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COMMENTARY
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Can microbiota research change our understanding of neurodegenerative diseases?

“In the desperate battle against these devastating disorders we should be open for theories that are free of the legacy of previous approaches, but instead examine the problem from a new perspective.”

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On the clinical and scientific level, most neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS) share common problems such as:

- Their etiology is unknown;
- Their pathogenesis is relatively poorly understood;
- As a consequence, no effective disease modifying treatments are available.

Nevertheless, our understanding of the underlying molecular processes has improved in recent years and, again, several shared features have emerged:

- The vast majority of cases is sporadic and the neurodegenerative process likely arises from an interaction of genetic and environmental factors;
- Misfolding and accumulation of certain neuronal proteins (amyloid-β [Aβ] and tau in AD, α-synuclein [α-syn] in PD and TDP-43 in ALS) is a pathological hallmark of the diseases;
- Disease-related pathology may spread across the nervous system in a self-propagative fashion;
- A neuroinflammatory component is present;
- There can be clinical and pathological overlap between different neurodegenerative disorders.

However, after decades of intensive research we are not even close to answer the most elusive, and probably most important question of all, namely “What initiates the pathological cascade that leads to progressive neurodegeneration?” In the desperate battle against these devastating disorders we should be open for theories that are free of the legacy of previous approaches, but instead examine the problem from a new perspective.

KEYWORDS
• Alzheimer’s disease • amyloid • amyotrophic lateral sclerosis • biomarker • gut–brain-axis • microbiome • microbiota • neuroinflammation • Parkinson’s disease

“For gastroenterologists, microbiota-based treatment approaches are already routine procedures, namely fecal microbiota transplantation for the treatment of refractory Clostridium difficile infection.”

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**Commentary**  Scheperjans

**An unknown organ**

It is nowadays an exceedingly rare event that a previously unknown organ of the human body is discovered and awaiting scientific exploration. The contemporary biomedical research community can regard itself privileged for being granted such a unique opportunity. The newly discovered organ of interest, that progressively entered the spotlight of mainstream research during the last decade, has a weight of approximately 1.5 kg, encompasses about 90% of the cells of the human body and has a genetic repertoire exceeding that of the remaining organism by a factor of 100–200 [1]. This organ has been named ‘microbiota’ and is the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space [2,3]. The term ‘microbiome’ refers to the collective genomes of these microorganisms, but both terms have been used largely synonymously.

In recent years, microbiota research has gained huge momentum through the development of next-generation sequencing approaches allowing rather unbiased assessment of all microbial taxa in a given sample with unprecedented detail and efficacy. The Human Microbiome Project found the diversity and abundance of signature microbes of different habitats (e.g., gut, skin, vagina) to vary widely even among healthy subjects, with strong niche specialization both within and among individuals [4]. The gut harbors by far the highest concentrations of microbiota and is the best studied habitat. Initial studies have focused on the mere description of microbiota composition, for example, measuring the abundances of different microbial taxa in samples. From these studies we have learned that clear alterations of gut microbiome community structure are associated not only with gastrointestinal diseases such as inflammatory bowel disease or colonic cancer, but also with other disorders such as diabetes, obesity, arthritis, allergy and cardiovascular disease. More recently, using multiomics and experimental approaches, the scope of microbiota research has expanded toward functional aspects of our microbial ecosystem. Together with integrative systems biology these studies are beginning to elucidate how gut microbiota interacts with the human host and may be related to health and disease.

**Microbiota, the brain & neurodegeneration**

Possible infectious etiologies of neurodegenerative diseases have been studied for a long time. In AD, connections to for example, Herpes simplex virus 1, spirochetal and *Chlamydiaphila pneumoniae* infections have been reported [5,6]. Recently, one group found signs of fungal infection in the brains of AD and ALS patients [7,8]. *Helicobacter pylori* has been suspected as a causal agent in PD [9]. Also bacterial toxins have been discussed as a possible cause of neurodegenerative diseases [10]. However, so far, no single infectious agent has emerged as being unequivocally associated with any of these disorders and it has been discussed that instead overall infectious burden and resulting systemic inflammation could predispose to neurodegeneration [11,12]. Furthermore, it has become clear that invasive infection by a pathogen is not the only mechanism by which microbes can cause disease. Possibly even more relevant are remote systemic influences of commensal microbiota mediated through numerous physiological pathways such as the immune system, autonomic nervous system (in particular the enteric nervous system and vagal nerve), metabolic routes as well as intercellular signaling locally and through the blood stream.

Importantly, there is a strong bidirectional interaction between the gut microbiota and the CNS, a connection recently termed the ‘microbiota–gut–brain axis’. While the effects of the autonomic nervous system on gut physiology have been known for a long time, we are just beginning to understand that gut microbiota has strong effects on CNS physiology as well. For example, in animal models, changes in gut microbiota composition rapidly affect behavior, neurotransmitter balance and expression of neuronal growth factors which has sparked great interest in the possible connection between gut microbiota and mental disorders [13,14]. Microbiota alterations early in life may have permanent structural and physiological neurodevelopmental consequences fueling hope for better understanding of neurodevelopmental problems such as autism spectrum disorders.

In terms of adult neurology, we have recently seen first concrete examples supporting a role of microbiota in neurological disorders. In 2011, Berer et al. demonstrated that in the relapsing-remitting mouse model of spontaneously developing experimental autoimmune encephalomyelitis, an animal model of MS, commensal gut microbiota is essential for the development of CNS autoimmunity since germ-free mice were protected from encephalomyelitis [15]. Later,
a Japanese study reported alterations of gut microbiota in MS patients [16]. Furthermore, gut microbiota changes have been recently associated with atherosclerosis and ischemic stroke and this association may be mediated through bacterial metabolism of ingested nutrients [17].

The vast number of ways through which gut microbiota affects the host shows intriguing overlaps with pathways previously implicated in neurodegeneration, for example, mitochondrial function, neuroinflammation and microglial activation, growth factors. Microbiota research in neurodegenerative diseases is still in the earliest phases and so far mainly descriptive in nature. In PD, there is a strong and early involvement of the gastrointestinal tract. It has long been speculated that in PD an environmental factor could act via the gut and initiate the pathological process leading to neurodegeneration, but no specific agent could so far be identified [18]. PD patients have a higher prevalence of small intestinal bacterial overgrowth which has been linked to worse motor function and fluctuations, as has been *H. pylori* infection [19].

Recently, Forsyth *et al.* demonstrated that the colonic mucosa of PD patients shows signs of bacterial invasion, oxidative stress and an impaired barrier function leading to an increased systemic exposure to proinflammatory bacterial lipopolysaccharide [20]. The first comprehensive study of fecal microbiota composition in neurodegenerative disease revealed clear differences between PD patients and control subjects even after adjustment for potential confounders such as medication and constipation [21]. The most striking finding was a 78% lower abundance of Prevotellaceae in the feces of PD subjects while several other bacterial families were more abundant than in controls. Furthermore, microbiota was related to the motor phenotype of PD patients. This study suggested that gut microbiota could be implicated in PD and might be a promising biomarker. Recently, two independent studies confirmed microbiota differences between PD and control subjects [22,23]. Both studies found decreased Prevotellaceae abundance in PD, but not all differences reached statistical significance which may at least in part be explained by differences in sample size, subject characteristics and methodology.

Not much is known about pathophysiological microbiota–host interactions in PD. An interesting initial finding pointing to interactions between the host genome and microbiota is the reported association of common variants in peptidoglycan recognition protein genes with PD risk in two independent studies [24]. These proteins maintain healthy gut microbial flora by regulating the immune response to both commensal and harmful bacteria. Variants in these genes could thus influence microbiota composition and the immunological reaction to it. According to one hypothesis, local inflammation in the gut mucosa, which may be related to microbiota and barrier dysfunction, could promote prion-like behavior of *α*-syn as an initial event in the neuropathological cascade that subsequently progresses along the vagal and sacral autonomic nerves leading eventually to CNS neurodegeneration [25]. A comparable process has also been proposed for the nasal route through the olfactory bulb, referring to the early olfactory dysfunction and related neuropathology in PD subjects. Following a similar line of thought, it was hypothesized that, in diseases such as AD and PD, amyloid proteins produced by bacteria could induce misfolding of host proteins and thus initiate a prion-like cascade leading to progressive neurodegeneration, possibly enhanced by amyloid-induced inflammation and oxidative stress [26,27]. Finally, although not yet confirmed by specific studies, it has been speculated based on a literature review that gut microbiota could play a role in mediating the protective effects of coffee and tobacco use on PD risk [28].

