Neuroinflammation in Huntington's Disease

Roland G.W. Staal and Thomas Möller

Abstract Huntington's disease (HD) is a progressive, eventually terminal, neurodegenerative disease caused by autosomal-dominant mutations in the huntingtin gene (*HTT*). The early symptoms of HD typically include subtle changes in mood and/or cognition, as well as poor coordination and unsteady gait. These symptoms progressively worsen until coordinated movement is virtually impossible and mental abilities have declined to a state of dementia. There is no cure and patients generally succumb to comorbid complications within 20 years of onset. The mutation is an expansion of the CAG triplet repeat stretch in the *HTT* gene, resulting in an expanded poly-glutamine (polyQ) stretch in the huntingtin protein (HTT). The length of this CAG repeat correlates strongly with the age of onset as well as the rate of disease progression. The ability to identify at-risk individuals by genetic testing enabled researchers to conduct clinical studies and learn about early events in the development of HD. One of the earliest pathological changes observed in the CNS of HD patients is the appearance of neuroinflammation, preceding overt neurodegeneration or protein aggregation. Here we will review the data implicating neuroinflammation in all stages of HD, from initiation to progression. We will also explore the most recent advances in our understanding of neuroinflammation in HD including a potential role for the peripheral immune system. We will also discuss how these various biologies may lead the way to discovery of novel, innovative, and urgently needed therapies.

Keywords Huntington's disease • Huntingtin • CAG repeat • PolyQ • Neurodegeneration • Neuroinflammation • PK-11195 • Microglia • Astrocytes • Monocytes • T cells

R.G.W. Staal (✉) • T. Möller
Neuroinflammation Disease Biology Unit, Lundbeck Research USA,
215 College Road, Paramus, NJ 07652, USA
e-mail: staa@lundbeck.com; tmoe@lundbeck.com

P.K. Peterson and M. Toborek (eds.), *Neuroinflammation and Neurodegeneration*,
DOI 10.1007/978-1-4939-1071-7_10,

List of Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ApoE	Apolipoprotein epsilon
ApoJ	Apolipoprotein J
ATP	Adenosine triphosphate
B cell	Bone marrow-derived lymphocyte
BBB	Blood–brain barrier
BDNF	Brain-derived neurotrophic factor
CB2R	Cannabinoid receptor 2
CCL5	Chemokine (C–C motif) ligand 5 (also CCL5)
CNS	Central nervous system
CXCL1	Chemokine (C–X–C motif) ligand 1 (GROα, KC)
EAAT2	Excitatory amino acid transporter 2
GABA	Gama-aminobutyric acid (inhibitory neurotransmitter)
GFP	Green fluorescent protein
HD	Huntington's disease
HTT	Huntingtin's protein
HTT	Human huntingtin's gene
Htt	Mouse huntingtin's gene
Iba1	Ionized Ca^{2+}-binding adapter molecule 1 [Allograft inflammatory factor 1 (AIF1)]
IKK	IκB kinase
IL-10	Interleukin 10
IL-4	Interleukin 4
IL-6	Interleukin 6
KCNN4	K^+ intermediate/small conductance Ca^{2+}-activated channel, subfamily N, member 4
KMO	Kynurenine 3-monooxygenase
LRP	Lipoprotein receptor-related proteins
MCP-1	Monocyte chemotactic protein-1 [Chemokine (C-C motif) ligand 2 (CCL2)]
MCP-4	Monocyte chemotactic protein-4 [Chemokine (C–C motif) ligand 13 (CCL13)]
MIP-1β	Macrophage inflammatory protein-1β, CCL4 [Chemokine (C-C motif) ligand 4]
MS	Multiple sclerosis
NAD^+	Nicotine adenine dinucleotide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	*N*-methyl-d-aspartate
NO	Nitric oxide
P2X4	P2X purinoceptor 4
P2X7	P2X purinoceptor 7
PD	Parkinson's disease

PolyQ	Poly-glutamine stretch
PrP	Prion protein
Q	Glutamine
RANTES	Regulated on activation, normal T cell expressed and secreted
ROS	Reactive oxygen species (superoxide, hydrogen peroxide, etc.)
SSRI	Selective serotonin reuptake inhibitor
T cell	Thymus-derived lymphocyte
TGF-β	Transforming growth factor β
TLR4	Toll-like receptor 4
TRPM2	Transient receptor potential cation channel, subfamily M, member 2
VGCC	Voltage-gated Ca^{2+} channels

