Major Neurodegenerative Diseases: A Literary Review of Cellular Immunotherapy in Alzheimer's, Parkinson's, and Huntington's Diseases

By

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Abstract

This thesis paper will focus on the most prominent applications in recent research of immunotherapy in neurodegenerative diseases (specifically Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease) with a focus on the cellular level on inflammatory pathways (such as, but not limited to: NFkB, JNK and anti-inflammatory pathways such as NRf2) present in the human body.

The literary analysis will begin with a basic overview of some of the major homeostatic pathways present in cellular organelles within the human body (such as the Unfolded Protein Response/UPR, present in the endoplasmic reticulum of eukaryotes) and how they normally act within a healthy human body. The paper will then delve deeper into how these cellular processes are altered, including the root causes of these neurodegenerative diseases commonly researched today, and what mutations in the normal homeostatic processes mean for the phenotypic display of these illnesses (how symptoms appear).

The final parts of this literary analysis thesis paper will delve into current research methodologies being performed in the neurodegenerative disease therapy field and how immunotherapy is quickly becoming a lead topic of interest amongst researchers and universities and the benefits (and negatives, if any discovered yet) of using immunotherapy compared to the alternative (and maybe currently less effective) therapeutic treatments.

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Inflammation within Homeostatic Parameters

One of the first responses to infection is inflammation. This can be in the form of external inflammation such as puffy eyes, closed sinuses, and a runny nose or internal inflammation such as the accumulation of lymphocytes in a local lymph node, causing swelling. Neurodegenerative diseases such as Alzheimer's Disease (AD), Parkinson's Disease (PD), and countless others have internal inflammation as an integral part of their pathology, which is often associated with neuronal cell death in the central and peripheral nervous systems.

Inflammation is often a sign of a healthy immune system, contrary to the unregulated propagation and damage caused by irregular, chronic inflammation present in neurodegenerative diseases. Regular inflammation is a means by which the human body creates an environment inhospitable by microbes, such as bacteria or viral particles. The most apparent physical representation of inflammation in the body is a fever, the human body's method of increasing the blood pressure to allow the delivery of immune cells to the site of infection more rapidly and efficiently.

A fever, however, is a response that is largely the first resort, meaning that it is a natural defense considered to be part of the innate immune system's response to foreign invaders/bodies. The entire immune system is split into two divisions: the innate and the adaptive. These two divisions are still largely intertwined and immunologists today still don't really have an explanation for all the ways that the two parts of our immune systems work together. Fever is only one example of inflammation that occurs in the body. Inflammatory responses related to fever, when not

properly regulated by the body's homeostatic mechanisms, can lead to the neurodegenerative diseases later explained in this thesis.

The Adaptive and Innate Immune Systems

The innate immune system is crude. It is considered the first line of defense against infection. Individuals are born with their innate immunity, and it does not change or alter throughout one's life. Innate immunity does not learn from microbe to microbe, it only acts on a set of predetermined instructions for each foreign invader. The innate immune system includes phagocytes (such as the macrophages that come up and eat away dead cells/tissue after infection), natural killer cells (cells that are key in the natural defense against tumorous cancer cells), chemical factors (such as pH and secreted fatty acids), Pathogen Associated Molecular Patterns (PAMPs) (Toll-like receptors, set of instructions that innately recognize parts of bacteria/viral particles), natural skin barriers (skin, mucous), neutrophils and mast cells. The innate immune system does not have a memory like the adaptive immune system, which means it does not learn from repeated exposures on how to better react to the same antigen/foreign body. It is non-specific and reacts generally to any foreign entities recognized as non-self by the immune system.

The adaptive immune system is the more complex of the two and is present only in vertebrates. It is composed of both B cells and T cells (B and T lymphocytes), both of which are antigen-specific and only react to very specific foreign substances. When compared to the innate immune system, which only takes minutes to hours to respond to infection, the adaptive immune

system requires days to weeks to fully react to infection from foreign bodies. Antigen-specific cells (B cells and T cells that have recognized an antigen) have a more rigorous response time. Mature memory B lymphocytes recognize a specific antigen or part of a foreign body and generate antibodies via clonal expansion. By the creation of these antibodies, which recognize and tag these antigens for removal innate immune system, the adaptive immune system develops a memory-like response to these antigens if exposed again to them in the future. It is the mechanism of creation of antibodies by memory B cells that allows for vaccines to be so effective. The adaptive immune system can be mediated by the humoral response (B lymphocyte response), the cell-mediated response (T lymphocyte response), antigen-presenting cells (APCs, such as B lymphocytes, dendritic cells, and macrophages), natural killer cells (NK cells), neutrophils, and mast cells. This is why immunologists often say the two divisions of the immune system are so interlinked; it is common and expected for parts of the innate immune system to affect and help the adaptive immune system, whether it be in the form of antigen-presenting cells present in its division such as macrophages or by the early response of neutrophils that lessen the strength of the infection. Even in cancerous cells or in serious infections (such as the herpes simplex virus), the natural killer cells of the innate immune system can act as a backup system for when viruses or tumor cells block the ability of T lymphocytes from recognizing its antigens and attacking them. Since natural killer cells are not a part of the adaptive immune system, they are not affected by these types of tricks on the adaptive immune system.

In a normal, healthy individual, immune cells such as the ones listed above cannot cross the blood-brain barrier. The brain heavily relies on microglia, a type of neuroglia cell that supports neurons in the brain. These microglia act as the 'immune system' for the cerebrospinal fluid (CSF) by sweeping the area of debris and serving as the line of defense for surrounding neuroglia and neural bodies. It is important to understand how the immune system both in the body and in the brain work on a regular basis in order to best showcase how neurodegenerative diseases stray from the norm.

