*Inflammation, Vol. 37, No. 3, June 2014 (© 2013)*
DOI: 10.1007/s10753-013-9780-2

# Inflammatory Mediators as Biomarkers in Brain Disorders

**Domenico Nuzzo,[1] Pasquale Picone,[1] Luca Caruana,[1] Sonya Vasto,[1,2] Annalisa Barera,[2] Calogero Caruso,[3] and Marta Di Carlo[1,4,5]**

*Abstract*—Neurodegenerative diseases such as Alzheimer, Parkinson, amyotrophic lateral sclerosis, and Huntington are incurable and debilitating conditions that result in progressive death of the neurons. The definite diagnosis of a neurodegenerative disorder is disadvantaged by the difficulty in obtaining biopsies and thereby to validate the clinical diagnosis with pathological results. Biomarkers are valuable indicators for detecting different phases of a disease such as prevention, early onset, treatment, progression, and monitoring the effect of pharmacological responses to a therapeutic intervention. Inflammation occurs in neurodegenerative diseases, and identification and validation of molecules involved in this process could be a strategy for finding new biomarkers. The ideal inflammatory biomarker needs to be easily measurable, must be reproducible, not subject to wide variation in the population, and unaffected by external factors. Our review summarizes the most important inflammation biomarkers currently available, whose specificity could be utilized for identifying and monitoring distinctive phases of different neurodegenerative diseases.

**KEY WORDS:** Alzheimer disease; Parkinson disease; amyotrophic lateral sclerosis; Huntington disease; inflammatory biomarkers.

## INTRODUCTION

In response to tissue damage caused by trauma or infection, the inflammatory response sets in as a complex network of molecular and cellular interactions directed to facilitate a return to physiological homeostasis and tissue repair. Inflammation is well defined as a localized response, but with systemic consequences caused by injury or tissue damage, which helps to destroy, reduce, or sequester both the harmful agent and the damaged tissue. Inflammation is well defined as a localized response, but with systemic consequences elicited by injury or tissue damage, which helps to destroy, reduce, or sequester both the harmful agent and the wounded tissue. It is characterized in the acute form by the classical signs of pain (dolor), heat (calor), redness (rubor), swelling (tumor), and loss of function (functio laesa). From a histological point of view, it involves a complex series of events, including vasodilatation of post-capillary veins, with increased permeability and blood flow, exudation of fluids, and plasma proteins and leukocyte migration into the inflammatory focus. Inflammation becomes a chronic condition that continuously damages the surrounding tissues in the cases in which the healthy tissue is not restored or in response to stable low-grade irritation. Neurodegenerative diseases are a group of chronic, progressive disorders characterized by the gradual loss of neurons in discrete areas of the central nervous system. Particular portions of the brain, spinal cord, or peripheral nerves are affected. Depending on the neurodegenerative disease, it can disturb cognition, movement, strength, coordination, sensation, or autonomic control. Inflammation is a common feature in neurodegenerative diseases. While inflammation may not be the event initiating neurodegeneration, there is evidence that chronic inflammation involving microglia and astrocytes activation

Domenico Nuzzo and Pasquale Picone contributed equally to this article.

[1] Institute of Biomedicine ed Molecular Immunology, National Research Council of Italy, Palermo, Italy
[2] Department of Biologic, Technology, Chemistry and Pharmaceutical Science, University of Palermo, Palermo, Italy
[3] Department of Biopathology and Medical and Forensic Biotechnology, University of Palermo, Palermo, Italy
[4] Istituto di Biomedicina ed Immunologia Molecolare (IBIM) Alberto Monroy, CNR, via Ugo La Malfa 153, 90146 Palermo, Italy
[5] To whom correspondence should be addressed at Istituto di Biomedicina ed Immunologia Molecolare (IBIM) Alberto Monroy, CNR, via Ugo La Malfa 153, 90146 Palermo, Italy. E-mail: marta.dicarlo@ibim.cnr.it

contributes to disease progression. The inflammatory response has been implicated in several neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS). Currently, a major question concerns whether inhibition of the inflammatory response can reverse or slow the symptoms of disease [1, 2]. In general, inflammation may have beneficial and/or injurious effects in some particular disease and/or in a particular phase of a disease. However, in neurodegenerative diseases chronic inflammation causes death/loss of neurons. In fact, there is evidence that brain inflammation may contribute to triggering off the pathology of neurodegenerative diseases including AD, PD, HD, and ALS [3–5]. These pathologies have different causes and consequences, and there is evidence that blocking inflammation can either delay onset or reduce symptoms [6]. Microglia can have three different morphologies: resting, activated, and amoeboid/phagocytic. The healthy, non-inflamed brain contains almost entirely "resting" microglia which are highly ramified, with a small, static cell body, but with dynamic and branched processes actively seeking out pathogens and damage in the brain [7]. Depending on the pathology, different pathways contribute to neurodegenerative processes activated by inflammation. Factors released from damaged neurons such as α-synuclein in PD, deposits of amyloid aggregates in AD, and SOD1 in ASL trigger activation of microglia and astrocytes which, in turn, release pro-inflammatory molecules. Furthermore, inflammation leads to enhanced levels of oxidative stress; astrocytes release ROS and NO that, together with NADPH oxidase stimulation, provoke microglia activation. Subsequently, activated microglia may secrete signals to recruit $CD4^+$ $CD25^+$ T cells, which directly affect neurons via Fas/Fas–ligand interaction. However, other events, such as mitochondrial dysfunction, protein aggregation, glutamate excitotoxicity, and loss of trophic factor support, may promote neuronal cell death. For instance, tumor necrosis factor-α (TNF-α), a major pro-inflammatory cytokine, activates microglia and cause neurotoxicity in motor neurons. The inflammatory mediators such as TNF-α, IL-1β, and IL6 derived from non-neuronal cells including microglia modulate the progression of neuronal cell death in neurodegenerative disease. Apoptosis and necrosis of neurons result in the release of ATP, which further activates microglia through the purinergic P2X7 receptor [1] (Fig. 1). Due to the extensive involvement of the inflammatory process in brain disorder, it is clear that the identification of biomarkers within the inflammatory process is of paramount importance. Biomarkers are a most important tool for an accurate diagnosis and prognosis of a disease and for monitoring the effect of therapeutic intervention [8]. Many unsuccessful efforts have been done to find a biomarker that fulfills the criteria of an ideal biomarker specific for neurodegenerative disease. There are some technical limitations in identifying low abundant cytokines, and many factors likely influence immune markers, such as concomitant infection and inflammatory illness. Furthermore, the levels of many biomarkers such as cytokines have been shown to display daily variation and different alterations caused, for example, by the storage of the samples, and standardization of pre-analytical procedures is fundamental to obtain reproducible results. On the basis of this experience, in the research and validation of new biomarkers some criteria must be considered and standardized such as healthy controls, clinical diagnosis and verification, publication, and validation by multicenter studies. Finally, the development of new technologies is now permitting to overcome some of these problems for screening and discovering new biomarkers. Different biomarkers identified in the main neurodegenerative diseases are summarized in Table 1.