1 Huntington's Disease

Huntington's disease (HD) is a progressive, terminal, neurodegenerative disease of monogenetic origin. The first signs of disease include subtle changes in mood and cognition as well as poor coordination and unsteady gate. Cognitive, behavioral, and psychological problems as well as the uncoordinated, jerking movements (chorea) continue to deteriorate and become more pronounced, eventually leading the patient to seek medical advice. Collectively, abnormal motor movements (dystonia and chorea) accompanied by cognitive decline and personality changes suggest the possibility of Huntington's disease, a diagnosis which can be confirmed by genetic testing. The disease and symptoms continue to progress until coordinated movement becomes extremely difficult and mental abilities decline to a state of dementia. Patients eventually succumb to bulbar dysfunction and accompanying complications such as pneumonia or heart disease within 20 years of disease onset [1, 2]. There are currently no disease-modifying treatments, only medications to manage the motor symptoms (e.g., Xenazine® (tetrabenazine), neuroleptics, benzodiazepines) and psychiatric symptoms (medications used to treat similar psychiatric symptoms in the general population, i.e., SSRIs, atypical antipsychotics).

One of the earliest neuropathological changes observed in HD patients is proteinacious inclusions rich in huntingtin protein (HTT) that are found in both the nucleus and cytoplasm of striatal GABAergic neurons. Whether or not these inclusions are themselves toxic or represent a protective sequestration mechanism remains unresolved [3, 4]. The CNS pathology of HD is characterized by severe atrophy of the caudate nucleus and putamen due to extensive loss of GABAergic medium spiny neurons that project to the globus pallidus. As the striatopallidal projections are lost, secondary degeneration of the globus pallidus sets in. The basal ganglia are key regulators of motor control, mood, and higher cognitive function which accounts for the clinical manifestations. As with most neurodegenerative diseases, neuronal death and atrophy of anatomical structures are not restricted to just one area, especially as the disease reaches later stages. Cerebral cortical atrophy is also commonly observed later in disease while the cerebellar Purkinje cells are generally spared [2].

The recognition that HD was an autosomal-dominant disorder enabled scientists to discover the underlying mutation in the huntingtin gene (*HTT*). Researchers identified the disease-causing mutation as an expansion of a CAG trinucleotide repeat stretch. As CAG encodes for glutamine (Q), the expansion in the protein is also known as polyQ region [2]. They determined that the length of the expansion predicts the age of onset, which while typically around 35–45 years of age can manifest itself as early as infancy and as late as 85 years. Individuals not affected by HD have fewer than 36 CAG repeats; however, people with 27–35 repeats have a slightly increased risk that their children could pass the 36 CAG repeat threshold due to de novo repeat expansion. Carriers of 36–39 repeats have a significant risk of manifesting HD, and carriers with more than 40 repeats will get the disease with certainty. As an autosomal-dominant disease, children of HD patients have a 50 % risk of receiving the gene with the CAG expansion.

Identification of the disease-associated gene was an important step in understanding HD, but determining the molecular function(s) of the huntingtin (HTT) protein and the biological processes it initiates, coordinates, or regulates remains largely unknown. The autosomal-dominant inheritance of mutated HTT suggests a toxic gain of function since HD patients usually still have one wild-type allele and normal protein is still produced. For example, mutant HTT can recruit normal HTT into insoluble aggregates in vitro and in vivo. In contrast, ablation of *Htt* leads to embryonic lethality, suggesting a critical role of normal HTT function in development. Other in vitro and in vivo studies suggest that loss of HTT function may contribute to disease as shown by decreased cell survival and dysfunction of neurons. Because HTT is expressed throughout the body, its expression pattern fails to provide any insight into its function or the increased susceptibility of the medium spiny neurons in the striatum. The ubiquitous expression, however, implies that the polyQ repeat expansion may affect any cell type in which it is expressed (including astrocytes, microglia, and oligodendrocytes in the CNS). The impact of the mutation upon a given cell type may also depend on the specific function of HTT within that particular cell. The effects of the mutation may also manifest itself at varying stages of the disease depending on the levels of mutant HTT expression. Thus, any cell in the CNS has the potential to contribute to the etiology of HD, suggesting that the underlying disease mechanism may be non-cell autonomous as has been suggested for other neurodegenerative diseases such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) [5–7].

2 Neuroinflammation in Huntington's Disease

One type of nonneuronal cells that garnered interest in early neuropathological studies of brains from patients with neurological disease is microglia, the resident immune cells of the CNS. These cells were observed to have profoundly altered morphology (less ramified and more amoeboid) and increased expression of immune cell markers (e.g., HLA-DR, CD68) in neurological diseases including HD [8, 9].

Numerous studies have since been published that firmly establish altered microglial morphology and phenotype (commonly called "activated microglia") as a pathological hallmark of HD. In addition, mediators of inflammation, such as cytokines and increased oxidation products, were found to be increased in HD [10, 11]. Hence, the term "neuroinflammation" was coined to describe the *inflammation* observed in the brains of patients with *neuro*logical disease.