Cytokines

Cytokines are proteins secreted by a broad range of cells within the human body and are the main factors that cause inflammatory responses, both internally and externally. They are produced when immune cells such as macrophages, neutrophils, T-cells, or B-cells are triggered by external factors or proteins that bind to their cell surface. An example of one of these external factors is Lipopolysaccharide (LPS), which is a major component in the outer membrane of gram-negative bacteria, which is recognized by Toll-like receptors (PAMPs) in innate cells.

Another example of one of these external factors is the protein Amyloid-beta. When folded properly and present in the brain at balanced amounts, the Amyloid-beta protein triggers cytokine production at a rate that doesn't disrupt the homeostatic balance present in the brain that is needed for effective communication between immune cells and neurons alike. However, in AD patients, this protein is misfolded/present in too high amounts, causing aggregation of it in thick, plaque-like structures between neurons. These plaques effectively block and severely limit

cell-cell communication. This block of communication eventually leads to major neuronal cell death, leading to dementia in AD patients. Many developing therapeutics today measure the release and expression of cytokines between immune cells to determine the strength of an immune response in both *in vitro* and *in vivo* models to best predict the way the human immune system would react to up and coming therapeutic drugs.

These cytokines are produced in the rough endoplasmic reticulum (RER) of the cell. In the RER, they are folded, later altered post-translationally in the Golgi, and then sent to be released by the cell. Some of the major cytokines involved in inflammatory immune responses include (but are not limited to) the interleukins (such as interleukin-6, IL-6 or interleukin-8, IL-8), interferon-γ (IFN-γ), and tumor necrosis factor-β (TNF-β).

Major Inflammatory and Anti-Inflammatory Cellular Pathways (NF-κB, JNK, NRf2, etc.)

Cell signaling pathways that moderate control over gene transcription and cytokine production such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), JNK c-Jun N-terminal kinases (JNK), and nuclear factor erythroid 2–related factor 2 (NRf2) are just a few of the pathways present in cells that have an important role in inflammation.

As far as NF-κB and JNK, NF-κB activation can have pro-survival, antiapoptotic effects while JNK signaling has been implicated in the induction of either cell proliferation or apoptosis (Nikolaou, K. *et al.,* 2013). Short-term activation of JNK pathway induces genes involved in proliferation control of the cell, specifically in the proliferation of cyclin D1, a protein required

for progression through the G1 phase of the cell cycle (Nikolaou, K. *et al.,* 2013). Both cell-signaling pathways are pro-inflammatory in nature and have been shown to work in tandem. An example of this relationship between the two pathways is their vital role in cancer research in the determination the cellular factors contributing to inflammation resulting in hepatocellular carcinoma (HCC), which is the third most common cause of cancer-related mortality worldwide as well as the most extensively investigated inflammation-based carcinogenic process (Nikolaou, K. *et al.,* 2013).

While NF-κB has been heavily researched for its role in inflammation leading to cancer, NRf2 has played a major role as an anti-inflammatory cell-signaling pathway in the same field. In a normal body, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are accumulated as a result of the many metabolic processes present within cells, such as proteasomal activity (breakdown of proteins). ROS and RNS contribute to oxidative stress and when not properly taken care of can lead to chronic inflammation and eventually cancer/tumorigenesis, neurodegenerative diseases, and more. NRf2 is a cell-signaling pathway that has been found to combat the accumulation of ROS and RNS in the cell by protecting the cell by binding to specific antioxidant response elements (AREs) in the promoter of antioxidant enzymes as well as defense protein genes (Walters, D. M. *et al.,* 2008).

While these cell-signaling pathways are an integral part of the cell's homeostatic processes, they can easily become a source of problems instead. An example of this is NRf2 being possibly utilized by cancer cells or tumors as a protective measure against anti-cancer drugs, creating the

perfect environment for these tumors to grow and eventually metastasize (Kensler, T. W. *et al.,* 2010). Although JNK has been seen to work with NF-κB in cancer research surrounding HCC, chronic JNK activation induces a Bax/Bak-dependent apoptotic pathway, via the mitochondrial release of cytochrome c (Nikolaou, K. *et al.,* 2013). This means that although activation of JNK helps to propagate the activation of NF-κB and pro-survival pathways, continuous and abnormal activation of JNK is seen to lead to apoptosis of the cell, leading to inflammation and liver fibrosis.

While it is important to note that inflammation can lead to negative consequences such as chronic inflammation, cancer, and a host of other ailments, inflammation is normally a regular part of the cell's homeostasis. Without inflammation, the cell is not living and not undergoing metabolic processes. It is when these inflammatory and anti-inflammatory cell-signaling pathways are not properly working in conjunction with each other and the genes that induce them that issues arise in the cell, disrupting homeostasis and acting as precursors to disease.

Precursors to Chronic Inflammation

While there are many factors that compose the precursors to developing chronic inflammation, such as environment or genetic predisposition, there are biological processes on the cellular level that serve as clear indicators for possible future issues with the homeostatic balance of inflammation within the body.

Unfolded Protein Response (UPR)

The Unfolded Protein Response (UPR) is a natural regulatory process within the RER of cells. The UPR is activated when the lumen of the RER is placed under significant amounts of stress due to accumulating quantities of misfolded proteins amongst other factors, resulting in the RER entering a condition of endoplasmic reticulum (ER) stress.

