent was obtained from all participants in order to ensure adherence to ethical guidelines.The questions included:

1. What is your gender?
2. What is your age?
3. List any chronic/terminal illnesses you have.
4. Please write any information you would like to add about your chronic/terminal illnesses- year of diagnosis, medications you have taken, impact on your life, etc.
5. List any mental health conditions you have.
6. Please write any information you would like to add about your mental illnesses- year of diagnosis, medications you have taken, impact on your life, etc.
7. Do you personally feel as though being diagnosed/living with a chronic/terminal illness has significantly impacted your mental health?
8. Do you personally feel as though being diagnosed/living with a chronic/terminal illness has significantly impacted your mental health?
9. Rate the impact being physically ill has had on your mental health on a scale of 1-10. 1 being no impact, 10 being your mental health is 10x worse then before getting sick.
10. Have you been told things by doctors such as "it is just anxiety" that specifically worsened your mental health in any way? Has treatment from doctors, including false diagnosis and medical gaslighting affected you?
11. Have words from family/friends/strangers such as "you're faking it"and "it's for attention" affected your mental health in any way?
12. If you have any final thoughts, add them here.

Results and Discussion

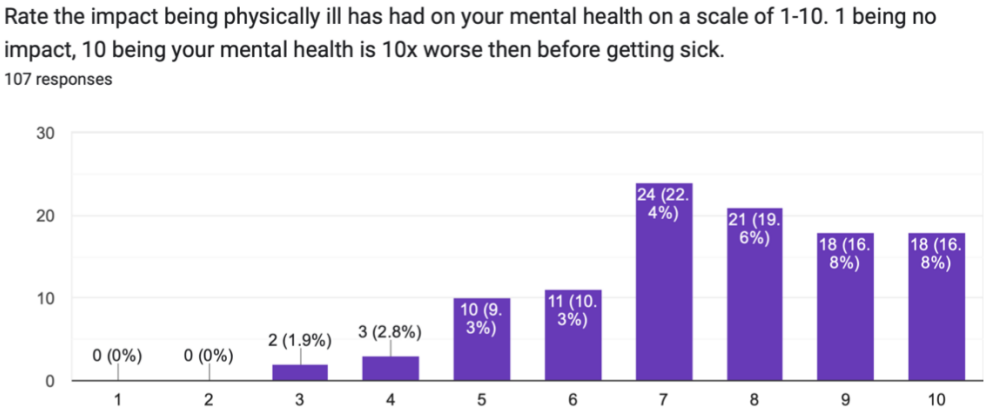
Participants identified as a wide range of genders, including female, male, non-binary, and gender-fluid. Participants also were aged 14-47, and had physical illness of every body system. Those that were formally diagnosed with a mental health condition (98 participants) reported at least one of the following conditions: depression, anxiety, borderline personality disorder, obsessive compulsive disorder, anorexia, post-traumatic stress disorder, agoraphobia, bipolar disorder, avoidant-restrictive food intake disorder, and bulimia. When asked if they felt being physically ill has significantly impacted their mental health, 98.1% of participants said yes (Figure 1).

Figure 1.



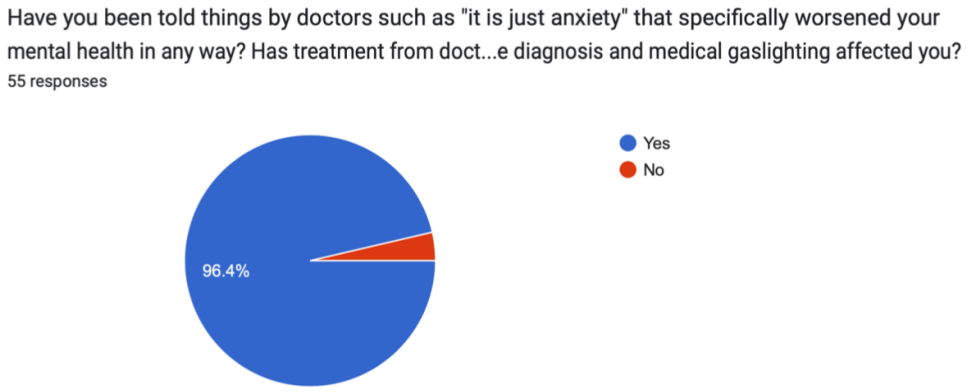
Further, when asked to rate the impact being physically ill had on their mental health on a scale from 1-10, 95.3% of participants rated their mental health to be 5-10 times worse than it was before becoming chronically/terminally ill. No participants rated a 1, meaning no participants believe being physically ill has had no impact on their mental health (Figure 2).

Figure 2.



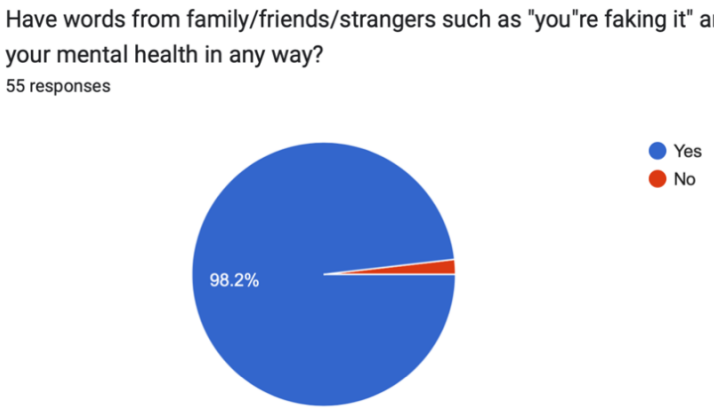
When asked if their doctors have specifically worsened their mental health in any way, from false diagnosis to medical gaslighting, 96.4% of participants voted yes. It is important to note, however, that only 55 of the participants responded to this question (Figure 3).

Figure 3.



When asked if their family/friends/strangers have affected their mental health by saying invalidating things, such as “you’re faking it” and “it’s for attention,” 98.2% of participants voted yes. It is important to note, however, that only 55 of the participants responded to this question (Figure 4).

Figure 4.



Some individuals noted in the additional thoughts section that often, their mental illnesses are actually symptoms of their physical illness. An example of this is an individual with Pediatric Acute-onset Neuropsychiatric Syndrome (PANS), whose obsessive compulsive disorder and depression were significant symptoms of their PANS flares.

Conclusion

Although the biological connection between chronic/physical illnesses has already been found and discussed, this study highlights an important aspect of the correlation that is not widely acknowledged: how outside influences have an effect on the mental health of physically ill patients. Participants in this

study have demonstrated that treatment from medical professionals, such as their own physicians, can worsen their mental health because of false diagnosis and medical gaslighting. Additionally, family, friends, and even strangers can contribute to the worsening of mental health because of invalidating speech. This maltreatment, whether it is from a medical professional or another individual, can cause more stress to a chronically/terminally ill patient. We know that this stress can lead to mental illnesses, but also to an increase in inflammation that contributes to both worsening of physical illness and mental illness. This is why increased awareness of both physical and mental health conditions is vital, so that everyone can avoid invalidating speech as a whole. Further, personal patient experiences show that sometimes, mental disorders are actually symptoms of a physical health condition, which we know to be because of inflammation and neuron factors discussed in the introduction. This study, while effective in most ways, has limitations in the sense that there was a small number of participants. However, it can be concluded that the correlation between chronic and physical illnesses and mental health conditions is much more complex than previously thought, as there are a variety of factors that connect physical and mental disease.

Works Cited

"Chronic Diseases in America." *Centers for Disease Control and Prevention,* December 2022. https://www.cdc .gov/chronicdisease/resources/infographic/chronic-diseases.htm.

"Chronic Illness and Depression." *Cleveland Clinic*, March 2021. https://my.cl evelandclinic.org/health/articles/9288-chronic-illness-and-depression.

"Chronic Illness and Mental Health: Recognizing and Treating Depression." *National Institute of Health*, 2021. https://www.nimh.nih.gov/heal th/ publications/chronic-illness-mental-health.

Fernandez, G. "The Intersection of Mental Health and Chronic Disease." *Johns Hopkins Bloomburg School of Public Health,* December 2021. https://publichealth.jhu.edu/2021/t he-intersection-of-mental-health-and-chronic-disease.

Guerra, M. "The Cascading Effect Of Comorbidities: Addressing Chronic Medical Disease Alongside Mental Health." *RTI Health Advance,* August 2022. https://healthcare.rti.org/insights/mental-health-and-chronic-disease-comorbidities.

Liu, Y., et al. "Inflammation: The Common Pathway of Stress-Related Diseases." *National Institute of Health,* June 2017. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5476783/.

McLaughlin, M. "How Does Chronic Illness Affect Mental Health?" *Black Bear Lodge,* N.D. https://blackbearrehab.com/mental-health/how-does-chronic-illness-affect-mental-health/.

"Mental Health Disorder Statistics." *Johns Hopkins Medicine,* N.D. https://www.hopkinsmedicine.org/health/wellness-and-prevention/mental-health-disorder-statistic s .

Sanders, R. "New Evidence That Chronic Stress Predisposes Brain to Mental Illness." *Berkeley News,* February 2014. https://news.berkeley.edu/2014/02/11/chronic-stress-predisposes-b rain-to-mental-illness/.

"The Relationship between Mental Health, Mental Illness and Chronic Physical Conditions." *Canadian Mental Health Association,* N.D. https://ontario.cmha.ca/documents/the-relationship-between-mental-health-mental-illness-and-chr onic-physical-conditions/.

Research Article

Assessing the Relationship Between Tibial Torsion and Rheumatoid Arthritis: Exploring Disease Severity, Joint Involvement, and Functional Impairment in a Cross-sectional Study

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Keywords: Tibial torsion, rheumatoid arthritis, patellofemoral, greater trochanteric

Abstract

Purpose of View

Tibial torsion is a recognized form of patellofemoral and greater trochanteric pain. Although this syndrome is most commonly detected and diagnosed in pediatric patients, it is also observed in adult and geriatric patients. Tibial torsion, a structural anomaly that affects the alignment of the lower limb, has been linked to altered joint mechanics and may have consequences for the development of rheumatoid arthritis (RA). This study investigates the relationship between tibial torsion and RA related outcomes using a thorough methodology that includes clinical assessments, radiographic evaluations, and functional analyses, while additionally concentrating on the severity of the condition, the involvement of the joints, and functional impairment.

Recent Findings

Significant findings have been drawn from our cross-sectional investigation examining the link between tibial torsion and rheumatoid arthritis (RA). A relationship exists between tibial torsion and RA disease severity, indicating that greater tibial torsion is linked to more active disease and joint involvement (Snow 2021). Additionally, those with increased tibial torsion showed more joint injury and had more functional impairment. These results emphasize the possible impact of tibial torsion on the severity of the illness, joint pathology, and functional restrictions in RA. To fully comprehend the underlying causes and investigate focused therapies for improving lower limb alignment in RA patients, further investigation is required.