As the CNS resident immune cell, microglia surveil the environment for danger signals (foreign or endogenous) and continuously communicate and interact with the other cells in the brain (astrocytes, oligodendrocytes, and neurons). One mechanism is the release of molecules that are traditionally considered to be immunological signaling molecules such as cytokines and chemokines [12]. Another modality is via release of growth factors, neuropeptides, and transmitters (neuro- and glio-transmitters, such as norepinephrine, glutamate, ATP). The cells of the CNS work in concert to initiate and then modulate neuroinflammation whose goal is to remove the initial cause (infection, disease, and trauma) and ultimately restore homeostasis. The term "neuroinflammation" has therefore been extended beyond the CNS resident immune cells and come to include all cells in the CNS that contribute to the inflammatory response to neurological disease or infection.

2.1 Microglia

The primary function of microglia is to survey the CNS for any signs of danger (either endogenous or foreign) [13]. In the surveilling state (formerly referred to as the "resting" state), microglia have small cell bodies with long, thin ramified processes that constantly extend and retract, making contacts with neurons and other cells. Upon detection of danger signals, microglia can rapidly migrate to the site of injury. They become less ramified and increase the expression of cell surface antigens, resulting in the "activated" morphology observed in virtually all neurological diseases and injuries. The specific phenotype of "activated microglia," however, can be very different from one another even though they morphologically "appear" similar. Microglia have a wide-ranging arsenal of executory functions, including phagocytosis of pathogens or cellular debris, secretion of enzymes that break down the extracellular matrix, secretion of proteins that opsonize dying or damaged cells and mark them for phagocytosis, release of pro- and/or anti-inflammatory cytokines and chemokines, release of growth factors (or downregulation of their release), generation of reactive oxygen species (ROS) to destroy phagocytized pathogens, as well as release of glutamate, toxic kynurenine metabolites, ATP, and nitric oxide [13]. The nature of the insult, as well as the immediate milieu of the microglia, will all impinge on the microglia and shape their response. Hence, even if "activated" microglia are observed, one can only conclude that their phenotype is no longer surveying and that they are now "activated." One needs to be cautious in making predictions about what these microglia are actually producing or doing (or have stopped producing/doing).

Microglia can respond quickly to danger, setting in motion fast, potent mechanisms such as phagocytosis, and production of ROS to kill or neutralize phagocytized pathogens, dangerous substances, or dying cells [13, 14]. Over time, other mechanisms are engaged, such as changes in gene expression, transcription, and release of pro-inflammatory and later anti-inflammatory substances including cytokines and growth factors [13, 15]. Especially pro-inflammatory mediators (as well as withdrawal of trophic support) can easily damage or kill nearby cells (bystander damage), so that tight control and effective resolution of neuroinflammation is critical. This resolution of neuroinflammation is accomplished not only by ceasing to produce the pro-inflammatory mediators but by producing anti-inflammatory mediators (IL-4, IL-10, TGF-β) [13]. Thus, microglia are key cellular participants in all stages of neuroinflammation, initiation, resolution, and the following tissue repair. One needs to keep in mind, however, that either this can be a temporal sequence of phenotypes through which microglia pass or it could be a sequential response mediated by distinct subsets of microglia. If the inflammatory response is overshooting in amplitude or length, cells can be damaged or killed, resulting in the spillage of intracellular molecules (chemokines, ATP, heat shock proteins, mitochondrial proteins some of which are formylated like their bacterial forefathers). Since these intracellular molecules are not normally "seen" by microglia, they act as additional danger signals that activate microglia or continue to maintain the microglia in an activated state. This cascade can propagate the neuroinflammatory response or prevent its resolution, potentially starting a vicious cycle. Unrelated inflammatory diseases or conditions (including peripheral inflammation or infection) may directly or indirectly "alert" microglia in the CNS. In this alerted state, microglia are primed such that when a subsequent danger signal comes along, the response is potentiated, leading to greater bystander damage. Thus, microglia activation can be a double-edged sword, one side slaying the microbe, the other the neuron [13, 14, 16].