## ALZHEIMER'S DISEASE

Alzheimer's disease is a heterogeneous and progressive neurodegenerative disease that in western societies accounts for 60 to 80 % of all dementia cases. Age is the first and primary risk factor in AD. Prevalence of AD increases exponentially with age, rising from 3 % among individuals between 65 and 74 years to almost 50 % among those from 85 years or older [9]. Hebert *et al*. estimated that the total number of people with AD dementia in 2050 would be 13.8 million, with 7.0 million aged 85 years or older [10]. The genetics of AD is complex and heterogeneous. Most cases are "sporadic" with no apparent familial recurrence of the disease. However, a small percentage of AD cases (1–2 % of all cases) have an early onset, with symptoms appearing before 65 years of age [11]. Neuropathological hallmarks are neuritic amyloid plaques and neurofibrillary tangles. Amyloid is a general term for protein fragments that the body produces normally. Beta amyloid (Aβ) is a protein fragment obtained from the amyloid precursor protein (APP) after cleavage of two specific secretases. In a healthy brain, these protein fragments are broken down and eliminated. In Alzheimer's disease, the fragments are misfolded and aggregate to form oligomers, fibrils and form hard, insoluble plaques. Extracellular senile plaques result from the accumulation of several

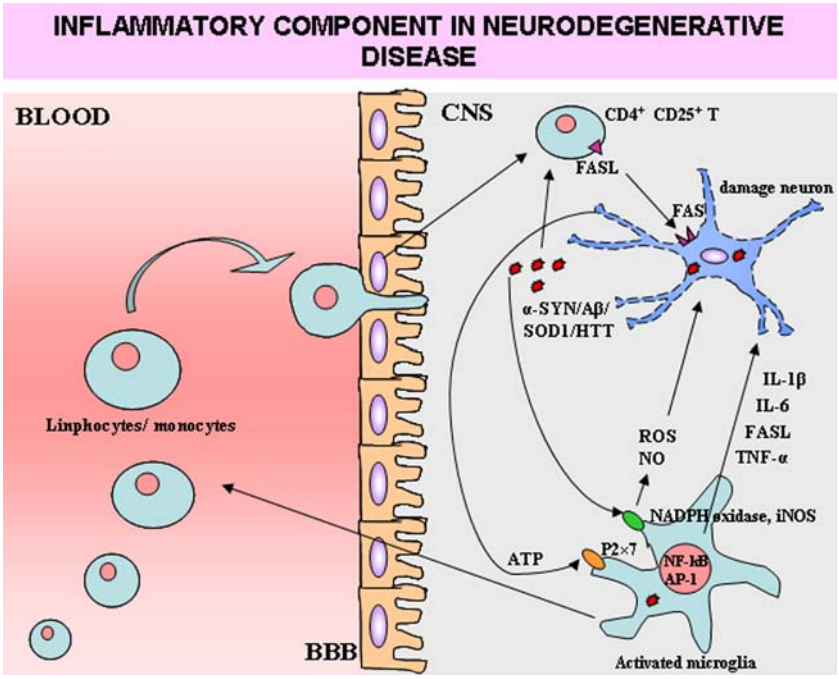


**Fig. 1.** A model of the different inflammatory pathways involved in neurodegenerative disease. Factors involved in neurodegenerative diseases such as α-synuclein in PD (*α-SYN*), deposit of amyloid aggregates in AD (*Aβ*), SOD1 in ASL (*SOD1*), and Huntingtin (*HTT*) in HD trigger activation of microglia. Activated microglia release pro-inflammatory molecules (IL-1β, IL6, FASL, and TNF-α). Inflammation leads to oxidative stress and release of ROS and NO, through NADPH oxidase and iNos stimulation, inducing neuronal death via apoptosis and/or necrosis. A consequence is the release of ATP, which activates microglia through the purinergic P2X7 receptor. Activated microglia secretes signals to recruit $CD4^+$ $CD25^+$ T cells, which directly affect neurons via Fas/Fas–ligand interaction. *BBB* blood–brain barrier.

proteins and an inflammatory reaction around deposits of amyloid proteins. Neurofibrillary tangles are intracellular deposits of hyperphosphorylated degenerate filaments, which result from aggregations of the microtubular protein tau. As these cellular changes progress, neurons are lost in the hippocampus, entorhinal cortex, and associated areas of the neocortex. The clinical hallmarks of AD are progressive impairment in memory, judgment, decision-making, orientation to physical surroundings, and language together with changes in personality, behavior or comportment, which cause a significant interference in the ability to function at work or in usual daily activities [12]. Immunohistochemical studies have shown that the plaques and tangles of AD are heavily infiltrated with activated glial cells and inflammatory factors. Cytokines, chemokines, complement components, and acute phase proteins are co-localized as secondary components in senile plaques or are over-produced in AD brains [13]. Activated glial cells surround the depositions of amyloid attempting to phagocyte and degrading amyloid component. Microglia cells recruit astrocytes too, activating them. Following activation, both cell types produce acute-phase proteins, complement components, prostaglandins, and cytokines. Actually, microglia cells are a high producer of free radicals and generation of reactive oxygen species (ROS)

**Table 1.** Inflammatory Biomarker Studies of Prospective Cognitive Decline in Neurodegenerative Disease

| Biomarker | Study cohort | Reference |
|---|---|---|
| Alzheimer disease | | |
| Interleukin-8 | 48 patients | Zhang 2008 [28] |
| s100B | 31 patients | Petzold 2003 [19] |
| 18 markers in blood | 2,007 patients | Doecke 2012 [29] |
| Complement component, Serpin G1 Ser/cYS protease inhibitor, Serpin G1 plasma protease C1 inhibitor | 7 patients | Henkel 2012 [31] |
| Parkinson disease | | |
| IL-6 | 84 patients | Chen 2008 [43] |
| Fibrinogen | 61 patients | Wong 2010 |
| miRNA | 7 patients | Margis 2011 [45] |
| Amyotrophic lateral sclerosis disease | | |
| TDP-43, cycl A, ERp57 | 23 patients | Nardo 2011 [62] |
| MMP-9 | 25 patients | Beuche 2000 [65] |
| Huntington disease | | |
| Cytokine IL-6 | 194 patients | Björkqvist 2008 [72] |