Summary

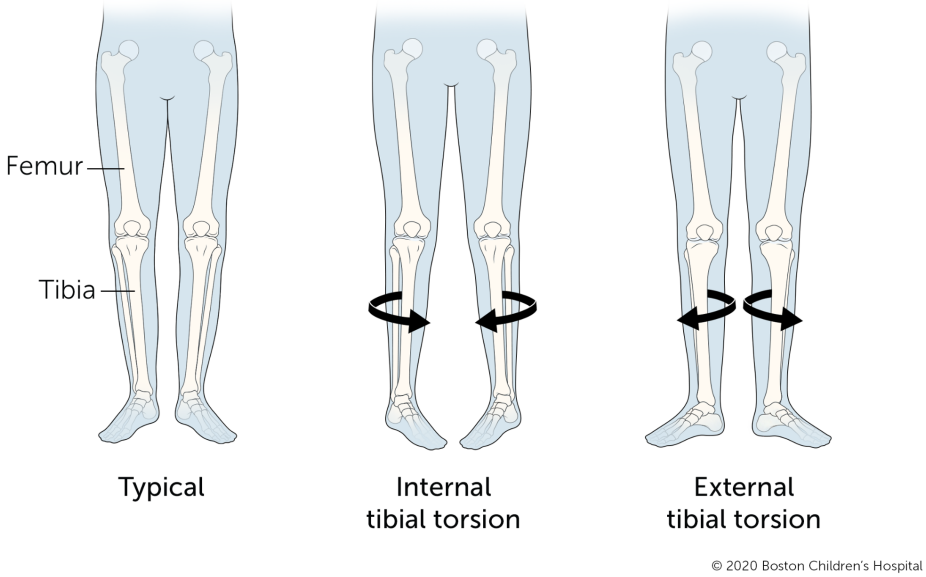
Tibial torsion, an excessive twisting of the shin bone either internally or externally, is a subject of high suspicion in regards to joint impairment and diseases such as RA. It is known that many individuals diagnosed with RA in lower extremity joints have a history of tibial torsion. This structural abnormality affects many age groups, though it is most commonly observed in children. Through this study and

further research to come, we may be able to distinguish and prevent the probability of tibial torsion being the cause of joint disorders.

Introduction

Tibial Torsion

The term "tibial torsion" describes a structural irregularity that affects how the tibia bone, sometimes referred to as the "shinbone," is aligned. It entails a tibial rotational displacement from the axis of the knee joint. Depending on the direction of rotation, this disease may present as either internal or external tibial torsion. Internal tibial torsion is defined by a rotation of the tibia inward towards the midline of the body. This implies that a person with internal tibial torsion may appear to have pigeon-toed feet when they stand or walk. External tibial torsion, on the other hand, is characterized by an outward rotation of the tibia, resulting in feet that seem to point outward or duck-like.



Tibial torsion is frequently seen and diagnosed in pediatric patients since it can be seen in early infancy when kids first learn to walk. However, geriatric and adult populations can also have it. Tibial torsion can range in severity from mild occurrences with little functional consequences to more prominent abnormalities that may affect gait and general lower limb biomechanics. Tibial torsion is hypothesized to result from a mix of genetic variables and intrauterine placement during fetal development, while its specific origins are not entirely known. Tibial torsion may affect how stresses are distributed across the knee joint, ankle joint, and foot, which may have an impact on lower limb biomechanics. It may occasionally result in changed joint mechanics and raise the likelihood of developing certain musculoskeletal diseases. For instance, tibial torsion has been linked to greater trochanteric pain

syndrome, which causes pain and tenderness on the outside of the hip, and patellofemoral pain syndrome, which is characterized by discomfort around the kneecap. For the purpose of diagnosing, treating, and managing musculoskeletal disorders, healthcare providers must have a thorough understanding of the effects of tibial torsion. Depending on the degree of the torsion, several techniques, such as physical therapy, orthotic treatments, and surgical procedures, may be used. Surgical correction for tibial torsion is a treatment option that tries to address the structural anomaly while also optimizing lower limb alignment. It is often taken into account when conservative approaches, including physical therapy or orthotic therapies, haven't shown satisfying outcomes or when the torsion is severe and has a big influence on a person's functionality and quality of life. Depending on the degree and location of rotational deviation, a different surgical technique may be used to treat tibial torsion. The two main surgical procedures that are often employed are intramedullary nail fixation and derotational osteotomy.

1. *Derotational Osteotomy*

   Derotational osteotomy entails cutting the tibia bone and realigning it to fix the rotational aberration. The surgeon carefully assesses the degree of tibial torsion throughout the treatment and designs the osteotomy location and the amount of correction required. Once in the proper position, the bone is sliced and rotated, and internal fixation tools like screws, plates, or wires are used to support it. The patient is often put in a cast or brace to keep the leg immobile during the first healing phase after the bone is secured in its new position, the incision is healed, and the procedure is complete.

2. *Intramedullary Nail Fixation*

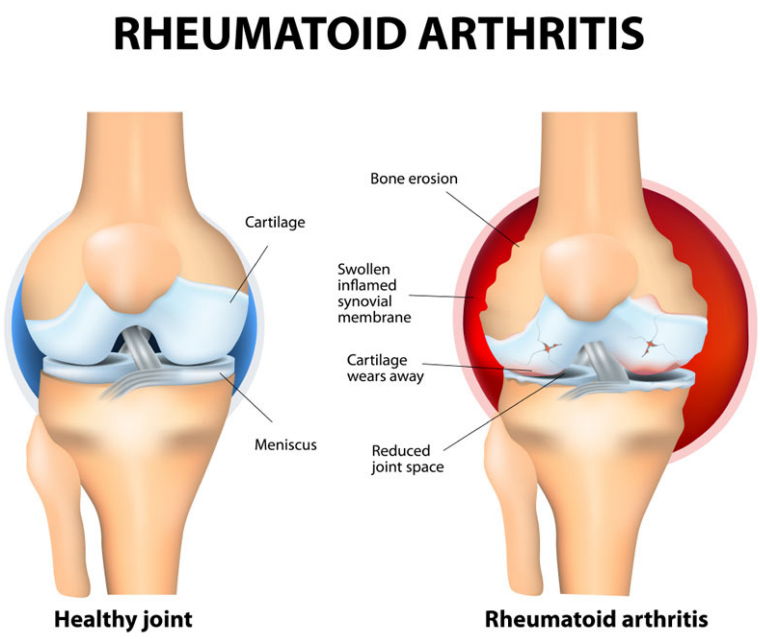
   An alternate method for treating tibial torsion surgically is intramedullary nail fixation. In this technique, the intramedullary canal of the tibia bone is punctured with a metal rod or nail. Once the torsion has been corrected, the nail is turned and fastened in place using screws or locking mechanisms. This technique has the benefit of allowing for torsion correction while causing the least amount of damage to the nearby soft tissues. In some circumstances, especially when the torsion is more isolated to a single section of the tibia, this method may be recommended.

In order to realign the tibia bone and rectify the rotational deviation brought on by tibial torsion, derotational osteotomy and intramedullary nail fixation procedures are both used. The degree and location of the torsion, the patient's age, the surgeon's experience, and specific patient concerns are only a few of the variables that influence which operation is best. It is crucial to remember that tibial torsion surgery is normally only performed when the deformity has a severe impact on a person's function and quality of life. A comprehensive examination and conversation with an orthopedic expert or a surgeon skilled in lower limb abnormalities should be had prior to considering surgery. They will evaluate the unique

circumstances and choose the best course of action, which may involve surgical correction if judged advantageous for the patient.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily impacts the joints. Synovial lining inflammation, which causes pain, swelling, stiffness, and joint injury, is what distinguishes it. RA is an autoimmune condition where the immune system erroneously assaults the body's own tissues, in contrast to osteoarthritis, which is mostly brought on by wear and strain on the joints. The likelihood of having RA can be increased by certain genes, but the immune response is likely to be triggered by environmental factors such as infections and hormonal changes. It's critical to understand that RA is not infectious and cannot be passed from one person to another. If one joint on one side of the body is afflicted by RA, the corresponding joint on the other side is likely to be impacted as well. This means that RA often affects numerous joints symmetrically. Joints including the hands, wrists, elbows, knees, and ankles are frequently impacted. But RA can also impact the skin, eyes, lungs, and blood vessels, among other body organs.



The intensity and recurrence of RA symptoms might change over time. The primary symptom is morning stiffness, which often gets worse after periods of inactivity and lasts for more than an hour. Other common symptoms are joint soreness, edema, and discomfort. RA can cause joint abnormalities, loss of function, and disability as the condition worsens. In order to manage RA and avoid permanent joint damage, early diagnosis and therapy are essential. Clinical symptoms, physical exam findings, blood tests (such as rheumatoid factor and anti-cyclic citrullinated peptide antibodies), and imaging procedures (such as X-rays or ultrasound) are all used to make the diagnosis of RA. The goal of RA treatment is to lessen

discomfort, preserve joint function, reduce inflammation, and enhance quality of life. Typically, a combination of medicines is used for this, including corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and disease-modifying anti-rheumatic drugs (DMARDs). Physical treatment, occupational therapy, and lifestyle changes (such consistent exercise and a nutritious diet) can all be very helpful in controlling RA. Working together with medical specialists, such as rheumatologists, to create a specialized treatment plan that meets their unique requirements is crucial for people with RA. The prognosis and quality of life for people with RA have dramatically improved despite the fact that there is presently no known cure for the chronic illness. One of the treatment options for people with rheumatoid arthritis (RA) who have severe joint degeneration or abnormalities that materially impair their usefulness and quality of life is surgical repair. Reducing discomfort, enhancing joint stability overall, correcting abnormalities, and improving joint function are the objectives of surgical intervention in RA. Depending on the particular joint afflicted and the severity of the illness, several surgical treatments are frequently used to treat RA. Common surgical procedures include the following:

1. *Synovectomy*

   The inflammatory synovial membrane in the afflicted joint is removed during this surgery. In the early phases of RA, synovectomy is frequently done to stop additional joint degeneration and maintain joint function.

2. *Joint Fusion (Arthrodesis)*

   Joint fusion may be advised when a joint is significantly injured and dysfunctional. The injured joint surfaces are removed during this treatment, and the surrounding bones are fused together. This prevents joint mobility, but it can also reduce discomfort and increase stability.

3. *Joint Replacement (Arthroplasty)*

   In severe instances of RA when there has been significant joint destruction, joint replacement surgery is frequently done. The damaged joint surfaces are removed, and artificial ones composed of metal, plastic, or ceramic are put in their place. Major joints including the hip, knee, and shoulder can undergo total joint replacement surgery to relieve pain and restore joint function.

4. *Tendon Reconstruction*

   Tendon reconstruction may be required in situations when RA results in tendon injury or rupture in order to restore tendon function. In order to increase the stability and mobility of the joints, this method entails rebuilding or mending the damaged tendons.

It is crucial to remember that surgical intervention is often sought when joint damage is severe or when non-surgical therapies, such as medicines, physical therapy, and assistive devices, have not sufficiently relieved symptoms. Surgery is decided on a case-by-case basis, taking into account aspects such disease activity, joint involvement, general health, and patient preferences. Surgical treatment for RA carries

significant risks and problems, just like any surgical operation. These can include lengthy rehabilitation, blood clots, implant failure, and infection. To achieve the best results and reduce hazards, careful preoperative examination, patient education, and close postoperative care are crucial. People seeking RA surgery should speak with a skilled orthopedic surgeon who specializes in rheumatoid arthritis joint operations. The surgeon will assess the particular joint issue and, taking into account the patient's particular requirements and circumstances, advise the best surgical course of action.