2.2 Microglia in HD

The first observation of activated microglia in autopsy brains from patients with HD, reported an altered microglial morphology and increased expression of HLA-DR (a component of the antigen-presenting "machinery") [8, 9]. This observation was interpreted as evidence of inflammation in the brains of these patients since HLA-DR expression is also increased on antigen-presenting cells during inflammation. Since then, other studies have demonstrated that in addition to increased expression of HLA-DR, other markers of microglial activation are also increased in HD brain, supporting the hypothesis that there is inflammation in the brains from patients with neurologic disease. These observations are complemented by PET imaging studies demonstrating increased microglial activation in HD patients (see below) [17, 18]. The first study to suggest that microglia might

actually participate in the pathological processes already in motion was the finding that there was an increased number of microglia in the caudate putamen from HD patients [19]. A subsequent study then showed an increase in the number of morphologically activated microglia in the neostriatum, cortex, and globus pallidus and adjoining white matter of HD brains vs. controls [20]. The numbers and density of microglia increased with the grade of HD pathology (i.e., Vonsattel rating scale [21]) and increasing neuronal loss. The findings were confirmed in additional studies with human HD tissue and extended to the R6/2 mouse model of HD where the number of activated microglia was also found to be increased in the striatum [20]. Importantly, this study was also the first to demonstrate the expression and, in some cells, aggregation of HTT in microglia. Aggregation of HTT is considered to be a hallmark of HD, and the observation that microglial cells contain HTT aggregates might indicate a direct cell-autonomous effect of HTT on microglial cells.

More recently, it was demonstrated that wild-type microglia localize to neurons expressing mutant HTT fragments, specifically, along dystrophic neurites but not to somata with mutant huntingtin inclusions [22]. Concurrent with neurodegeneration, microglia increased their expression of Iba1, increased in number, underwent morphological alterations (more amoeboid, less ramified), and increased the expression of the proliferation marker Ki67. Surprisingly, the inflammatory mediators IL-1β, TNFα, and IFNγ were unchanged. In contrast to other studies, however, IL-6 and complement 1q were increased once overt neurodegeneration set in, suggesting that neuroinflammation was still occurring even though different effector molecules were released [22]. Together, the data suggest that microglia recognize and respond to neurons expressing mutant HTT, perhaps to remove dysfunctional synapses or neurites.

A study by Singhrao et al. showed that in addition to an increased number of microglia, there was increased complement biosynthesis by microglia and an increase in complement activation on neurons [19]. For a long time, the complement system was ignored by neuroscientists who considered it a part of the peripheral innate immune system that provides powerful cytotoxic and cytolytic activities against a large variety of pathogens [23]. Over the years, it has become increasingly clear that complement is not only synthesized in the CNS but also participates in most CNS pathologies from acute stroke to chronic neurodegenerative diseases such as HD [24–26]. In HD postmortem samples, neurons, astrocytes, and myelin show increased deposition of C1q, C4, and C3, iC3b-neoepitope and C9-neoepitope compared with non-HD controls [19]. The authors hypothesized that increased levels of complement in HD brains contributes to disease progression, by either contributing to increased inflammatory signaling or the elimination of synapses or neurons by phagocytosis by microglia. Furthermore, the complement receptors, C3aR and C5aR, are also strongly expressed in HD caudate. Interestingly, activation of these receptors in microglia resulted in a reorganization of the actin cytoskeleton and subsequent increase in motility [27]. The increase in expression of complement

proteins in HD brains could not be recapitulated in the R6/2 mouse model. Furthermore, crossing the R6/2 mice with C3 KO mice did not change their phenotype [28]. Intriguingly, the complement system has been shown to play an important role in synaptic pruning in CNS development, homeostasis and disease (reviewed in [29]), suggesting that perhaps the complement system has a function (such as synaptic pruning or phagocytosis) not revealed in the R6/2 mouse model of HD. Thus, the role of increased complement proteins and receptors in brains from HD patients remains to be determined.

Our understanding of the role of neuroinflammation in HD was advanced in 2006 by studies using the radioligand PK-11195 that labels microglia [30, 31]. PK-11195 binds to the peripheral benzodiazepine receptor whose expression is significantly increased in activated microglia and, some studies suggest, reactive astrocytes [31]. Pavese and colleagues demonstrate increased binding of PK-11195 and presumably increased peripheral benzodiazepine receptor in the striatum of HD patients [17, 30]. The increase in PK-11195 binding was correlated with disease progression as assessed by the loss of dopamine D2 receptor binding sites in the striatum. Since most HD is inherited, genetic testing can be used to identify at-risk individuals years before disease onset. In a follow-up study, the same investigators were able to demonstrate that microglial activation is already evident as much as 15 years before onset of overt symptoms which was predicted based on CAG repeat length. The appearance of activated microglia over a decade before disease onset suggests that neuroinflammation is an early event in the disease. Furthermore, higher levels of microglial activation and decreased levels of D2 receptors were associated with a higher probability of developing clinical HD (as determined by onset of symptoms). The findings that neuroinflammation preceded neurodegeneration put the spotlight on microglia and neuroinflammation as a disease biology that had to be more than just a coincidental consequence of disease and the degenerative processes in play.