contributes to damage neurons. Since Aβ represents a pathogenetic molecule, the innate immune system makes an initial attempt to protect the brain by clearing these potentially toxic products. The hypothesis is that the complex nature of the plaques and tangles stimulates a chronic inflammatory reaction to scavenge this debris. Chronically activated microglia and astrocytes can kill adjacent neurons by the release of highly toxic products such as reactive oxygen intermediates, nitric oxide, proteolytic enzymes, complement factors or excitatory amino acids. Microglia cells activation in AD can be due to the binding of Aβ to the CD14 receptor and its co-receptor TLR4 [14]. Cerebrospinal fluid (CSF) is a fluid that circulates throughout the central nervous system (CNS) and is located between the brain and skull. It is produced in the brain and can therefore be considered a window onto the brain. The CSF is in direct contact with the extracellular space of the brain and can reflect biochemical changes occurring in the latter. Attention to CSF biomarkers is important in the treatment of patients to assess *in vivo* pathology, even if the use of CSF is limited because it is considered an invasive collection method. In particular, interleukins-2, -3, and 6, transforming growth factor β1 (TGFβ1), interferon-α, heparin binding growth-associated molecule, nitric oxide synthase, macrophage-colony stimulating factor, interleukin-8 receptor B, monocyte chemoattractant protein-1, the beta-chemokine receptors CCR3 and CCR5 and macrophage inflammatory protein-1β (MCP1), fibroblast growth factor-9, vascular endothelial growth factor, and the interferon γ-inducible chemokine IP-10 were found [15]. A studied inflammatory biomarker is α1-antichymotrypsin (A1ACT). It is secreted by astroglial cells and can co-localize with Aβ in plaques of AD, and is observed either increased [16] or unchanged [17] in CSF samples of AD patients. However, the contradictory results suggest that more studies must be conducted before that A1ACT can be regarded as an effective biomarker. A study comparing levels of s100B, a protein secreted by astrocytes, in the CSF of AD patients and healthy controls found increased levels of s100B in mild-to-moderate AD, but not in severe AD [18]. This raises the possibility that s100B is an index of astrocytic process involved in the earlier stages of AD, possibly accompanying plaque maturation. In another study, s100B was increased in CSF to a similar extent in AD and fronto-temporal dementia relative to controls [19]. Interleukin-8 (IL-8) receptor has been localized in dystrophic neurites, suggesting that IL-8 mediates glial interactions with neurons and thereby contributes to neuronal damage [20]. IL-8 was significantly increased in CSF of AD patients compared to healthy controls [21], whereas plasma levels of IL-8 in late-onset AD and vascular dementia did not differ from controls [22]. Transforming growth factorβ (TGFβ) is an important astrocyte-derived cytokine, induced by IL-1, that manifests both pro-inflammatory and anti-inflammatory properties. Brain tissue levels of TGFβ and TGFβ mRNAs increase in AD [23]. A number of reports published between 1990s and early 2000s describe alterations in the levels of various cytokines, markers of oxidative stress and inflammatory molecules in CSF. However, results were very inconsistent, probably owing to differences in methodology, CSF collection and processing, assays, subject ascertainment, prevalence of comorbidities and methods of diagnosis. Unbiased proteomics methods have more recently been used to identify molecules that diverge between dementia of the Alzheimer type and control CSF. These studies have consistently identified a plethora of inflammatory markers that differ in abundance between clinical groups [24–26]. However, even in these unbiased screens, the direction of reported difference in abundance has not been consistent. In contrast to CSF, blood samples are much easier to obtain, but concentrations of most potential neuronal biomarkers are several fold lower in blood than in CSF [27]. Besides such practical problems, there are intrinsic difficulties encountered when blood serum or plasma samples are analyzed because the blood proteome contains large quantities of high-abundance proteins, which have most likely no direct reference to the pathological process. Given the multiplicity of pathophysiological processes implicated in AD, a combination of biomarkers related to different mechanisms might increase diagnostic accuracy. Several studies focusing on combinations of blood-based biomarkers have yielded positive results. Ray *et al*. used combined multivariate analysis of 18 plasma signaling and inflammatory proteins to identify AD patients and predict future AD with high accuracy in mild cognitive impairment (MCI) patients [28]. This protein panel was identified after screening of a large number of known proteins using a filter-based protein array. More recently, two research groups have found multi-marker panels able to distinguish unhealthy from healthy individuals. In one report, Martins and colleagues [29] found that a set of 18 markers in blood had sensitivity and specificity of more than 80 % for distinguishing patients with Alzheimer's disease from healthy controls. In a biomarker panel, significantly increased levels of cortisol, pancreatic polypeptide, insulin-like growth factor binding protein 2, β2 microglobulin, vascular cell adhesion molecule 1, carcinoembryonic antigen, matrix metalloprotein 2, CD40, macrophage inflammatory protein 1α, superoxide

dismutase, and homocysteine, and decreased levels of apolipoprotein E, epidermal growth factor receptor, hemoglobin, calcium, zinc, interleukin 17, and albumin were found in samples of AD patients. The other report, by Holly Soares and colleagues, indicated that a mainly different set of markers associated with the apolipoprotein E genotype such as eotaxin 3, pancreatic polypeptide, and N-terminal protein B-type brain natriuretic peptide, C-reactive protein, cortisol, interleukin 13, apolipoprotein B, and gamma interferon were increased both in patients with Alzheimer's disease and MCI. Plasma biomarker results confirm CSF studies reporting increased levels of pancreatic polypeptide and N-terminal protein B-type brain natriuretic peptide in patients with AD and MCI. Incorporation of plasma biomarkers yielded high sensitivity with improved specificity, supporting their usefulness as a screening tool [30]. Recently, new analytical proteomic technologies like MS coupled with protein separation or protein microarrays, which can be applied on CSF and other body fluids, have been developed to study proteins in neuroscience. Henkel *et al*. removed 12 high-abundance proteins from plasma and labeled the depleted samples from AD patients and disease controls with different fluorescent dyes, mixed the samples, and separated them by anionic exchange and RP chromatography. The resulting chromatography fractions were analyzed on 2D gels. They identified 20 significant differentially expressed proteins through MS analysis, some known to be involved in inflammatory processes: three members of the complement system (C1S, C6 complement component six precursor, and CFH isoform one of complement factor H), SerpinG1 Ser/Cys protease inhibitor, and Serpin G1 plasma protease C1 inhibitor [31]. However, in the research of new biomarkers for AD, it will become extremely important in the future to find also markers able to differentiate AD from other forms of dementia.

## PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive neurodegenerative pathology characterized by motor manifestations. Its prevalence increases with age, being approximately 1 % in people over the age of 60 and increasing to about 4 % over the age of 85 [32]. The etiology is still unknown [33], but it has been hypothesized that the onset of PD may be the result of a complex interaction among environmental factors, genetic susceptibility, and aging [34, 35]. The pathological hallmark of PD comprises loss of nigrostriatal dopaminergic neurons in the substantia nigra (SN) pars compacta (SNc) and the presence of insoluble protein inclusions, termed Lewy bodies (LBs) and Lewy neurites (LNs), located in either the neuronal cell bodies or neuronal processes, respectively [35–37]. The major constituent of LBs and LNs is a misfolded version of the protein alpha-synuclein (α-syn) [37]. Diagnosed patients are characterized by motor and non-motor clinical manifestations. The motor symptoms include resting tremor, bradykinesia, akinesia, muscular rigidity, and a loss of balance [38]. These symptoms are predominantly attributed to lack of dopamine (DA) in the striatum and to the resulting dysfunction of the basal ganglia, a cluster of nuclei involved in the initiation and execution of movement [38, 39]. Several lines of evidence suggest that inflammatory mediators such as ROS, NO, TNF-α, and interleukin (IL)-1β derived from non-neuronal cells including microglia modulate the progression of neuronal cell death in PD [40]. A direct effect of a-synuclein on microglia (as opposed to a TLR-dependent pathway as observed following LPS treatment) has been demonstrated. In fact, extracellular α-synuclein is phagocytized by microglia, resulting in activation of NADPH oxidase and ROS production. NADPH oxidase activation and ROS production are a crucial mechanism for microglia activation after exposure to α-synuclein as the toxic effect was less strong in mice lacking NADPH oxidase [41]. The oxidative stress induced by α-synuclein is a potent inducer of microglia *in vitro* and *in vivo* and is associated with activation of NFkB-related genes and increased expression of neurotrophins such as NFKB1, TNF, TNFRSF1A, BDNF, and GDNF [42]. Furthermore, activated microglia may secrete signals to recruit $CD4^+$ T cells, which directly affect neurons via Fas/Fas–ligand interaction. Moreover, nitrated α-synuclein may activate $CD4^+$ and $CD4^+$ $CD25^+$ T cells, and then they interact with microglia to initiate microgliosis and immune responses. Some other events, such as mitochondrial dysfunction, protein aggregation, glutamate excitotoxicity, and loss of trophic factor support, may also promote death of dopaminergic neurons. Several biomarkers have been associated with cognitive impairment in PD in longitudinal studies. Two recent studies have attempted to determine the predictive value of prediagnostic levels of inflammation on future PD risk. In one study [43], investigators observed an association between IL-6 concentrations and higher PD risk but no found any association with other markers of inflammation including C-reactive protein (CRP), fibrinogen, or TNF-α. In the other study, higher levels of fibrinogen were associated with higher PD risk among men of Japanese ancestry [44]. Chen and co-workers concluded that men

with high plasma concentrations of IL-6 have an increased risk of developing PD. An innovative study of miRNAs in blood samples from a healthy control group, untreated early-onset PD, and PD patients undergoing treatment identified six differentially expressed miRNAs. Whereas miR-1, miR-22, and miR-29 expression levels distinguished untreated PD from healthy patients, miR-16-2, miR-26a2, and miR30a differentiated treated PD patients from untreated patients [45], suggesting a potential approach to evaluate drug response. Moreover, careful CSF studies using a sophisticated metabolomics analysis platform have revealed PD-specific fingerprints with an increased xanthine/homovallinic acid ratio in PD compared with controls. This ratio correlated with disease severity making it a good candidate for both diagnostic and surveillance purposes [46, 47]. The search for effective biomarkers for diagnosis and surveillance of PD continues.

## AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis is an incurable neurodegenerative disorder of unknown cause arising from progressive degeneration of motor neurons and resulting in paralysis and death, usually within 3–5 years from diagnosis. Its incidence is between 1.5 and 2.5 per 100,000 per year: approximately 90 % of cases are sporadic and the remaining 10 % are familial. In the familial ALS (fALS), a number of causative genes were identified. The first gene identified was Cu/Zn superoxide dismutase 1 (SOD1), which has numerous different mutations [48]. Other genes implicated in fALS are fused in sarcoma protein (FUS) [49] and tar-DNA binding protein of molecular weight 43 kDa (TDP 43) [50]. For the diagnosis of ALS, there are strict clinical definitions [51] that involve the finding of a combination of upper and lower motor neuron signs. Moreover, the clinical course varies widely. At first presentation, some patients do not fulfill these strict criteria, but as time goes by they develop additional signs that confirm the diagnosis [52]. Neuro-inflammation is a prominent pathological feature in ALS. The importance of glial cell activation and pro-inflammatory cytokines in ALS has been confirmed by numerous studies. For instance, TNF-α, a major pro-inflammatory cytokine, activates microglia and causes neurotoxicity in motor neurons. Expression of mutant SOD1 in microglia leads to ROS production and secretion of TNF-α and the metalloproteinases (ADAM10–17) suggesting a role for oxidative stress in neuro-inflammation in ALS [53]. It is possible that degenerating neurons in the spinal cords of ALS patients release ATP activating glial cells and inducing astrocytic purinergic (P2X7) receptors to release IL-1β [54]. In addition, FUS/TLS is a coactivator of NF-κB, which may be involved in the inflammatory response [55, 56]. Therefore, TLRs and purinergic receptors may serve as sensors for candidate transcription factors, including activator protein-1 (AP-1) and NF-κB. Increased levels of ROS, inflammatory mediators such as COX-2, and proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 characterize glia activation [57]. Further investigations are required to better understand the role of inflammation in the CNS of ALS patients. Moreover, there have been numerous studies investigating peripheral immune abnormalities in ALS. With respect to T-cell abnormalities, in the blood of individuals with ALS, it has been reported that there is an increased number of $CD4^+$ T-helper cells and expression of HLA class II molecules on monocytes and macrophages, suggesting activation of immune system [58]. Another study found increased $CD4^+$ cells, reduced regulatory T cells (Treg), and expression of HLA DR in monocytes [59]. There are increased levels of circulating chemokines and cytokines in ALS. Other markers of inflammation are also abnormal in ALS. There is also evidence of low-level systemic inflammation with increased levels of C-reactive protein and ESR in patients with ALS compared to controls, with the levels correlating to disability as measured by the ALS functional rating scale [60]. All these studies demonstrate the presence of a systemic immune response in people affected by ALS. No ALS biomarkers and molecular diagnosis are currently in clinical use, but they would be valuable to support early diagnosis, monitor disease progression, and assess the efficacy of any new treatment [61]. Nardo *et al*. used a proteomic approach (using peripheral blood mononuclear cells, PBMC) to identify a panel of protein biomarkers that are closely associated with ALS. Their results indicate that PBMC multiprotein biomarkers could contribute to ALS diagnosis, severity, and progression. Validation and a longitudinal study were performed by immunoassays on a selected number of proteins. The same proteins were also measured in PBMC and spinal cord of a G93A SOD1 transgenic rat model. Their multiprotein biomarkers can distinguish ALS patients from healthy controls (98 %) and from patients with neurological disorders that may resemble ALS (91 %). They demonstrated that TDP-43, cyclophilin A, and ERp57 were associated with disease progression in a longitudinal study. Moreover, there are higher levels of the chemokine MCP-1 in patients with a shorter diagnostic delay, which is a marker of more severe disease progression disease [62]. Ching-Hua Lu showed