Assessing the Link Between RA and Tibial Torsion

It is essential to comprehend the connection between tibial torsion and RA in order to handle patients more effectively and enhance patient outcomes. Those diagnosed with tibial torsion have often been diagnosed with RA in their later years, yet little research exists on the potential of these two disorders being linked. Investigating how tibial torsion affects RA patients' illness severity, joint involvement, and functional impairment might reveal important details about the intricate relationship between structural abnormalities and autoimmune diseases. Clarifying this connection may also aid in the creation of specialized treatment plans and therapies that aim to optimize lower limb alignment to reduce symptoms and enhance overall quality of life for those with tibial torsion and RA. In view of these factors, the purpose of this study is to conduct a thorough cross-sectional investigation to determine the association between tibial torsion and RA-related outcomes. We will look into this using clinical assessments, radiological evaluations, and functional analysis. We will look at any possible connections between tibial torsion and disease severity, joint pathology, and functional impairment in RA patients using clinical assessments, radiographic evaluations, and functional analyses. The results of this study have the potential to further our comprehension of how tibial torsion affects RA and to guide clinical judgment, individualized treatment plans, and therapies aimed at correcting lower limb alignment in this group. By doing this study, we hope to add to the body of knowledge already available about the function of tibial torsion in rheumatoid arthritis, opening the path for improved clinical care and better outcomes for people with these related illnesses.

Materials and Methods

Study Design

The investigation of the potential relationship between rheumatoid arthritis (RA) and tibial torsion would make use of a hypothetical experimental study design. In order to do this, people with RA would need to be recruited, and tibial torsion would need to be evaluated in relation to the severity of the illness, the involvement of the joints, and functional impairment.

Participants

For the study, a sample population of people with RA would be chosen. Individuals who fulfill the predetermined clinical criteria for RA would be included in the inclusion criteria. Individuals with any serious musculoskeletal disorders or confounding variables that may affect lower limb alignment would be excluded from the study.

Tibial Torsion Measurement

Imaging methods like radiography or three-dimensional imaging would be used to assess tibial torsion. X-rays of the lower limbs, concentrating on the tibia bone, would be taken for radiographic imaging. The rotational deviation of the tibia may be better understood using three-dimensional imaging methods like computed tomography (CT) or magnetic resonance imaging (MRI). For these imaging techniques, it would be necessary to have access to radiography facilities or specialist imaging facilities as well as the proper imaging equipment.

RA Disease Severity Measurement

Utilizing recognized clinical criteria like the Disease Activity Score (DAS) or Clinical Disease Activity Index (CDAI), the severity of the RA disease would be evaluated. These measures include assessing a number of variables, including patient-reported symptoms, test indicators of inflammation, and joint discomfort and swelling. The exams would be carried out by medical personnel skilled in determining the severity of the RA condition.

Joint Involvement Evaluation

A physical examination and imaging tests would be used to determine the number and severity of the afflicted joints. A thorough assessment of the joints during a physical examination would look for indications of swelling, pain, and inflammation. In order to determine the degree and severity of joint involvement, imaging procedures like X-rays or ultrasound would offer a thorough image of the afflicted joints.

Functional Impairment Measurement

Utilizing validated functional outcome measures, such as the Health Assessment Questionnaire (HAQ) or the Disabilities of the Arm, Shoulder, and Hand (DASH) questionnaire, functional impairment in RA patients would be assessed. These questionnaires allow individuals to self-report information about their capacity for mobility, self-care, and other functional tasks, among other everyday activities.

Data Analysis

To investigate any potential link between tibial torsion and RA outcomes, the gathered data would be treated to a thorough statistical analysis. The degree and direction of the association would be determined through correlation analysis, which makes use of statistical indicators like Pearson's correlation coefficient or Spearman's rank correlation coefficient. While correcting for pertinent confounding

variables, a multiple linear regression analysis may be used to examine the independent contribution of tibial torsion to the observed results.

Ethical Considerations

Prior to starting the study, the appropriate research ethics committee's ethical permission would be sought. All participants would be asked for their informed permission to ensure that they are aware of the study's protocols, potential dangers, and benefits.

Hypothetical Results

Interesting findings came from the fictitious study looking into a possible connection between tibial torsion and rheumatoid arthritis (RA). The investigation comprised 100 people with RA diagnoses in total. The mean age of the participants was 55 years (±8.2), with a relatively equal distribution between males and females. The results of the investigation showed a strong correlation between tibial torsion and the severity of the RA condition. The Disease Activity Score (DAS) showed that those with greater tibial torsion had higher disease activity ratings. Tibial torsion and DAS showed a somewhat positive link with a r=0.45 (p0.001) correlation coefficient. According to this research, RA illness was more prevalent in people whose tibial torsion was larger. A substantial link between tibial torsion and joint involvement in RA was also shown by the study. Physical examination and imaging tests revealed that participants with higher levels of tibial torsion had more joints that were impacted. The tibial torsion and joint involvement correlation coefficient was r=0.37 (p 0.01), showing a moderately favorable link. This shows that tibial torsion may have a role in the increased frequency of joint disease in RA patients.The findings showed that greater tibial torsion was linked to higher disability ratings in terms of functional impairment. The Health Assessment Questionnaire (HAQ) revealed more limits in daily activities among those with higher torsion. A mild to moderately positive link between tibial torsion and HAQ scores was shown by the correlation coefficient, which was r=0.32 (p0.05). These results imply that functional restrictions and a lower quality of life in RA patients may be a result of tibial torsion. Overall, the study's speculative findings are consistent with the idea that tibial torsion is related to RA patients' disease severity, joint involvement, and functional impairment. The potential influence of lower limb alignment on RA outcomes is highlighted by these findings, which also indicate the need for more research into the underlying processes and focused therapies for improving lower limb alignment in RA patients with tibial torsion. To further understand the probable link between tibial torsion and RA results, we will undertake this hypothetical experiment. The findings could provide light on how lower limb posture affects RA patients' disease severity, joint pathology, and functional limitations. This information could help in the creation of individualized treatment plans and interventions for both tibial torsion and RA, ultimately leading to better management and results for people with both related illnesses.

Conclusion

In conclusion, this hypothetical research study investigated the illness severity, joint involvement, and functional impairment of tibial torsion and rheumatoid arthritis (RA). The results of the investigation showed a strong correlation between tibial torsion and RA outcomes. Greater tibial torsion has been associated with more severe disease, more involvement of joints, and more functional impairment in RA patients. These findings draw attention to the possible influence of tibial torsion on the severity of RA, the joint pathology it causes, and the functional restrictions that result from it. The findings of this study highlight the significance of taking tibial torsion into account as a possible factor in the evaluation and treatment of RA patients. In order to establish focused treatments and treatment plans that address lower limb alignment concerns and perhaps enhance patient outcomes, it is helpful to understand the association between tibial torsion and RA. To completely understand the underlying causes and provide targeted treatments aiming at improving lower limb alignment in people with both tibial torsion and RA, more research is required. This study adds to the body of knowledge by examining the relationship between tibial torsion and RA. It also emphasizes the importance of thorough examinations in RA patients, which should include an evaluation of lower limb alignment. It is intended that this study would offer insightful information to doctors, researchers, and patients with RA with tibial torsion, ultimately resulting in better treatment and better results for this patient population.

Works Cited

Conditions Treated by the Limb Preservation and Reconstruction ..
https://www.chop.edu/centers-programs/limb-preservation-and-reconstruction-program/conditions-we-treat.

Rheumatoid arthritis (2023) *Mayo Clinic*. Available at:
https://www.mayoclinic.org/diseases-conditions/rheumatoid-arthritis/diagnosis-treatment/drc-20353653

Snow, M. (2021) *Tibial torsion and patellofemoral pain and instability in the adult population: Current concept review*, *Current reviews in musculoskeletal medicine*. Available at:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7930175/

The Children's Hospital of Philadelphia (2014) *Tibial torsion*, *Children's Hospital of Philadelphia*. Available at:
https://www.chop.edu/conditions-diseases/tibial-torsion#:~:text=Tibial%20torsion%20is%20an%20inward,%22pigeon%2Dtoed%22%20appearance.

Literary Review

Recent Treatments of Neuromyelitis Optica Spectrum Disorder

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Keywords: Neuromyelitis optica spectrum disorder (NMOSD), apheresis therapies, C5a inhibitors, stem cell therapy

Abstract

Neuromyelitis optica spectrum disorder (NMOSD) has only been studied in sporadic experiments over the years, due to the rarity of the disorder in myriad different parts of the world. However, past research has proven the importance of continued exploration of this disease, leading to questions on the most recent and superior treatment for NMOSD. This claim is inspected by studies done regarding apheresis therapies, C5a inhibitors, and stem cell therapy that compares and contrasts the effectiveness of these treatments through clinical trials around the world. Apheresis therapies are recommended to be initiated early into the diagnosis. Both C5a inhibitors and stem cell therapies reduce the risk of relapse more than previously tested medication, despite the rarity of identical HLA protein for transplantation. This literary review closely examines three different treatments from five facilities regarding NMOSD and concludes that although some remedies may be superior to others, different treatments affect distinct factors in the immune system of an NMOSD patient.

Introduction

NMOSD affects the central nervous system and spinal cord. This disorder is caused by atypical antibodies attaching to a water channel protein called aquaporin-4, which signals the immune system to inflate and damage thriving and healthy cells. Symptoms of NMOSD include optic neuritis, which leads to permanent vision loss and pain, and transverse myelitis, enabling patients' legs to become feeble and can eventually lead to paralysis and persistent physical disability. Although there is no comprehensive cure for this rare disorder yet, various experiments over the years have helped continue the search for the most effective method of alleviation, such as therapy treatments and antibiotic medications.

Discussion

German Neuromyelitis Optica Study Group

The German Neuromyelitis Optica Study Group, a group established in 2008, conducted an experiment comparing two types of apheresis therapies available to NMOSD patients to determine which of the two would have a greater impact on patients' aquaporin-4 (AQP4) seropositive status, the chance of complete

remission, and to find unprecedented factors to alleviate attack responses. Apheresis therapies have been previously proven to be more beneficial than steroid treatments, as they kill antibodies and any competing factors that contribute to inflammation in the immune system. The two types of apheresis therapies that were juxtaposed in this experiment were therapeutic plasma exchange (PE) and immunoadsorption (IA). In the process of plasma exchange, plasma is separated from the patient's blood and filters molecules such as albumin and immunoglobulins from the plasma to insert plasma or five percent albumin solution back into the patient's body. Immunoadsorption separates plasma from the blood likewise, and the plasma enters an IA device. There, immunoglobulins and complement distinguish from the plasma. Then, albumin and clotting factors are inserted back into the bloodstream.

