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The Immune System and Neuroinflammation as Potential Sources of Blood-Based Biomarkers for Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease

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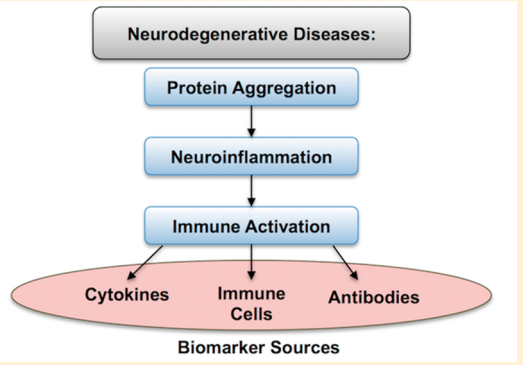
ABSTRACT: Neurodegenerative diseases are characterized by a loss of neurons that leads to cognitive and behavioral dysfunction. Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting millions of people in the United States and worldwide, followed by Parkinson's disease (PD). While some early onset forms of AD and PD are hereditary, the sporadic or late-onset cases are believed to result from lifestyle and environmental factors. On the contrary, Huntington's disease (HD) is a neurodegenerative disease solely caused by mutations in the gene for huntingtin protein. The disease mechanisms at play for all three disorders remain elusive, hampering efforts to develop effective therapeutic interventions. In light of this, the discovery of robust biomarkers is crucial in order to identify people at risk for AD and PD, preferably before symptoms arise. For all three diseases, the identification of biomarkers would not only allow development of treatments but also evaluation and adjustment of these with disease progression. It is now understood that neuroinflammation plays a crucial role in neurodegenerative diseases, along with subsequent immune activation. Therefore, research is actively ongoing to discover and evaluate inflammatory and immune-related biomarkers. Recent progress in this area for AD, PD, and HD is presented here.



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Neurodegenerative diseases are characterized by a loss of neurons, accumulation of aggregated and misfolded proteins, cognitive decline, and locomotive dysfunction.[1] The most common neurodegenerative disorder is Alzheimer's disease (AD), which features hallmark pathology of extracellular β-amyloid (Aβ) deposits and intracellular neurofibrillary tangles composed of phosphorylated tau (p-tau), eventually leading to the characteristic memory loss associated with this disorder.[2,3] Parkinson's disease is the second most common neurodegenerative disorder, characterized by aggregation of α-synuclein into Lewy bodies and Lewy neurites as well as loss of dopaminergic neurons in the substantia nigra pars compacta.[4−6] As a result, PD patients exhibit distinctive symptoms including resting tremors, bradykinesia, stooped posture, and in some cases dementia.[7] While both familial and sporadic forms of AD and PD are possible, Huntington's disease (HD) is an autosomal dominant neurodegenerative disease that is caused by mutations in the huntingtin gene.[8] The resulting clinical manifestations of HD include chorea, as well as cognitive and behavioral decline.[9]

The common thread of AD, PD, and HD is the absence of biomarkers to accurately determine disease progression and success of therapeutic interventions. Furthermore, there is a lack of clinical biomarkers for AD and PD to identify individuals at risk during the prodromal phase, before symptoms arise. Current research is focused on accomplishing the goal of identifying suitable biomarkers for all three neurodegenerative disorders, given the unmet need of reliable biomarkers to identify individuals at risk (with the exception of HD) and to accurately monitor disease progression. Considering the severity of the symptoms that accompany AD, PD, and HD, which lead to a dramatic drop in quality of life, accurate and reliable biomarkers would be invaluable in order to gauge therapeutic outcome.

Imaging, cerebrospinal fluid (CSF), and blood-based biomarkers are all potential candidates for assessing risk and progression of neurodegenerative diseases. For example, imaging technologies such as MRI and PET scans can provide insight into brain changes that accompany AD, PD, and HD but these technologies are expensive, require labor-intensive interpretation, are not widely available, and may not be covered by insurance.[10,11] Additionally, the presence of Aβ aggregates does not always correlate with AD as some cognitively healthy individuals exhibit Aβ buildup, while some AD patients lack Aβ aggregates.[12,13] This suggests other pathological pathways are at play that lead to AD, rather than just the presence of Aβ alone. CSF biomarkers have great appeal since the proteins and metabolites present in the CSF are a direct reflection of the environment in the brain.[14] However, it is not feasible to repeatedly subject at-risk patients to the invasive lumbar