With respect to AD, no detailed study of human microbiota composition or function has been published yet. However, some studies have examined a possible connection between oral microbiota and AD. It has been reported that serum antibodies to oral bacteria have been associated with AD and risk for developing incident AD [29,30]. In one study, next-generation sequencing was used to compare subgingival plaque microbiota between elderly subjects with and without dementia, without specifying what type of dementia was present [31]. A higher level of Fusobacteriaceae and a lower level of Prevotellaceae was seen in subjects without dementia, although the difference did not reach statistical significance, possibly because of the small sample size. While no state-of-the-art assessment of microbiota in AD has been published, yet, interesting findings have been recently reported from an AD
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“...there is a great interest in the potential of microbiota research for reshaping our understanding and treatment of enigmatic diseases.”

animal model. According to a not yet peer-reviewed manuscript on arXiv, Harach et al. studied gut microbiota in APPPS1 mice that coexpress the KM670/671NL Swedish mutation of human amyloid precursor protein and the L166P mutation of human presenilin 1 under control of the Thy-1 promoter and show age-dependent accumulation of cerebral Aβ plaques [32]. They found that, in comparison to wild-type mice, gut microbiota of APPPS1 mice showed reduced levels of Firmicutes, Verrucomicrobia, Proteobacteria and Actinobacteria with a concurrent increase in Bacteroidetes and Tenericutes. Furthermore, they generated a germ-free version of the APPPS1 mouse model and observed that these mice showed clearly less Aβ amyloid pathology in the brain than the conventionally raised APPPS1 mice. Colonization of germ-free APPPS1 mice with harvested microbiota from conventionally raised APPPS1 mice increased cerebral Aβ pathology. In contrast, colonization with microbiota from wild-type mice was ineffective in increasing cerebral Aβ levels. This important study suggests that, at least in this mouse model of AD, commensal gut microbiota plays a causal role in promoting cerebral Aβ amyloid pathology and thus provides an important proof-of-concept warranting further studies of microbiota in AD.

With respect to ALS, no comprehensive assessment of microbiota has been published. One small study in transgenic a mouse model (G93A) expressing mutant superoxide dismutase reported alterations of intestinal tight junctions and increased intestinal permeability associated with microbiota alterations [33].

Conclusion & future perspective

For gastroenterologists, microbiota-based treatment approaches are already routine procedures, namely fecal microbiota transplantation for the treatment of refractory Clostridium difficile infection [34]. In endocrinology, translational studies for microbiota-based interventions targeting diabetes and obesity are underway. Also in many other specialties there is a great interest in the potential of microbiota research for reshaping our understanding and treatment of enigmatic diseases. Such new treatments frequently aim at changing microbiota composition and thus shifting the equilibrium of microbiota–host interactions to a state that is beneficial for the host. One way is to replace patients’ gut microbiota by that of a healthy donor (fecal microbiota transplantation). Alternatively, the abundance of beneficial microbes can be increased by supplementing such live organisms directly (probiotics) or instead by using compounds that promote their growth (dietary interventions or prebiotics). Another approach is to specifically identify molecules excreted by such beneficial microbes and to use them pharmacologically (postbiotics).

For neurologists, gut microbiota is still rather unknown territory and its connection to neurological diseases may appear counterintuitive. It should not. As outlined above, recent developments in sequencing techniques and molecular biology allow studies of microbiota in unprecedented detail and the theoretical framework for relevance of the microbiota–gut–brain axis in neurological disorders is actually very strong. Although evidence for involvement of microbiota in neurodegenerative diseases is still very preliminary, initial findings are extremely promising. Future research will need to establish how stable and specific these findings are and whether microbiota changes precede or follow neurodegeneration. Such associations per se could indicate a possible biomarker role of microbiota. More difficult to answer will be the question of causality and what mechanisms drive the interaction with the host and other environmental factors such as diet. Ahead is a long scientific journey that could fundamentally reshape our understanding of neurodegenerative diseases. The time to embark is now. If this endeavor is successful, we may end up with completely new therapeutic approaches that could hopefully turn the ship around toward effective disease modification or even prevention.

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Can microbiota research change our understanding of neurodegenerative diseases?  

**COMMENTARY**

References


Ischemic, traumatic and neurodegenerative brain inflammatory changes

Rachelle Dugue & Frank C Barone

This review serves to link the role of the immune system in the neuropathology of acute ischemic stroke, traumatic brain injury and neurodegenerative disease. The blood–brain barrier delineates the CNS from the peripheral immune system. However, the blood–cerebrospinal fluid barrier acts as a gate between the periphery and the brain, permitting immune activity crosstalk and modulation. In acute ischemic stroke, traumatic brain injury and other neurodegenerative diseases, the blood–brain barrier is compromised and an influx of inflammatory cells and plasma proteins occurs, resulting in edema, demyelination, cell dysfunction and death, and neurobehavioral changes. The role of the complement system, key cytokines, microglia and other neuroglia in brain degenerative pathology will be discussed.

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The innate immune system in the periphery versus the CNS

- Innate immunity in the periphery

The innate immune system of the periphery acts as a first responder to pathogens. Also known as the nonspecific immune system, the components of innate immunity provide general, nonspecific defense mechanisms to control infection. In addition to anatomical barriers like the skin and GI tract to physically prevent pathogen invasion, innate immunity provides two important initial functions: it immediately recognizes pathogens, pathogenic by-products, pathogen-altered human cells and proteins, and tissue injury; and it rapidly recruits and initiates effector cells to eliminate/regulate these intruders/changes [1].

In order to recognize pathogens and other markers of bodily harm, the innate immune system utilizes an arsenal of receptor types (referred to as ‘pattern recognition receptors’ [PRRs]) that are expressed on cell membranes, within the cytoplasm of cells, or secreted in soluble forms by effector cells. Different PRRs and cell systems can detect self-MHC class I molecules, pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs) [2]. PAMP detecting receptors include Toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors (NLRs), RIG-like receptors, AIM2-like receptors, scavenger receptors and the soluble collagen-containing C-type lectins (i.e., collectin receptors) [3,4]. Many of these same receptors, including TLRs, NLRs, as well as the scavenger receptor, receptor for advanced glycation end products, detect DAMPs as well [5]. DAMPs are produced when tissue is damaged or cells lyse during injury/inflammation [2–3,6]. DAMP receptors are of particular importance to neurodegenerative pathologies like acute ischemic stroke (AIS) and traumatic brain injury (TBI) where noninfectious...
tissue injury occurs.

In response to DAMPs and PAMPs detected by their receptor complexes, effector cells of the peripheral innate immune system are activated. Thus, macrophages, dendritic cells, neutrophils, mast cells, eosinophils, natural killer cells and γδ T cells collectively contribute to host defense via phagocytosis of pathogens, the destruction of compromised host cells, cell toxin and cytokine release, and the activation of the adaptive immune response via antigen presentation to naïve T cells [1,2].

Inflammatory cytokines are small cell signaling proteins such as interleukins, TNF-α, interferons and chemokines (e.g., chemotactrant cytokines such as MCP-1). Cytokines contribute both locally and systemically to pro-inflammatory and anti-inflammatory responses [7]. The imbalance of pro- versus anti-inflammatory cytokines contributes to vasodilation, edema, the development, activation and recruitment of immune cells, and apoptotic cell death [1,8–9]. In cooperation with the innate immune system’s cellular and molecular immune defense system, is the complement system (see ‘The complement system’ section). The complement system is comprised of plasma proteins and proteases that tag pathogens for destruction, initiate cell death, recruit inflammatory cells and induce inflammation [1,10].

In summary, the rapid, highly organized and interlocked system of innate immunity in the periphery showcases three main players: the effector cell bound and soluble PRRs; the effector cells (macrophages, dendritic cells, neutrophils, mast cells, eosinophils, natural killer cells and γδ T cells), which act in response to their PRRs, binding to PAMPs and/or DAMPs to produce both pro- and anti-inflammatory cytokines, activate the adaptive immune response via antigen presentation, recruit cells and induce cell death via phagocytosis, toxin release and other mechanisms; and the complement system, which contains both PRRs to PAMPs and DAMPs and mechanisms to tag damaged cells, pathogens and induce cell death and other cellular (e.g., synaptic) changes following injury or infection.

