● INVITED REVIEW

Promises and pitfalls of immune-based strategies for Huntington's disease

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Abstract

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disease characterized by the selective loss of neurons in the striatum and cortex, leading to progressive motor dysfunction, cognitive decline and behavioral symptoms. HD is caused by a trinucleotide (CAG) repeat expansion in the gene encoding for huntingtin. Several studies have suggested that inflammation is an important feature of HD and it is already observed in the early stages of the disease. Recently, new molecules presenting anti-inflammatory and/or immunomodulatory have been investigated for HD. The objective of this review is to discuss the data obtained so far on the immune-based therapeutic strategies for HD.

Key Words: *Huntington's disease; treatment; disease modifying therapy; inflammation*

Introduction

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease. It is pathologically characterized by selective loss of neurons in the striatum and cortex, which leads to progressive motor dysfunction, cognitive decline and behavioral symptoms (Tabrizi et al., 2009). HD is caused by an unstable CAG trinucleotide repeat expansion in exon 1 of the *Huntingtin* gene (*HTT*) encoding a mutant form of the huntingtin protein (HTT). The presence of more than 40 CAG repeats is translated into the mutant HTT (mHTT) which causes the disease within a normal lifespan, while longer repeats can accelerate disease onset. The onset of HD usually occurs in midlife, followed by 15 to 20 years of disease progression (Langbehn et al., 2010).

The HTT is ubiquitously expressed and plays several roles in human neurons, including embryonic development. The mechanisms of neuronal cell toxicity by mHTT have not been clearly established, possibly involving multiple pathways such as abnormal protein aggregation, mitochondrial dysfunction, excitotoxicity, among others (Labbadia and Morimoto, 2013).

Currently, there is no effective disease-modifying therapy for HD and only symptomatic approaches are available. New agents have been investigated for HD and some have focused on immunomodulatory and/or anti-inflammatory mechanisms. Herein, we will discuss the data obtained so far on the immune-based therapeutic strategies for HD.

Immune Dysfunction in HD

During the last decade, great attention has been drawn to the involvement of neuroinflammation in the pathogenesis of HD. Although the primary cause of HD is the mHTT expression in neurons leading to neuronal death, different pathophysiological mechanisms participate in this process. In this context, immune mechanisms could be activated by neuronal cells in degeneration, with subsequent release of mediators responsible for amplifying neuronal toxicity and, therefore, contributing for disease progression. Besides this, mHTT is highly expressed in immune cells where it can promote cell-autonomous immune activation (Weiss et al., 2012). Accordingly, the immune system can be directly or indirectly activated in HD.

Indeed, several studies have reported immune activation in patients with HD. For instance, neuropathological and positron emission tomography (PET) studies showed accumulation of reactive microglia in the brain of HD patients, a finding that is significantly correlated with the severity of the disease (Pavese et al., 2006). Microglia are central nervous system (CNS) resident myeloid cells with phagocytic activity, and have traditionally been seen as innate immune cells mediating inflammatory responses in the brain. When activated, microglial cells produce pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β). These cytokines, in turn, promote further activation of microglia, resulting in an inflammatory flow that contributes to neuronal toxicity (Kreutzberg, 1996). Interestingly, activation of microglia is already evident in pre-manifest subjects, *i.e.*, subjects carrying more than 40 CAG repeats of the *HTT* gene but not showing neurological symptoms. Microglial activation has been detected up to 15 years before disease onset (Björkqvist et al., 2008). Post-mortem studies have also shown elevated levels of inflammatory markers, such as interleukin-6 (IL-6), interleukin-8 (IL-8) and TNF-α in the brain of patients with HD (Silvestroni et al., 2009). Higher levels of IL-6, IL-8 and TNF-α have also been found in the cerebrospinal fluid (CSF) of patients with

HD in comparison with controls (Björkqvist et al., 2008).

In parallel with these changes in the CNS, there is a significant increase in the circulating (blood) levels of inflammatory cytokines, such as IL-6, IL-8, and TNF-α, and chemokines like eotaxin/CCL11 and monocyte chemotactic protein 1 (MCP-1/CCL3) in patients with HD compared to controls. Increased circulating levels of inflammatory cytokines are also observed in pre-manifest subjects (Rocha et al., 2016).