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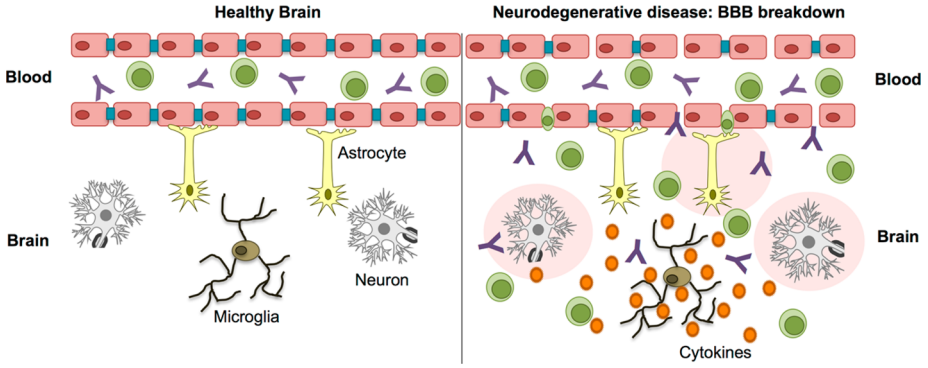

Figure 1. Breakdown of the blood-brain barrier allows infiltration of immune mediators. In healthy brains, passage through the blood-brain barrier (BBB) occurs via tightly regulated transport. Breakdown of the BBB, which presumably occurs during neurodegenerative diseases, allows unhindered access into the brain, including immune mediators such as antibodies and lymphocytes. Over time, this either causes or contributes to neuroinflammation.

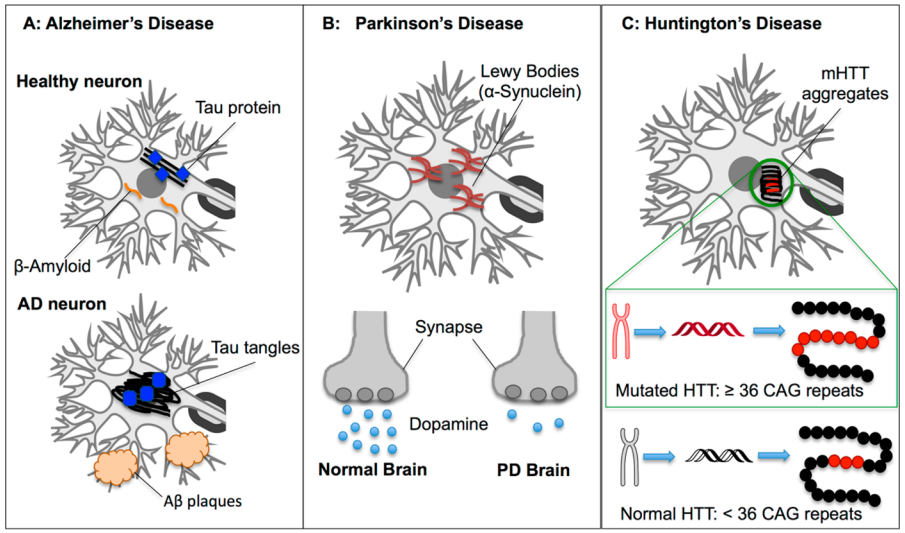

Figure 2. Hallmark pathologies of AD, PD, and HD. (A) Alzheimer's disease: In healthy neurons, tau protein is associated with microtubules and monomeric β-amyloid is present. In AD, neurons contain intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein and extracellular plaques composed of Aβ. (B) Parkinson's disease: α-Synuclein aggregates form Lewy bodies inside neurons. Neuronal loss leads to less dopamine production in PD patients. (C) Huntington's disease: Huntingtin protein (HTT) containing an extended polyglutamine repeat caused by ≥36 CAG repeats in the huntingtin gene leads to intraneuronal aggregates. In all three diseases, the pathological events ultimately result in neuronal death. Over time, this either causes or contributes to neuroinflammation.

puncture procedure. Given the limitations of biomarker searches via imaging and CSF, it would be highly desirable to identify reliable blood-based biomarkers for diagnosis of AD, PD, and HD since they are easily accessible and analyzable, as well as inexpensive.

