The role of microglia in Poly Q diseases

Bachelor thesis

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Abstract

Poly Q diseases are a group of age-related neurodegenerative diseases. They are caused by a CAG repeat expansion in the disease-specific gene. Poly Q proteins are prone to aggregate and are toxic to neurons causing them to die. This in turn leads to a progressive loss of motor function and cognitive decline, among other symptoms. It is now thought that multiple cell types influence disease progression, including astrocytes and microglia. Microglia, the main immune cells of the central nervous system, are typically neuroprotective, however in poly Q diseases they mediate a chronic state of inflammation, as in many other age-related neurodegenerative diseases. Chronic inflammation contributes to the degenerative process. There are multiple ways by which microglia can cause this state pro-inflammatory activation. Neurons that die due to expression of the poly Q protein release danger associated molecular patterns (DAMPs), which activate pro-inflammatory transcription pathways in microglia. Aged microglia also show a slight increase in pro-inflammatory cytokine mRNA expression. In Huntington's disease, the poly Q protein is expressed in microglia as well, stimulating the transcription of more pro-inflammatory cytokines. Their contribution to the degenerative process makes microglia a putative therapeutic target to alleviate disease symptoms.

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Introduction

Poly glutamine (poly Q) diseases are a series of age-related, heritable neurodegenerative diseases caused by a genetic expansion, like Huntington's disease and multiple forms of spinocerebellar ataxia (SCA).

General symptoms include involuntary rapid eye movement and progressive loss of motor function and cognitive abilities.[1,3] While the diseases can be diagnosed even before onset because of its genetic origin, there is currently no treatment available. Patients live with these diseases for 10-30 years after onset, depending on type and severity, but require a lot of healthcare in the later stages of the disease mainly because of loss of motor function.

Each poly Q disease is caused by a poly glutamine (CAG) expansion in the disease-specific gene, for example huntingtin in Huntington's disease. Translation of this gene leads to the formation of unstable proteins with a toxic gain of function.[2] These proteins form aggregates inside neurons[1,2] and eventually the neurons die, leading to the clinical symptoms and eventually death. The diseases generally appear in late adulthood, dependent on the length of the CAG repeat: a longer repeat leads to an earlier disease onset and more severe symptoms.[3,4] The neurons affected are very specific to each disease, while the disease-causing proteins are generally expressed throughout a large part of the central nervous system (CNS) and are not even restricted to neurons.[4] This has led to the suspicion that other cell types might also be involved in the disease process, including astrocytes and microglia.[4] This thesis will focus on microglia, for several reasons, described below.

Age-related neurodegenerative diseases, including poly Q diseases, are known to coincide with chronic neuro-inflammation.[5] Microglia are the main immune cells of the CNS and they are also the main contributor to this inflammatory response. Activated microglia can mediate inflammation for example by the release of pro-inflammatory molecules.[6] Microglia are activated in patients with age-related neurodegenerative diseases in general where an increase in pro-inflammatory cytokines can been measured.[7] In these diseases this inflammation is chronic, it does not stop on its own or resolve the cause. Chronic inflammation is damaging to neurons, eventually leading to a potential increase in the degenerative process instead of protecting against it. This in turn leads to more neurons dying which activates the microglia even further.[8]

Microglia are typically neuroprotective. To understand what goes wrong in poly Q diseases and maybe neurodegenerative diseases in general, it is vital to understand their normal role and the factors that can play a part in their activation in these diseases.

The role of microglia

The central nervous system is separated from the rest of the body by the blood-brain barrier (BBB). Microglia are the only cells in the CNS that are of hematopoietic origin, but once present, they are capable of self-renewal.[9,10]

Microglia are normally present in their "resting" state, in which they are actually very busy with homeostatic maintenance. When they detect molecules of pathogenic origin or signals from neurons that indicate damage or injury they quickly migrate to this location and become fully activated microglia. There are two main activated states: phagocytic (neuroprotective) microglia and inflammatory (neurotoxic) microglia.[4,6]