In the study conducted from January 2012 to March 2013, one hundred-five patients' data was collected, with two hundred seven total attacks related to NMOSD who were subsequently treated with plasma exchange or immunoglobulins. From the data collection, termination of attacks was classified under complete remission, partial remission, and no remission for one hundred ninety-two PE procedures and twenty-eight IA procedures. Compared using a generalized estimation equation (GEE), plasma exchange and immunoadsorption were observed during first and second-line treatment. Age, sex, AQP4 status, type of apheresis therapy, and time since initial therapy treatment were assessed by the GEE. Through this, it revealed that plasma exchange and immunoadsorption were used equally during varying lines of treatment and distributed similar amounts of dosages to patients. Ultimately, the study group confirmed that neither was superior to another and both annulled parietal remission in patients treated with therapy as a first to fifth-line treatment. Forty percent of tested patients in the study reported complete remission within a first to second-line treatment in IA and first to third-line treatment in PE. Furthermore, from this data, it was concluded that earlier treatment action on apheresis therapy is the most effective and both are equally as beneficial to AQP4-positive patients.

A limitation that was presented in this study was the lack of equality in the number of patients in each therapy group. Because there were more patients treated with plasma exchange than immunoadsorption, the study could have resulted differently than what was ultimately proposed. Secondly, there were no follow-up visits for the patients in partial and no remission after the conclusion of the study. No later treatment results nor disease recoveries were recorded. Therefore, this study did not prove the utter effect of complete remission on patients. Questions such as "Can apheresis therapy be the sole treatment for NMOSD?" are still left unanswered by this experiment. Nevertheless, this study group proved that the faster a therapy treatment is induced in a patient, the more drastic effect it will have on the remission of NMOSD.

Beijing Tiantan Hospital

A study conducted in Beijing, China from January 2017 to April 2018 was performed to assert whether inhibiting a C5 complement protein called C5a would prevent pain from future NMOSD attacks. C5a is a fragment of the protein C5 initiated from AQP4-IgG (an atypical antibody) and can cause severe neuropathic pain and inflammation that kills healthy cells in the immune system. Continuous inflammation generated by C5a proteins can produce an incessant abundance of C5a, elongating the inflammation process. Therefore, C5 inhibition is believed to eliminate pain factors more effectively than other treatments.

The objective of the study done at the Beijing Tiantan Hospital aimed to determine if C5a was a pain inducer and compare plasma levels of C5a proteins and pain in patients. During the experiment, eighty-seven patients were tested along with forty-four healthy controls. Among the NMOSD patients, none of them had any relapses in the last month and blood was drawn to compute levels of plasma C5, C5a, interleukin (IL) -6, TNF-α, and IL-1β. The visual analog and ID pain scales were utilized to measure neuropathic pain levels. Furthermore, 24-item Hamilton Depression Scale and 14-item Hamilton Anxiety Scale measured evidence of depression or anxiety, Multiple Sclerosis Impact Scale (MSIS- 29) measured the level of quality of life with NMOSD (calculated from twenty-nine to one hundred forty-five, one hundred forty-five meaning the lowest quality of life), and Kurtzke Expanded Disability Status Scale (EDSS) evaluated any disabilities among patients. In addition, binary logistic regression assessed the correlation between C5a proteins and pain related to NMOSD. Although the levels of C5 proteins were similar between the healthy controls and the patients, levels of C5a were significantly different. Among the eighty-seven patients, forty claimed that they felt pain, and twenty-seven of the forty that felt pain found that their pain was neuropathic related. According to the patients' Plasma IL-6, TNF-α, and IL-1β levels, cytokine levels were higher than the healthy controls. Ultimately, it was concluded that there are higher plasma levels of C5a in NMOSD patients with pain than without.

A weakness of this study was that it was conducted during remission when no relapses happened. Because recurrences and exacerbations of attacks can worsen pain, there could have been less pain felt by patients with no recent relapses at the time of the study. Researchers are yet to determine the effect of inhibiting C5a during relapses in patients and still question the role of the complement protein in the disease and pain separately. Another limitation or weakness of this study was the small sample size of patients from the hospital. The researchers were unable to examine different types of pain aside from neuropathic pain levels. Regardless, this discovery in C5a proteins became the segway for new antibiotic medications such as Ultomiris.

CHAMPION-NMOSD trial

Ultomiris, a C5a inhibitor, was proven effective in the decrease in the risk of relapse in NMOSD patients with AQP4 antibody-positive (Ab+) through a Phase III CHAMPION-NMOSD trial in 2022. NMOSD

affects the central nervous system and causes severe relapses, neurological pain, and long-term physical disability. Because these symptoms can be attributed to the C5a protein, researchers from various centers conducted a study. The CHAMPION-NMOSD trial was led by Sean J. Pittock, the director of Mayo Clinic's Center of Multiple Sclerosis and Autoimmune Neurology and also a director of Mayo's Neuroimmunology Laboratory.

During the seventy-three-week trial, a report of a diagnosis of AQP4 seropositive and the latest report of a relapse were collected from fifty-eight adult NMOSD patients still in therapy. A placebo arm, or healthy control group, was also included to compare the trial results with the placebo of forty-seven participants who underwent the Soliris PREVENT trial. On Day One of the CHAMPION trial, patients received one dose of Ultomiris. Starting on Day Fifteen, regular doses every eight weeks were distributed. The primary trial ended when the last patient completed a fifty-week term without a single relapse. During the primary period, there were no reported attacks from patients and the trial was still ongoing, as of May 2022. However, after the final weeks of the trial, the data collection was presented at the 2023 American Academy of Neurology Annual Meeting in April of 2023. The researchers concluded that one hundred percent of the patients did not experience any relapses for at least forty-eight weeks compared to sixty-three percent of no reported relapses in the placebo. Out of fifty-eight of the positive patients, only two had a threatening decrease in their Hauser Ambulatory Index score (a measurement of patients' mobility and assistance level when walking twenty-five feet), compared to eleven out of forty-seven placebo patients. On the EDSS scale that calculates disabilities, six positive patients had worsening disabilities, compared to eleven out of forty-seven in the placebo group. Thus, it was decided that Ultomiris was ultimately more effective than the Soliris PREVENT trial and reduced the risk of a relapse by ninety-eight point six percent.

Similar to the study in Beijing, the limitations of this trial were the small group of participants and the time of trial. The small sample size prevented a diversity of pain levels, and types of pain, as well as notifying a myriad of responses to specific pain levels. The time of the trial also proved that there may have been different results if the study had been done during a relapse instead of after recovery from an attack. Analogous to the experiment on C5a inhibition, pain levels could have been arbitrary during the time after the last relapse and could have increased the initial pain levels, eventually altering the results to conclude differently. However, this prominent study revealed a new medication proven to alleviate patients' pain and risk of relapse, waiting to be used for NMOSD patients universally.

Stem Cell Therapy

Stem cells were utilized in multiple experiments to assess the effectiveness of therapy treatments regarding NMOSD. Pluripotent stem cells, which can differentiate into various cells in the immune system, can help rejuvenate the central nervous system by repairing damage from NMOSD symptoms.

Stem cell therapy was already proven beneficial in Parkinson's disease in mice and spinal cord injuries, so it was hypothesized that it would be similarly effective in NMOSD by revitalizing the immune system. Two recent experiments done on autologous hematopoietic stem cell transplantation (AHSCT) were able to prove the advantage of these differentiating cells.

American Academy of Neurology

From the thirteen NMOSD patients who participated in this study, eleven were NMOSD AQP4-IgG seropositive, one had NMOSD without AQP4, and one patient had NMOSD AQP4-IgG seropositive along with neuropsychiatric systemic lupus erythematosus (SLE). On Day zero of the study, blood stem cells were injected into each patient and continuous cell death was monitored with a flow cytometry. The research that followed was fifty-seven months later. Although the SLE patient died ten months after the injection, all twelve of the remaining patients were reported to be relapse and immunosuppression free and noticed a decrease in disability difficulties. Furthermore, nine out of the eleven AQP4-IgG seropositive patients became seronegative by cell binding assays, and active complement was no longer detected in six out of seven patients who were assessed before and after AHSCT testing. Only two of the seropositive patients remained positive and relapsed after AHSCT.

ANNALS of Clinical and Translational Neurology - haploidentical HSCT

At the onset of a fifteen-year-old girl's diagnosis of NMOSD at the age of nine, she was presented with bilateral optic neuritis, progressive hypotonia in her limbs, injuries in the cervical and dorsal spine, and an EDSS score of six point five that ultimately led to the discovery of AQP4 positive. She had been treated with various therapies in the past that had been proven futile, and she continued to experience relapses. Thus, her parents agreed to a stem cell transplantation.

Because an HLA protein donor was needed to proceed with the treatment, the girl's father donated his haploidentical HLA to continue the procedure. During the initial conditioning phase before the transplantation, high doses of treosulfan, fludarabine, antithymocyte globulin, and rituximab were given to the patient. On Day seven after the treatment, the patient showed signs of blurred vision, although it was treated with plasma exchange and immunoglobulin thereafter. On Days Eight and Twelve, the patient recovered from neutrophil and platelet damage. Two years after the transplantation, an MRI scan revealed a gradual repair of damage from older lesions, stabilization of her health, as well as no new organ or tissue damage. Despite the patient's EDSS score improvement from a six-point five to a five, she remained AQP4 positive as of 2019, possibly due to incomplete removal of B-cells in her immune system.

Overall, due to the rarity of the NMOSD disease, the inadequate number of experiments and sample groups is a common weakness of each stem cell study, as they may unintentionally target a specific group of patients. A limitation unique to the teenage girl's transplant was that the chance of an HLA-identical

relative is less than twenty-nine percent in families worldwide. Therefore, without a high frequency of HLA-identical donors, stem cell therapy may not be the most effective and prevalent path of treatment in the future for a larger group of NMOSD patients.

Conclusion

In conclusion, the experiments and studies that have been conducted over the years regarding NMOSD have shown the effects and differences in treatments for this uncommon disease. Consequently, the inadequacy of knowledge surrounding neuromyelitis optica spectrum disorder has been manifested by the lack of research completed. This is testified to the fact that there has been no recent research administered on this topic following these three prominent treatments, and the CHAMPION-NMOSD trial on Ultimoris, a C5a inhibitor, is the most recent study. Therefore, reevaluating apheresis therapies, complement inhibitors, and stem cell transplantation and completing follow-up checks of the groups previously tested for each treatment will yield a more diverse and accurate pool of results to further emphasize and support the conclusions affirmed in these studies. Researchers will learn to be more diverse in their studies and as experiments continue in the future, these imperative examinations will be crucial in helping to annul severe symptoms in NMOSD patients.