• **Innate immunity in the CNS**

Similar to the anatomical barriers of innate immunity in the periphery, the CNS blood–brain barrier (BBB) generally isolates the brain from the cells and molecules of the periphery. However, the blood–cerebrospinal fluid barrier (BCSFB), formed by the choroid plexus, arachnoid and arachnoid villi, lines each ventricle and provides regulated communication between the brain and the periphery [11]. It filters out metabolic waste, excess neurotransmitter and foreign substances from the cerebrospinal fluid (CSF), in addition to conditionally permitting immune cell trafficking [12]. The choroid plexus of the BCSFB acts as a gate, allowing leukocytes to enter the CNS in specific physiological and pathological conditions [13]. These mechanisms provide immune cells that can contribute to injury as well as brain protection and repair [12]. The CNS also contains a meningeal lymphatic system, recently discovered along the dural sinuses, through which CSF contents can be transported to and activate immune responses in the deep cervical lymph nodes [14]. The pathways through which immune cells are transported may relate to their function [15].

Like the peripheral innate immune system, the CNS contains its own effector cell types, in addition to all complement components. Astrocytes, microglia, oligodendrocytes, mast cells, and NG2 chondroitin sulfate and PDGF-α receptor-positive oligodendrocyte precursor cells (NG2-OPCs), in addition to complement components produced locally within the brain itself, contribute to the brain’s immune response [16,17]. Microglia act as the brain’s phagocytes and patrol the brain tissue for any threats (see ‘The role of microglia in AIS, TBI & neurodegeneration’ section), while astrocytes regulate the concentrations of ions, glutamate and water, store glycogen and offer metabolic support to neurons, in addition to regulating synaptogenesis, forming the BBB and defending against oxidative damage [1–4,18]. Astrocytes and microglia produce many pro- and anti-inflammatory cytokines following DAMP and/or PAMP detection. For example, microglia, astrocytes, neurons, mast cells and oligodendrocytes all express TLRs. TLRs 3, 7 and 8 are present on neurons and, when activated, can inhibit post-injury axonal growth and repair. The accumulation of TLR-activating DAMPs after neuronal injury may prevent axon regeneration and repair by inducing TLR3-mediated axonal growth cone collapse and/or TLR7- and 8-mediated neuronal death [4].

In the CNS and peripheral immune system (see Figure 1), while infection causes the release of PAMPs, injury causes the release of DAMPs, both of which can drive inflammasome
The innate immune response in the periphery, like the innate immune response of the CNS, is activated by PAMPs related to infection and DAMPs related to tissue injury. Pattern recognition receptors like TLRs, NLRs, RLRs and ALRs detect these signals and induce a cellular and molecular response from the effector cells containing these receptors. In the periphery, major effector cells are natural killer cells, macrophages, neutrophils and dendritic cells. Natural killer cells induce apoptosis of infected cells, while neutrophils use a respiratory burst response involving the release of ROS to destroy phagocytosed material. Effector cells like dendritic cells and macrophages detect PAMPs and act as antigen-presenting cells. These antigen-presenting cells travel to the nearest draining lymph node and trigger the maturation of naive T cells and the more effective, long-term adaptive immune response of the peripheral immune system. In contrast, the CNS innate immune response mainly utilizes microglia, astrocytes and neurons. These cells express PRRs like TLRs and NLRs to detect PAMPs and DAMPs. In response to these signals, astrocytes are activated leading to astrogliosis, glial scar formation, and the release of complement, cytokines and growth factors. Microglia become activated in response to these signals and via their interaction with neurons, phagocytose debris, and release cytokines, complement, growth factor, reactive oxygen species, glutamate, histamine and chemokines. Cytokines and chemokines recruit and activate glial and immune cells, increase vascular permeability, and participate in cell death and survival signaling. The interaction between PAMPs or DAMPs and PRRs leads to the formation of inflammasomes, multicomplexes that permit the release of active pro-inflammatory cytokines IL-1β and IL-18, as well as apoptosis; AIM-2 inflammasomes form in neurons in response to DNA released from dying and injured cells and contribute to pyroptotic neuronal death. A variety of NLR inflammasomes (NLRP1, NLRP2, NLRP3) are expressed in neurons, microglia and astrocytes. The formation of the NLR inflammasome involves an initial activation of the NF-κB pathway via pattern-recognition receptors like TLRs (signal 1). The NF-κB pathway transcribes pro-IL-1β and pro-IL-18, which are cleaved to their pro-inflammatory active form by caspase-1. NLRs are activated by ATP (signal 2) and form inflammasomes that recruit and activate caspase, thereby catalyzing this reaction [4]. Inflammasomes can form in both the periphery and the CNS. Furthermore, frequent communication occurs between the periphery and the CNS via the BCSFB and meningeal lymphatic system. ALR: AIM-like receptor; BBB: Blood–brain barrier; BCSFB: Blood–cerebral spinal fluid barrier; DAMP: Damage-associated molecular pattern; NLR: Nucleotide oligomerization domain-like receptor; PAMP: Pathogen-associated molecular pattern; ROS: Reactive oxygen species; RLR: RIG-like receptor; TLR: Toll-like receptor.
The complement system in the CNS

● The complement system

The complement system is heavily integrated in the CNS, contributing to neuronal development, synaptic remodeling and neuropathology. The complement system has three main activation pathways: the alternative pathway, the lectin pathway and the classical pathway (see Figure 2 for a pictorial summary of the complement cascade). The alternative pathway is spontaneously activated via hydrolysis of complement protein C3 and in response to pathogen-induced changes in the physiochemical environment. The lectin pathway is activated by mannose-binding lectin binding to mannose-containing glycoproteins and carbohydrates of pathogens and PAMPs. The classical pathway is activated when the initiating complement protein, C1q, binds to any of the following: antigen–antibody complexes, apoptotic cells, certain coagulation cascade factors, serum pentaxin proteins like CRP, and serum amyloid P [22,23].

The central event within the complement cascade is the cleavage of complement protein C3 to C3a and C3b. C3b tags pathogens and deposits on membranes in a process called opsonization to encourage phagocytosis, while C3a is an anaphylatoxin, an inflammatory modulator that recruits effector cells to complement-targeted sites and increases effector cell activity [22]. The formation of C3b and C3a from C5 permits the complement system to respond rapidly and robustly; this is the primary amplification step in the cascade.

All activation pathways lead to the formation of C3 convertases. The C3 convertases of the classical and lectin pathways form from cleavage of C4 and C2, while the C3 convertase of the alternative pathway forms from C3b and Factor B’s cleavage product, Bb. C3 convertases bind and cleave C3, creating a positive feedback loop, in addition to contributing to the formation of the terminal C5 convertase. C5 convertase is the substrate for the terminal complement proteins C5b-9. All complement pathways converge to form the membrane attack complex (MAC) from terminal complement proteins for membrane lysis of pathogens and abnormal cells, in addition to the production of anaphylatoxin, C5a (see Figure 2) [10,24–25].

Complement proteins can also activate cytokines and intracellular signaling pathways. For example, C3 can trigger TNF-α release, which intensifies inflammation by leading to the release of IL-1, IL-6 and IL-18 [10]. To regulate this cascade, inhibitory proteins such as Factor H, C1-inhibitor, decay-accelerating factor, membrane cofactor protein (i.e., CD46) and MAC-inhibitory protein (i.e., CD59) oppose complement cascade promoting factors [1,26].

Complement factors and receptors are synthesized throughout the CNS by neurons, microglia, astrocytes, oligodendrocytes, as well as endothelial cells of the microvasculature. Most neurons express C1q, C5, the C5a receptor (i.e., CD88) and CD59, but have the potential to produce other complement proteins when stimulated to do so [27]. Astrocytes produce C1q, C1r, C1s and C2–4 of the classical pathway, C3, Factor B and Factor D of the alternative pathway, and C5–9 of the terminal part of the pathway. Astrocytes also express complement receptors...
Ischemic, traumatic & neurodegenerative brain inflammatory changes

C1q, CR2, C3aR and C5aR, while producing regulatory proteins C1-inhibitor, Factor H, Factor I, clusterin, CD59, decay-accelerating factor, membrane cofactor protein and CR1 [26]. Microglia express the same complement components of the classical pathways as astrocytes, but only produce C3 of the alternative pathway, while expressing the receptors C1qR, C3R, C3aR, CR4, C5aR and the regulatory proteins C1-inhibitor, CD59 and CR1 [16,26]. In contrast to microglia and astrocytes, oligodendrocytes produce complement components C1q, C1s, C4, C2, C3, C5–9, but have no regulatory proteins; oligodendrocytes are therefore more susceptible to complement-mediated attack.