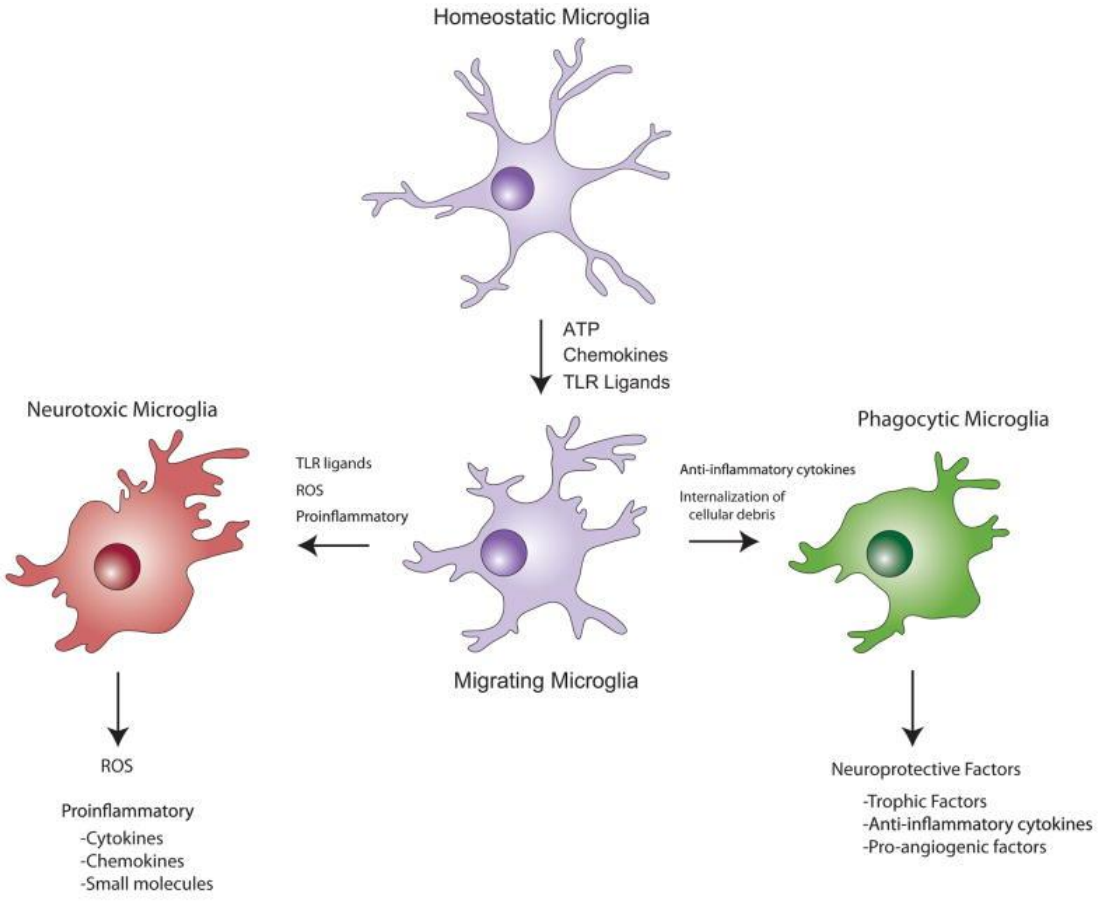

Figure 1. Microglia normally scan their environment and perform homeostatic functions. They can be activated by a variety of signals resulting in a pro-inflammatory or phagocytic state depending on the signals.[4]

"Resting" state

In their resting state microglia have a small cell body with long processes (figure 1). They remain in place and continuously scan their surrounding for cellular debris, foreign material, dead cells and any indication of injury and respond quickly when necessary by clearing debris and attracting other microglia.[6]

Microglia play an important part in neuronal development and maintenance of homeostasis of the extracellular environment in the CNS. They release several trophic factors that promote survival and function of the surrounding neurons, for example insulin-like growth factor 1, brain-derived neurotrophic factor, fibroblast growth factor.[4,6]

Activated state

When activated, microglia show morphological changes: they retract their processes causing the cell body to increases in size. They migrate to the area where the activation signals come from and subsequently respond to the threat. Activated microglia can be distinguished from their resting state because their membrane receptor expression pattern is different.[4,11]

Attempts have been made to classify the activated states of microglia but this has proven very difficult as their response is very insult-specific.[9,14] It generally comes down to a pro-inflammatory or phagocytic/neuroprotective state. The pro-inflammatory microglia will produce pro-inflammatory cytokines and ROS to damage any invading pathogens or infected neurons.[4] Microglia will also send signals to attract more microglia to the location to do the same. This inflammatory process can cause a lot of collateral damage to healthy neurons.[4,12]

Microglia can also be neuroprotective by releasing anti-inflammatory cytokines as well as trophic factors and growth factors to mediate recovery.[6] They can phagocytose cellular debris and are capable of doing so without releasing pro-inflammatory cytokines.[4] The state of activation is dependent on the threat that microglia are exposed to. They are capable of recognizing these threats by the expression of a variety of receptors.