that plasma neurofilament heavy chain (NfH) levels closely reflect later stages of disease progression and therapeutic response in the SOD1 G93A mouse model of ALS and may potentially be a valuable biomarker of later disease progression in ALS [63]. Mitchell *et al*. reported a cytokine profile in the CSF of ALS patients using luminex multiplex assay. A five cytokines panel could predict ALS with 89 % accuracy in a set of 74 subjects. However, since altered cytokines expression occurs during inflammation, further verification and validation studies with additional disease mimics are required to determine the specificity of this CSF cytokines panel for ALS [64]. Finally, Beuche et al. demonstrated that matrix metalloproteinase-9 (MMP-9) is elevated in serum of patients with ALS, indicating a potentiality to be employed as biomarker [65].

## HUNTINGTON'S DISEASE

Huntington's disease or Huntington's chorea is a devastating neurodegenerative disorder whose main hallmark is brain atrophy. It is characterized by various clinical symptoms and signs including cognitive dysfunction with a particular impairment of executive functioning; psychiatric and behavioral traits with frequent suicidal ideation; and motor abnormalities, the so-called choreic movements which are continuous, random, brief, involuntary limb and orofacial muscle contractions [66]. Typical onset is between 35 and 45 years of age, but onsets from 2 to 85 years of age have been reported. Patients usually die 10–15 years after the beginning of symptoms due to bulbar dysfunction and complications. HD is of genetic origin and caused by a mutation in the huntingtin gene, by expansion of a CAG repeat, which is translated into a polyglutamine (polyQ) stretch in exon-1 (HDx1) of Huntingtin (Htt). In most animal and cellular models of HD, the neurotoxicity of mutant Htt is enhanced by the cleavage and production of N-terminal fragments, which are generated by various enzymes including caspases and calpains. The N-terminal mutant Htt fragments also form amorphous intracellular aggregates and accumulate in HD brain, but the role of these aggregates in the pathobiology of HD remains a mysterious area of investigation. Wild-type Htt is also cleaved by similar proteases, which can lower its level and interfere with its vital function in neurons. Although expansion of polyQ is a determinant in HD, the age of disease onset is variable among patients with similar polyQ length, thus, other genetic or environmental factors may regulate the onset and progression of HD [67]. Several mechanisms have been implicated in HD pathogenesis, including (1) disruption of axonal transport, (2) excitotoxicity via NMDA receptors, (3) Htt exp-mediated cytoplasmic sequestration of transcription factors, and (4) mitochondrial/bioenergetic dysfunction [68–71]. None of them are mutually exclusive, and most likely a combination of processes leads to the pathology observed in HD. By use of predictive genetic testing, it is possible to identify individuals who carry the gene defect before the onset of symptoms, providing a window of opportunity for intervention aimed at preventing or delaying disease onset. However, without robust and practical measures of disease progression (i.e., biomarkers), the efficacy of therapeutic interventions in Huntington's disease population cannot be readily evaluated.

Although neuroinflammation, signified by activated microglia and elevated levels of pro-inflammatory cytokines, is a component of major neurodegenerative disorders, its role in these disorders is not well characterized, but the possibility to develop neuroinflammation biomarkers has been explored. HD patients express increased levels of the inflammatory cytokine IL-6 in the serum and CNS many years before the onset of symptoms, and its level correlates with disease development [72]. Furthermore, post-mortem studies of HD brains also indicate abnormal levels of several inflammatory mediators, including CCL2, IL-10, IL-6, IL-8, and MMP-9 in various brain regions. The IKK/NF-κB pathway, the main inducer of these inflammatory mediators, is deregulated in HD. IKKβ in neuroinflammation may cause both elevated cytokine levels in the CNS and abnormal level of inflammatory mediators in HD patient's serum. Curiously, the relationship between peripheral inflammation and CNS pathology in HD is unknown. Inflammatory changes in the CNS and peripheral tissues in HD may be due to independent effects of mutant huntingtin in both compartments, causing analogous derangements centrally and peripherally; or inflammatory activation may begin peripherally and spread to the CNS, or vice versa, through the passage of immunomodulatory molecules across the blood–brain barrier.

In the CNS of a R6/2 genetic mouse model of HD, which expresses a toxic N-terminal fragment of mutant Htt (HDx1), elevated IKKβ activity is widespread [73]. These animals also express high levels of inflammatory cytokines including IL-6, IL-1β, and TNF-α in the serum and CNS, which is consistent with a deregulated IKKβ/NF-κB pathway [72]. A recent study [74] supports a role for IKKβ in neuroinflammation and highlights that imbalances in IKKβ activity may be the underlying cause of elevated cytokines in the CNS of HD patients. In these works, the authors observed that the inhibition of caspase-mediated maturation of IL-1β enhances neuroinflammation and neurotoxicity in a mouse model and the lowering of IKKβ in microglia reduces inflammation and neurotoxicity in to kainic acid-induced excitotoxicity model of HD. Indeed,

they display exaggerated IKKβ/NF-κB activity when stimulated with cytokines known to activate IKKβ. The dominant evidence indicates that suppressing IKKβ activity may prevent disease progression in HD and may lower the production of inflammatory cytokines. Thus, exploring the effects of IKKβ inhibitors in animal models of HD could lead to development of novel therapeutics. However, the strategies should be developed to selectively inhibit IKKβ in the brain due to the importance of IKKβ in immune cell development and survival. Some changes have been noted in the CSF of HD patients, including monoamine metabolites and a tryptophan pathway metabolite [75–78]. However, current progress in the development of biomarkers has been done and their potential in clinical use and value in the development of future treatments for patients with HD is encouraging.