One source of such markers might be molecules involved in immune system function. Research involving animal models and patient studies indicate a connection between neurodegenerative diseases, neuroinflammation, and immune system activation.[15] This process is initiated by activation of microglia and astrocytes, the resident immune cells of the brain, and leads to a subsequent release of proinflammatory and immune mediators, such as cytokines and chemokines. Microglia activation also promotes production of reactive oxygen species (ROS) and nitric oxide (NO). The belief that the central nervous system (CNS) is immune privileged and thus protected from infiltration of immune mediators from the periphery has been challenged over recent years. In addition to the presence of activated microglia and astrocytes, neurodegenerative diseases also often exhibit lymphocyte infiltration from the periphery, as demonstrated by the presence of both CD4+ and CD8+ T lymphocytes in brains of PD patients. It is believed that access of immune mediators from the periphery results from breakdown of the blood-brain barrier (see Figure 1). All of these events contribute to chronic inflammation and eventually result in neuronal dysfunction and cell death.[1,5,16,17] In light of the pivotal role of inflammatory and immune mediators in neurodegenerative diseases, their potential utility as biomarkers for AD, PD, and HD is discussed in this Review with focus on their application as blood-based biomarkers.

■ ALZHEIMER'S DISEASE BIOMARKERS

Overview. AD is the most common cause of dementia and affects 13% of people over the age of 65 and nearly 50% of people over the age of 85 years.[18] Currently, approximately 5.3 million people in the United States have AD and this number is estimated to increase to 13.8 million by 2050.[19] Early onset or familial AD leads to disease onset before the age of 65 and is caused by mutations in the genes for Aβ precursor protein (APP), and in genes encoding proteins involved in Aβ peptide

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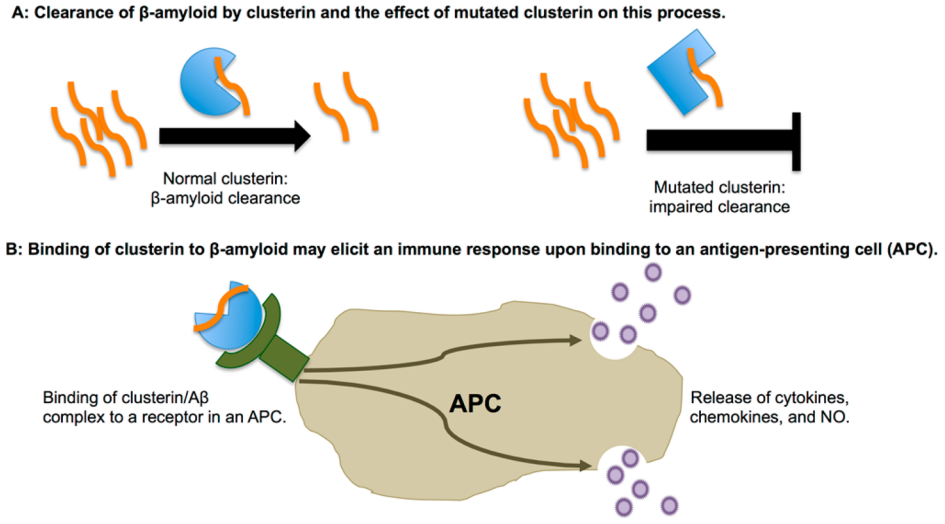

Figure 3. Possible roles of clusterin in AD. (A) Mutated clusterin may affect clearance of β-amyloid, contributing to its aggregation. (B) Alternatively, binding of clusterin to β-amyloid may elicit an immune response if this complex is presented to an antigen-presenting cell.

cleavage, presenilin-1 PSEN1, and presenilin-2 (PSEN2).[20] The greatest genetic risk factor for late-onset or sporadic AD is the presence of the ε4 allele of the apolipoprotein E (APOE) gene but environmental risk factors and advanced age also drive the development of AD.[21] Current diagnosis of AD relies solely on neuropsychological tests and the absence of other forms of dementia.[22] The projected increase in AD patients from a current worldwide estimate of 34 million people to 90 million people by 2050 will undoubtedly place a heavy financial burden on society if reliable biomarkers and therapeutics remain elusive.[18]