2.3 Astrocytes in HD

As with microglia, the idea that astrocytes might play a prominent role in the etiology or progression of HD took several years to gain traction even though the expression of HTT in astrocytes was demonstrated soon after the discovery of the gene mutation that caused HD [32, 33]. It was not until much later that genetic studies in mice implicated astrocytes in the pathogenesis of mutant HTT [34–38]. Restriction of mutant HTT expression to select neuronal populations, as opposed to pan-expression, actually resulted in a marked reduction of motor deficits and neuropathology in the striatum [39]. When the mutant HTT transgene was expressed in neurons and astrocytes, progression of disease like symptoms and neuropathology was exacerbated vs. those mice in which expression was restricted to neurons [40, 41]. This suggested that astrocytes, like microglia, are important contributors to neuroinflammation and pathology in HD animal models and likely in patients as well.

Like microglia, astrocytes can respond to danger signals, endogenous and foreign, in a process termed reactive astrogliosis. Their responses are also a continuum of changes that depend on the nature of the insult and signals from other cells in the CNS. These changes can result in changes to tissue structure, scar formation, and altered blood flow. Upon activation, the gene expression profile of astrocytes changes dramatically as does their phenotype and their portfolio of signaling molecules [42]. In animal models of Huntington's disease, mutant HTT expressed in astrocytes can accumulate in their nuclei and decrease the expression of glutamate transporters [35–37]. In these models, researchers demonstrated that the uptake of glutamate as well as the release of CCL5/RANTES and brain-derived neurotrophic factor (BDNF) was diminished [43]. While mRNA for the astrocyte-expressed glutamate transporter (EAAT2) was reported to be altered in HD brains in one report [44], another study failed to find any alteration in synaptosomal glutamate transport [45]. While decreased BDNF levels in HD have been largely ascribed to neuronal loss [46], BDNF is also expressed in astrocytes (and microglia) where expression of HTT decreases its release via transcriptional regulation [47]. In support of a role for astrocytic BDNF in HD, targeting BDNF overexpression to astrocytes delayed disease in animal models of HD, suggesting that this may be a therapeutic strategy for disease intervention [48, 49]. BDNF is one among dozens of genes whose transcription is regulated by NF-κB, a transcription factor found throughout the body. NF-κB plays a critical role in both microglia and astrocytes, by positively and negatively regulating transcription of various signaling molecules. For example, NF-κB can increase the transcription of pro-neuroinflammatory genes, such as cytokines, while leading to decreased transcription of neurotrophic factors. One mechanism of activating the NF-κB pathway is via stimulation of the toll-like receptor 4 (TLR4) with lipopolysaccharide (LPS). A recent study demonstrated that two HD mouse models (Hdh(150Q) and R6/2) both responded more robustly to systemic LPS with greater systemic inflammation and by producing more pro-inflammatory cytokines in the brain [50]. The hypothesis that the increased response to LPS was due to enhanced NF-κB activation was supported by observations that activated NF-κB levels were elevated in HD patients and that astrocytes from R6/2 mutant mice express higher IκB kinase (IKK) activity, which prolongs NFκB activation [50]. Similar increases in astrocytic NF-κB levels in mouse models of HD as well as HD patients suggest that enhancement of the NF-κB signaling pathway in astrocytes could contribute to neuroinflammation and HD.

Astrocytes also play a vital role in cholesterol synthesis, transport, and metabolism. Cholesterol is a vital molecule for the CNS, yet it does not cross the BBB and thus has to be synthesized locally. Almost 25 % of a person's cholesterol is within the CNS, and 70 % of that is incorporated in oligodenroglial myelin sheaths enwrapping axons [51]. Lipid imbalance as a potential cause of HD was first proposed in the 1970s but was highly controversial [52–54]. Much later it was demonstrated that expression of mutant HTT reduced the expression of genes involved in cholesterol biosynthesis in vitro and in vivo (R6/2 model) [55, 56]. However, over time dysfunction in the cholesterol synthesis pathway was replicated across 4 different HD rodent models (R6/2, YAC, Hdh^{Q111}, transgenic HD rats) [57–59].