In addition to its immunoregulatory functions within the brain, the complement cascade is involved in neurogenesis, cell migration and survival, and synaptic development and elimination [25,28]. For example, adult hippocampal neurogenesis was discovered to be regulated by CR2, a receptor that binds C3 fragments and

**Figure 2. The complement cascade.** The complement system is comprised of three pathways: the classical pathway, the lectin pathway and the alternative pathway. These pathways are activated by different signals, but all culminate in the production of C3 and later the formation of the MAC, which lyses abnormal cell membranes and pathogens. C3b deposits on pathogens and cell membranes to encourage phagocytosis via phagocytes containing CR3 in a process termed opsonization. Anaphylatoxins C5a and C3a, recruit and increase the activity of effector cells and are a by-product of C3 and C5 cleavage, respectively. Various proteins such as DAF, MCP and C1-inhibitor protein regulate the complement cascade.

DAF: Decay-accelerating factor; MAC: Membrane attack complex; MASP: Mannose-binding lectin-associated serine protease; MBL: Mannose-binding lectin; MCP: Membrane cofactor protein, i.e., CD46 complement regulatory protein; PAMP: Pathogen-associated molecular pattern; SAP: Serum amyloid P.
IFN-α, and is therefore sensitive to inflammatory changes [29]. Complement proteins are also utilized in the elimination of synapses in the developing brain. C1q, the initiating complement protein in the classical pathway, is upregulated in developing neurons during periods of pruning and is hypothesized to localize to synapses destined for elimination through communication with immature astrocytes [30,34]. The presence of C1q on such synapses triggers C3 activation and opsonization for phagocytosis by C3R bearing microglia, as well as the formation of the terminal MAC [31]. The increased presence of activated microglia during periods of developmental pruning supports this idea [10].

These mechanisms of synaptic modification have also been implicated in TBI, AIS and neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and multiple sclerosis (MS). The involvement of complement in neurodegenerative pathologies will be explored in the following section.

The complement system in AIS, TBI & neurodegeneration

The basic pathophysiology of TBI involves the initial traumatic insult followed by a complex cascade of events in response to the primary injury; the release of excessive amounts of excitatory neurotransmitters and increases in intracellular calcium, immune activation and dysfunction, free radical release, ion flux and metabolic imbalances, BBB disruption, and vasogenic edema, all contribute to disruptions in homeostasis leading to cell death via necrosis and apoptosis [32,33]. Similarly, the basic pathophysiology of ischemic stroke involves an initial event – the interruption of blood flow to the brain via embolism, hypoperfusion, or arterial or venous thrombosis, producing a severely hypoperfused area termed the core and a moderately perfused surrounding area termed the penumbra; the core of the lesion undergoes widespread necrosis, while the penumbra is at risk of cell death via neurotoxic factors from the core [34]. The ischemic event leads to a cascade of responses: excitotoxicity, inflammation, oxidative damage, apoptosis, angiogenesis and changes in ionic balance [35].

The expression of complement components in the brain is highly upregulated following ischemic stroke, TBI and other forms of brain injury. Reactive astrocytes and microglia produce high levels of C3 and complement proteins, in addition to cytokines and other inflammatory signals. For example, the anaphylatoxins C3a, C4a and C5a are produced by complement system activation in AIS and TBI. These anaphylatoxins increase vascular permeability and recruit and activate inflammatory cells. Because the BBB is breached in these conditions, immune cells from the periphery quickly enter the brain in response to such signals; C5a induces chemotaxis of basophils, neutrophils, macrophages and microglia to the site of injury. In ischemic stroke, C5a is increased in ischemic neurons, likely driving neuronal apoptosis, while C3a and C5a receptors, C1q and C3 are increased in the infarct core [27,36].

Complement depletion has been shown to improve neurological function in cerebral ischemia [37]. C3 is detrimental in ischemic stroke. For example, following AIS, C3−/−mice had smaller infarct volumes, less granulocyte infiltration into the brain, decreased oxidant stress levels, and better neurological function than wild-type mice. Similar results were obtained in wild-type mice when a C3R antagonist was administered following ischemic stroke [38]. In patients with MS, C3 has also been shown to positively correlate with disease severity, and neurofilament light levels, a biomarker of nerve injury [39].

C5−/−mice were also protected from brain injury [40]. In neurons, C5a receptor (i.e., CD88) expression and the production of C5a are increased in response to ischemic stress. CD88−/−mice and neuronal cultures treated with CD88 receptor blockers were significantly protected in models of AIS and TBI, suggesting that C5a and its receptor contribute significantly to neuronal death [27]. C5a, in addition to mediating the release of pro-inflammatory cytokines, upregulates the expression of coagulation cascade-activating tissue factor in endothelial cells. Importantly, C3 and C5 can be proteolytically activated by plasmin, thrombin and other coagulation cascade factors [22].

Regulators of the complement system, such as C1 inhibitor, have exhibited protection in AIS models. C1 inhibitor-treated mice had lower degrees of neuronal degeneration, improved neurological deficit scores, smaller infarct volumes and decreased leukocyte infiltration [41–43]. This could occur through the upregulation of the anti-inflammatory cytokines IL-10 and IL-6 [42]. A lack of mannose-binding lectin has also been associated with improved outcomes following stroke in mouse models and in patient cases [22].
Complement activation also acts as a mediator of injury in TBI. C1q, C3b, C3d and MAC levels are increased in TBI brains. In TBI brains, MAC levels have been observed to correlate with the level of BBB disruption [44]. In stroke patients, serum levels of MAC components C5b-9 have been positively correlated with ischemic stroke severity [45]. These levels rise and exacerbate injury in the absence of complement regulator, CD59 [3].

Unlike astrocytes and microglia, neurons and oligodendrocytes do not express as much CD59, the complement regulatory protein responsible for inhibiting the formation of the terminal MAC. They are therefore more susceptible to complement-mediated damage and death. This is especially true of apoptotic neurons that lose complement regulatory proteins, permitting their progression toward cell lysis and phagocytosis [46]. The absence of protective CD59 in oligodendrocytes and some neurons could explain the selective white matter damage of diffuse axonal injury in some forms of TBI. Furthermore, mice overexpressing complement inhibitor Crry had fewer neurological deficits following TBI [3].

Although complement-mediated synaptic pruning is a normal aspect of neuronal development, the elimination of synapses is likely to contribute to neurodegeneration in AIS and TBI, as well as in other neurological diseases such as MS, AD and PD. Reactive astrocytes upregulated following injury are of a similar composition to immature astrocytes involved in signaling related to C1q upregulation at synapses during developmental synaptic pruning [34]. Following injury, C1q is upregulated in microglia and neurons, while reactive astrocytes begin to appear and downstream components of the complement cascade are activated. Supporting the concept of complement-mediated synaptic elimination in neurodegenerative disease, is the recent observation of a combination of hypoxia and the inflammatory stimulus lipopolysaccharide (LPS) inducing long-term depression (LTD) in the hippocampus. The LTD observed was triggered by microglial CR3 activation and involved NADPH oxidase production of the potent reactive oxygen species superoxide, leading to AMPA receptor internalization [47]. Long-lasting LTD at a synapse can result in the elimination of that synapse by microglia. Furthermore, CRP (i.e., a serum pentraxin that activates the classical complement cascade via binding to C1q) levels positively correlate with infarct volume in AIS patients [48]. Thus, an excess of CRP could trigger C1q upregulation and contribute to synaptic elimination via mechanisms similar to those occurring during developmental synaptic pruning.

In MS, synaptic loss was shown alongside increases in synaptic C1q and C3 deposition in MS hippocampi with and without lesions and demyelination. C1q deposition positively correlated with synaptophysin staining for synaptic density, as well as mHSP70 density, a marker of neuronal stress, in the hippocampi of patients with progressive MS. These findings, in addition to observations of C1q and C3 deposition co-localized with CR3 in synapses within microglial processes, support complement-mediated synapse elimination in MS [49].

In a study on age-related cognitive decline, C1q levels increased with age and were far higher in aged mice, as well as in aged human brains, especially in the dentate gyrus molecular layer; C1q-deficient aged mice had greater cognitive flexibility than wild-type aged mice [50]. In a transgenic mouse model of AD, the absence of C1q protected neuronal integrity and decreased astrocytic and microglial activity [51]. A model of PD showed increased C1q expression in activated microglia surrounding degenerating dopaminergic neurons in the substantia nigra. However, C1q-deficient PD mice had the same degree of nigrostriatal degeneration as wild-type mice [52]. Furthermore, in humans, genetic variants of C1q are not likely to influence cognitive decline in PD [53]. Therefore, C1q may not be detrimental in PD pathology.