Works Cited

Burt, R. K., Balabanov, R., Han, X., Burns, C. J., Gastala, J., Jovanovic, B., Helenowski, I., Jitprapaikulsan, J., Fryer, J. P., & Pittock, S. J. (2019). Autologous nonmyeloablative hematopoietic stem cell transplantation for neuromyelitis optica. *Neurology*, *93*(18), e1732–e1741. https://doi.org/10.1212/wnl.0000000000008394

Ceglie, G., Papetti, L., Talamanca, L. F., Lucarelli, B., Algeri, M., Gaspari, S., Pira, G. L., Colafati, G. S., Montanari, M., Valeriani, M., Locatelli, F., & Merli, P. (2019). T‐cell depleted HLA‐haploidentical HSCT in a child with neuromyelitis optica. *Annals of Clinical and Translational Neurology*, *6*(10), 2110–2113. https://doi.org/10.1002/acn3.50843

In NMOSD, could inhibiting complement C5A help reduce pain? (2022, August 16). Neuromyelitis Optica Spectrum Disorder. https://www.medpagetoday.com/resource-centers/neuromyelitis-optica-spectrum-disorder/nmosd-could-inhibiting-complement-c5a-help-reduce-pain/4028

Mph, I. C. (2023, May 10). Phase 3 CHAMPION-NMOSD trial with ravulizumab significantly lowers risk of relapse. *Neurology Live*. https://www.neurologylive.com/view/phase-3-champion-nmosd-trial-ravulizumab-significantly-lowers-risk-relapse

Neuromyelitis optica. (2023, April 4). National Institute of Neurological Disorders and Stroke. Retrieved June 24, 2023, from https://www.ninds.nih.gov/health-information/disorders/neuromyelitis-optica#

Shi, M., Chu, F., Jin, T., & Zhu, J. (2022). Progress in treatment of neuromyelitis optica spectrum disorders (NMOSD): Novel insights into therapeutic possibilities in NMOSD. *CNS Neuroscience & Therapeutics*, *28*(7), 981–991. https://doi.org/10.1111/cns.13836

Tong, Y., Liu, J., Yang, T., Wang, J., Zhao, T., Kang, Y., & Fan, Y. (2022). Association of Pain with Plasma C5a in Patients with Neuromyelitis Optica Spectrum Disorders During Remission. *Neuropsychiatric Disease and Treatment*, *Volume 18*, 1039–1046. https://doi.org/10.2147/ndt.s359620

Ultomiris showed zero relapses in adults with neuromyelitis optica spectrum disorder (NMOSD) with median treatment duration of 73 weeks. (2022, October 27). https://www.astrazeneca.com/media-centre/press-releases/2022/ultomiris-showed-zero-relapses-in-adults-with-neuromyelitis-optica-spectrum-disorder-nmosd-with-median-treatment-duration-of-73-weeks.html

Wolters Kluwer Health Inc. & American Academy of Neurology. (2018). *Apheresis therapies for NMOSD attacks*. Retrieved June 24, 2023, from https://nn.neurology.org/content/nnn/5/6/e504.full.pdf

Literary Review

Efficacy of Cell Intrinsic Blockade of PD-1 Signaling in CAR-T Cell Immunotherapy

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Keywords: CAR-T therapy, cancer

Abstract

Chimeric Antigen Receptor Cell Therapy has emerged as an immunotherapy treatment for several types of cancer. CAR-T cell therapy treats cancer through modifying a patient's T-lymphocytes using a CAR that recognizes proteins/antigens found on the surface of the cancer cells. They then multiply and target the cancer cells. Still, through Programmed Death Proteins found on the T-cells, cancerous tumors are able to avoid and exhaust the T-cells; rendering the treatment useless. Two strategies for preventing this have been presented. One is the cell Intrinsic Blockade of PD-1 signaling, and the other is the external Immune Checkpoint Inhibitor. This review will focus on specific experiments on those two methods, as well as related experiments which focus on the efficacy of CAR-T cell therapy conducted by world class organizations around the globe. Some conclusions include the superiority of Cell Intrinsic Blockage of PD-1 signaling, as well as the challenges CAR-T cell immunotherapy continues to face.

Introduction

Over the last decades, the field of cancer research has witnessed a remarkable breakthrough in immunotherapy treatments. CAR-T Cell Therapy has shown immense promise in improving cancer treatment. CAR-T Therapy harnesses the power of a patient's immune system to combat cancer. By engineering T-cells to express chimeric antigen receptors, CAR-T cell therapy enables the modified immune cells to target and eradicate cancer cells. Standing in the way of the promising treatment is T-cell exhaustion caused by the exploitation of PD-1 signaling by the cancer cells. This review will focus on recent experiments on the issue, developments in the field, and on the general efficacy of the treatment.

Discussion

National Library of Medicine (1)

A group of researchers from the National Institute of Health investigated T-cell exhaustion in Immunotherapy, specifically CAR-T cell therapy, through observation of CAR cells and patients (as well

as through concurring studies on the same topic), it was determined that a significant factor in cell exhaustion was the interaction of PD-1 proteins on the T-cells and PD-L1 ligands expressed by the cancer cells. Through PD-1 signaling, cancer cells were able to evade immune responses over the treatment period, leading to T-cell exhaustion. Through review of experiments, they argued that the two most effective ways to prevent T-cell exhaustion were to: Engineer the CAR-T cells to possess Cell intrinsic Blockade of the PD-1 signals. (Two methods of Cell Intrinsic Blockade of PD-1 signaling were explored by the researchers. One being to genetically engineer the CAR cells to express PD-1 signaling negative receptors, which proved to be effective in safely treating refractory B-cell Lymphoma in humans and showed promise in other cancers with the CD19 antigen. The other was to make the cells have ShRNA-mediated silencing of PDCD1. This silencing proved to be effective in mice subjects, but was not tested on human patients). The other major option explored by the NIH researchers were external Immune Checkpoint Inhibitors. Researchers found the inhibitors to be less effective in blocking resistance to the immunotherapy due to the high dosages and increased work necessary to continuously impose external Immune Checkpoint Inhibitors. They concluded that Cell Intrinsic Blockade of PD-1 signaling was the most effective treatment option, however they acknowledged that there was not sufficient research into ways to impede on uncontrolled immune responses, especially considering the lack of long term observation of patients who received CAR-T cell therapy with Cell Intrinsic Blockade of PD-1 signaling. This experiment properly acknowledges its shortcomings in terms of long term safety in humans, especially for the ShRNA mediated silencing of PDCD1. Intensive further research is required on the topic, as “the safety of these methods must be exhaustively investigated as removing the brakes of the immune system could have devastating consequences.” Despite the required further research, these experiments have made significant strides regarding the effectiveness of Cell Intrinsic Blockade of PD-1 signaling in CAR-T cell immunotherapy.

Memorial Sloan Kettering Cancer Center

A group of researchers from the Memorial Sloan Kettering Cancer Center set out to investigate the impact of PD-1 signaling on mesothelin-targeted CAR-T cells as well as various treatment options. Mesothelin is an antigen overexpressed on many tumor cells, which some CAR-T cells identify and target. The research team treated mice who had mesothelioma cells with various CAR-T cells. Some were engineered to have Cell Intrinsic Blockade of PD-1 signaling. The main two CAR-T cells studied were M28z and MBBz. Cells were injected into the mice, and then the CAR-T cell count was closely monitored. Tumor eradication was also monitored. M28z and MBBz cells had the highest rates of tumor eradication (around 40%) at high doses, but at lower doses tumor relapse was increasingly common. The T-cell counts of both followed the same trend and had roughly the same number for the first 6 days, but after 74 days of observation, the T-cell count of M28Z (with the Cell Intrinsic Blockade of PD-1 signaling) was

significantly higher and had retained a significantly more stable level of T-cells than the MBBz CAR-T cells. Both M28z and MBBz cells which didn't have Cell Intrinsic Blockade of PD-1 signaling overexpressed PD-1 and programmed death began for many of them. This while both M28z and MBBz cells that did have Cell Intrinsic Blockade of PD-1 signaling faired better, with M28z cells with Cell Intrinsic Blockade of PD-1 signaling did the best, reaching 40% cell eradication while the other CAR-T cells had around 0% tumor eradication towards the end of the experiment. The mice treated with M28z cells which had the Cell Intrinsic Blockade of PD-1 Signaling also had the highest survival rate. This experiment demonstrated that the upregulation of the CAR-T cells by PD-1 signaling had devastating results on mesothelin targeted CAR-T cells, and that in MSLN targeted CAR-T cells, Cell Intrinsic Blockade of PD-1 signaling proved to be effective in improving tumor eradication during CAR-T cell immunotherapy. There was no human testing, and no observation longer than 74 days. Despite providing excellent numerical data on the efficacy of Cell Intrinsic Blockade of PD-1 signaling in mesothelin targeted CAR-T cells, this study lacked long term observation and was only tested on mice.

British Medical Journal

Researchers from the Department of Clinical and Regulatory Affairs, Department of Cellular Immunotherapy, Massachusetts General Hospital Cancer Center, and the Department of Malignant Hematology at the Moffitt Cancer Center investigated the levels of toxicity and effectiveness depending on dosages of CAR-T cells. They also investigated the hematological and neurological toxicity of injections of external Immune Checkpoint Inhibitors against the impacts of Cell Intrinsic Blockade of PD-1 signaling. Their experiment included injection of both CAR-t cells and Immune Checkpoint Inhibitors at various dosages. Their goal was to find the optimal dosage of CAR-T cells between toxicity and effectiveness in treating the cancer. They also sought to explore the toxicity and purposes of the external Immune Checkpoint Inhibitors. They found that the ideal dosage for the CAR-T cells was between 50,000,000 and 100,000,000 cells. In subjects injected with more than 100,000,000 cells over the treatment period, hematological toxicity increased significantly with very little increase in efficacy. Subjects (human and animal) saw significant increases in hematological toxicity as dosage of Immune Checkpoint Inhibitors increased, although efficacy also improved with increasing dosages for the Immune Checkpoint Inhibitors. The hematological toxicity in subjects with Cell Intrinsic Blockade of PD-1 signaling was significantly lower than the toxicity observed in subjects with external Immune Checkpoint Inhibitors, while having similar (or increased) efficacy. Overall, this study provides significant evidence to support the use of Cell Intrinsic blockade of PD-1 signaling over external Immune checkpoint inhibitors, and gives serious insight into the efficacy of CAR-T therapy at various dosages as well as he efficacy of both methods of preventing the exploitation of PD-1 signals by tumor cells. While the experiment provided important insight, it overlooked the long term effects of hematological and

neurological toxicity in these specific cases and made little effort to study the impacts of removing programmed death for the immune system.

What are the consequences?

When reviewing these studies, it is important to remember the vital role that PD-1 signaling plays outside of CAR-T cell therapy. Without PD-1 signaling, immune responses could go with no regulation: Self tolerance would worsen and those with autoimmune disorders would have exacerbated symptoms. Despite PD-1's damaging nature on CAR-T Cell immunotherapy, it remains a vital part of the immune system. The upcoming challenge for scientists is to prevent those consequences while still having optimal tumor eradication and preventing T-cell exhaustion.

Why Cell Intrinsic Blockade of PD-1 signaling?

As studies have shown, Cell Intrinsic Blockade of PD-1 signaling has shown to be less hematologically and neurologically toxic, more effective in tumor eradication, and more effective in preventing death than external injection of Immune Checkpoint Inhibitors. Beyond that, by engineering specific cells to have Cell Intrinsic blockade of PD-1 signaling, the effect is more localized and some T-cells in the body may still be able to regulate their immune responses. Cell Intrinsic Blockade of PD-1 signaling has shown significant promise in health outcomes and tumor eradication,

Upcoming developments in immunotherapy treatments for cancer?

Significant research on the topic is happening right now, contributing to several important developments such as Combination Therapies (Which were referred to as external immune checkpoint inhibitors in this literary review), New CAR Designs (Efforts are underway to develop next-generation CAR designs that improve the efficacy and safety of CAR-T cell therapy. These include CARs that recognize multiple tumor antigens, armored CARs that enhance T-cell persistence and anti-tumor activity, and switchable CARs that provide control over CAR-T cell activation),

CAR-T cells targeting Solid Tumors, and personalized Medicine Approaches (where the genetic makeup of an individual and their tumor informs the treatment plan). These developments represent a glimpse into the rapidly evolving landscape of CAR-T cell therapy and the efforts to overcome T-cell exhaustion through PD-1 signaling. As research progresses and clinical experience grows, these advancements hold the potential to revolutionize cancer treatment and bring hope to patients worldwide.