Neurons make cholesterol much less efficiently than glia, but it is an absolute necessity for their survival and ability to function. All glial cells contribute to the overall cholesterol pool in the CNS, but the majority of neuronal cholesterol originates from astrocytes. The ABCA1 transporter on astrocytes loads cholesterol onto ApoE which carries it to neurons (and oligodendrocytes). Neurons have an array of receptors to take up the cholesterol-rich ApoE molecules. It is interesting to note that ApoE4/4 genotype is a significant risk factor for Alzheimer's and Parkinson's disease, with deficits in cholesterol transport (potentially due to reduced ability to bind to LRP class of receptors) as one proposed mechanism [60]. Oligodendrocytes (discussed below) also produce cholesterol. If the production of cholesterol is inhibited, myelination cannot occur, causing a profound phenotype, including ataxia and tremor [61]. It is interesting to note that a close relative of ApoE is ApoJ, also known as clusterin. Clusterin plays an important role in complement activation and innate immune responses, raising the question whether cholesterol metabolism and these pathways may have additional roles in neuroinflammation. Thus, cholesterol metabolism and transport is another vital metabolic process connecting the main propagators of neuroinflammation; however, little is known about neuroinflammation-dependent changes in cholesterol in HD.

2.4 Central and Peripheral Cytokines

As mentioned earlier, peripheral inflammation, infection, or disease states can alter the phenotype of microglia in the CNS and, more generally, affect the neuroinflammatory status of the CNS [34]. It is also becoming increasingly clear that the peripheral immune system significantly impacts neurological disease [62, 63]. The first evidence that the immune system may be dysregulated in HD was presented by Lebhuber et al. in 1998 [64]. In their study of 12 patients and 10 controls, they reported increased serum levels of IgA, soluble TNF receptor, soluble IL-2 receptor, neopterin, and complement C3 [64]. Another group showed that chemokines were elevated in the plasma of HD patients [65]. Eotaxin, eotaxin-3, MIP-1β, MCP-1, and MCP-4 were significantly elevated in HD patients. Of these, three (eotaxin-3, macrophage inflammatory protein (MIP)-1β, and eotaxin) correlated with advancing disease stages [65]. Björkqvist and colleagues demonstrated that HD gene carriers had elevated IL-6 levels, on average, 16 years before the predicted onset of clinical symptoms [66]. Interestingly, a more global increase in cytokine transcripts was also detected in the striatum of HD patients [11]. The Björkqvist study also examined the response of monocytes from HD subjects, in order to determine if they might be the source of the elevated cytokines observed in the plasma of patients. The monocytes not only expressed mutant HTT but also release significantly more IL-6 in response to stimulation by lipopolysaccharide. Similar patterns of cytokine release were observed in macrophages and microglia from HD mouse models [66]. Finally, IL-6, IL-10, CXCL1, and interferon-γ were significantly elevated in the serum

of HD vs. wild-type mice but were normal in HD mice receiving a bone marrow transplant from WT mice [67]. Together, these data suggest that there is dysregulation of the peripheral immune system that might parallel the neuroinflammation in the CNS and that, perhaps, this could be a contributing factor to HD pathology.

2.5 Peripheral Immune Cells

The observation that peripheral cytokine release is dysregulated in HD led people to investigate further the role of HTT in immune cells as well as HD. Studies that quantified mutant as well as total HTT protein levels in leukocytes from patients with HD demonstrated robust changes in mutant HTT expression between carriers and noncarriers and also between asymptomatic and symptomatic carriers of the *HTT* mutation in monocytes as well as T and B lymphocytes [68]. The investigators also demonstrated a significant correlation between mutant HTT levels and disease burden scores and caudate atrophy rates in monocytes and T cells in patients with HD. However, total HTT levels in leukocytes were not different between HD patients and controls or between different disease stages within the same patient. In contrast to monocytes and T cells, mutant HTT was not altered in buccal cells between any group, suggesting that the increased expression and dysfunction are specific to at least some cells of the immune system [68]. Chemotaxis was another immune cell function that was determined to be dysregulated by mutant HTT in leukocytes (white blood cells including monocytes, T and B cells, basophils, neutrophils, eosinophils, and dendritic cells) from the HD mice. Leukocytes from mutant HTT mice as well as carriers of the *HTT* mutation had a blunted chemotactic response [69]. Evidence that the peripheral cells could impact the CNS pathology in HD mouse models came from a study in which bone marrow from wild-type mice was transplanted into lethally irradiated transgenic mice (YAC128 and BACHD mice) [67]. While the bone marrow transplant only partially attenuated the hypokinetic and motor deficits in HD mice, the investigators observed increased levels of synapses in the cortex of these mice. This suggests that transplantation of peripheral immune cells could influence some of the pathophysiology in HD models. Interestingly, the group observed that in the brain of irradiated HD mice, many more microglia were positive for Iba1 as well as green fluorescent protein (GFP) than in normal irradiated mice. Since only the transplanted bone marrow cells were GFP positive, the Iba1/GFP double positive cells in the brains of these mice must have come from the periphery. The implication is that more bone marrow-derived cells could migrate into the CNS of irradiated HD mice than in irradiated wild-type mice. Once in the CNS, the bone marrow-derived cells can directly influence neuroinflammation and the pathological processes in mouse models of HD, and perhaps in patients as well [67].