The initial objective of the UPR is to reestablish homeostasis and alleviate ER stress through two mechanisms: increasing folding capacity via expression of protein-folding chaperones and downregulation of ER protein client load via inhibiting general protein translation and promoting the degradation of misfolded proteins (Ozcan, L. *et al.,* 2012). There are many chaperones that are present within the endoplasmic reticulum. One example of a major protein folding chaperone is 78-kDa glucose-regulated protein (Grp78), also known as binding immunoglobulin protein (BiP). The UPR serves as a type of safe-brake for the RER, resorting to the degradation of proteins that cannot be properly folded after multiple attempts and eventually apoptosis (programmed cell death) if the UPR is prolonged and the cell cannot settle back into homeostasis.

There are three major branches of UPR, which are each activated by a dedicated ER-localized transmembrane molecule: protein kinase RNA-like ER kinase (PERK); inositol-requiring protein–1 (IRE1); and activating transcription factor–6 (ATF6) (Ozcan, L. *et al.,* 2012). These pathways are discussed below in the relative depth needed to understand the basic implications

of chronic stress or inflammation on the RER and its later discussed role in neurodegenerative diseases.

Out of the three pathways by which the UPR is activated, IRE1 is the most heavily referenced in literature utilized. IRE1 is usually associated with the BiP/Grp78 chaperone protein in the normal environment of the RER, and it is when the RER undergoes significant stress that IRE1 is dissociated from BiP and activated via trans-autophosphorylation. This activation of the IRE1 pathway leads to the mRNA translation of X-box binding protein 1 (XBP1), a transcription factor which upregulates the genes in the UPR that simultaneously code for higher RER protein folding capacity and an expanded RER membrane surface for folding. IRE1 has also been found to show associating with TNF receptor-associated factor-2 (TRAF2), which promotes the JNK pathway. The JNK pathway, as mentioned before, is a major cell-signaling pathway involved in apoptosis as well as the inflammatory response in cells and a major contributor to chronic inflammation present in neurodegenerative diseases.

The PERK pathway of the UPR response is a serine-threonine kinase. Like the IRE1 pathway, PERK is also activated by trans-autophosphorylation. The activated PERK pathway phosphorylates eukaryotic translation initiation factor 2 alpha (eLF2α), resulting in a reduced RER protein load by promoting the translation of activating transcription factor-4 (ATF4) and inducing the activity of C/EBPα-homologous protein, also known as GADD153 (CHOP). CHOP expression is similar to JNK activation in that its prolonged activity can trigger apoptosis due to chronic stress in the RER.

Out of the three major pathways of the UPR, ATF6, a basic leucine zipper transcription factor, is the only pathway that directly involves the Golgi complex during its activation. The complex of ATF6 is moved to the Golgi to be cleaved by Site 1 and Site 2 proteases that break it down enough to be used as a new, resultant transcription factor. This new transcription factor is returned to the nucleus of the cell experiencing RER stress to upregulate the genetic expression of chaperone proteins like BiP/Grp78.

Aggresome/Plaque Formation

When the UPR response is not enough to mitigate the large amounts of misfolded proteins accumulating in the lumen of the RER, plaques of proteins may form. These plaques form because while folding, the misfolded protein may assume a three-dimensional structure that exposes its hydrophobic amino acid side chains to the outside, hydrophilic environment. This causes misfolded proteins with their hydrophobic side chains exposed as well to aggregate together, much like oil does in water. Often, there are biological mechanisms in place to prevent such plaques from forming, such as chaperone proteins (i.e. BiP/Grp78) separating these misfolded proteins as a function of the UPR. It is when the RER is under immense stress and the UPR fails that these plaques accumulate at a rate and size that is not easily prevented or corrected by protein chaperones.

These plaques, termed aggresomes (Johnston, J. *et al.,* 1998), can have a long-lasting negative effect on the RER by making the stress that activated the UPR pathways in the first place to

heighten. When proteasome activity (one of the responses of the UPR to degrade misfolded proteins that cannot be folded properly) was inhibited, these plaques were shown to accumulate even more (Johnston, J. et al., 1998).

This plaque formation due to heightened misfolded protein accumulation and the failure of the UPR to mitigate these symptoms occurs in many, if not all, neurodegenerative diseases.

An Overactive Immune System

In the case of many neurodegenerative diseases, plaque formation, when not controlled by the UPR or proteasomal activity properly, irritates neuroglial populations present within the brain. This irritation results from plaques hindering cell-cell signaling, causing a response from microglial populations.

Microglial cells are macrophage derivatives and act as the ‘clean-up crew’ for the brain, sweeping debris from the surrounding area after a successful defense. Plaque formation triggers microglial activation, which triggers the release of inflammatory cytokines such as IL-6, tumor-necrosis factor alpha (TNF-α), etc. In normal amounts, these cytokines simply act as ways of communication between neuroglial cells. However, when the microglial population surrounding an area is consistently activated due to the accumulation of aggresomes, the cytokines can cause chronic inflammation of the surrounding area, inflaming neural bodies and causing physical damage to both neuroglia and neuron cells alike. It is this mechanism that is often a part of the pathology of many neurodegenerative diseases, such as AD or PD. It is also

this area that is of intense focus in the field of immunotherapy when researching the many factors causing neurodegenerative diseases.

Long-Term Chronic Inflammation (Neuroinflammation) is a Topic of Concern in Neurodegenerative Diseases

Chronic inflammation contributes to a whole host of health issues, including but not limited to cancer, autoimmune disorders, and neurological disorders. Even some viruses, such as herpes simplex-1, are linked with chronic inflammation. Major neurodegenerative diseases are heavily associated with chronic inflammation, such as AD and other dementias, PD, Huntington's Disease (HD), Amyotrophic lateral sclerosis (ALS), Motor Neuron disease, and a whole host of others.