**Key cytokines & immune players in brain injury & neurodegeneration**

- Neuroglia & BBB disruption in AIS, TBI & neurodegeneration

Brain injury puts extreme stress on the regulatory function of astrocytes and other brain cells. The destruction of neurons and glial cells causes the release of ATP and many DAMPs, thus activating microglia, neurons, mast cells and glial cells to produce cytokines, chemokines and complement [17,26]. Tissue injury-induced stress disturbs astrocyte-driven ion and glutamate homeostasis resulting in the substantial release of glutamate, K+ ions and reactive oxygen species, all of which contribute to neurotoxicity [54]; astrocytes downregulate their glutamate transporters, thus...
neurodegenerative diseases such as PD, AD and MS (i.e., reactive astrogliosis, an excessive proliferation and activation of astrocytes, a necessary response to all CNS injury) [56–59]. Microglia also play a key role in neurodegenerative disease pathology. When stimulated in these conditions, microglia may switch to a state in which they phagocytose dead or dying cells and debris, while producing cytokines and neurotoxic substances that can exacerbate cell damage [23].

The common disruption of the BBB in AIS and TBI results in vasogenic edema, permits the influx of immune cells from the periphery and signals the immediate migration of microglia and NG2-OPCs to BBB leak sites and sites of tissue injury [60,61]. Chemokines and cytokines recruit immune and glial cells, in addition to increasing vascular permeability, while adhesion molecules drive neutrophils and other leukocytes to adhere to endothelial cell layers and infiltrate both injured and nearby brain tissue [62,63]. Activated neutrophils produce neurotoxic free radicals, in addition to upregulating inflammatory cytokines and oxidative enzymes like NADPH oxidase and inducible nitric oxide synthase through their oxidative burst response [64]. Mast cells promote BBB changes and promote neuroinflammation in AIS and TBI via the release of substances (histamine, proteases, cytokines) that degrade matrix, recruit and communicate with other immune cells, and alter cerebral blood flow [17]. Infiltrating macrophages and resident microglia of the ‘M1 type’ are also thought to mediate injury, while macrophages/microglia of the ‘M2 type’ are hypothesized to be beneficial in ischemic injury (see “The role of microglia in AIS, TBI & neurodegeneration” section). A decrease in the number of infiltrating macrophages via deletion of MCP-1 (i.e., CCL2) or its receptor results in smaller infarcts and fewer pro-inflammatory molecules (Figure 3) [65,66].

By binding to activated endothelium and entering the brain via BBB injury, CD4+ and CD8+ T cells contribute to damage in both TBI and AIS within the first 24 h post-injury. This implies that T cells are not exerting an adaptive immune response attack, which normally takes days to mobilize, but are contributing to neuroinflammation immediately via cytotoxic mechanisms of perforin-mediated apoptosis and the secretion of IFN-γ [67]. CD4+/CD28+ T cells are increased and associated with higher stroke reoccurrence risks and death [68]. Upregulation of MHC-I and MHC-II on neurons facilitates the destructive action of T cells. However, some T-cell types produce protective molecular mediators, assisting in defense and repair [69].

In accordance with the interaction between the peripheral and CNS immune responses in neurodegenerative pathologies, splenectomy has conferred protection in AIS when conducted prior to stroke, decreasing lesion volume and reducing the number of T cells, neutrophils, macrophages and pro-inflammatory cytokines [70]. Splenectomy has also reduced mortality and improved cognition in a rat model of severe TBI [71]. Brain injury itself also interacts with the spleen and other organs. For example, post-TBI, inflammatory cytokines are released in the gut and post-stroke, a reduction in the size of the spleen and thymus has been observed [72]. A recent study using CD74-deficient mice showed neuroprotection, further evidencing peripheral–CNS immune system interactions and a role for peripheral adaptive immunity in TBI pathology [73]. Neuroinflammatory responses observed peripherally could potentially be used as noninvasive clinical biomarkers in AIS and other neurodegenerative diseases, using gene-expression profiling obtained from blood samples [74,75].

In MS, demyelinating white matter lesions are invaded by many immune cells including T and B lymphocytes, macrophages, microglia, plasma cells and MHC class II-positive dendritic cells. It is hypothesized that peripheral autoreactive T cells infiltrate the CNS and induce an inflammatory milieu that further recruits immune cells that destroy myelin and glial cells while exacerbating inflammation [76]. Therefore, treatments suppressing peripheral immunity help to control symptoms of MS. However, such immunosuppression is detrimental in other neurodegenerative diseases, such as AD, as immuno-regulatory cells from the periphery may aid the brain in such diseases [12].

Although a host of molecules facilitate neuroinflammation, not all molecules produced in response to brain injury are bad. For example, although activated astrocytes form a glial scar that later inhibits axon regrowth, it initially serves to wall off the site of injury from healthy tissue to protect healthy tissue from pro-inflammatory mediators and debris from
Activated astrocytes also produce neuroprotective molecules such as NGF, TGFβ1 and BDNF to assist in repair/growth [5,41]. Additionally, some cytokines are also known to exert protective effects (see ‘Key cytokines in AIS, TBI, and neurodegeneration’ section).

**Figure 3. Microglial activation.** Microglial activation occurs in response to infection or tissue injury leading to the production of PAMPs and DAMPs, respectively. This activation results in the production of activated microglial phenotypes like M1 and M2. The M1 phenotype is promoted by IFN-γ, while the M2 phenotype is promoted by IL-13 and IL-10 production. The M2 phenotype promotes neuroprotective factors like neurotrophins BDNF and NGF, and anti-inflammatory cytokines. The M1 phenotype promotes neurodegeneration via the release of pro-inflammatory cytokines, ROS, RNS, nitric oxide, excitotoxic factors like glutamate, and results in increased BBB permeability and immune cell recruitment and activation. However, microglial activation is not limited to these two activation types, but is broad, with a spectrum of activation states that continue to be characterized. Synaptic elimination (‘synaptic stripping’) is the removal of dysfunctional synapses by activated microglia. The occurrence of LTD in weaker synapses as well as the presence of molecular signals of cell damage, allow microglia to target such dysfunctional synapses for elimination via phagocytosis. It is possible that some of these interactions are mediated by complement receptor CR3 and the tagging of synapses via C1q, similar to synaptic pruning in early development.

The role of microglia in AIS, TBI & neurodegeneration

Microglia are activated within minutes of an insult to the brain. These cells are the main source of pro-inflammatory cytokines and chemokines. Microglia express PRRs that sense molecular signals indicating harm (DAMPs and PAMPs), enabling them to initiate the appropriate inflammatory response. For example, ATP released from dying and dysregulated cells serves as a chemotactic signal to microglia. In response to neuronal damage in TBI and AIS, ramified microglia are activated to their amoeboid form, producing cytotoxic factors like reactive oxygen species, NO, glutamate [77] and histamine, pro-inflammatory cytokines like IL-1, TNF-α [78], IL-6 and IFN-γ [79], in addition to neurotrophic and anti-inflammatory cytokines like IL-4, IL-10, BDNF, neurotrophin-3, -4 and -5, and NGF, depending on the signals encountered [80–82]. A protective microglial phenotype can be induced in response to IL-10 and IL-13 stimulation, while a pro-inflammatory M1 microglial phenotype can be induced via IFN-γ exposure. Pro-inflammatory cytokines drive apoptosis and cell death, excitotoxicity, increases in BBB permeability and leukocyte infiltration, to contribute to neurodegeneration (see Figure 3 for schematic of microgli activation) [83]. Anti-inflammatory M2 microglial phenotypes can help to remove obstructive myelin debris, deliver trophic factors and recruit oligodendrocyte precursor cells to lesion sites in order to promote remyelination [17,84]. However, such microglia activation states operate on a continuum, expressing different degrees of pro- versus anti-inflammatory and immunoregulatory action [85]. For example, when microglia incubated with TGF-β, IFN-γ or IL-10 were added to organotypic hippocampal slice cultures, there was a significant decrease in neuronal death implicating these cytokines in the induction of a protective microglia phenotype [67,86]. Similarly, IL-4 as well as low concentration IFN-γ-activated microglia primarily promoted oligodendrogenesis and neurogenesis from adult stem cells, respectively. The roles of these different phenotypes can be even more complex for neurodegeneration in AD, as reviewed very recently [87].

The DNA-binding protein HMGB1 is actively released from injured or dying cells and inflammatory cells, binds to the microglial receptor for advanced glycation end products, and thus promotes neurodegeneration via NO and prostaglandin E2 release [88]. In a mouse model of AIS, the HMGB1 ligand was elevated in ischemic brain tissue, in addition to being elevated in the serum of stroke patients, thus evidencing the role of this ligand in the pathophysiology of stroke [5]. TLR2 and TLR4 on microglia sense damage in ischemic stroke and exacerbate the infarct via the production of pro-inflammatory cytokines. TLR2 activation mainly results in the production of IL-6 and IL-1β release from microglia. TLR4 detects the presence of amyloid beta (Aβ; peptides significantly involved in Alzheimer’s disease plaques and aging), as well as HSP60 from dying cells in the CNS leading to the production of cytokines and reactive oxygen species that worsen injury [89].