Recognizing threats

Microglia are very plastic cells, they have a very specific response to various types of insult. In order to respond accordingly they need to identify the threat first. They possess a wide array of receptors that allow them to recognize any potential threat or indication of damage. Among them are a series of receptors known as the pattern recognition receptors. These recognize patterns that are of foreign origin, the so-called pathogen-associated molecular patterns (PAMPs). These consist of foreign material like proteins specific to bacterial membranes or double-stranded RNA.[9,13]

More recent research has shown that immune cells, including microglia, can also recognize endogenous molecules released from cells that indicate something is wrong. These are called DAMPs, danger-associated molecular patterns. These DAMPs consist of proteins normally located within the cells, like heat shock proteins. They can also consist of other molecules like DNA fragments or ATP. [4,14,15] Presence of these molecules outside of a cell would generally indicate cell damage.

There are multiple receptors that can recognize DAMPs. Among them are the Toll-like receptors (TLRs), which are a sub-group of the pattern recognition receptors. TLRs are well known mediators of inflammation.[15] Upon binding of a ligand they dimerize and induce a intracellular response.[9] They can form both homo- and heterodimers which contribute to the specificity of the response. TLRs mainly mediate pro-inflammatory responses, although certain TLRs can also mediate an anti-inflammatory response.[9,]

All TLRs (except TLR 3) signal through the Myeloid differentiation factor 88- (MyD88) dependent pathway, which in the end activates the transcription NFκB (nuclear factor kappa B). NFκB induces the transcription of pro-inflammatory cytokines. TLR 3 signals through the TRIF- (TIR-domain-containing adoptor inducing IFNβ) dependent pathway. TRIF is an adaptor protein which upon activation eventually leads to the production of anti-inflammatory cytokines. TLR 4 can signal through both pathways depending on which adaptor proteins are present.[9] (figure 2).

Another receptor recently discovered to be capable of recognizing DAMPs is NLRP3, a NOD-like receptor. NOD-like receptors are another sub-group of pattern recognition receptors and were only recently discovered to be involved in immunity in the CNS. NLRP3 is normally kept in a suppressed state by chaperone proteins, but can be activated in the presence of signals including PAMPs and DAMPs. NLPR3 upon activation will activate the inflammasome. The inflammasome is a complex of proteins which upon activation will lead to the release pro-inflammatory cytokines[9,16] (figure 2).