All these diseases have the disruption in the homeostasis of cell-signaling pathways, genes, and regular inflammation in common, resulting in the breakdown of neural bodies. While only AD, PD, and HD are discussed in this literary analysis, many of the immunotherapeutic techniques mentioned have good future prospects in many, if not all, neurodegenerative diseases.

Alzheimer's Disease (AD)

AD is a neurodegenerative disease characterized by the formation of amyloid-beta plaques (aggresomes) and tau tangles surrounding neural bodies within the brain.

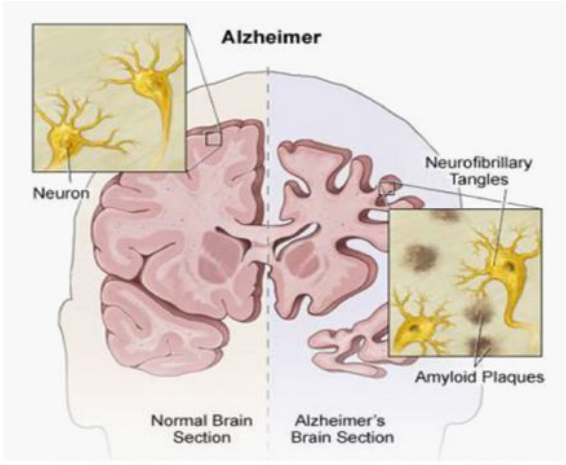

Figure 1: This Photo by Dario Rusciano is licensed under CC BY-NC. Image source: Rusciano, D. (2014). Critical Review on the Relationship between Glaucoma and Alzheimers Disease. *Advances in Ophthalmology & Visual System, 1*(4). doi:10.15406/aovs.2014.01.00024

In a healthy brain, neuron cell-cell signaling progresses normally, allowing for a regulated environment within the CSF. In patients with AD or other forms of dementia, this environment is slowly riddled with neurofibrillary (tau) tangles and amyloid-beta plaques over time. These amyloid-beta and tau aggresomes result in a disruption of the cell-cell signaling of neurons which eventually leads to the breakdown of the neural bodies and, in later stages of AD and other forms of dementia, neuron cell death (Figure 1).

Normally, the tau and amyloid-beta proteins are folded correctly within the brain and do not pose an issue. It is when they are folded improperly, with their hydrophobic side chains exposed so that they cluster together to form rafts within the CSF, that an issue such as dementia can eventually arise. This clustering of proteins causes chronic inflammation within the brain, resulting in a hyperactive microglia population and the release of more inflammatory cytokines by these neuroglia, such as IL-6. The overcompensation of cytokine release in an effort to communicate with other microglia for the need to clean the ‘debris’ (the plaques and tangles) results in a chronically irritated immune system. This only propagates the issue of chronic inflammation further and leads to the worsening of dementia/AD symptoms, including but not limited to memory loss.

Alzheimer's Disease: Immunotherapeutic Advances

Many of the immunotherapeutic approaches to AD include passive immunization, active immunization, the use of microglia to sweep debris and target misfolded amyloid-beta plaques and tau tangles, as well as the administration of neuroprotective molecules (derived usually from plant sources).

Passive immunization, as explained before, is the placement of readily-made antibodies towards a specific antigen placed into the host/individual to be immunogenized. Active immunization involves the body's cells direct reaction and creation of antibodies within the body to antigens presented by APCs such as B lymphocytes, which elicit a reaction from T lymphocytes (either CD4 T-helper cells or CD8 cytotoxic T-cells). In the first study of anti-amyloid beta immunization ever, transgenic mice models of AD were immunized against amyloid-beta via a vaccine that supported both passive and active immunization, meaning that antibodies were readily in the vaccine as well as antigens of amyloid-beta to allow for the mice's immune system to create their own antibodies against the molecule (Schenk *et al.,*1999). The use of both active and passive immunization is a prevalent area of research when it comes to immunotherapy of AD despite frequently running into new health concerns that appear during later stages of *in vivo* human studies. An example of this issue is a phase II clinical trial utilizing active immunization against amyloid-beta that was discontinued because of the development of severe meningoencephalitis (meningitis of the brain) in 6% of the patients (Hung *et al.,* 2017). In recent clinical trials, anti-amyloid beta monoclonal antibodies are being used (such as solanezumab) against amyloid-beta plaque aggresomes in AD patients and seen to have some improvement in

the cognition in those with mild AD symptoms (Rygiel, 2016). This clinical trial with solanezumab unfortunately did not meet its primary endpoint in phase III and is now being tested in a prevention study with older subjects who show amyloid-beta deposits but no AD symptoms (Hung *et al.,* 2017). Other drugs, such as crenezumab, a monoclonal antibody with a higher affinity for oligomeric/fibrillar amyloid-beta plaques than the usually asymptomatic monomeric amyloid-beta, are in Phase III clinical trial studies and show promise for treatment of amyloid-beta aggresomes via passive immunization (Hung *et al.,* 2017). One drug currently still active in phase III clinical trials, aducanumab, has demonstrated an ability to significantly reduce the number of amyloid-beta aggresomes in AD patients (Sevigny, J. *et al.,* 2016). Targeting neurofibrillary tangles (NFTs; hyperphosphorylated tau aggresomes) is also a route of interest. However, most of the current clinical trials on clearing tau tangles involve active immunization, an example being the AADvac1 drug (currently in phase 2 of clinical trials), which aims to treat patients with mild Alzheimer's disease (Panza, F. *et al.,* 2016).