## CONCLUSION

Neurodegenerative diseases represent major unmet challenges for therapeutic interventions. Characterization and targeting of the processes initiating specific pathologies act primarily at the level of neurons and are clearly an important area for continued investigation. The emerging evidence for both protective and pathogenic roles of microglia and astrocytes and the activation of common inflammation pathways in these cells in several neurodegenerative diseases supports the concept that glia-induced inflammation is an amplifier of the pathology. Given the extensive involvement of the inflammatory process, the identification of biomarkers from inflammation process might be useful not only for diagnosis but also for prediction of individual response to medications and the rate of progression. Combination of inflammatory biomarkers might be required to achieve high sensitivity and specificity.

## ACKNOWLEDGMENTS

This work was partially supported by the Italian Ministry of Economy and Finance with the "PNR-CNR Aging Program 2012-2014" project.

## REFERENCES

1. Glass, C.K., K. Saijo, B. Winner, M.C. Marchetto, and F.H. Gage. 2010. Mechanisms underlying inflammation in neurodegeneration. *Cell* 140(6): 918–934.
2. Amor, S., F. Puentes, D. Baker, and P. van der Valk. 2010. Inflammation in neurodegenerative disease. *Immunology* 129: 154–169.
3. Zhang, Y.Y., Y.C. Fan, M. Wang, D. Wang, and X.H. Li. 2013. Atorvastatin attenuates the production of IL-1β, IL-6, and TNF-α in the hippocampus of an amyloid β1-42-induced rat model of Alzheimer's disease. *Clinical Interventions in Aging* 8: 103–110.
4. Nolan, Y.M., Sullivan, A.M., Toulouse, A. (2013) Parkinson's disease in the nuclear age of neuroinflammation. *Trends in Molecular Medicine, 19,* 187–196.
5. Hsiao, H.Y., Chen, Y.C., Chen, H.M., Tu, P.H., Chern, Y. (2013) A critical role of astrocyte-mediated nuclear factor-κB-dependent inflammation in Huntington's disease. *Human Molecular Genetics*, *22*, 1826–1842.
6. Wyss-Coray, T. 2006. Inflammation in Alzheimer disease: driving force bystander or beneficial response? *Nature Medicine* 12: 1005–1015.
7. Singh, S., A.S. Kushwah, R. Singh, M. Farswan, and R. Kaur. 2012. Current therapeutic strategy in Alzheimer's disease. *European Review for Medical and Pharmacological Sciences* 16(12): 1651–1664.
8. Aluise, C.D., R.A. Sowell, and D.A. Butterfield. 2008. Peptides and proteins in plasma and cerebro spinal fluid as biomarkers for the prediction, diagnosis, and monitoring of therapeutic efficacy of Alzheimer's disease. *Biochimica et Biophysica Acta* 1782(10): 549–558.
9. Reitz, C., C. Brayne, and R. Mayeux. 2011. Epidemiology of Alzheimer disease. *Nature Reviews Neurology* 7(3): 137–152.
10. Hebert, L.E., Weuve, J., Scherr, P.A., Evans, D.A. (2013) Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. *Neurology*, *80*, 1778–1783.
11. Guerreiro, R.J., D.R. Gustafson, and J. Hardy. 2012. The genetic architecture of Alzheimer's disease: beyond APP, PSENs and APOE. *Neurobiology of Aging* 33(3): 437–456.
12. McKhann, G.M., D.S. Knopman, H. Chertkow, B.T. Hyman, C.R. Jack Jr., C.H. Kawas, W.E. Klunk, W.J. Koroshetz, J.J. Manly, R. Mayeux, R.C. Mohs, J.C. Morris, M.N. Rossor, P. Scheltens, M.C. Carrillo, B. Thies, S. Weintraub, and C.H. Phelps. 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers & Dementia* 7(3): 263–269.
13. Wyss-Coray, T., and J. Rogers. 2012. Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. *Cold Spring Harbor Perspectives in Medicine* 2(1): a006346.
14. Balistreri, C.R., G. Colonna-Romano, D. Lio, G. Candore, and C. Caruso. 2009. TLR4 polymorphisms and ageing: implications for the pathophysiology of age-related diseases. *Journal of Clinical Immunology* 29(4): 406–415.
15. Mrak, R.E., and W.S.T. Griffin. 2005. Potential inflammatory biomarkers in Alzheimer's disease. *Journal of Alzheimer's Disease* 8(4): 369–375.
16. Harigaya, Y., M. Shoji, T. Nakamura, E. Matsubara, K. Hosoda, and S. Hirai. 1995. Alpha 1-antichymotrypsin level in cerebrospinal fluid is closely associated with late onset Alzheimer's disease. *Internal Medicine* 34(6): 481–484.
17. Pirttila, T., P.D. Mehta, H. Frey, and H.M. Wisniewski. 1994. α1Antichymotrypsin and IL-1β are not increased in CSF or serum in Alzheimer's disease. *Neurobiology of Aging* 15(3): 313–317.
18. Peskind, E.R., W.S. Griffin, K.T. Akama, M.A. Raskind, and L.J. Van Eldik. 2001. Cerebrospinal fluid s100B is elevated in the earlier stages of Alzheimer's disease. *Neurochemistry International* 39: 409–413.
19. Petzold, A., R. Jenkins, H.C. Watt, A.J. Green, E.J. Thompson, G. Keir, N.C. Fox, and M.N. Rossor. 2003. Cerebrospinal fluid s100B correlates with brain atrophy in Alzheimer's disease. *Neuroscience Letters* 336: 167–170.
20. Xia, M., S. Qin, M. McNamara, C. Mackay, and B.T. Hyman. 1997. Interleukin-8 receptor B immunoreactivity in brain and neuritic plaques of Alzheimer's disease. *American Journal of Pathology* 150(4): 1267–1274.