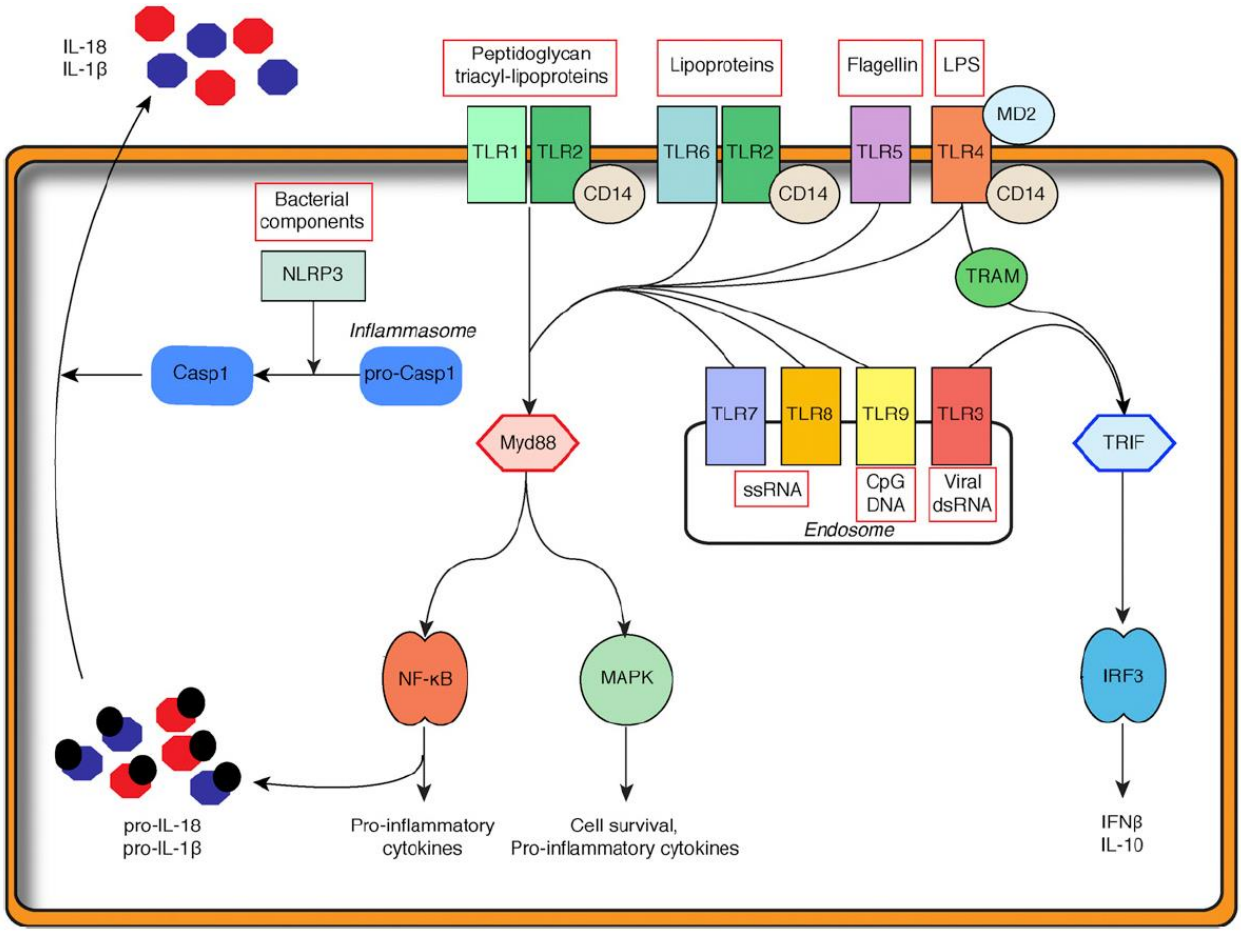

Figure 2. DAMPs binding to TLRs cause the expression of pro-inflammatory cytokines through the NFκB pathway. DAMPs can also activate NLRP3 causing activation of the inflammasome, which in turn also causes the release of pro-inflammatory cytokines.[9]

Lastly, RAGE (receptor for advanced glycation endproducts), is a receptor also capable of recognizing DAMPs.[14] RAGE also signals through the NFκB pathway, leading to the production of pro-inflammatory cytokines.

Pro-inflammatory cytokines can attract other microglia, causing them to contribute to the inflammatory response producing more pro-inflammatory cytokines and ROS.[17] They also activate astrocytes to help in the inflammatory process and can attract macrophages from the bloodstream.[9]

Inflammatory loop

Neurons are relatively vulnerable to damage. An inflammatory response of microglia, as stated before, can be neurotoxic and can cause a lot of collateral damage to healthy neurons and even neuronal death. Dead neurons will lead to more microglial activation. Damaged neurons will release DAMPs and this will also cause more microglial activation, further increasing the inflammatory response and thus the release of neurotoxic factors. This will lead to a self-sustaining inflammatory loop. (figure 3)

The inflammatory loop in this way can be a strong contributor to the severity and the chronic aspect of the inflammation seen in many neurodegenerative diseases.[18]

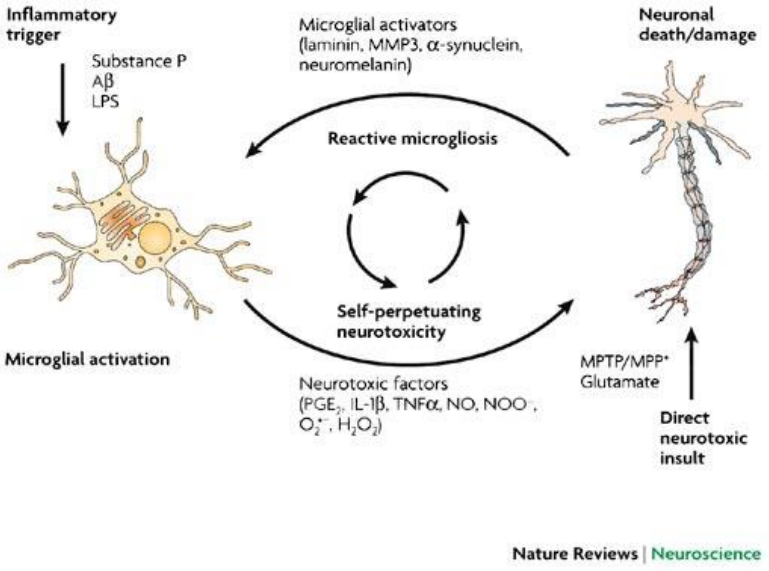

Figure 3. Damage to neurons leads to pro-inflammatory microglia activation through the release of DAMPs. Pro-inflammatory microglia release factors with neurotoxic properties that cause more neuronal damage, resulting in an inflammatory loop.[18]