For these reasons, much research is dedicated to identifying suitable biomarkers. Since the hallmark pathology of AD involves the extracellular accumulation of Aβ aggregates released during neuronal death and neurofibrillary tangles composed of phosphorylated tau, both Aβ and tau (including p-tau) have undergone extensive investigation for their utility as biomarkers (Figure 2).[14] In AD patients, a reduction in the CSF levels of Aβ is generally observed, which is believed to be due to increased Aβ plaque accumulation in the brain. Additionally, high CSF levels of tau and p-tau in AD patients may denote neuronal damage.[14] However, biomarker collection from CSF is not feasible due to the invasiveness of the sample collection procedure, although it may be useful to rule out other diseases. Studies on the utility of Aβ and tau collected from the blood as biomarkers for sporadic AD have so far produced disappointing results. In the case of Aβ, some studies reported higher levels of Aβ in plasma compared to controls, while others found no change.[23] Similarly, findings of tau protein plasma levels have yielded contradictory findings since some studies found a decrease in tau plasma levels in AD patients, while another study observed an increase.[24−26] These inconsistent results could be due to low levels of Aβ and tau within plasma and the high concentration of total plasma protein that render detection of low levels of a specific protein problematic.[14] These difficulties highlight the necessity to identify more suitable blood-based biomarkers for AD. Recent advances in the discovery of neuroinflammatory and immune system biomarkers are discussed here.

Identification of Inflammatory and Immune System Biomarkers for AD. AD was not initially perceived as an inflammatory or immune-related disorder, but research over the past few years has begun to change this point of view. Both genome-wide association studies (GWAS) and longitudinal patient studies contributed to the idea that neuroinflammation might not be the result but possibly the cause or an early development of neurodegenerative diseases.[1]

In 2013, a mutation within the TREM2 gene, which encodes the triggering receptor expressed on myeloid cells 2, was independently identified by two GWAS led by Guerreiro and Jonsson to confer an increased risk for developing sporadic AD.[27,28] TREM2 is part of the immunoglobulin superfamily of receptors that is primarily expressed in osteoclasts and microglia. In association with its coreceptor DAP12, TREM2 regulates various signaling pathways in immune cells, including phagocytosis and anti-inflammatory activity.[29] The two 2013 GWAS studies applied genome-sequencing techniques to populations of AD patients from various geographical regions that led to the identification of the rare R47H mutation in TREM2. While the role of TREM2 in AD is not fully elucidated, it is possible that the R47H mutation affects the phagocytic activity of microglia and therefore contributes to accumulation of Aβ. Impaired function of mutated TREM2 may also promote inflammatory responses, including the production and secretion of cytokines that eventually lead to the characteristic neuronal death observed in AD.

Another candidate biomarker that was initially discovered through GWAS is clusterin, also known as apolipoprotein J.[30,31] Clusterin is a 75 kDa heterodimeric protein and is expressed in almost all tissue types. It functions as a molecular chaperone and has been associated with a variety of cellular processes, including sequestering of the Aβ40 isoform of the Aβ peptide.[32] Clusterin is a heavily glycosylated protein with a carbohydrate content that amounts to 20−25% of the total protein mass at six N-linked glycosylation sites.[33] Building upon the link discovered between clusterin and AD through GWAS, Liang and co-workers examined the utility of glycosylated plasma clusterin as a candidate biomarker for AD.[34] The authors examined 37 plasma samples from AD and mild cognitively impaired (MCI) subjects with low and high hippocampal atrophy. Clusterin from plasma was initially captured by immunoprecipitation, followed by Western blot/SDS-PAGE and subsequent mass spectrometry (MS) analysis. A correlation between changes in glycoforms at glycosylation site β64N and

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hippocampal atrophy was observed, as eight glycoforms exhibited a significant reduction in high atrophy patient samples. This study demonstrates that not only clusterin itself but possibly certain glycoforms may function as diagnostic and prognostic AD biomarkers. However, additional studies with healthy controls and at-risk patients should be included to determine the utility of glycosylated clusterin as a biomarker, especially since previous studies on plasma and CSF clusterin as an AD biomarker have yielded contradictory results.[35] For example, it may be useful to determine a certain base value of glycosylated clusterin healthy individuals that allows determination of likely disease onset as this value decreases. Clusterin is generally not considered to be involved in the immune response; however, its exact function remains to be determined and thus it could have an as of yet undiscovered role in immunity. It is known that peptides chaperoned by heat-shock proteins can elicit an immune response upon interaction with antigen-presenting cells that leads to production of cytokines, chemokines, and nitric oxide.[36] Since clusterin functions as a molecular chaperone and exhibits functional homology to heat-shock proteins,[32] it may contribute to onset of AD through one of two mechanisms: mutations in the CLU gene may compromise its association with Aβ, therefore playing a part in the accumulation of Aβ plaques. Alternatively, binding of clusterin to Aβ peptides may elicit an immune response that contributes to the inflammation commonly seen in AD patients (see Figure 3). Further research on the role of clusterin in AD is needed to fully understand its role in AD pathogenesis.