Anti-tau passive immunization could be a potential path of AD immunotherapy in the future, but few studies have been done on anti-tau immunization that is not active immunization. That is not to say that all AD immunotherapy involving anti-tau treatment involve only active immunization. Passive immunization of tau tangles by the treatment of triple-transgenic AD mice with 43D and 77E9 antibodies against multiple forms of tau and amyloid-beta resulted in a reduced pathology of tau, hyperphosphorylated tau tangles, amyloid precursor protein (APP), and amyloid-beta aggresomes (Dai, C. *et al.,* 2017).

Other methods of passive immunization include plaque disaggregation, Fc receptor (FcR) mediated phagocytosis, and the Peripheral Sink Mechanism (Figure 2). All three of these

methods involve the injection of antibodies to the protein being targeted, whether it be hyperphosphorylated tau or amyloid-beta.

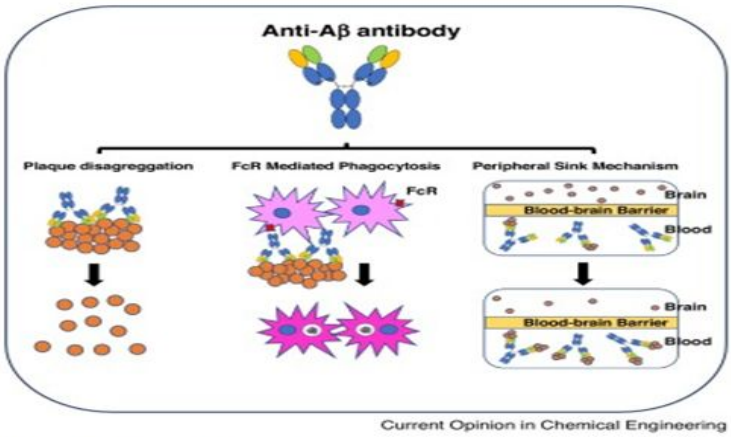

Figure 2: Routes of Passive Immunization in Neurodegenerative Disease Models (Anti-amyloid beta). Image Source: Montoliu-Gaya, L., & Villegas, S. (2018). Immunotherapy for neurodegenerative diseases: The Alzheimers disease paradigm. Current Opinion in Chemical Engineering,19, 59-67.

Plaque disaggregation involves Fab-fragment binding of anti-amyloid-beta to amyloid-beta protein and subsequent proteasomal degradation of these marked antigens. Inhibition of the formation of fibrillar, cytotoxic amyloid-beta aggresomes usually involves targeting a certain terminus of misfolded amyloid-beta species with monoclonal antibodies to discourage plaque aggregation. This method was used in the prevention of the formation of amyloid-beta aggresomes with antibodies that targeted the C-terminal of two forms of amyloid-beta (Montañés, M. *et al.,* 2013). With this method, it's important for researchers in the future to carefully consider exactly which terminus is selected for antibody formation. One study showed that an anti-amyloid antibody that targeted the N-terminus of amyloid-beta species encouraged the amyloid-beta to form into neurotoxic oligomer aggresomes rather than having the opposite, intended effect of alleviating such plaque formation (Liu, Y. *et al.,* 2015). FcR-mediated Phagocytosis involves the recruitment of microglial cells to amyloid-beta aggresomes plaques and degradation via activation of the FcR microglia receptor sites by IgGs, a subtype of antibody. The peripheral sink mechanism involves flushing/off-loading of CSF amyloid-beta

populations through the blood-brain barrier, where antibodies on the other side can mark the proteins for immune response. While the peripheral sink hypothesis is, in theory, a method that could allow for the treatment of AD symptoms without having to design medicine that is able to cross the blood-brain barrier, it has shown mixed results in practice. Some treatments utilizing the peripheral sink hypothesis work, and some do not. For instance, treatment of Tg2576 mice with enoxaparin, an anticoagulant medication, resulted in amyloid-beta aggresome pathology worsening, which was indicated by a significant increase in the amyloid-beta-42/amyloid-beta-40 ratios in the brain as well as encouraged aggresomes of both amyloid-beta species *in vitro* (Cui, H. *et al.,* 2016). Other studies show that the while the peripheral sink hypothesis can sometimes result in a decrease in amyloid-beta levels in the brain due to an inhibition of the gene β-secretase (BACE)1 via inhibitors in the peripheral immune system, the reduction is simply not enough to indicate that BACE1 inhibition is the rate-limiting enzyme for amyloid-beta degradation in the CSF or that the peripheral sink hypothesis is anything other that just that: a hypothesis (Georgievska, B. *et al.,* 2014). A similar study was performed analyzing the effect that neprilysin, an amyloid-beta degrading enzyme, had on amyloid-beta populations within the brain compared to the CSF (Henderson, S. J. *et al.,* 2013). This study also found that while the molecule studied was highly effective at decreasing the prevalence of amyloid-beta aggresomes in the periphery, it did not alter CSF amyloid-beta levels, therefore also disproving the peripheral sink hypothesis. While there is significant evidence that the peripheral sink hypothesis is currently nothing more than an ideal concept, there is still research being done on its possibility as a new immunotherapeutic strategy in AD in the future (Menendez-Gonzalez, M. *et al.,* 2018).