Poly Q diseases

Multiple cell types play a part in the pathogenesis of poly Q diseases. Expression of the poly Q protein that causes the disease is not just restricted to neurons.[4] Immune cells also play a part by creating a pro-inflammatory environment and can contribute to the degenerative process. Degeneration in poly Q diseases is partially mediated in a cell non-autonomous manner.[4,23] Cell non-autonomous neurodegeneration means that the expression of the pathogenic protein in one particular group of cells leads to the degeneration of another group of cells.

Cell non-autonomous degeneration

Recent research indicates that the neurodegeneration seen in poly Q diseases is not just cell-autonomous. A protein containing a poly Q expansion in neurons alone does lead to degeneration, but does not explain the complete disease phenotype, like the activated state of microglia prior to disease onset. Neither does cell-autonomous degeneration alone explain why only a certain population of neurons is affected while the pathogenic protein is expressed widely throughout the CNS.

A study investigating neurodegeneration in a mouse model for Huntington's disease has shown that huntingtin aggregates can also be formed in astrocytes, although a longer CAG repeat (160Q) was necessary compared to neurons. This expression of the poly Q protein in astrocytes alone was enough to cause degeneration of neurons. Even with a shorter repeat length (98Q), which did not cause aggregates to form in astrocytes, neurons degenerated faster when the poly Q expanded gene was expressed in both astrocytes and neurons then when it was expressed in neurons alone.[19]

In a mouse model for SCA7, a poly Q disease resulting in the degeneration of the Purkinje cells in the cerebellum, cell non-autonomous degeneration also plays an important role in the disease process. Here too expressing the mutant gene in Bergmann glia (astrocytes of the cerebellum) was enough to cause degeneration of Purkinje cells.[20]

An important task of astrocytes is the re-uptake of the neurotransmitter glutamate. Impaired glutamate re-uptake will lead to a higher amount of extracellular glutamate, which is toxic to neurons.[21] Interestingly, in both the Huntington's disease and SCA7, neuronal damage can be caused by glutamate toxicity. In both cases there was impaired astrocytes function due to the disease protein,[20,21] leading to impaired glutamate re-uptake and excitotoxicity. Excitotoxicity can be caused by overstimulation of glutamate receptors, which will cause high numbers of calcium ions to enter the neurons. This will lead to neuronal damage and which in turn can cause an immune response.

Microglia also possess a glutamate receptor (mGlu2) that upon stimulation causes microglia to exhibit neurotoxic properties by the release of TNFα (tumor necrosis factor α),[22] which is a cytokine capable of inducing cell death.

Microglia

In poly Q diseases microglia can be activated in a variety of ways and there are multiple factors involved in their activation. The activation of microglia is likely to be, at least partially, a response to dead and dying neurons due to poly Q protein expression. Damaged neurons will release DAMPs, triggering an inflammatory response mediated by microglia.[4] However there are more factors that can cause microglia to activate in pro-inflammatory manner, described below.

An aspect that can play a part in the microglial component of the disease process is microglial aging. Microglia present in CNS are capable of self-renewal, proliferating when necessary.[9,10] Aged microglia show replicative stress: their mitotic abilities become impaired after a certain number of cell divisions. Also their processes showed less complexity,[24] indicating that aged microglia might be less capable of scanning their surroundings and performing their protective tasks. The mRNA levels of pro-inflammatory cytokines are increased in aged microglia, which could lead to a chronic state of slight inflammation called inflammaging.[25] Exposure of neurons to this constant slightly inflammatory environment could potentially make them more susceptible to insults.

Microglia might also play a part in the cell non-autonomous degeneration of neurons in Huntington's disease. In Huntington patients, the poly Q-expanded huntingtin protein is expressed in microglia. In a mouse model researchers found that expression of the poly Q expanded huntingtin protein promotes the activity of transcription factors, which cause an increase in transcription of mRNA of pro-inflammatory cytokines in the absence of extracellular pro-inflammatory stimulation. They showed that microglia can indeed cause cell non-autonomous neurodegeneration. When healthy neurons were co-cultured with microglia expressing poly Q-expanded huntingtin, the microglia were capable of inducing neuronal cell death.[26]

This increase in expression of pro-inflammatory cytokines by microglia due to expression of the poly Q-expanded huntingtin protein may be an explanation for why there are signs of microglial activation and increased pro-inflammatory cytokine production in patients, years before the onset of disease.