Diffuse TBI injury leads to the presence of activated microglia and elevated cytokine and chemokine levels chronically, for at least 7 days. To induce the inflammatory response from activated microglia, intracellular signaling pathways are activated in response to injury signals. For example, a recent study examined the role of p38α MAPK, the inflammatory cytokine arm of MAPK signaling, in a model of diffuse TBI. In microglia, p38α MAPK is important in the production of IL-6 and TNF-α and in the phagocytosis of Aβ and axonal debris. In the study, TBI-injured p38α- deficient mice were protected from TBI-related motor deficits and synaptic protein loss, but exhibited less activated microglia morphology following injury, and had an increase in the early peak of cytokine and chemokine levels that decreased by day 7 post-TBI. The mixed actions of p38α in chronic microglia activation and the control of cytokine levels attest to the dynamic role of microglia in the immune response following injury [90].

Interestingly in TBI, a distinct rod-like microglial morphology is found throughout the cortex aligning and proliferating in a unique ‘train formation’ in response to injury. Rod microglia, microglia with narrow cell bodies and few polar processes, previously associated with infection, have recently been described in the sterile inflammatory condition of TBI. In a model of diffuse TBI, rod microglia appeared at 1 day post-injury and remained detectable for up to 28 days post-injury. These microglia become significantly elongated on day 1 post-injury and return to sham-injured lengths by day 2. The primary and secondary processes of rod microglia also retracted over the course of...
7 days post-injury [91]. These dynamic morphological changes in response to injury probably reflect the active involvement of this microglia type in brain injury. In another study examining microvascular pathology and delayed white matter damage in TBI, activated microglia accumulated within the site of injury and migrated over time into neighboring and distal white matter outside of the lesion site. These activated microglia were present as late as 3 months post-injury and found near focal microbleeds despite the fact that the initial injury was localized [92]. In TBI patients, activated microglia and neuroinflammation potentially associated with axonal degeneration and tissue atrophy has been observed in the corpus callosum up to 18 years post-injury. This chronic neuroinflammation may contribute to the higher incidence of Alzheimer’s disease and dementia in TBI survivors [93].

It has been suggested that with aging, microglia are ‘primed’ by previous infections and pathology to elicit a more intense inflammatory response to inflammatory stimuli encountered later in life [77]. Increases in microglial activation have generally been observed in the aging brain and manifest as abnormal, often extreme responses to activating stimuli, thus contributing to neuropathology in the elderly. Some studies have observed increases in the expression of CR3 on microglia, but this is a variable result, perhaps due to region-specific increases in CR3 expression [94]. Increased synaptic loss in neurodegenerative diseases of the aged could be related to an increase in the activation of microglia via CR3 and to the potential interaction of microglial CR3 with C1q complement to eliminate synapses [31].

### Key cytokines in AIS, TBI & neurodegeneration

Cytokines are small cell signaling proteins encompassing a wide range of family members including the tumor necrosis factor (TNF-), lymphokines, interleukins (IL-) and interferons (IFN-). In AIS, IL-1β, IL-6, IL-10 and TNF-α are secreted in ischemic brain tissue within the first 24-h. Additional cytokines known to be involved in AIS are IFN-γ, IL-2, IL-4, IL-16, IL-17, granulocyte–macrophage colony stimulating factor, TGF-β (also considered to be a growth factor) and recently, IL-9 [2,6,35,48]. In TBI, IL-1β, IL-6, IL-10 and TNF-α are among the first cytokines upregulated. Other cytokines involved in the pathophysiology of TBI include IL-8, granulocyte colony stimulating factor, FAS ligand and MCP-1 [1–2,99]. Many of these cytokines influence the production of other cytokines, alter the BBB and recruit inflammatory cells [67]. Furthermore, cytokine concentrations have been associated with behavioral changes following brain injury, such as post-stroke depression and apathy, as well as post-traumatic stress disorder and anxiety [1,8,96–99]. For example, cytokines can affect neurotransmitter metabolism of monoamines, serotonin, dopamine and glutamate [100].

#### Interleukins

Interleukins, IL-1β and IL-18 play a prominent role in the pathology of TBI and AIS. Both IL-1β and IL-18 are activated by the protease caspase-1, activated by the formation of inflammasomes via a host of PRRs, including NLRP1, NLRP3, AIM-2 and RIG-1. The NLRP3 inflammasome is most often associated with ‘sterile’ or DAMP-associated inflammation, as occurs in brain injury [101]. However, the inflammasomes NLRP1, AIM-2 and NLRC4 have also been implicated in brain injury pathology. A recent study elucidated important contributions of the inflammasomes NLRC4 and AIM-2, as well as the inflammasome component ASC, to ischemic brain injury through the use of knockout mice missing various inflammasome components. NLRC4−/− mice, AIM-2−/− mice and ASC−/− mice showed functional improvement and decreased microglia activation, leukocyte recruitment and infarct volume post-middle cerebral artery occlusion [101]. These observations highlight additional players in sterile injury and the resultant inflammasome-activated interleukins and caspase.

IL-1β and IL-18 also initiate transcription through NF-κB via their respective receptors on microglia, astrocytes, neurons and other cells. IL-18 helps regulate IFN-γ signaling in T cells and natural killer cells; an IL-18 inhibitor administered 1 h after brain injury improved subsequent recovery [24,102]. IL-1β helps to activate microglia and astrocytes, while also promoting the production of cytokines like IL-6 and TNF-α [1,103]. IL-1β has also been shown to inhibit long-term potentiation in the hippocampus, evidencing its ability to interfere with synaptic activity [104].

In AIS, IL-1β is released by activated microglia as early as 30 mins after injury. By contrast,
mRNA and protein expression studies show a delayed rise and peak in IL-18 post-stroke, at 24–48 h and 6 days respectively [105]. Activated IL-1β stimulates the release of VEGF from locally reactive astrocytes and the release of MMP-9 from NG2-OPCs during the acute phase of injury. IL-1β-specific monoclonal antibodies and IL-1R antagonists (IL-1Ra) have shown protection against brain injury in ischemic stroke. Specifically, IL-1β concentration in relation to the concentration of its endogenous inhibitor, IL-1Ra, has been hypothesized to be an important factor in the degree of neuroinflammation. IL-1β levels positively correlate with increased severity in TBI, AIS and neurodegeneration, and IL-1Ra has exhibited significant brain protective effects [61,106–107]. For example, a small Phase II, double-blind, randomized controlled study indicated that IL-1Ra can cross the BBB to achieve CSF concentrations in humans previously shown to be neuroprotective in rats, and reduce inflammation following hemorrhagic stroke [108]. Phase II clinical studies using IL-1Ra in severe TBI also proved safety and BBB penetration, while characterizing treated versus control patient cytokine responses [109,110].

The interleukin IL-6 helps to regulate the inflammatory response and has been associated with both neuroprotection and neuroinflammation. IL-6 regulates transcription via the JAK/STAT pathway and is a known agonist of VEGF, which alters tight junction proteins to promote disruptions in the BBB and NO production [6,61,111]. The JAK/STAT pathway is integral to NMDA receptor-triggered long-term depression and may contribute to synapse elimination, while another cytokine-activated pathway, the PI3K/Akt pathway may contribute to synapse survival via NMDA receptor-triggered long-term potentiation [54,112]. The balance between such pathways may contribute to synapse survival and improved outcome in TBI and AIS.

Injection of IL-6 in a model of ischemic stroke resulted in a reduction in ischemic damage. Clinical studies have demonstrated that lower IL-10 levels are associated with an increased risk of stroke [35,55]. IL-10 administration reduces ischemic stroke injury and suppresses the expression of TNF-α and IFN-γ [23,113–114]. IL-33, an interleukin cleaved into its active form by inflammasome-induced caspase-1, also confers protection following AIS by supporting a beneficial Th2 response and decreasing detrimental Th1 and Th17 upregulation; when IL-33 was administered to mice, pre-AIS, the neurological score, infarct volume, edema levels, as well as the balance between detrimental and beneficial T-helper cells improved at 24 and 72 h post-stroke, but not at 6 h post-stroke [115,116]. IL-33 levels assayed in patients within 4.5 h post-stroke were extremely low, while the endogenous IL-33 receptor inhibitor ST2 was high and could be used as an outcome predictor, correlating with worse outcome at 3 months; there was a trend toward better recovery at 24 and 48 h in patients with lower ST2 levels [117].