Another area of interest in terms of treatment of AD includes the use of neuroprotective molecules to combat neuroinflammation and decrease plaque aggresomes cytotoxicity in AD brain models. Asiatic acid (AA), a triterpene of a pennywort plant called Centella (*Centella asiatica*) has been shown to mitigate oxidative stress in the CSF of an *in vivo* $AlCl_3$-induced AD-model mice (Thenmozhi, A. J., 2018).

Parkinson's Disease (PD)

Parkinson's Disease (PD) is characterized by the loss of dopaminergic neurons within the Substantia nigra Pars Compacta (SnPC), a cluster of brain tissue that runs from the top of the pons in the spinal cord through the midbrain (Figure 3). The death of these dopaminergic neurons is caused by an increased presence of Lewy bodies-- aggresomes of a protein exclusive to neurons called α-Synuclein—within the CSF, leading to reduce dopamine production and a subsequent decrease in cell-to-cell communication between neurons within the SnPc (Kalia et al., 2015).

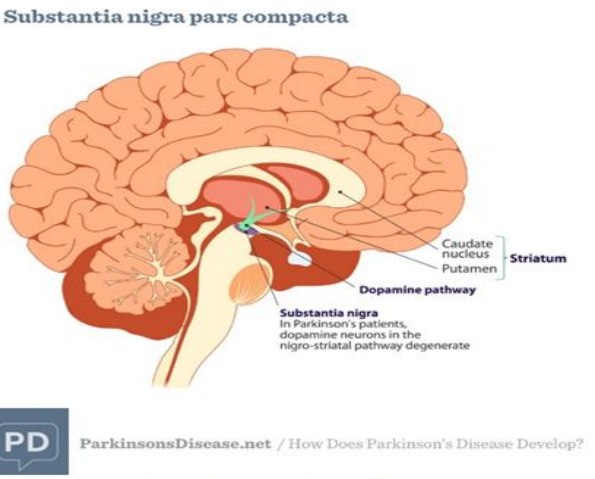

Figure 3: Substantia nigra pars compacta in the brain. **Image Source:** Downward, E. (n.d.). How Does Parkinson's Disease Develop? | Parkinson's Disease. Retrieved from https://parkinsonsdisease.net/basics/pathophysiology-what-is-it/

The natural response of astrocytes and microglia present in the brain is to try and clean out these aggresomes plaques of α-Synuclein to restore homeostasis within the CSF so that communication between the neurons through neurotransmitters is restored to normal. However, as is the case with most neurodegenerative diseases, this neuroinflammatory response can

sometimes exceed homeostatic control and become unregulated—overactive microglia and astrocytes can contribute to the accumulation of cytokine proteins within the brain's environment, further propagating the chronic inflammation resulting from the presence of Lewy bodies around dopaminergic neurons. This is due to the microglia's ROS pathway being overstimulated due to a high presence of misfolded proteins within the CSF, resulting in a higher presence of oxidative species, the activation of pro-inflammatory pathways like NF-κB and more cytokine production and neuroinflammation.

Parkinson's Disease: Immunotherapeutic Advances

The treatment of neuroinflammation is a major area of immunotherapy for Parkinson's disease. Nonsteroidal anti-inflammatory medicine (NSAIDs) has shown to have a statistically significant relation to a reduced risk of PD—a risk reduction of approximately 17%— via meta-analysis of published research studies (Noyce *et al.,* 2012). This indicates a relation between decreased inflammation or the treatment of possible inflammation that the precursors or risk factors of PD could cause within the SnPC by anti-inflammatory drugs could be a possible preventative measure (or treatment) for chronic inflammation resulting from PD-like symptoms.

Neuroinflammation is related to an increase in oxidative stress by the overactivation of the ROS cellular pathway in microglia. Mitochondrial dysfunction, which is also a symptom of PD, can result in an increase in oxidative stress due to the disruption of homeostatic parameters of calcium channels within the neuron cells. Urate (a salt of uric acid) is known to have antioxidant properties and has been shown to reduce oxidative stress from neuroinflammation and mitochondrial dysfunction in cellular models as well as reduce the oxidation of dopamine in the SnPC neurons within actual PD patients (Cipriani *et al.,* 2010). Urate was found to act as both a

biomarker (predictor of disease-like symptoms and development) for progression of PD but also as a neuroprotectant molecule for SnPC neurons within PD patients (Chen *et al.,* 2012).

Plant-based derivatives and alternatives as neuroprotective biomolecules is also a prevalent area of research. Mulberrin, a key component of a Chinese herb *Ramulus mori,* was used in both *in vivo* via the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to Sprague Dawley rats and *in vitro* via the administration of LPS as a positive control to BV-2 cells, an immortalized murine microglial cell line as a neuroprotector and was found to have suppressed both neuroinflammation and oxidative stress in both experiments (Cao *et al.,* 2019). Thymol, a phenol derivative of the spice thyme, was administered to a PD model (induced by rotenone, causing rotenone (ROT) induced neurodegeneration in mice) of Male Wister rats and found to have significantly reduced dopaminergic neuronal death, neuroinflammation, and oxidative stress in the rats via thymol's action as an antioxidant against ROT-induced neurotoxicity, amongst other mechanisms (Javed *et al.,* 2019). A similar *in vivo* experiment involved treating aged, ROT-induced PD model rats with kolaviron (a derivative of Garcinia kola seeds) indicated that treatment with kolaviron resulted in an improved mobility and coordination of the affected rats, exhibiting the derivative's neuroprotective, anti-inflammatory, and antioxidant properties (Farombi *et al.,* 2019).