21. Zhang, J., I. Sokal, E.R. Peskind, J.F. Quinn, J. Jankovic, C. Kenney, K.A. Chung, S.P. Millard, J.G. Nutt, and T.J. Montine. 2008. CSF multianalyte profile distinguishes Alzheimer and Parkinson diseases. *American Journal of Clinical Pathology* 129(4): 526–529.
22. Zuliani, G., G. Guerra, M. Ranzini, L. Rossi, M.R. Munari, A. Zurlo, A. Blè, S. Volpato, A.R. Atti, and R. Fellin. 2007. High interleukin-6 plasma levels are associated with functional impairment in older patients with vascular dementia. *International Journal of Geriatric Psychiatry* 22(4): 305–331.
23. Luterman, J.D., V. Haroutunian, S. Yemul, *et al*. 2000. Cytokine gene expression as a function of the clinical progression of Alzheimer disease dementia. *Archives of Neurology* 57(8): 1153–1160.
24. Zhang, J., D. Goodlett, J. Quinn, *et al*. 2005. Quantitative proteomics of cerebrospinal fluid from patients with Alzheimer's disease. *Journal of Alzheimers Disease* 7: 125–133.
25. Hu, Y., A. Hosseini, J. Kauwe, *et al*. 2007. Identification and validation of novel CSF biomarkers for early stages of Alzheimer's disease. *Proteomics Clinical Applications* 1: 1373–1384.
26. Castano, E., A. Roher, C. Esh, T. Kokjohn, and T. Beach. 2006. Comparative proteomics of cerebrospinal fluid in neuropathologically-confirmed Alzheimer's disease and non-demented elderly subjects. *Neurological Research* 28: 155–163.
27. Lewczuk, P., and J. Wiltfang. 2008. Neurochemical dementia diagnostics: state of the art and research perspectives. *Proteomics* 8: 1292–1301.
28. Ray, S., M. Britschgi, C. Herbert, Y. Takeda-Uchimura, A. Boxer, K. Blennow, L.F. Friedman, D.R. Galasko, M. Jutel, A. Karydas, *et al*. 2007. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nature Medicine* 13: 1359–1362.
29. Doecke, J.D., S.M. Laws, N.G. Faux, W. Wilson, S.C. Burnham, C.P. Lam, A. Mondal, J. Bedo, A.I. Bush, B. Brown, K. De Ruyck, K.A. Ellis, C. Fowler, V.B. Gupta, R. Head, S.L. Macaulay, K. Pertile, C.C. Rowe, A. Rembach, M. Rodrigues, R. Rumble, C. Szoeke, K. Taddei, T. Taddei, B. Trounson, D. Ames, C.L. Masters, and R.N. Martins. 2012. Blood-based protein biomarkers for diagnosis of Alzheimer disease. *Archives of Neurology* 69(10): 1318–1325.
30. Soares, D.H., *et al*. 2012. Plasma biomarkers associated with the apolipoprotein E genotype and Alzheimer disease. *Archives of Neurology* 69: 1310–1317.
31. Henkel, A.W., K. Muller, P. Lewczuk, T. Muller, K. Marcus, J. Kornhuber, and J. Wiltfang. 2012. Multidimensional plasma protein separation technique for identification of potential Alzheimer's disease plasma biomarkers: a pilot study. *Journal of Neural Transmission* 119: 779–788.
32. Crosiers, D., J. Theuns, P. Cras, and C. Van Broeckhoven. 2011. Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes. *Journal of Chemical Neuroanatomy* 42(2): 131–141.
33. Reichmann, H. 2011. View point: etiology in Parkinson's disease. Dual hit or spreading intoxication. *Journal of the Neurological Sciences* 310(1–2): 9–11.
34. Lesage, S., and A. Brice. 2012. Role of Mendelian genes in "sporadic" Parkinson's disease. *Parkinsonism & Related Disorders* 18(supplement 1): S66–S70.
35. Taccioli, C., Maselli, V., Tegn'er, J., *et al*. (2011) ParkDB: a Parkinson's disease gene expression database. *Database*, vol. 2011, article bar007.
36. Crosiers, D., J. Theuns, P. Cras, and C. Van Broeckhoven. 2011. Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes. *Journal of Chemical Neuroanatomy* 42(2): 131–141.
37. Dunning, C.J., J.F. Reyes, J.A. Steiner, and P. Brundin. 2012. Can Parkinson's disease pathology be propagated from one neuron to another? *Progress in Neurobiology* 97(2): 205–219.
38. Shulman, J.M., P.L. De Jager, and M.B. Feany. 2011. Parkinson's disease: genetics and pathogenesis. *Annual Review of Pathology* 6: 193–222.
39. Obeso, J.A., M.C. Rodriguez-Oroz, C.G. Goetz, *et al*. 2010. Missing pieces in the Parkinson's disease puzzle. *Nature Medicine* 16(6): 653–661.
40. Hirsch, E.C., and S. Hunot. 2009. Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurology* 8: 382–397.
41. Zhang, W., T. Wang, Z. Pei, D.S. Miller, X. Wu, M.L. Block, B. Wilson, W. Zhang, Y. Zhou, J.S. Hong, *et al*. 2005. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB Journal* 19: 533–542.
42. Reynolds, A.D., I. Kadiu, S.K. Garg, J.G. Glanzer, T. Nordgren, P. Ciborowski, R. Banerjee, and H.E. Gendelman. 2008. Nitrated alpha-synuclein and microglial neuroregulatory activities. *Journal of Neuroimmune Pharmacology* 3: 59–74.
43. Chen, H., E.J. O'Reilly, M.A. Schwarzschild, and A. Ascherio. 2008. Peripheral inflammatory biomarkers and risk of Parkinson's disease. *American Journal of Epidemiology* 167: 90–95.
44. Wong, K.T., J.S. Grove, A. Grandinetti, J.D. Curb, M. Yee, P. Blanchette, *et al*. 2009. Association of fibrinogen with Parkinson disease in elderly Japanese-American men: a prospective study. *Neuroepidemiology* 34: 50–54.
45. Margis, R., and C.R. Rieder. 2011. Identification of blood microRNAs associated to Parkinson's disease. *Journal of Biotechnology* 152: 96–101.
46. LeWitt, P. 2012. Recent advances in CSF biomarkers for Parkinson's disease. *Parkinsonism & Related Disorders* 18(Suppl 1): 49–51.
47. LeWitt, P., L. Schultz, P. Auinger, and M. Lu. 2011. CSF xanthine, homovanillic acid, and their ratio as biomarkers of Parkinson's disease. *Brain Research* 140: 88–97.
48. Orrell, R.W., J.J. Habgood, A. Malaspina, *et al*. 1999. Clinical characteristics of SOD1 gene mutations in UK families with ALS. *Journal of Neurological Science* 169: 56–60.
49. Blair, I.P., K.L. Williams, S.T. Warraich, *et al*. 2010. FUS mutations in amyotrophic lateral sclerosis: clinical, pathological, neurophysiological and genetic analysis. *Journal of Neurology, Neurosurgery, and Psychiatry* 81(6): 639–645.
50. Kabashi, E., P.N. Valdmanis, P. Dion, *et al*. 2008. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nature Genetics* 40: 572–574.
51. World Federation of Neurology Research Group on Neuromuscular Diseases Subcommittee on Motor Neuron Disease. Airlie House guidelines. 1995. Therapeutic trials in amyotrophic lateral sclerosis. Airlie House, "Therapeutic Trials in ALS," Workshop Contributors. *Journal of Neurological Science* 129: 1–10.
52. Traynor, B.J., M.B. Codd, B. Corr, C. Forde, E. Frost, and O.M. Hardiman. 2000. Clinical features of amyotrophic lateral sclerosis according to the El Escorial and Airlie House diagnostic criteria: a population-based study. *Archives of Neurology* 57: 1171–1176.
53. Liu, Y., W. Hao, A. Dawson, S. Liu, and K. Fassbender. 2009. Expression of amyotrophic lateral sclerosis-linked SOD1 mutant increases the neurotoxic potential of microglia via TLR2. *Journal of Biological Chemistry* 284: 3691–3699.
54. Yiangou, Y., P. Facer, P. Durrenberger, I.P. Chessell, A. Naylor, C. Bountra, R.R. Banati, and P.Y. Anand. 2006. COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurology* 6: 12.
55. Amit, I., M. Garber, N. Chevrier, A.P. Leite, Y. Donner, T. Eisenhaure, M. Guttman, J.K. Grenier, W. Li, O. Zuk, L.A. Schubert, B. Birditt, T. Shay, A. Goren, X. Zhang, Z. Smith, R. Deering, R.C. McDonald, M. Cabili, B.E. Bernstein, J.L. Rinn, A. Meissner, D.E. Root, N. Hacohen, and A. Regev. 2009. Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* 326: 257–263.