The degeneration of neurons can further increase the pro-inflammatory response through the release of DAMPs by damaged neurons, eventually leading to a chronic state of inflammation through an inflammatory loop.

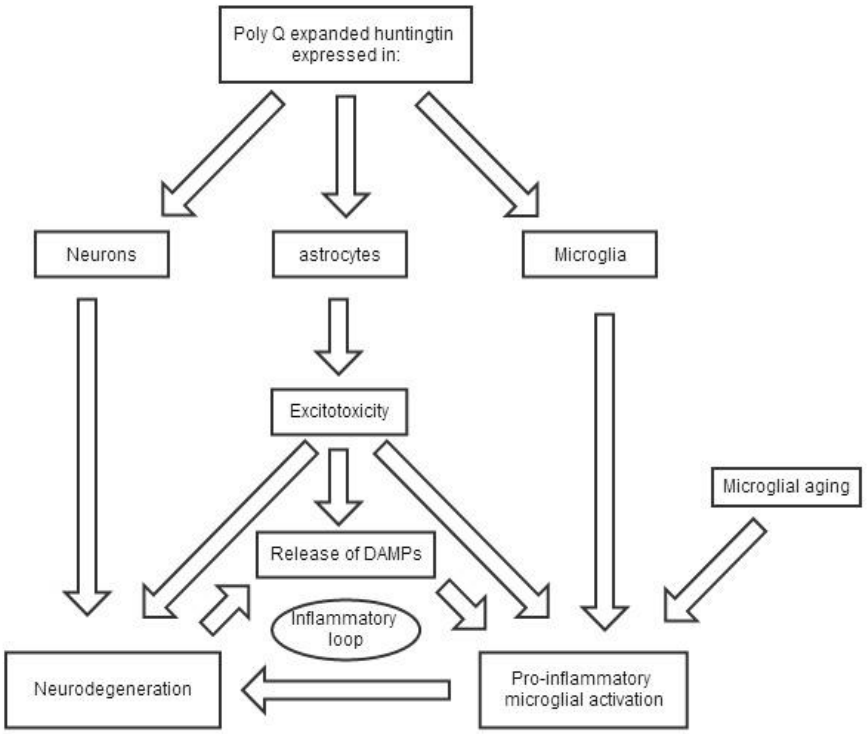

Figure 4. Neurodegeneration in Huntington's disease is a complex network of cell-cell interactions. These either contribute to neurodegeneration directly, or through pro-inflammatory activation of microglia.

Discussion

Poly Q diseases were long thought to be caused by the expression of the disease-dependent expanded protein in neurons. Research has shown that other cell types also play important parts in a cell non-autonomous manner, including microglia. The chronic state of neuroinflammation that accompanies these diseases contributes to the degenerative process, which makes it an interesting target for therapy. There are multiple ways in which microglia can generate a pro-inflammatory environment in the CNS in poly Q diseases. These include exposure to DAMPs or glutamate, expression of the disease protein and microglial aging. Complete suppression of the immune system however does not seem to slow the degenerative process. Specific activation of microglia however, to preserve or promote protective functions, may be an option.

It is possible to induce specific microglial responses that might prove effective. Specific stimulation of TLR4 for example led to an increase in phagocytosis of extracellular debris by microglia in an Alzheimer's disease model, with minimal production of pro-inflammatory cytokines.[27] For poly Q diseases a similar strategy may prove effective as well, slowing down the degenerative process by putting a halt to the inflammatory process and stimulating beneficial phagocytic activity. Perhaps it is possible to induce the expression of trophic factors for neurons as well.

The immune system naturally serves a protective role, although at which point it starts contributing to degeneration in poly Q diseases is not clear. This could be specific for each poly Q disease depending on what causes the microglial activation. Research to at which time a turning point occurs when the immune system starts being harmful would also be very important in this approach.

Even if microglia can be turned to a non-inflammatory state it does not cure the disease because the poly Q protein is still formed. Perhaps in combination with other potential therapies for slowing down the degenerative process we may be able to further delay or slow down the degeneration to improve the quality of life for these patients. Stopping the detrimental effects of chronic neuroinflammation while preserving the protective functions of microglia would at least be a big step in the right direction.

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