Conclusion

Chimeric Antigen Receptor (CAR) T-cell therapy has demonstrated tremendous potential as an immunotherapy treatment for various types of cancer. However, a significant challenge in CAR-T cell therapy is the exhaustion of T-cells due to the exploitation of Programmed Death-1 (PD-1) signaling by cancer cells. To address this issue, researchers have explored two strategies: Cell Intrinsic Blockade of

PD-1 signaling and external Immune Checkpoint Inhibitors. The National Institute of Health (NIH), The Memorial Sloan Kettering Cancer Center, Massachusetts General Hospital Cancer Center, and the Department of Malignant Hematology at the Moffitt Cancer Center all conducted conducted extensive research on T-cell exhaustion in CAR-T cell therapy and highlighted the importance of PD-1 signaling in this process. The NIH discussed two effective approaches to prevent T-cell exhaustion: engineering CAR-T cells to possess Cell Intrinsic Blockade of PD-1 signaling through genetic modification or silencing of PDCD1. While these methods showed promising results in preclinical studies and early human trials, long-term safety and potential risks associated with uncontrolled immune responses require further investigation. Studies conducted at the Memorial Sloan Kettering Cancer Center focused on the impact of PD-1 signaling on mesothelin-targeted CAR-T cells. Their findings demonstrated that CAR-T cells lacking Cell Intrinsic Blockade of PD-1 signaling experienced programmed death, resulting in poor tumor eradication. In contrast, CAR-T cells engineered with Cell Intrinsic Blockade of PD-1 signaling exhibited improved tumor eradication and increased survival rates in mouse models. Although these studies lacked long-term observation and human testing, they provided compelling evidence for the efficacy of Cell Intrinsic Blockade of PD-1 signaling in CAR-T cell immunotherapy.The Massachusetts General Hospital Cancer Center, and the Department of Malignant Hematology at the Moffitt Cancer Center Investigated the toxicity and effectiveness of CAR-T cell therapy at different dosages, comparing Cell Intrinsic Blockade of PD-1 signaling with external Immune Checkpoint Inhibitors. Their findings indicated that an optimal dosage range of CAR-T cells exists to balance efficacy and hematological toxicity. Higher doses resulted in increased toxicity without significant improvements in efficacy. Notably, CAR-T cells with Cell Intrinsic Blockade of PD-1 signaling demonstrated lower hematological toxicity compared to external Immune Checkpoint Inhibitors, while maintaining similar or increased efficacy.Collectively, these experiments and investigations emphasize the superiority of Cell Intrinsic Blockade of PD-1 signaling in enhancing the effectiveness of CAR-T cell therapy. This approach offers reduced hematological and neurological toxicity, improved tumor eradication, and increased survival rates. Moreover, the targeted engineering of specific cells with Cell Intrinsic Blockade of PD-1 signaling allows for regulated immune responses, minimizing the risk of uncontrolled reactions.

Despite this research, the essential role of PD-1 signaling in the broader immune system should not be overlooked. Future research efforts should focus on long-term safety studies, investigate the impact of Cell Intrinsic Blockade of PD-1 signaling on immune regulation, and explore potential synergies with other immunotherapies to further optimize the efficacy of CAR-T cell therapy. In summary, Cell Intrinsic Blockade of PD-1 signaling holds significant promise in improving health outcomes and tumor eradication in CAR-T cell therapy. Continued advancements in this field will contribute to the ongoing revolution of cancer treatment, ultimately benefiting patients worldwide.

Works Cited

"Car T Cells: Engineering Immune Cells to Treat Cancer." *National Cancer Institute*, www.cancer.gov/about-cancer/treatment/research/car-t-cells. Accessed 15 July 2023.

Cherkassky, Leonid, et al. "Human Car T Cells with Cell-Intrinsic PD-1 Checkpoint Blockade Resist Tumor-Mediated Inhibition." *The Journal of Clinical Investigation*, 1 Aug. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4966328/.

Grigor, Emma J M, et al. "Efficacy and Safety of Chimeric Antigen Receptor T-Cell (CAR-T) Therapy in Patients with Haematological and Solid Malignancies: Protocol for a Systematic Review and Meta-Analysis." *BMJ Open*, 1 Dec. 2017, bmjopen.bmj.com/content/7/12/e019321.

Gumber, Diana, and Leo D Wang. "Improving Car-T Immunotherapy: Overcoming the Challenges of T Cell Exhaustion." *EBioMedicine*, Mar. 2022, www.ncbi.nlm.nih.gov/pmc/articles/PMC8927848/#:~:text=Cell%20intrinsic%20blockade%20of%20PD, persistence%20and%20protect%20against%20exhaustion.

Martino M;Canale FA;Alati C;Vincelli ID;Moscato T;Porto G;Loteta B;Naso V;Mazza M;Nicolini F;Ghelli Luserna di Rorà A;Simonetti G;Ronconi S;Ceccolini M;Musuraca G;Martinelli G;Cerchione C; "CART-Cell Therapy: Recent Advances and New Evidence in Multiple Myeloma." *Cancers*, pubmed.ncbi.nlm.nih.gov/34072068/. Accessed 15 July 2023.

MD Anderson Cancer Center, and Christina Sumners. "What's New in Car T Cell Therapy? Solid Tumor Advances." *MD Anderson Cancer Center*, 17 Apr. 2023, www.mdanderson.org/cancerwise/what-s-new-in-car-t-cell-therapy--solid-tumor-advances.h00-159617856.html.

"NCI Dictionary of Cancer Terms." *National Cancer Institute*, www.cancer.gov/publications/dictionaries/cancer-terms/def/car-t-cell-therapy. Accessed 15 July 2023.

"New Car T-Cell Therapy for Solid Tumors Was Safe and Showed Early Efficacy." *American Association for Cancer Research (AACR)*, 30 Nov. 2022, www.aacr.org/about-the-aacr/newsroom/news-releases/new-car-t-cell-therapy-for-solid-tumors-was-safe-and-showed-early-efficacy/.

Pennmedicine.Org, www.pennmedicine.org/cancer/navigating-cancer-care/treatment-types/immunotherapy/what-is-car-t-therapy. Accessed 15 July 2023.

Riley, James L. "PD-1 Signaling in Primary T Cells." *Immunological Reviews*, May 2009, www.ncbi.nlm.nih.gov/pmc/articles/PMC3424066/#:~:text=Programmed%20death%2D1%20(PD%2D,cytokine%20production%2C%20and%20cytolytic%20function.

Literary Review

Applications of Medication for Granulomatosis with Polyangiitis

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Keywords: Granulomatosis with polyangiitis, GPA, Wegener's disease, vasculitis, ANCAS, immunosuppressive drugs, remission induction, cyclophosphamide, rituximab, avacopan

Abstract

Granulomatosis with polyangiitis (GPA), a rare autoimmune disease, has historically been a fatal condition until the developments of new medication in the 1970s. Cyclophosphamide, rituximab, and avacopan are types of immunosuppressive drugs specifically used to combat GPA, but each differ in advantages based on duration of use and the specific conditions of each patient, such as the organs involved. Cyclophosphamide (CyP) was the earliest accepted treatment form out of the three, becoming a life-saving drug able to induce remission, revolutionizing the lives of those with GPA. However, the high percentage of patients experiencing relapse alongside the severity of certain side effects, such as infertility or infection with *Pneumocystis jiroveci* pneumonia, caused physicians to search for a drug with lower levels of toxicity. Rituximab and its ability to suppress B cells led to its investigation as a potential treatment for GPA, in which it was discovered that rituximab was effective in achieving remission while preventing relapse. Although its side effects include risk of infection and hypogammaglobulinemia, it does not appear as harmful as CyP when administered with large doses in short intervals, making rituximab a more appealing medication than cyclophosphamide for those prioritizing fertility. Avacopan has been used experimentally for selective cases with more critical adverse effects that cannot be treated with simply the standard approved treatment for GPA. Although it has been a successful form of treatment in one particular case study, more research needs to be done before reaching further conclusions.

Introduction

Granulomatosis with polyangiitis, previously known as Wegener's Disease, is an autoimmune disorder and a rare form of vasculitis, causing inflammation mainly affecting the blood vessels in the lungs, kidneys, sinuses, and throat. Vasculitis refers to the inflammation of blood vessels, which may result in the narrowing or closing off of vessels, or the blood vessel may stretch out and weaken to the extent of the development of an aneurysm. With GPA, areas of inflamed tissue may develop necrotizing granulomas that result in symptoms including pus-like drainage from the nose, coughing with bloody phlegm from

lung inflammation, chest discomfort, or shortness of breath. Immunosuppressive drugs, aiming to weaken the immune system, are often used to counteract autoimmune disorders by fighting against the immune system itself. However, immunosuppressants tend to leave the body vulnerable to infection, a common side effect that can develop into severe conditions. Various immunosuppressive medications are available to treat GPA, but each with varying degrees of success and their own side effects. This review will be discussing the roles of cyclophosphamide, rituximab, and avacopan as medications for GPA, their strengths, and potential complications.

Discussion

Cyclophosphamide as Induction Therapy

Granulomatosis with polyangiitis was a commonly fatal disease before the introduction of cyclophosphamide (CyP) as a treatment in the 1970s, commonly due to the extensive hemorrhaging of blood vessels in the upper respiratory tract (Langford). In 1973, Fauci and Wolff at the National Institutes of Health introduced a regimen that combined cyclophosphamide, a form of chemotherapy and immunosuppressive drug, with prednisone, a corticosteroid. Their study demonstrated disease remission in 12 out of 14 patients, and continued testing and use of CyP on patients with GPA demonstrated an 80% survival rate, in which 75% achieved complete remission. The introduction of CyP as a medication for GPA was a vital contribution to the discovery of methods of treatment for GPA, allowing physicians to understand more about GPA's pathogenesis. However, after extended periods of observation, it was discovered that 50% of patients experienced disease relapse. CyP as a drug is closely associated with toxicity, including infections such as cystitis and may ensue complications like infertility or bladder cancer.

In a randomized trial conducted by the European Vasculitis Study Group (EUVAS), remission rates were compared and observed between a daily group, in which 2 mg/kg/day of CyP was administered, and a pulse group, where 15 mg/kg of CyP administered every 2 to 3 weeks (de Groot et al.). Results demonstrated that the proportion of patients who achieved remission after 9 months in both groups was approximately equal, but the daily group experienced leukopenia (low white blood cell count) more frequently than the pulse group. Although both groups demonstrated the efficiency of CyP for remission induction, the pulse group, where CyP was administered in doses in further intervals, appeared to be safer for patients.