A brief literature search easily reveals several studies undertaken at the protein level that have identified various candidate AD biomarkers, many of which are associated with immune system activation and inflammation. For example, a study performed by Laske and co-workers examined a panel of immunologic biomarkers including cytokines, chemokines, soluble receptors, and ligands representing various inflammatory mechanisms.[37] The authors wanted to determine whether a single or a panel of inflammatory biomarkers in peripheral blood could distinguish between AD and age-matched healthy controls. Using a bead-based multiplexed sandwich immunoassay followed by multivariate data analysis, sTNF-R1 was identified as the best discriminatory marker out of the 25 serum markers investigated. This marker distinguished AD patients from controls with 90% accuracy (88% sensitivity and 93% specificity) and, interestingly, the addition of any of the other markers did not improve discrimination of AD and control sample sets. Since TNF-R1 functions in apoptosis, the authors hypothesized that increased sTNF-R1 serum levels may correspond to the level of neuronal damage in the brain. This study is not only interesting because it contributes to a connection between peripheral inflammatory processes and AD, but it is also one of few that identified a single marker as sufficient to discriminate between AD and healthy controls, as generally a panel of markers is required to accurately distinguish disease and control samples.

A number of studies have also researched the utility of antibodies as biomarkers for AD, which illustrate that mining of the adaptive immune system may provide novel biomarker candidates for AD.

The Nagele lab investigated whether autoantibodies could be exploited for diagnostic purposes after discovering that human sera contain brain-reactive autoantibodies regardless of age and disease status.[38,39] The authors used human protein microarrays to study expression patterns of serum autoantibodies from AD and nondemented control groups. Binding to the array was analyzed by statistical algorithms, which led to the identification of 10 autoantibody biomarkers that could distinguish AD from control sera with a sensitivity of 96% and specificity of 92.5%. Furthermore, this method could also distinguish AD patients from PD and breast cancer patients. Restrepo and co-workers demonstrated that the application of plasma from AD patients and healthy controls to microarrays containing 10 000 random-sequence peptides yielded distinct binding patterns or "immunosignatures".[40] The authors speculated these immunosignatures resulted at least in part from binding of autoantibodies to the microarray peptides. Work in our laboratory involving screening of combinatorial peptoid libraries against AD also identified autoantibodies that selectively bound to specific peptoids.[41] Peptoids are oligomers of N-substituted glycines and thus differ from peptides because the side chain emerges from the nitrogen rather than from the α carbon.[42] Libraries of peptoids are synthesized via submonomer synthesis that yields thousands of molecules with conformations not found naturally.[43] Therefore, this approach of serum antibody discovery does not seek to identify native antigens but instead allows for unbiased identification of synthetic compounds capable of binding to the antigen recognition pocket of the antibody. In our study, peptoids were immobilized on glass microarray slides and incubated with serum from AD, PD, and age-matched controls. Three peptoids bound much higher levels of IgG antibodies from the AD patients compared to the two sets of controls. More recent unpublished results employing ELISA with the ADP3 peptoid, which had shown the highest diagnostic sensitivity and selectivity in the original study, indicate however that it is not a sufficient marker, mostly because of a large number of false positives. Nonspecific binding of IgG antibodies to the peptoid-containing ELISA plates, batch-to-batch variations of microarrays, and differences in samples collected at different institutions possibly contributed to this result.[44]

In summary, much evidence exists that supports a role of the immune system in AD pathogenesis and this can be exploited to identify candidate AD biomarkers. Biomarkers that allow monitoring of disease progression and success of therapeutic interventions are certainly necessary but the true challenge of blood-based biomarkers for AD is the identification of those that can predict disease onset before the symptomatic stage. Therefore, while many research studies have found a single or a panel of potential biomarkers, it is pertinent to verify these in studies involving much larger cohorts and samples from presymptomatic at-risk patients. Only then can effective therapeutic agents be developed in order to prevent or slow down AD pathogenesis.