IL-2, IL-4, IL-17, IFN-γ and TGF-βs have also distinguished themselves in the pathophysiology of AIS. IL-2 activates immunomodulatory regulatory T cells that may be potentially beneficial in AIS [60–61,118]. IL-4 also activates astrocytes to produce BDNF, decreases the levels of TNF-α produced by microglia, and increases TGF-β2, while promoting an anti-inflammatory microglial phenotype that may be reliant on TGF-β signaling (inhibition of TGF-β receptor I also reduced IL-4-induced microglia activation, while addition of TGF-β1 upregulated IL-4 receptor α on microglia) [69,119]. Excessive microglia activation was decreased via the TGF-β1:microglial TGF-β receptor I interaction in an in vitro model of PD, while another in vitro model demonstrated the ability of TGF-β1 to reduce IFN-γ-induced neurodegeneration and IFN-γ signaling/microglia promotion [120,121].

In a study by Stoll et al., TGF-β1 rapidly rose post-ischemia (within 16 h and was sustained for at least 7 days) along with phagocytic microglia, in contrast to TGF-β2, which did not increase until day 14 and 28 post-injury and appeared in active astrocytes along the infarct perimeter post-microglia and macrophage invasion; when TGF-β2 was applied to an assay for Wallerian degeneration in the CNS, the ability of local microglia to phagocytose myelin was reduced [122]. As with all cytokines involved in neurodegenerative disease pathology, time course is an important factor in assessing whether a molecule is contributing positively or negatively to recovery from injury and should be considered when determining appropriate inflammation-modifying treatments and their dosing. The time course of TGF-βs’ expression contribute
to the timing of microglia activation states as evidenced by these observations and may be important when determining when and how to employ treatment strategies that modulate TGF-βs, such as minocycline, a drug now in Phase I clinical trials for severe TBI [123].

IL-9, granulocyte colony stimulating factor and granulocyte–macrophage colony stimulating factor may also potentially benefit AIS pathology via anti-apoptotic mechanisms [65,124–125]. In contrast, IL-17 is largely present expressed more IL-17 and advanced age. However, the decrease in calpain activation and treatments blocking IL-17 in an animal model of AIS resulted in a decrease in infarct size and neurological improvements, in addition to a decrease in calpain activation [62,126]. Clinical evaluation of γδ T cells in stroke showed a large decrease (~50%) in the number of circulating γδ T cells; decreases were also associated with hypertension (~65%), coronary artery disease and advanced age. However, the γδ T cells present expressed more IL-17 [127].

TNF-α

TNF-α is produced by microglia, astrocytes, endothelial cells and neurons. It binds to TNFR1, a TNF receptor expressed on all cell types that mediates cell death via activation of caspase-8, caspase-10 and pro-apoptotic proteins through the formation of complex II. Complex II formation is the TNF receptor-associated death domain adaptor protein ‘TRADD’ complexed with TNFR1 and Fas-associated death domain protein. In contrast, TNF-α can also mediate cell survival via NF-κB-mediated transcription of anti-apoptotic proteins initiated by complex I formation (i.e., TRADD associated with TNFR1, TNF receptor-associated proteins 2/5, cellular inhibitor of apoptosis proteins 1/2, ubiquitin-conjugating enzyme 13 and receptor-interacting protein); TNF-α also binds to the less abundant TNFR2 which induces antiapoptotic signaling pathways via NF-κB-mediated transcription [128].

TNF-α has been implicated in the regulation of microglial activation, as well as glutamatergic glial and synaptic transmission [129]. Even peripherally produced TNF-α can affect inflammation in the CNS; a recent study showed that peripheral TNF-α induced the microglial production of MCP-1/CCL2 to recruit monocytes into the CNS [130]. Peripheral TNF-α is also known to induce leukocyte rolling and adhesion in cerebral vasculature via E- and P-selectins [131]. When administered by intracerebroventricular injection in an experimental model of AIS, TNF-α exacerbated the infarct in a dose-related manner that was reversed by administration of TNF-α monoclonal antibody. Administration of soluble TNFR1 alone, applied to the brain to sequester released TNF-α, also reduced AIS injury [4,6,132]. AIS-induced TNF-α in endothelial cells and leukocytes upregulates MMP-9, thus exacerbating BBB permeability, allowing leukocytes and serum proteins like proteases, immunoglobulins and thrombin to enter the CNS and contribute to extracellular matrix destruction and brain injury [133–137]. Normalization of TNF-α levels by treatment with an analog of thalidomide in a rat model of chronic neuroinflammation resulted in decreased TLR-mediated signaling associated with microglia activation and improved cognitive function as indicated by improved performance in the Morris water maze task and the novel object recognition task [4,138–139]. Experiments using a TNF-α decoy receptor resulted in reduced infarct volumes and neural deficits post-ischemia, evidencing the destructive role of TNF-α. Furthermore, improvements in post-stroke cognitive dysfunction and TBI have been reported from the recent off-label clinical use of etanercept, an anti-TNF-α monoclonal antibody, when administered perispinally [26,140].

The loss of neurons in AIS and TBI results in a loss of inhibitory signals that help to control the production of pro-inflammatory cytokines and neurotoxins [26]. Numerous cytokines are produced and can be detected clinically in cerebrospinal fluid and serum; clinical studies still seek to determine definitive cytokine and molecular biomarkers for injury severity [48]. A number of studies are now examining how single nucleotide polymorphisms (SNPs) in cytokines and other markers can affect the duration and severity of the neuroinflammatory response in addition to informing diagnoses and prognoses in TBI and AIS [141–144].

Associations between clinical outcomes as assessed by the Glasgow outcome scale 6 months
following TBI and cytokine gene polymorphisms found that TNF-α -308 SNP allele 2 carriers had a worse outcome at 6 months post-injury than noncarriers. This SNP is found in the promoter region of the gene and increases TNF-α transcription [144]. The investigation of cytokine actions, as well as variations in cytokine genes and concentrations, show the importance of these immunomodulatory molecules in TBI and AIS. Further research on cytokines, genetic polymorphisms influencing inflammation, and mechanisms of neuroinflammation will assist in our understanding of neurodegenerative disease, its pathological sequel, and individualized approaches to treatment.

- Timing & location as factors in post-injury recovery

Key factors in facilitating recovery post-injury are the spatiotemporal nature and relative contributions of inflammatory cells and molecules [145]. These factors should also be considered in identifying effective treatments. For example, a study on the role of TNF-α in TBI using TNF-α−/− mice showed better motor function within the first 48 h post-injury than wild-type mice, but demonstrated larger amounts of cortical tissue loss and comparatively worse motor function at 2 and 4 weeks post-injury as compared with wild-type mice [146]. These results can inform how TNF-α inhibitors could be used post-injury.

The close evaluation of timing and location of inflammatory responses gives insight into the development of cell- or location-specific targeted treatments, in addition to the timing and dosing of drugs. Clinical evaluation of high-dose corticosteroids for the treatment of TBI in the CRASH study, proved that total inhibition of neuroinflammation could be harmful [147]. Drugs attempting to target inflammation must carefully consider timing, dosing and duration of drug administration, as well as the injury-dependent heterogeneity of the inflammatory response. These factors could have contributed to the failure of drugs attempting to target inflammation, such as the PROTECT III and SyNAPSe clinical trials assessing progesterone for TBI treatment and the Enlimomab Acute Stroke Trial.

Conclusion

This review has discussed the immune system in the periphery versus the CNS, and has examined the role of complement, inflammasomes and immune cells in AIS, TBI and neurodegenerative diseases. We hope that by providing a focus on the roles/actions of immune mediators and cells, this review helps to provide a framework to identify opportunities for reducing brain injury and neurodegeneration in the future. Some examples of approaches for use in the clinic have been included above and follow (see the ‘Future perspective’ section).

Future perspective

It will be important to address neuroinflammatory cascades in the development of future therapeutics for brain protection, as well as in the determination of effective biomarkers of injury severity and post-injury dysfunction for early clinical monitoring. In order to elucidate the pathology of TBI and AIS, as well as other neurodegenerative diseases, the role of immune cells and mediators (both positive and negative) in the CNS and periphery must be considered. Clearly there are issues with early versus delayed interference with neuroinflammation. Furthermore, due to the complex role of inflammatory mediators like cytokines and chemokines in the evolution of brain injury, it is necessary to characterize the time course, concentrations, multiple receptor effects and interactions of such substances. Generally, blocking early inflammatory changes can protect the brain, but later interventions might interfere with reparative processes that require inflammatory mediators. Post-injury changes in circulating immune cells might also provide additional, novel opportunities for intervention. More research needs to be done to understand this.