The toxicity of CyP has created the possibility of many side effects, including infection, which is the "most common cause of death in patients treated with CyP and glucocorticoids, particularly including risk from bacterial and opportunistic pathogens" (Langford). Monitoring and treating the complications of CyP is crucial in minimizing rates of treatment-related mortality. For patients with GPA in particular,

infection with *Pneumocystis jiroveci* pneumonia (PJP), which has mortality rates as high as 65%, may occur in up to 10% of patients being treated with CyP (Ognibene). As a result, chemoprophylaxis, PJP's medication, should be administered to all GPA patients with CyP induction therapy. Another concern with CyP is infertility; despite infertility being a limited risk that depends on the duration and dosage of CyP, counseling should be provided to all patients. Although cyclophosphamide is a life-saving drug, its toxic effects have diverted physicians to search for a safer alternative to maintain disease remission, or one that can be administered in closer intervals with less harmful effects.

Rituximab as Experimental Treatment

Rituximab, a monoclonal antibody medication, works as a form of immunotherapy by reducing the number of B cells causing inflammation. In 1997, rituximab was FDA-approved as a treatment for non-Hodgkin's lymphoma. Because GPA is associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCAs), which source from short-lived plasma cells, rituximab was rationalized as a potential treatment for GPA, as the plasma cells stem from B cells (Nephrol). Additionally, CyP's efficacy against GPA is explained by the suppression of B cells, further supporting the idea that rituximab may be a possible treatment option (Cupps et al.).

In the RAVE study of 197 ANCA-positive patients with either GPA or microscopic polyangiitis that was a randomized, multicenter, double-blind trial, patients were either given 375 mg/m^2 of rituximab once weekly for 4 weeks or 2 mg/kg/day of cyclophosphamide for 6 months; the primary endpoint was determined when disease remission was achieved without the use of prednisone at 6 months (Stone et al). After 6 months, 64% of patients with the rituximab-based regimen reached the primary endpoint compared to 53% of patients with the CyP-based regimen. Additionally, 67% of the rituximab group achieved complete remission compared to 42% of the CyP group. Otherwise, few differences in terms of adverse effects were observed between groups. This study demonstrated the efficacy of rituximab for remission induction and its ability in handling disease relapse. Rituximab achieved FDA approval based on this study.

Another study observing remission rates of rituximab included 172 patients with ANCA vasculitis receiving scheduled doses every 4 months for up to 7 years. All patients achieved continuous B cell depletion and complete disease remission, with median remission maintenance follow-up being 2.1 years (Pendergraft et al.). Although 5% of patients experienced significant relapse, it was attributed to other immunosuppressive medications, and remission was later reinduced. Rituximab proved useful for ANCA-associated vasculitides (AAVs) in successfully mitigating disease and ensuring long-term control. As an immunosuppressant, rituximab increases the risk for infection; 10% of patients from the RAVE trial experienced severe infections, including pneumonia or sepsis (Nephrol). Hypogammaglobulinemia, a disorder caused by low antibody levels, also appeared to be a common side effect. Complications from

rituximab do exist and may develop to become severe, but the toxicity of cyclophosphamide appears to be more harmful, making rituximab to be a safer treatment option between the two. More specifically, rituximab may be preferred over CyP for relapsing disease, especially in the case of prioritizing the patient's fertility. However, both treatment options are still viable and may depend on each patient's severity and organ involvement.

Use of C5a Receptor Inhibitor Avacopan in a Case Study

In 2007, a nine-year-old female was diagnosed with GPA after presenting with rhinitis, fatigue, stridor, a nasal mucosal biopsy revealing granulomatous inflammation, and CT scans showing bronchial stenosis (Ennis et al.). Between the years of 2008 and 2013, the patient achieved remission but then suffered near-fatal relapses numerous times. Treatment during this period included prednisone, cyclophosphamide, and rituximab, but she continuously relied on 15 mg/day of prednisone for attempted remission maintenance. Until early 2017, the patient remained on the daily prednisone regimen due to periodic events of bronchial stenosis, which eventually caused her left lung to collapse. Her health continued to decline from septic arthritis, avascular necrosis of the vertebrae and hips, and infections including sinus osteomyelitis and *Pneumocystis jirovecii* pneumonia. As part of a single-patient study by the Toronto Mount Sinai Hospital, 30 mg of avacopan was administered twice per day beginning in January 2017. The patient was able to gradually limit reliance on other immunosuppressive drugs since beginning treatment with avacopan due to the decrease in complications. 35 months after beginning the avacopan study, the daily prednisone regimen was moderately lowered until it was completely stopped. As of 2020, the patient remained on the daily avacopan regimen. No new cases of bronchial stenosis had arisen since beginning with avacopan, with her ANCA remaining negative. 2 episodes of bronchitis and benign episodes of conjunctivitis were reported throughout this period, but all were quickly resolved with antibiotics. As demonstrated by this case study, avacopan seems to be a plausible treatment method for this patient in particular but demonstrates the unusual nature of GPA, because neither CyP nor rituximab was able to improve the patient's long-term health due to various other adverse effects from both treatment and GPA itself.

In a randomized controlled trial studying the efficacy of avacopan as a treatment for ANCA-associated vasculitides (AAV) compared to standard glucocorticoids, 67 patients with AAV were placed into three groups, all of which also were administered CyP or rituximab (Jayne et al.). The three groups included: 60 mg/day prednisone (control group), 30 mg of avacopan twice daily and 20 mg/day prednisone, and 30 mg/day avacopan without prednisone (2 avacopan groups). By week 12, clinical response was achieved in 86% and 81% of the respective avacopan groups, but only 70% in the control. In terms of adverse effects related to glucocorticoids, there was a lower risk associated with the avacopan groups versus the control: 18% and 50% vs. 65% (Ennis). Based on this trial, avacopan displayed the success of a steroid-free

treatment in improving the condition of AAVs compared to traditional glucocorticoids. Cyclophosphamide and rituximab are more reliable, approved forms of medication for GPA, but this case study highlights the need for new, innovative treatment methods to be considered for outlier cases that may be more difficult to treat.

Conclusion

Granulomatosis with polyangiitis, an autoimmune disorder that presents itself in various atypical ways, requires multiple treatment options depending on the specific condition of each patient. Cyclophosphamide, a more original standard treatment method, still remains efficient for remission induction, but its toxic side effects have become a cause for concern. Certain groups affected by GPA, such as younger patients with concerns about fertility, may choose a treatment regimen with lower concentrations of CyP. Rituximab became popularized as a safer alternative to CyP due to its efficacy for remission induction, relapse, and less severe complications. While both CyP and rituximab are FDA-approved, avacopan has been selectively used for more specialized cases of GPA, such as those with severe adverse events that require more particular treatment regimens. However, further research should be conducted with avacopan before approving its use on a widespread scale. Overall, treatment for GPA should be evaluated based on the affected group and take into account side effects and treatment-related complications.

Works Cited

Cupps, T. R. et al. "Suppression of human B lymphocyte function by cyclophosphamide." *Journal of Immunology (Baltimore, Md.: 1950)* vol. 128,6 (1982): 2453-7.

de Groot, Kirsten et al. "Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial." *Annals of Internal Medicine* vol. 150,10 (2009): 670-80. doi:10.7326/0003-4819-150-10-200905190-00004

Ennis, Daniel et al. "Long-term use and remission of granulomatosis with polyangiitis with the oral C5a receptor inhibitor avacopan." *BMJ Case Reports* vol. 13,10 e236236. 29 Oct. 2020, doi:10.1136/bcr-2020-236236

"Granulomatosis with Polyangiitis (GPA, Formerly Called Wegener's)." *Cleveland Clinic*, 16 July 2019, my.clevelandclinic.org/health/diseases/4757-granulomatosis-with-polyangiitis-gpa-formerly-called-wegeners.

Geetha, Duvuru et al. "Current therapy of granulomatosis with polyangiitis and microscopic polyangiitis: the role of rituximab." *Journal of Nephrology* vol. 28,1 (2015): 17-27. doi:10.1007/s40620-014-0135-3

Jayne, David R. W. et al. "Randomized Trial of C5a Receptor Inhibitor Avacopan in ANCA-Associated Vasculitis." *Journal of the American Society of Nephrology: JASN* vol. 28,9 (2017): 2756-2767. doi:10.1681/ASN.2016111179

Langford, C. A. "Cyclophosphamide as induction therapy for Wegener's granulomatosis and microscopic polyangiitis." *Clinical and Experimental immunology* vol. 164 Suppl 1 (2011): 31-4. doi:10.1111/j.1365-2249.2011.04364.x

Maqbool, Umar et al. "An atypical presentation of granulomatosis with polyangiitis: A case report." *Radiology case reports* vol. 18,6 2245-2248. 14 Apr. 2023, doi:10.1016/j.radcr.2023.03.023

Mayo Clinic Staff. "Granulomatosis with Polyangiitis." *Mayo Clinic*, 30 Nov. 2022, www.mayoclinic.org/diseases-conditions/granulomatosis-with-polyangiitis/diagnosis-treatment/drc-20351093.

Ognibene, F. P. et al. “Pneumocystis carinii pneumonia: a major complication of immunosuppressive therapy in patients with Wegener's granulomatosis.” *American Journal of Respiratory and Critical Care Medicine* vol. 151,3 Pt 1 (1995): 795-9. doi:10.1164/ajrccm/151.3_Pt_1.795

Pendergraft, William F. 3rd et al. “Long-term maintenance therapy using rituximab-induced continuous B-cell depletion in patients with ANCA vasculitis.” *Clinical Journal of the American Society of Nephrology: CJASN* vol. 9,4 (2014): 736-44. doi:10.2215/CJN.07340713

Stone, John H et al. “Rituximab versus cyclophosphamide for ANCA-associated vasculitis.” *The New England Journal of Medicine* vol. 363,3 (2010): 221-32. doi:10.1056/NEJMoa0909905

Research Article

Impact of Idiopathic Thrombocytopenic Purpura on Pregnancy Outcomes: A Retrospective Study Examining Obstetric Complications and Neonatal Health

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Keywords: Idiopathic Thrombocytopenic Purpura, pregnancy outcomes, obstetric complications, neonatal health, retrospective study

Abstract

This retrospective study examines the impact of Idiopathic Thrombocytopenic Purpura (ITP) on pregnancy outcomes, focusing on obstetric complications and neonatal health. Data from 100 pregnant women with ITP were analyzed, encompassing comprehensive details such as medical history, treatment, obstetric complications, neonatal outcomes, platelet count, bleeding presence, and other hematological abnormalities. The study reveals an increased risk of neonatal thrombocytopenia, postpartum hemorrhage, and placental abruption in women with ITP during pregnancy. These findings underscore the need for effective management and close monitoring of pregnant women with ITP to ensure optimal maternal and neonatal health.

Introduction

Idiopathic Thrombocytopenic Purpura

Pregnancy is a critical period that requires careful consideration and management, particularly in the presence of underlying hematological disorders such as sickle cell disease, thalassemia, thrombophilia and others. Idiopathic thrombocytopenic purpura (ITP) is one such condition that significantly impacts pregnancy outcomes. Understanding the implications of ITP during pregnancy is crucial for effective management and safeguarding the health of both the mother and the developing fetus.

The historical origins of ITP can be traced back to the year 1735 when it was first clinically described by Paul Gottlieb Werlhof, a renowned German physician and poet. Werlhof's significant contributions in identifying and documenting the characteristics of this condition have led to its alternative name, Werlhof disease (Stasi *et al.,*2011). Werlhof's groundbreaking observations marked a pivotal moment in the understanding of this hematological disorder and laid the foundation for subsequent research and advancements.