56. Uranishi, H., T. Tetsuka, M. Yamashita, K. Asamitsu, M. Shimizu, M. Itoh, and T. Okamoto. 2001. Involvement of the pro-oncoprotein TLS (translocated in liposarcoma) in nuclear factor-kappa B p65-mediated transcription as a coactivator. *Journal of Biological Chemistry* 276: 13395–13401.
57. McGeer, P.L., and E.G. McGeer. 2008. Glial reactions in Parkinson's disease. *Movement Disorders* 23: 474–483.
58. Zhang, R., R. Gascon, R.G. Miller, *et al*. 2005. Evidence for systemic immune system alterations in sporadic amyotrophic lateral sclerosis (sALS). *Journal of Neuroimmunology* 159: 215–224.
59. Mantovani, S., S. Garbelli, A. Pasini, *et al*. 2009. Immune system alterations in sporadic amyotrophic lateral sclerosis patients suggest an ongoing neuroinflammatory process. *Journal of Neuroimmunology* 210: 73–79.
60. Keizman, D., O. Rogowski, S. Berliner, *et al*. 2009. Low-grade systemic inflammation in patients with amyotrophic lateral sclerosis. *Acta Neurologica Scandinavica* 119: 383–389.
61. Turner, M.R., M.C. Kiernan, P.N. Leigh, and K. Talbot. 2009. Biomarkers in amyotrophic lateral sclerosis. *Lancet Neurology* 8: 94–109.
62. Nardo, G., S. Pozzi, M. Pignataro, E. Lauranzano, G. Spano, S. Garbelli, S. Mantovani, K. Marinou, L. Papetti, M. Monteforte, V. Torri, L. Paris, G. Bazzoni, C. Lunetta, M. Corbo, G. Mora, C. Bendotti, and V. Bonetto. 2011. Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. *PLoS One* 6(10): e25545.
63. Lu, C.H., A. Petzold, B. Kalmar, J. Dick, A. Malaspina, and L. Greensmith. 2012. Plasma neurofilament heavy chain levels correlate to markers of late stage disease progression and treatment response in SOD1(G93A) mice that model ALS. *PLoS One* 7(7): e40998. doi:10.1371/journal.pone.0040998.
64. Mitchell, R.M., W.M. Freeman, W.T. Randazzo, H.E. Stephens, J.L. Beard, Z. Simmons, *et al*. 2009. A CSF biomarker panel for identification of patients with amyotrophic lateral sclerosis. *Neurology* 72: 14–19.
65. Beuche, W., M. Yushchenko, M. Mader, M. Maliszewska, K. Felgenhauer, and F. Weber. 2000. Matrix metalloproteinase-9 is elevated in serum of patients with amyotrophic lateral sclerosis. *Neuroreport* 11: 3419–3422.
66. Walker, F.O. 2007. Huntington's disease. *Lancet* 369(9557): 218–228.
67. Moller, T. 2010. Neuroinflammation in Huntington's disease. *Journal of Neural Transmission* 117(8): 1001–1008.
68. Li, S.H., and X.J. Li. 2004. Huntingtin–protein interactions and the pathogenesis of Huntington disease. *Trends in Genetics* 20: 146–154.
69. Orr, H.T., and H.Y. Zoghbi. 2007. Trinucleotide repeat disorders. *Annual Review of Neuroscience* 30: 575–621.
70. Imarisio, S., *et al*. 2008. Huntington disease: from pathology and genetics to potential therapies. *Biochemistry Journal* 412: 141–209.
71. Roze, E., *et al*. 2008. Pathophysiology of Huntington's disease: from huntingtin functions to potential treatments. *Current Opinion in Neurology* 21: 495–503.
72. Björkqvist, M., E.J. Wild, J. Thiele, A. Silvestroni, R. Andre, N. Lahiri, *et al*. 2008. A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. *Journal of Experimental Medicine* 205: 1869–1877.
73. Khoshnan, A., J. Ko, E.E. Watkin, L.A. Paige, P.H. Reinhart, and P.H. Patterson. 2004. Activation of the IkappaB kinase complex and nuclear factor-kappaB contributes to mutant huntingtin neurotoxicity. *Journal of Neuroscience* 24: 7999–8008.
74. Cho, I.H., J. Hong, E.C. Suh, J.H. Kim, H. Lee, J.E. Lee, *et al*. 2008. Role of microglial IKK beta in kainic acid-induced hippocampal neuronal cell death. *Brain* 131: 3019–3303.
75. Kurlan, R., E. Caine, A. Rubin, *et al*. 1988. Cerebrospinal fluid correlates of depression in Huntington's disease. *Archives of Neurology* 45: 881–883.
76. Schwarcz, R., C.A. Tamminga, R. Kurlan, et al. 1988. Cerebrospinal fluid levels of quinolinic acid in Huntington's disease and schizophrenia. *Annals of Neurology* 24: 580–582.
77. Montine, T.J., M.F. Beal, D. Robertson, *et al*. 1999. Cerebrospinal fluid F2-isoprostanes are elevated in Huntington's disease. *Neurology* 52: 1104–1105.
78. Jeitner, T.M., M.B. Bogdanov, W.R. Matson, *et al*\. 2001. N(epsilon)-(gamma-L-glutamyl)-L-lysine (GGEL) is increased in cerebrospinal fluid of patients with Huntington's disease. *Journal of Neurochemistry* 79: 1109–1112.