Together, the findings suggest that multiple functions of immune cells are dysregulated by mutant HTT. Furthermore, the dysregulation of immune cell function is

not restricted to a single immune cell lineage, but manifest in the myeloid, lymphoid lineages, as well as yolk sac-derived microglia of the CNS. While the studies do not identify the sequelae of increased mutant HTT in T or B cells, they do raise the question whether or not the increased mutant HTT levels cause T or B cell dysfunction, much like they do in monocytes. Could T and B lymphocytes also contribute to the initiation or progression of HD? Critics of the hypothesis point out that there is no large-scale infiltration of the CNS by T cells as in multiple sclerosis (MS). In MS, it has been demonstrated in animal models as well as the clinic that T cell infiltration into the CNS plays an integral role in the pathophysiology of the disease as illustrated by Tysabri® [70]. In other neurodegenerative diseases such as PD and ALS, evidence supporting a role for T cells in the pathological process is accumulating, even though large T cell infiltrates are generally not observed [71, 72]. Recent studies, however, have demonstrated that T cells patrol the CSF and subarachnoid spaces (and perhaps even the CNS parenchyma), supporting the hypothesis that T cells are able to get into the CNS and potentially respond to antigen presentation and participate or modulate neuroinflammation [73]. The ability of T cells to control parasitic (*Toxoplasma gondii*) infections of the CNS demonstrates not only that T cells patrol the brain parenchyma but that they are able to execute their immunological functions as well. It will be interesting to see if future studies will demonstrate that mutant HTT expression in T cells results in their dysfunction and if this dysfunction contributes to the pathophysiology of HD.

3 Targeting Neuroinflammation in Huntington's Disease

An area of active investigation, not just for HD but all major neurological diseases, is the therapeutic targeting of neuroinflammation. By expanding drug discovery, efforts from the traditional neuron-focused strategies (neuroprotection, neuroregeneration, neurotransmission) to include neuroinflammation greatly increase the diversity and number of targets amenable for therapeutic intervention using small molecules and biologic. Targets, such as toll-like receptors, cytokine and chemokine receptors, purinoceptors, neuro- and glio-transmitter receptors, kinases, glutamate transporters, and catalytic enzymes (proteolytic, reactive oxygen species generators, ATP hydrolyzing), may offer tractable novel approaches to treat HD. Microglia have many of the same receptors or signaling pathways as peripheral monocytes/macrophages. This, unfortunately, is a double-edged sword, in that it is target rich and may present repurposing opportunities but may also potentially result in unwanted side effects mechanistically coupled to the target's role in the peripheral immune system (e.g., increased risk of infection). Astrocytes and oligodendrocytes have the potential advantage that they have less overlap with cells of the immune system, decreasing the risk of unwanted immune-related side effects. Other glial targets, such as neurotransmitter receptors and amino acid transporters, may also be expressed on neurons, raising the possibility of significant adverse neuronal side effects.

Currently, small molecules are the best strategy for treatment of CNS diseases as CNS-penetrant molecules can be designed or selected. While many targets would likely require a biologic as a therapeutic, delivery technologies need to be developed to increase the brain penetration, or the biologics themselves need to be optimized to achieve significant brain penetration. Until these challenges are effectively solved, many promising targets that regulate neuroinflammation will remain intractable (including adhesion molecules, immunoglobulin signaling molecules, and other targets not currently amenable to modulation by small molecules [74]). Finally, as we learn more about the role of peripheral immune cells in HD perhaps, one could target them in the periphery, without the need for a CNS-penetrant agent. Such a therapeutic could alter the phenotype of peripheral cells before they migrate into the CNS. It could also block/augment the ability of peripheral immune cells to migrate into the CNS (depending on if they are desirable or pathological). Precedent for this idea comes through modulation of peripheral immune mechanisms in the treatment of MS.

One area of intensive exploration has focused on the mechanism of action of the antibiotic minocycline, which has been reported to ameliorate neuroinflammation and subsequent pathology in many animal models of neurological disease, including HD models [75]. Experiments using minocycline were believed to target neuroinflammation presumably via inhibition of NF-κB [75, 76]. In contrast, other studies have suggested that minocycline targets caspases and neuronal apoptosis [77, 78]. The actual mechanism of action or the target of minocycline is a matter of ongoing debate and is reviewed elsewhere [79–82]. While minocycline has not been used in a clinical trial for HD, it was tested in numerous other neurological diseases and disorders exhibiting neuroinflammation. Unfortunately, the promising findings failed to translate into the clinic, as the clinical trials to date have largely failed [75].