Another area of immunotherapy research in PD involves studying the effects of the mutation of the leucine-rich repeat kinase 2 (LRRK2) gene, which is heavily involved in PD pathology. The G2019s LRRK2 mutation is the most common one, increasing kinase activity and resulting in neuroinflammation from the cytokines (inflammatory ones such as TNFα) produced from this kinase activation (Connor-Robson *et al.,* 2019). In a study with *in vitro* BV-2 microglia,

microglia cells were activated by LPS (as a positive control) and activated LRRK2 kinase, resulting in a significant increase in TNFα release and subsequent neuronal cell death (Ho *et al.,* 2019). In the same study but with *in vivo* GS mice models, TNFα levels in brains of GS mice was significantly higher than mice without the GS mutation; only when the LRRK2 kinase gene was inhibited was this increase in pro-inflammatory cytokines resolved. In another *in vivo* study involving LRRK2 G2019S transgenic mice, α-Synuclein aggregation was found to have increased, resulting in the death of dopaminergic neurons in the SnPC of the mice and increased neuroinflammation (and related loss of coordination) (Bieri *et al.,* 2019). In the same study, researchers created a PD-like model of α-Synuclein aggregation with iPS (Induced Pluripotent Stem cells) derived neurons (iNs) that showcased an increase in α-Synuclein protein aggregation with the presence of the G2019S mutation and a decrease in α-Synuclein aggregation when the LRRK2 gene was inhibited. G2019S LRRK2 has also been found to activate the MKK4-JNK-c-Jun pro-inflammatory cellular pathway in the SnPC, resulting in loss of SnPC dopaminergic neurons in a PD model of 12-16-month-old (aged) GS-LRRK2 transgenic mice (Chen *et al.,* 2012).

Targeting α-Synuclein protein aggregation itself is also a prominent area of interest regarding immunotherapy of PD. Measuring the UPR that is triggered in the ER due to an increased prevalence of misfolded α-Synuclein and using neuroprotective molecules to target folding chaperones such as Grp78 and BiP can contribute to a decrease in neuroinflammation resulting from a prolonged, chronic activation of the UPR (Enogieru *et al.,* 2019). Another approach that targets α-Synuclein is the use of α-Synuclein antibodies. α-Synuclein monoclonal antibodies (mAbs) were shown to reduce α-Synuclein formation of Lewy bodies in culture, resulting in a

decrease of neuronal cell death and inhibiting the spread of misfolded α-Synuclein pathology (Tran *et al.,* 2014). One review highlighted the importance of antibodies against misfolded α-Synuclein so that microglial cells in the CSF can degrade them and prevent these misfolded proteins from transferring extracellularly and progressing PD pathology (George, S. *et al.,* 2015).

Huntington's Disease

Huntington's Disease (HD) is a neurodegenerative disease characterized by chorea (uncontrolled and sporadic movement and loss of motor control), dystonia, and parkinsonism (Gövert, F. *et al.,* 2013). HD results in an abnormally high occurrence of trinucleotide repeats (TNTs) in the genome of the patient, resulting in significant differences in genetic expression in the form of changes in phenotypic display (motor function). These TNTs occur in CAG repeats, nucleotide base-pairs in DNA consisting of cytosine, adenine, and guanine that code for glutamine (Kim, S. D. *et al.,* 2014) (Figure 4). Huntington's disease is passed on from generation to generation via autosomal dominant inheritance, meaning that the disease mutation does not skip generations and is present in all homozygous dominant and heterozygous offspring.

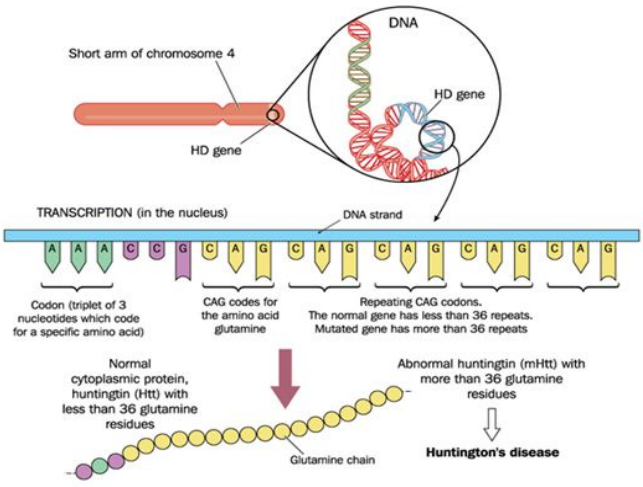

Figure 4: CAG Trinucleotide Repeats (TNTs) as pathology of Huntington's Disease (HD).

Image Source: National Institute of Health (NIH).

Huntington's Disease: Immunotherapeutic Advances

Much of the research done relating to immunotherapy for HD is done in conjunction with other major neurodegenerative diseases such as AD, PD, and ALS. Immunotherapy focused on

HD alone is a relatively new field, with many of the studies done so far being rather limited compared to that of other neurodegenerative diseases. The main areas of immunotherapy of HD include research done with neuroprotective molecules on neuronal cell bodies within the brain as well as passive immunization via Fab-fragment induced phagocytosis. Active immunization of mHTT research in HD is severely limited in quantity as well as results; the research done in this branch of immunization is rather superficial currently but has good prospects for the future.