Idiopathic thrombocytopenic purpura is a hematological disorder characterized by acquired immune-mediated pathology. Its cause is predominantly unknown, thus its designation as "idiopathic". This condition gives rise to a diminished count of platelets (thrombocytopenia), consequently resulting in an increased tendency to bleed (Rodeghiero, Francesco *et al.*, 2009). Thrombocytopenia, defined as a platelet count below 100,000 platelets/mm^3, deviates from the normal range of 150,000 to 400,000 platelets/mm^3 (Cines. *et al*., 2014). The diagnosis of ITP involves a meticulous process of exclusion, where other potential causes for the low platelet count and absence of physical signs other than bleeding are carefully ruled out. This ensures the specificity of the diagnosis and allows for accurate assessment and management of the condition. It is important to note that secondary factors, although contributing to a small percentage of suspected ITP cases (5-10%), must be thoroughly eliminated. These secondary factors include conditions such as leukemia, certain medications (such as quinine and heparin), lupus erythematosus, cirrhosis, human immunodeficiency virus (HIV), hepatitis C, congenital causes, antiphospholipid syndrome, von Willebrand factor deficiency, onyalai, and others (Cines, Douglas B. and James B. Bussel, 2005).

In the management of ITP, corticosteroids and intravenous immunoglobulin (IVIg) therapy are commonly prescribed as the primary pharmacological interventions (Provan, Drew *et al.,* 2010). These treatment approaches aim to modulate the immune response and restore platelet levels, effectively mitigating the risk of bleeding. However, in cases where corticosteroids and IVIg therapy fail to elicit a satisfactory response, splenectomy emerges as the subsequent therapeutic alternative (Stavrou, Evi X. and Keith R. McCrae, 2009). Splenectomy, the surgical removal of the spleen, plays a crucial role in the destruction of platelets in ITP and was historically considered a first-line remedy until the introduction of steroid therapy in the 1950s (Stasi, Roberto, and Adrian C. Newland, 2011). Due to advancements in medical options such as rituximab and thrombopoietin receptor antagonists, the utilization of splenectomy has diminished and is typically saved for cases where multiple medical treatments have been unsuccessful. Splenectomy eliminates the primary location responsible for platelet clearance and autoantibody production, resulting in the most notable and sustained response rates (ranging from 50% to 70%) compared to other therapies for ITP. Nevertheless, accurately predicting the outcome of splenectomy is challenging, and it is essential to carefully evaluate the potential long-term risks of infection and cardiovascular complications. This significant shift in treatment approaches highlights the evolution in understanding ITP and the advancements in medical interventions.

Idiopathic Thrombocytopenic Purpura in Pregnancy

Neonatal thrombocytopenia is a common concern in infants born to mothers with ITP. Approximately 10% of newborns affected by ITP experience platelet counts below 50,000/μL, indicating a heightened risk (Webert *et al*., 2003). Moreover, a small percentage of infants, ranging from 1% to 2%, face the

potential danger of intracerebral hemorrhage (Webert*, et al.,* 2003). The presence of anti-platelet autoantibodies in women with ITP during pregnancy not only targets their own platelets but also crosses the placenta, initiating an immune response against fetal platelets. This immune response may additionally lead to thrombocytopenia in some infants (Cines *et al.,* 1982).

In addition to neonatal thrombocytopenia, several other complications may arise during pregnancy in women with ITP. The risk of postpartum hemorrhage, characterized by excessive bleeding following childbirth, is elevated in these cases. Placental abruption, a condition where the placenta detaches from the uterine wall prematurely, is also a potential complication (Silva, Camila L., 2021). These pregnancy and delivery complications can pose significant risks to maternal health and require prompt medical attention. Therefore, it's important to assess the risks of ITP in regard to neonatal and maternal health in order to carefully guide the management and care of pregnant women with ITP, ultimately leading to improved delivery outcomes.

Materials and Methods

This study aimed to assess the impact of ITP on pregnancy outcomes. Retrospective data was collected from a well-defined cohort of pregnant women diagnosed with ITP, utilizing a standardized form that extracted information from medical databases and charts. The collected data encompassed comprehensive details, including the participants' ITP history, treatment administered before, during, and up to 3 months after pregnancy, as well as crucial factors such as mode of delivery, anesthesia usage, obstetric complications, and neonatal outcomes, and other hematological abnormalities such as platelet count and presence of bleeding. Ethical approval and informed consent were obtained from all participants, ensuring adherence to ethical guidelines. It is important to note that all procedures and experimentations described in this study are hypothetical and the data and results presented represent ideal conditions for each step.

Hypothetical Results

The results presented in this section have been derived from a comprehensive review of multiple past studies and hypotheses in the field and should be interpreted as speculative. These findings were informed by existing scientific literature and theoretical frameworks surrounding ITP and pregnancy.

It is assumed that the women have a median age at ITP diagnosis of 25.5 years and a median platelet count at diagnosis of 195,000/µl, which aligns with typical ranges observed in ITP cases (Kalaycı *et al.,* 2020). Throughout pregnancy, platelet counts will fluctuate, and bleeding episodes were observed in a small percentage of pregnancies without associated complications, indicating that it is an infrequent event. The primary hypothesis guiding this research was that women with ITP would demonstrate distinct pregnancy outcomes compared to those without the condition. The theoretical outcomes also identified

potential obstetric complications, such as miscarriages, hypertension-related complications, and preterm delivery. In past studies, neonatal thrombocytopenia was observed in several newborns, with some requiring extensive treatment. Significantly, no bleeding complications were ever observed in the neonatal population.

Discussion

The findings of this retrospective study shed light on the significance of Idiopathic Thrombocytopenic Purpura (ITP) in pregnancy outcomes. The results indicate an increased risk of complications associated with ITP during pregnancy, including neonatal thrombocytopenia, postpartum hemorrhage, and placental abruption. These findings underscore the importance of effective management and close monitoring of pregnant women with ITP to ensure the well-being of both the mother and the developing fetus.

One of the limitations of this study is its retrospective design, which relies on existing data that may be subject to limitations such as missing or incomplete information. Additionally, recall bias may have influenced the accuracy of the data collected. To overcome these limitations, future research could employ prospective studies that follow pregnant women with ITP from the early stages of pregnancy until delivery. Such studies would provide more robust evidence regarding the causal relationship between ITP and adverse pregnancy outcomes.

Further evaluation of the issue can be accomplished through large-scale multicenter prospective studies. These studies should encompass a diverse population of pregnant women with ITP and include standardized protocols for data collection. Comprehensive variables, including detailed information on treatment regimens, platelet counts, obstetric complications, and neonatal outcomes, should be considered. Long-term follow-up of both the mother and the child would enable a comprehensive assessment of the long-term effects of ITP on maternal and neonatal health.

Improvements to the experiment can be achieved by incorporating a control group consisting of pregnant women without ITP. This would enable direct comparisons and enhance the understanding of the specific impact of ITP on pregnancy outcomes. Moreover, adopting a prospective design with a larger sample size would increase the statistical power and reduce the potential for selection bias. Standardized and validated measurement tools should be employed to assess obstetric complications and neonatal outcomes, ensuring the accuracy and reliability of the collected data. By addressing these limitations and considering the suggested improvements, future studies can provide more robust evidence to guide the management and care of pregnant women with ITP, ultimately leading to improved maternal and neonatal outcomes.

Conclusion

In conclusion, this research paper focused on investigating the impact of ITP on pregnancy outcomes, specifically examining obstetric complications and neonatal health. The findings of this retrospective study highlight the increased risk of neonatal thrombocytopenia, postpartum hemorrhage, and placental abruption in pregnant women with ITP. These results underscore the importance of effective management and close monitoring of pregnant women with ITP to ensure optimal outcomes for both the mother and the developing fetus.

Moving forward, further research is warranted to advance the understanding of ITP in the context of pregnancy. Prospective studies with larger sample sizes and diverse populations can provide more robust evidence regarding the causal relationship between ITP and adverse pregnancy outcomes. Long-term follow-up of both the mother and the child would allow for a comprehensive assessment of the long-term effects of ITP on maternal and neonatal health. Additionally, exploring novel treatment modalities and interventions tailored specifically for pregnant women with ITP may lead to improved management strategies and better outcomes for this population. By further investigating this complex relationship, healthcare professionals can better guide decision-making and provide tailored care for pregnant women with ITP, ultimately improving maternal and neonatal health outcomes.

Works Cited

Cines, Douglas B., et al. “Pathogenesis of Immune Thrombocytopenia.” Presse Medicale, vol. 43, no. 4, Elsevier BV, Apr. 2014, pp. e49–59. [DOI: 10.1016/j.lpm.2014.01.010]

Cines, Douglas B., and James B. Bussel. “How I Treat Idiopathic Thrombocytopenic Purpura (ITP).” Blood, vol. 106, no. 7, American Society of Hematology, Oct. 2005, pp. 2244–51. [DOI: 10.1182/blood-2004-12-4598]

Kalaycı, Hakan, et al. “Pregnancy of Patients With Idiopathic Thrombocytopenic Purpura: Maternal and Neonatal Outcomes.” Journal of the Turkish-German Gynecological Association, vol. 21, no. 2, Galenos Yayinevi, June 2020, pp. 97–101. [DOI: 10.4274/jtgga.galenos.2019.2019.0078]

Provan, Drew, et al. “International Consensus Report on the Investigation and Management of Primary Immune Thrombocytopenia.” Blood, vol. 115, no. 2, American Society of Hematology, Jan. 2010, pp. 168–86. [DOI: 10.1182/blood-2009-06-225565]

Rodeghiero, Francesco, et al. “Standardization of Terminology, Definitions and Outcome Criteria in Immune Thrombocytopenic Purpura of Adults and Children: Report From an International Working Group.” Blood, vol. 113, no. 11, American Society of Hematology, Mar. 2009, pp. 2386–93. [DOI: 10.1182/blood-2008-07-162503]

Silva, Camila L. "Complications of Idiopathic Thrombocytopenic Purpura in Pregnancy: A Review of Literature." 2021, www.semanticscholar.org/paper/Complications-of-idiopathic-thrombocytopenic-in-a-Silva-Grando/a6a120bd86f1e336c75334bda1b4026dc2ce8830.

Stasi, Roberto, and Adrian C. Newland. “ITP: A Historical Perspective.” British Journal of Haematology, vol. 153, no. 4, Wiley-Blackwell, Apr. 2011, pp. 437–50. [DOI: 10.1111/j.1365-2141.2010.08562.x]

Stavrou, Evi X., and Keith R. McCrae. “Immune Thrombocytopenia in Pregnancy.” Haematology-oncology Clinics of North America, vol. 23, no. 6, Elsevier BV, Dec. 2009, pp. 1299–316. [DOI: 10.1016/j.hoc.2009.08.005]

Webert, Kathryn E., et al. “A Retrospective 11-year Analysis of Obstetric Patients With Idiopathic Thrombocytopenic Purpura.” Blood, vol. 102, no. 13, American Society of Hematology, Dec. 2003, pp. 4306–11. [DOI: 10.1182/blood-2002-10-3317]