PARKINSON'S DISEASE BIOMARKERS

Overview. The second most common age-related neurodegenerative disease is PD, which affects approximately 1% of people over the age of 65.[45] Its pathological features include the progressive loss of dopaminergic neurons in the substantia nigra pars compacta and aggregation of α-synuclein into Lewy body inclusions (see Figure 2). Characteristic symptoms of PD patients include resting tremor, bradykinesia, postural instability, gait imbalance, and in some cases dementia.[5,35] Similar to AD, the cause of PD is unknown, and as a result diagnosis relies on clinical evaluation of motor symptoms. Current treatment options fail to address all PD-related symptoms.[46] GWAS have linked mutations in 16 loci to disease onset, including the gene

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for α-synuclein (SNCA), in which point mutations, duplications, and triplications cause hereditary forms of PD. However, PD is considered to be the result of a combination of factors that involves not only genetics, but also lifestyle and environment.[45] Furthermore, studies on the utility of genetic biomarkers for PD have yielded inconsistent or mixed results, thus leaving the identification of a reliable biomarker elusive.[46] As with AD, a blood-based biomarker would be ideal in order to identify people at risk at an early, presymptomatic stage so that effective treatments can be developed and eventually implemented. Recent highlights in the area of potential inflammatory and immune system biomarkers are the focus of the following section.

Identification of Inflammatory and Immune System Biomarkers for PD. Aside from neuronal loss and α-synuclein aggregation, PD is also accompanied by an active immune response. This is evidenced by the presence of CD4+ and CD8+ T lymphocytes and proinflammatory cytokines such as IL-1β in the brains of PD patients.[5] Furthermore, it is known that aggregated α-synuclein leads to expression of inducible nitric oxide synthase, in turn leading to nitration of α-synuclein that further promotes proinflammatroy responses and leads to activation of microglia.[47] In light of these discoveries, the immune system is being actively explored as a potential source of biomarkers for PD.

Work by Nagele and co-workers previously identified autoantibody panels from human sera capable of distinguishing both mild-moderate AD and PD.[39,48] A more recent study by the Nagele laboratory investigated the utility of a different autoantibody panel as blood-based biomarkers for early detection and diagnosis of PD.[49] Serum samples from early stage PD and age- and sex-matched controls were first applied to a microarray containing nearly 9500 unique human protein antigens. The data obtained from the microarrays were then analyzed using different algorithms, which identified the top 50 autoantibodies differentially expressed in early stage PD patients compared to healthy controls. Most of the selected biomarkers were present in both early stage PD and control groups but show a several fold increase in expression in the early stage PD samples. Using the same approach, the researchers further determined that a minimum of four biomarkers was required to attain an overall diagnostic accuracy of 84%. The panel of 50 biomarkers and the reduced panel of 4 biomarkers were then employed to determine how well they could distinguish early stage PD sera from AD, MS, and breast cancer sera. In all cases, the two panels showed overall diagnostic accuracies of over 90%. Furthermore, a high level of specificity for early stage PD was demonstrated when both biomarker panels were applied to early stage and mild-moderate PD sera, which revealed an overall accuracy of about 97%. Interestingly, the biomarker panel identified in this study did not reveal any with an obvious connection to PD, though some play a role in neuronal migration while others represent proinflammatroy cytokines, thus suggesting unknown pathological pathways. This study by the Nagele laboratory, as well as their prior studies on PD and AD, demonstrate that immune-related protein biomarkers isolated from small serum samples can accurately distinguish disease samples from healthy ones or from samples of patients with other diseases. Future independent studies should focus on confirming the utility of these biomarkers.

A study published in 2013 suggested that the R47H variant of TREM2 might also be a risk factor in PD and frontotemporal dementia in addition to AD.[50] The authors extracted genomic DNA from patients with PD, frontemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy, ischemic stroke, and a healthy control group. The association between the R47H variant of TREM2 was then analyzed, which demonstrated a significant association of this TREM2 variant with FTD and PD. However, a study performed in 2015 was unable to replicate the results with PD nor in patients suffering from PD with dementia.[51] The authors explain that this could be due to differences in variation of minor allele frequencies between the different samples cohorts. Additionally, differences in statistical analysis methods may be responsible for the opposing results.