As mentioned, studies done on neuroprotective molecules and their effect on mHTT are the more prevalent variety of immunotherapy for HD. Eicosapentaenoic acid (Ethyl-EPA), an omega-fatty acid, was a phase III clinical trial held in 2005 that only produced improved chorea score (amongst other scores) in HD patients with a CAG lower than 45 TNTs (Denis, H. *et al.,* 2018). Despite this clinical trial not going as planned necessarily, EPA has been shown to inactivate both JNK and NF-κB pro-neuroinflammatory pathways as well as mark a reduction in the secretion of TNFα messenger ribonucleic acid (mRNA) in culture with murine BV-2 microglia (Moon, D. *et al.,* 2007). NF-κB inactivation has also occurred in human monocyte cells when treated with EPA (Zhao, Y. *et al.,* 2004). Inactivation of these pro-inflammatory cellular pathways is indicative of neuroprotection and EPA's potential as a future treatment for neuroinflammation resulting from aggresome plaque formation of mHTT accumulation within the CSF. Another clinical trial (phase 2) testing the effect of a neuroprotective molecule on neurodegeneration was completed in 2015 was with Epigallocatechin gallate (EGCG), a green tea polyphenol, but unfortunately never had results released for it (Denis, H. *et al.,* 2018). EGCG, like EPA, has also been shown to inactivate NF-κB activity; this was observed in a study

involving the monitoring of microglial activation in an ALS transgenic mice model (Xu, Z. *et al.,* 2006).

Passive immunization in HD involves targeting mHTT via mechanisms like Fab-fragment (from IgG monoclonal antibodies) induced phagocytosis, like the mechanism presented in the AD portion of this review. These intrabodies target a specific protein in a cell by antibody-antigen recognition. Targeting the N-terminus of HTT with monoclonal antibodies resulted in a decrease of mHTT plaque aggresomes and reduce cytotoxicity *in vitro* (Lecerf, J. *et al.,* 2001).

Active immunization in HD, as mentioned previously, is an area of research that still appears rather shallow and undeveloped in comparison to studies done on active immunization as an immunotherapy method in other, related neurodegenerative diseases. Very few studies have been cited in literature and those that have are usually more speculative in nature. A recent 2015 study by Ramsingh, A. I. *et al.* used a R6/1, zQ175 mice model to test the safety of active immunization through vaccines of peptide, protein and DNA plasmids. This study, however, did not produce any results regarding the actual pathology of HD in the mice model—it simply discussed the immune responses that these vaccines (in combination with certain adjuvants such as alum) caused in the transgenic mice.

Future Outlooks: Ethics and Safety with Immunotherapy

Despite immunotherapy having a lot of potential in terms of a new methodology of treatment for neurodegenerative diseases, it is still a relatively new field of research—in some diseases, such as AD, only having study data from over the past two decades.

This brings up a question of safety and health concerns regarding immunotherapy—a lot of the concepts surrounding many of immunotherapy and these neurodegenerative diseases is based on the speculation of relation-based science; 'this' molecule has neuroprotective properties against ROS' oxidative species *in vitro*, so it could lead to a decrease in pathology in 'this disease' *in vivo*. This is a realistic route to take, given that is the foundation for most medicine-related research. As long as the evidence of neuroprotection from the molecule in a disease-like model (whether it be cellular or in mice) is significant, then the next step *would* logically be to try it with more complex organisms to further gather data on its potential as a neuroprotective molecule or effective method of decreasing pathology. Unfortunately, this thought process when applied to immunotherapy often does not result in the preferred, optimal results—results being that a researched neuroprotective molecule *does not* result in a *clinically significant* decrease in pathology of a neurodegenerative disease; an example of this is was the drug *Avagacestat*, a γ-secretase inhibitor, in its phase II clinical trials for the treatment of AD, which was discontinued in 2012 due to trends of the disease pathology worsening in terms of cognition at higher doses (Coric, V. *et al.,* 2012).

This same trial (Coric, V. *et al.,* 2012) was also found to pose health risks to the patients, some even developing nonmelanoma skin cancer. In another clinical trial for Alzheimer's disease, the study was terminated in phase II due to 6% of the patients developing severe meningoencephalitis, which is counterintuitive to the whole goal of immunotherapy targeting neuroinflammation (Holmes, C. *et al.,* 2008). This showcases the critical issue of immunotherapy not only having the opposite of the intended effect in clinical studies, but also possibly leading to other adverse effects to patients' health.

Another topic of concern is something that all clinical trial studies are subjugated—the reaction of the treatment being tested with already FDA-approved, prescribed or over-the-counter (OTC) medications. While not much in terms of data showing that these neuroprotective derivatives react adversely with certain medications, this is a topic of concern that will only increase to garner more focus as new immunotherapies enter clinical studies. Nearly 50% of the approved drugs from the last three decades are somehow derived from natural products (whether it be plant-based or some other source) in cancer treatment alone (Veeresham, C., 2012). Many of the major neurodegenerative diseases are heavily associated with the elderly; it is both of a logical and ethical concern to consider the effects that future immunotherapeutic drugs might have on the patient(s) when mixed with drugs commonly taken by the older population, including but not limited to blood pressure medicine, Beta-blockers, Calcium channel blockers, and so on.

Conclusion

Immunotherapy is an up-and-coming field of research in neurodegenerative diseases that has a lot of promise—in terms of conceptual science and theories, the precursors of a valid and clinically significant alternative form of treatment are there amongst the many pathways currently being explored by researchers. Despite the setbacks that the field have experienced so far in terms of non-optimal results from clinical studies, immunotherapy is a relatively new field of interest. Many clinical trials regarding immunotherapy, whether it be the testing of neuroprotective molecules against disease pathology, or active/passive immunization against the proteins that propagate these diseases, have occurred only within the last decade. The field has a

lot of area to grow and explore, and many of the techniques being studied in immunotherapy are applicable to multiple neurodegenerative diseases, especially those regarding neuroinflammation.

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