It is worth noticing that different animal models of HD exhibit alterations in inflammatory markers and activated microglia, corroborating the findings from clinical studies (Franciosi et al., 2012). In sum, immune activation and enhanced inflammation are important features of HD, being already present in pre-clinical stages of the disease, *i.e.*, years before the onset of motor symptoms. In this context, the study of immune-related mechanisms as potential therapeutic targets for HD is warranted.
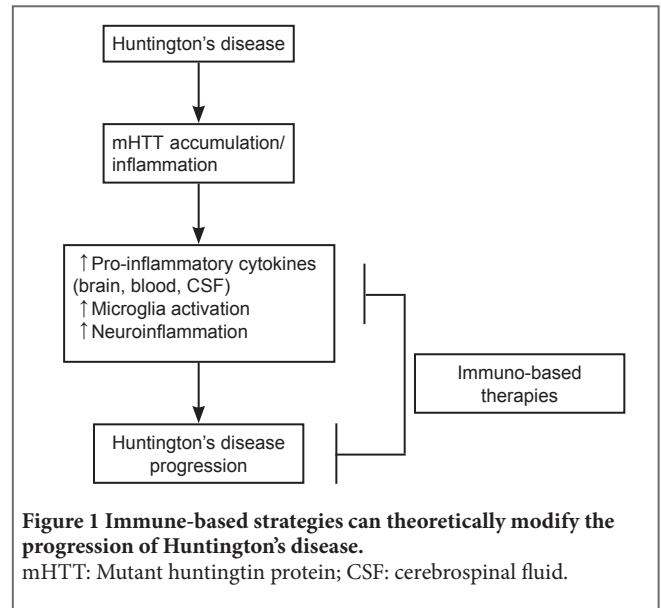
Treatment Strategies for HD

Even though the HD gene was identified over 20 years ago, there is no effective disease-modifying therapy for HD and only symptomatic treatments are currently available. Most therapies for HD target motor symptoms, such as chorea, *i.e.*, involuntary movements that can affect different parts of the body, interfering in activities of daily living. Although chorea is only one dimension of the constellation of motor symptoms of HD, it is the most recognizable and treatable characteristic of HD (Armstrong et al., 2012). Recently, tetrabenazine and deutetrabenazine were approved by the Food and Drug Administration for the treatment of chorea in HD. Psychiatric and behavioral symptoms such as aggression, irritability, impulsiveness, anxiety, depression and psychosis represent a significant burden for patients with HD and their caregivers (Teixeira et al., 2016). Accordingly, antidepressants, antipsychotics, and mood stabilizers are commonly prescribed for HD.

Different treatments have been evaluated as disease-modifying strategies for HD. Most studies have targeted intracellular pathways that are imbalanced in HD, such as protein synthesis and aggregation. Nevertheless, the strategies tested in clinical trials failed to show a significant change in motor, cognitive or functional decline (Wild and Tabrizi, 2014). Based on the emerging data on immune/inflammatory changes in HD, agents targeting immune mechanisms have been investigated (**Figure 1**).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been evaluated for behavioral and cognitive symptoms in neurodegenerative diseases with mixed results (Terzi et al., 2017). Celecoxib and meloxicam attenuated behavioral and biochemical changes present in a rat model of quinolinic acid-induced HD (Kalonia and Kumar, 2011). Conversely, another study failed to show neuroprotective effects of acetylsalicylate and rofecoxib in N171-82Q and R6/2 transgenic HD mice (Norflus et al., 2004). No clinical trial has been conducted to evaluate potential benefits of NSAIDs in patients with HD.

Minocycline is a second-generation, semi-synthetic tetra-

Figure 1 Immune-based strategies can theoretically modify the progression of Huntington's disease.
mHTT: Mutant huntingtin protein; CSF: cerebrospinal fluid.

cycline antibiotic. In addition to its antibiotic properties, minocycline can exert a variety of biological actions, including anti-inflammatory and anti-apoptotic activities (Noble et al., 2009). Minocycline has been shown to be neuroprotective in several animal models of CNS diseases, including HD (Chen et al., 2000; Wang et al., 2003). Minocycline readily crosses the blood-brain barrier (BBB) and attenuates inflammation associated with microglial activation. More specifically, minocycline inhibits cyclooxygenase-2 (COX-2) expression and reduces prostaglandin E2 (PGE2) levels in microglial cells, attenuating the accumulation of activated cells (Kim et al., 2004; Bye et al., 2007). Clinical trials in individuals with HD showed that minocycline is a well-tolerated and safe drug. In contrast to the natural course of HD, patients treated with 100 mg of minocycline for 24 months showed stabilization of motor and neuropsychological performance at the endpoint (24 months), after a significant improvement in the first 6 months of treatment. Moreover, there was a significant improvement of psychiatric symptoms at the endpoint that was not apparent in the first 6 months (Bonelli et al., 2004). However, in another clinical trial involving 87 patients treated with 200 mg of minocycline for 18 months, no clinical benefit was observed with the treatment (Huntington Study Group DOMINO Investigators, 2010). Therefore, the results with minocycline are still controversial, and further studies are needed to better define its potential role and mechanisms in HD.

Recently, laquinomod has been evaluated in HD. Laquinomod is a disease-modifying therapy approved for the treatment of multiple sclerosis, an autoimmune demyelinating disease of the CNS (Kolb-Sobieraj et al., 2014). Laquinomod is a small molecule that can be given orally with a good safety profile, exerting both immunomodulatory and neuroprotective effects. Laquinomod has been shown to upregulate brain-derived neurotrophic factor (BDNF) in patients with multiple sclerosis (Thone et al., 2012), a neurotrophic factor with reduced expression and secretion in HD, and

to reduce the levels of secreted pro-inflammatory factors, leading to neuroprotection (Varrin-Doyer et al., 2014). However, its immunomodulatory mechanisms are still not clear (Kolb-Sobieraj et al., 2014). In animal models of HD, laquinomod has shown neuroprotective effects, rescuing striatal and cortical neurodegeneration, and improving behavior in YAC128 mice (Garcia-Miralles et al., 2016). A clinical trial (NCT02215616) is currently recruiting participants for testing laquinomod in patients with HD.