Another potentially promising therapeutic strategy for HD is to regulate or normalize kynurenine metabolism which can produce both neuroprotective and neurotoxic metabolites. The kynurenine pathway is the primary route of l-tryptophan metabolism and the primary metabolic pathway for the formation of nicotinamide adenine dinucleotide (NAD^+) [83]. Several of the metabolites in this pathway have neuroactive properties. (For review, see [84, 85].) Schwarcz and colleagues were the first to suggest that the kynurenine pathway may play a role in the pathogenesis of HD by showing that an intra-striatal injection of quinolinic acid replicates many features of human HD in rodents [86]. Quinolinic acid is an N-methyl-d-aspartate (NMDA) receptor agonist and induces excitotoxicity [87]. Follow-up studies demonstrated that the levels of neurotoxic kynurenine metabolites were elevated in HD patients and mouse models, whereas the levels of neuroprotective metabolites were decreased [85, 88]. A subsequent report demonstrated that cultured microglial cells from the R6/2 HD mouse model synthesized increased levels of neurotoxic kynurenine metabolites [89]. The discovery that genetic ablation of kynurenine 3-monooxygenase (KMO) suppresses HTT-mediated toxicity and the fact that KMO is predominantly expressed in microglia, not neurons, made for a strong argument that microglial kynurenine metabolism might play a significant role in HD [89–91]. Microglial dysregulation of the kynurenine pathway was also the first

example of a potentially non-cell-autonomous mechanism in HD (from a neuronal point of view). KMO thus presents an attractive target for the treatment of HD as evidenced by the number of academic and nonprofit organizations working to develop KMO inhibitors [92].

Another strategy for targeting neuroinflammation is based on activation of the cannabinoid receptor 2 (CB2R) which in the CNS is only expressed on activated microglia [93]. The authors demonstrated the role of CB2Rs in regulating neuroinflammation by knocking out the CB2R in the R6/2 mouse model of HD which resulted in enhanced microglial activation, worsened disease symptoms, and shortened life span. Following injection of the neurotoxin quinolinic acid, edema and loss of medium spiny neurons were also exacerbated in the R6/2 mice lacking CB2Rs as compared to R6/2. Pharmacological activation of CB2R in R6/2 mice with intact CB2R attenuated the microglial activation and loss of medium spiny neurons following quinolinic acid lesioning [94]. The study also demonstrated that a CNS-penetrant CB2R agonist can extend the life span while suppressing motor deficits, synapse loss, and CNS inflammation in a mouse model of HD. Unexpectedly, a non-CNS-penetrant CB2R antagonist had similar effects. Since this compound does not reach the CB2R-expressing microglia in the brain, it suggested that peripheral cells were driving the neuroinflammation and pathology [95]. CB2 agonists were also protective against striatal malonate toxicity, another toxin model of Huntington's disease [96, 97]. While there is some debate about the expression of CB2 in microglial cells in vivo [93], these studies suggest that neuroinflammation in HD may be reduced by pharmacological activation of CB2 receptors.

Currently, there are no disease-modifying treatments for HD, only symptomatic medications. As discussed previously, there are many potentially promising therapeutic targets that could treat the neuroinflammation associated with HD. Even if blocking neuroinflammation in HD only would be able to slow disease progression, but not provide a cure, it would be considerable progress for a disease with currently very limited therapeutic options. While the target space for neuroinflammation in HD is rich, it still needs validation in the clinic. Since neuroinflammation is present in all neurological disease, there is the potential that discovery of a therapeutic that blocks or reduces neuroinflammation in HD may also have efficacy in other devastating neurological diseases.

4 Summary and Conclusion

Neuroinflammation is increasingly being recognized as a biological process that is intimately linked to the pathological cascades underlying HD. While symptoms are a manifestation of neuronal dysfunction or loss, neurons do not live and die in isolation within the CNS. They are in constant contact, not only with each other, but also with astrocytes, oligodendrocytes, and microglia. The polyQ expansions have been well documented to cause neurotoxicity and render neurons more vulnerable to toxic insults, but they have also been shown to alter microglial and astrocytic functions. The finding that microglial activation is increased years before overt

neurodegeneration suggests that neuroinflammation is involved early HD and, at minimum, an important contributor. This is supported by numerous studies showing that immune-related markers are similarly dysregulated in HD as well as the animal models. Furthermore, if these mechanisms are modulated, they result in robust changes in "disease" outcome in the models, suggesting that they also play a significant role in disease. Peripheral immune cells may also affect neuroinflammation upon migration into the CNS or by release of inflammatory mediators that indirectly alter neuroinflammation. While the interplay of these CNS resident and peripheral players presents a daunting complexity, it also provides a wealth of new targets for desperately needed therapeutics.

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