The contradictory results of the TREM2 studies illustrate the need for thorough follow-up work once a potential biomarker has been discovered. Ideally, a different laboratory would conduct these using large sample numbers from various repositories. Furthermore, it is pertinent that the samples undergo exactly the same processing procedure and analysis. Differences in these two areas are the most likely culprits for contradictory study results as observed for TREM2 in relation to PD or plasma Aβ in AD.

HUNTINGTON'S DISEASE BIOMARKERS

Overview. Huntington's Disease (HD) is a hereditary neurodegenerative disease caused by mutations in the gene encoding huntingtin (HTT) protein, which contains a polyglutamine expansion encoded by a CAG repeat. While healthy individuals contain 16−20 repeats, more than 36 are present within the huntingtin gene in HD patients.[9] The pathological hallmark of HD involves loss of neurons in the cortex and striatum that lead to the characteristic clinical manifestations including involuntary movements known as chorea, behavioral and psychiatric features, and cognitive dysfunction. It is estimated that HD affects 25 000−30 000 individuals in the United States. Genetic testing allows identification of individuals at risk for developing this disorder.[52] No cure is available for HD, and current treatment options provide only symptomatic relief at best.[53] Similar to AD and PD, toxic protein aggregates are also observed in HD patients, whose brains contain accumulations of mutated HTT protein.[54] Interestingly, a clinical trial focusing on removal of mutant HTT using antiaggregating molecules failed to produce symptomatic relief in HD patients, which is reminiscent of trials using antibodies targeting Aβ that failed to slow down cognitive decline in AD patients.[55,56] This suggests other pathological mechanisms are at play in HD in addition to aggregates of mutated HTT. There is great interest in biomarker discovery for HD due to the current lack of disease-modifying treatment for HD accompanied by the lack of biomarkers to assess success of therapeutic interventions. Given the armamentarium of biomarkers potentially provided by neuroinflammation and the immune system for HD, this section will focus on recent progress in this area.

Identification of Inflammatory and Immune System Biomarkers for HD. As is observed for AD and PD, immune activation is also present in HD patients. Mutant HTT leads to activation of microglia and complement, resulting in subsequent production and release of ROS, NO, and cytokines.[53] The involvement of the immune system may offer new avenues for both biomarker as well as therapeutic exploration to target at-risk individuals before symptoms arise and to gauge

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therapeutic intervention in order to provide an improved quality of life.

Since huntingtin expression is not limited to the brain but is also found in the periphery, a study led by Chang and co-workers set out to determine whether plasma inflammatory markers could correspond to the characteristic neuroinflammation observed in HD.[57] Particular focus was placed on microglia-derived inflammatory markers and plasma IL-6, which is present at higher levels in HD patients, all of which were assayed using enzyme-linked immunosorbent assay (ELISA) kits. The study cohort consisted of 20 HD patients, of which 5 were presymptomatic and 15 were symptomatic, as well as 16 age-matched healthy controls. The authors found increased levels of IL-6, MMP-9, VEGF, and TGF-β1 in HD patients as well as reduced levels of IL-18. These trends were additionally observed in a mouse model of HD. Notably, no differences were seen between presymptomatic HD carriers and controls. Thus, the panel of inflammatory markers identified in this study may be applicable to monitoring progression of HD once symptoms emerge but additional longitudinal studies with larger patient and control cohorts are needed to confirm this. Interestingly, TGF-β1 is known to also play a role in AD where impaired TGF-β1 signaling contributes to neurodegeneration.[58] Similarly, Chang and co-workers found decreased levels of the proinflammatory cytokine IL-18, while increased plasma levels of IL-18 are present in mild and moderate AD patients.[59] The common occurrence of inflammatory markers across various neurodegenerative diseases clearly demonstrates the pivotal role of inflammation in the cause and progression of these diseases, although divergent mechanisms may be at play.