Other compounds with anti-inflammatory and/or immunomodulatory properties have been investigated in HD, such as an inhibitor of soluble TNF-α (XPro1595) and an anti-SEMA4D monoclonal antibody. Semaphorin 4D (SEMA4D) is a transmembrane signaling molecule that modifies a variety of mechanisms central to neuroinflammation and neurodegeneration (Southwell et al., 2015). These compounds were only used in pre-clinical settings to date. In a transgenic mouse model of HD (R6/2), XPro1595 decreased TNF-α level in the cortex and striatum, enhanced motor function and reduced the burden of mHTT aggregates (Hsiao et al., 2014). Treatment with anti-SEMA4D improved neuropathological signs, cognitive deficits and a subset of behavioral symptoms including anxiety-like behavior in YAC128 mice (Southwell et al., 2015).

Stem cell therapy is a promising treatment for neurodegenerative diseases (Dutta et al., 2013; Colpo et al., 2015; Salem et al., 2016). The goals of stem cell therapy involve the replenishment of lost cells and/or the increase in cell survival, reversing the disease phenotype or delaying disease progression overtime. Among the potential mechanisms of action of stem cells, the release of growth factors, such as BDNF and glial cell-derived neurotrophic factor (GDNF), is of great relevance as they provide trophic support to different cell types in the damage areas. Besides that, stem cells exhibit immunomodulatory and anti-inflammatory properties which can contribute to decrease and/or control the inflammatory response in HD (Uccelli et al., 2011). Studies have proven the efficacy of stem cells in providing functional recovery in various pre-clinical models of HD (Rossignol et al., 2015; Kerkis et al., 2015). Although stem cells improved behavior and neuropathological signs, and increased the levels of neurotrophic factors in animal models of HD, information about the immunodulatory effects of stem cells is lacking in HD. As immunomodulatory and anti-inflammatory effects are important mechanisms by which these cells work, further studies addressing these effects are warranted in HD. One clinical trial (NCT01834053) is registered to test autologous stem cell in HD patients with no available results to date.

The attempt to develop a vaccine for HD occurred in the context of a larger effort to develop vaccines for a variety of neurological conditions. In the case of HD, they expected to prevent or reverse the long-term accumulation of a toxic protein, *i.e.*, mHTT, generating antibodies against particular epitopes of the HTT protein. However, the results with the vaccine were disappointing in pre-clinical studies, and, as consequence, the endeavor was abandoned in humans (Lu-

thi-Carter, 2003).

Promises and Pitfalls for HD

Changes in the immune system have been recognized in the physiopathology of HD. Accordingly, new treatment possibilities for HD could arise from these recent insights on immune dysfunction in HD. However, there are potential pitfalls in this process.

In first place, it is important to refine the understanding of the role played by the immune system in HD. It is not clear whether immune changes result from neurodegeneration and/or represent an independent pathological mechanism in HD. Evidence of elevated cytokine levels in pre-symptomatic patients, for example, certainly argues in favor of inflammation not being a direct consequence of brain disease, but rather an independent phenomenon or a precursor of other pathological events. It is also unclear regarding the role of the immune system in the different phases of the disease, and it remains to be defined whether this role changes from early to advanced stages of HD. Moreover, to define new therapeutic targets and design suitable drugs to alter the immune response efficiently is necessary, first, to address the different immune mechanisms that are elicited by HD pathology.

Other pitfall is the length of a determined intervention in clinical trials to confirm its disease-modifying potential. As a neurodegenerative disease, HD progresses slowly, requiring long clinical trials that demand much time and resources. Surrogate markers of outcome could help in this regard. Accordingly, the role of immune molecules as biomarkers needs to be explored. Another important issue related with immune-based therapies is the increased risk of infectious or neoplastic diseases as many of these therapies can decrease the efficiency of immune responses in general.

In conclusion, immune changes seem to play a role in the physiopathology of HD. However, there are several unanswered questions regarding the involvement of the immune system in HD. This is a largely unexplored area where studies addressing pathophysiological mechanisms, biomarkers and pharmacological targets can impact the clinical management of patients with HD in the real world.

Open peer review report:
Reviewer: Kyle D. Fink, University of California, USA.
Comments to authors: The present review addresses a very interesting area of neurodegenerative diseases, specifically Huntington's disease. The role of neuro-inflammation and the greater idea neuroimmunology and neurodegenerative diseases is a very interesting and timely topic to publish a review. The strength of this review lies in discussing the shortcomings in the field in terms of immune-bases strategies for HD and gives a future perspective regarding the unanswered questions in the field.

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