Another study by Politis and co-workers investigated levels of plasma cytokines in presymptomatic HD gene carriers using both multiplex ELISA and PET scanning.[60] The authors found increased peripheral plasma levels of the pro-inflammatory cytokine IL-1β in HD gene carriers compared to normal controls. Additionally, increased microglial activation in the somatosensory cortex was associated with increased plasma levels of IL-1β, IL-6, IL8, and TNF-α. This study further highlights the role of inflammatory markers in HD that could potentially be used to monitor onset and progression of disease in mHTT carriers. However, the authors point out that unlike in AD and PD, breakdown of the blood-brain barrier has not been observed in HD as of yet. Thus, the increased levels of cytokines observed in gene carriers may reflect concomitant but unrelated effects of mHTT.

The two studies described here illustrate the link between the innate immune response and elevated levels of pro-inflammatory markers in HD carriers. Additional studies with larger sample sizes should verify the utility of peripheral cytokine levels and whether treatments relying on anti-inflammatory agents could dampen disease progression. Furthermore, research studies should also focus on identifying other potential inflammatory and immune markers to increase the repertoire of reliable biomarkers for HD.

CONCLUSION

The role of the immune system in neurodegenerative diseases has become obvious over the past few decades as demonstrated by the presence of activated microglia and astrocytes in brains of affected patients. Additionally, inflammatory markers such as cytokines and chemokines, and immune mediators such as lymphocytes and brain-reactive antibodies can be found in affected brain regions. This offers a new avenue for biomarker exploration and many research studies have begun to identify potential inflammatory and immune system biomarkers for neurodegenerative diseases.

For AD, variants of the TREM2 and CLU genes may represent a risk factor and thus could serve as biomarkers, though it is unlikely that every AD patient is a carrier for either of these. Research into the clusterin protein itself as a marker for AD has accomplished some results, with certain clusterin glycoforms potentially allowing distinction between AD patients and healthy individuals.[34] Various proinflammatory cytokines and chemokines have also been identified as possible disease biomarkers, as illustrated in this review for AD and HD. However, it remains to be determined whether the elevated presence of a given inflammatory marker is a direct result of a specific disease mechanism, such as aggregation of a given protein, or a general response. This is illustrated by the discovery of elevated levels of TGF-β1 in plasma of HD patients in the study by Chang and co-workers.[57] This cytokine is also involved in AD where impaired signaling of TGF-β1 affects neurodegeneration.[58] Similarly, TREM2 has been implicated in both AD and PD, though its role in PD is disputed by some researchers.[27,28,50,51] Furthermore, the discovery of a biomarker that is later found to be either irrelevant or implicated in another disease implies that a single marker may not be sufficient. Thus, panels of biomarkers have also been researched as demonstrated by work performed by the Nagele group for both AD and PD.[39,48,49] These examples illustrate the urgent need for further thorough investigations that ideally would be conducted by multiple research laboratories using the same sample processing and analysis methods and large numbers of patient samples from presymptomatic, symptomatic, and healthy controls in addition to samples from patients with various other neurodegenerative diseases in order to conclusively determine the utility of a given marker.

The original belief that the CNS is immune privileged has been revised after discovery of inflammatory and immune mediators in the brain of patients with neurodegenerative diseases. This led researchers to conclude that some neurodegenerative diseases are accompanied by breakdown of the blood-brain barrier, such as AD and PD, leading to subsequent infiltration of inflammatory and immune mediators from the periphery. Interestingly, Louveau and colleagues discovered in 2015 in a landmark study the presence of CNS lymphatic vessels in mouse brains.[61] This provides an alternative mechanism of entry and exit of immune cells from the CNS into the periphery aside from blood-brain barrier breakdown and has implications for neurodegenerative diseases in humans. For example, protein aggregates such as Aβ in AD, α-synuclein in PD, and mHTT in HD may impede drainage of the brain lymphatic system, causing inflammation and immune activation. Additionally, entry of T lymphocytes into the brain from the periphery may utilize this pathway rather than openings in the blood-brain barrier.[62] Overall, the discovery of the brain lymphatic system offers new exploration of disease mechanisms and concomitant research into new biomarkers and treatments.

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Author Contributions

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■ ABBREVIATIONS

AD, Alzheimer's disease; Aβ, beta-amyloid; BBB, blood-brain barrier; CSF, cerebrospinal fluid; CNS, central nervous system; GWAS, genome-wide association studies; HD, Huntington's disease; NO, nitric oxide; PD, Parkinson's disease; ROS, reactive oxygen species

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