The role of the complement cascade and its degree of activation in individual neurodegenerative diseases warrants more detailed investigation. Research should also examine targeting infiltrating macrophages and endogenous microglia to promote reduced inflammatory/increased anti-inflammatory phenotypes. Patient data attempting to identify and correlate inflammatory biomarkers to brain injury and neurodegeneration should be based upon preclinical studies and interpreted alongside clinical data to maximize translation of results. Thus, inflammatory cells and their contents marking phenotypic changes could serve as prognostic/diagnostic markers in CNS disease. Additionally, the potential use of new...
or existing clinically used monoclonal antibodies and biologics could be further explored to modulate cytokine levels in brain injury and neurodegenerative diseases, as inhibition of specific pro-inflammatory cytokines may improve outcomes. Again, the timing of such interventions will be critical to their efficacious use. It will be important to identify combination therapeutic targeted approaches that provide improved outcomes. In order to better develop questions and discover meaningful answers to the challenge of brain ischemia, trauma and neurodegeneration, we must continue to integrate and translate findings from diverse fields of medical research, while examining diseases individually for appropriate new intervention opportunities.

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EXECUTIVE SUMMARY

The innate immune system in the periphery versus the CNS

- Innate immunity involves the detection of pathogen-associated molecular patterns and damage-associated molecular patterns by soluble and effector cell bound pattern recognition receptors in both the periphery and the CNS.
- The blood–brain barrier (BBB), the blood–cerebrospinal fluid barrier and the meningeal lymphatic system regulate the degree to which the peripheral immune system and the CNS interact.

The complement system

- The complement system has three main activation pathways (the classical, alternative and lectin pathways) that culminate in the formation of a membrane attack complex to lyse pathogens and abnormal cells. This system is implicated in development and neurodegenerative disease since it tags synapses with complement factors for elimination (termed ‘synaptic pruning’ in development).
- Following brain injury, complement factors are upregulated by both neurons and neuroglia. Knockout mice have elucidated the contribution of complement to brain injury and neurodegenerative diseases; C3−/− mice had smaller ischemic stroke infarcts and better neurological function after stroke; C5−/− mice also exhibited protection from brain injury. Complement downregulatory proteins and inhibitors protect the brain from injury, while increases in C1q and C3 levels correlate with increased injury.

Key cytokines & immune players in brain injury & neurodegeneration

- Tissue injury and BBB disruption in neurodegenerative disease disrupts brain homeostasis, causing astrogliosis (excessive proliferation/activation of astrocytes), activation of microglia and an influx of immune cells, all of which produce both pro- and anti-inflammatory substances. The balance and timing of pro- versus anti-inflammatory substances and actions influence the severity of injury.
- Microglia have a spectrum of activation states in response to pathogen-associated molecular patterns and damage-associated molecular patterns detected by their pattern recognition receptors. Microglial activation phenotypes can range from helping to remove debris from tissue injury, producing trophic factors and promoting axonal remyelination, to producing pro-inflammatory cytokines that encourage apoptosis, increased BBB permeability and detrimental neuroinflammation. These phenotypes are induced by microvascular injury, leukocyte infiltration and exposure to various cytokines and signaling molecules.
- Major inflammatory cytokines involved in traumatic brain injury, acute ischemic stroke and neurodegeneration include interleukins IL-1β and IL-18 and TNF-α. IL-1β and IL-18 are released upon activation via inflammasome formation (a multi-molecular complex that releases pro-inflammatory cytokines that promotes cell death via apoptosis and pyroptosis) and are linked to increased injury severity, BBB permeability and decreased synaptic activity. TNF-α primarily promotes cell death via pro-apoptotic caspase activation upon binding to its receptor, TNFR1. Other key cytokines that can play an anti-inflammatory role in traumatic brain injury, acute ischemic stroke and neurodegeneration include IL-33, IL-6, IL-10 and IL-4.
An exploration of ‘protective autoimmunity’ as a tool to attenuate acute and chronic inflammatory conditions; studies observing the peripheral autoimmune response against CNS antigens as a mediator of neuroprotection and the adaptive immune system’s targeted role in normal brain function and plasticity are discussed.


**Reviews recent work examining complement’s role in synaptogenesis, neural plasticity, microglia recruitment, synaptic tagging and neurogenesis in the healthy and injured CNS.**


Ischemic, traumatic & neurodegenerative brain inflammatory changes

Review


• Explores the pathophysiology of stroke with a focus on the contribution of the adaptive and innate immune system.


• Describes the pathophysiology and role of the innate and adaptive immune system in the early versus progressive stages of multiple sclerosis.


**A discussion of neuroinflammation in Alzheimer’s disease; the role of ‘CNS-resident immune cells’, especially microglia in the pathology of Alzheimer’s disease and neuroinflammatory targets for therapeutic intervention, many of which are produced by or interact with microglia, are reviewed**


**Explains traumatic brain injury pathophysiology, the role of microglia and the inflammatory state that may persist years postinjury. The protective versus harmful functions of microglia and biomarkers based on microglia cells are explored.**


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tau protein, ischemic injury and vascular dementia

Elizabeta B Mukaetova-Ladinska*,1, Mosi Li2 & Raj N Kalaria1

Clinical, neuroimaging and neuropathological studies have confirmed overlap between Alzheimer’s disease (AD) and vascular dementia (VaD). Classical neuropathological changes of AD (plaques and tangles) can be present in VaD. We review neuroimaging, biochemical and animal studies to consider the role of tau protein in ischemic injury and VaD pathogenesis. The evidence comes largely from transgenic animal studies that confirm that tau transgenes influence cerebral vasculature. Clinicobiochemical studies in the cerebrospinal fluid (CSF) have, similarly, confirmed alterations in both total and phosphorylated tau protein in VaD. These data suggest that tau protein not only serves as a potential diagnostic tool for differential diagnosis of VaD from other types of dementia, but may also be a therapeutic target in ischemic stroke.

Both Alzheimer’s disease (AD) and vascular dementia (VaD) contribute to the increased burden of dementia prevalence worldwide. Thus, the prevalence rates of VaD double every 5.3 years, ranging from 1.5% (70–75y) to 15% in >80y olds [1], and are very similar to those reported in older AD subjects [2]. More detailed epidemiological studies on dementia and its subtypes have confirmed regional differences in the prevalence estimates of VaD and AD from three continents, with the highest proportion of VaD in older Asian population, accounting for 38% of all dementia cases [3].

In contrast to AD, which has been extensively investigated at clinical and molecular levels, there is a paucity in advances in respect to treatments available for subjects with VaD. This is accompanied by enormous medical care costs VaD patients require worldwide. Remarkably, the longer life expectancy in developing countries, including China, has resulted in VaD, together with cerebral vascular diseases, to become leading challenges to their aging population. The epidemiological research on dementia in four major cities of China has provided informative results, indicating that China may have the largest population of people with VaD in the world [4], amounting to 14 million cases. The northern regions of China, in particular, are estimated to have higher prevalence of VaD (1.11 vs 0.97%) [5], which can be attributed to higher incidence of stroke, different lifestyle and diet in these northern areas.

Prevaling studies on AD have enriched our knowledge about the molecular substrates of dementia in general. The characteristic neuropathological changes in AD are the presence of amyloid plaques and neurofibrillary tangles with a predilection of the latter in the medial temporal lobe [6]. Amyloid (Aβ) deposition also occurs in the temporal lobe in VaD subjects [7] with greater age [8]. Some VaD subjects also bear neurofibrillary pathology, for example, tangles and neuritic plaques [9] over and above that in normally aging individuals without any overt neurological or psychiatric disease. Thus, the presence of some Aβ and neurofibrillary pathology in older VaD subjects indicates that both

KEYWORDS:
- Alzheimer’s disease
- cerebrospinal fluid biomarkers
- cerebral ischemia
- tau protein
- vascular dementia
AD and VaD share a certain level of vascular and neurodegenerative pathological features [8].

In correlative clinico-neuropathological and biochemical studies, the characteristic hallmarks of AD correlate with the clinical course of dementia [10]. Pathological advances have led to the development of novel diagnostic tools to visualize brain amyloid in vivo (e.g., PiB [31], florbetapir F18 [12]) and treatments to regulate the cholinergic deficits (e.g., cholinesterase inhibitors) and glutamatergic (NMDA antagonists) changes in AD subjects [13].

**What is the fate of tau protein in VaD?**

tau protein is a microtubule-associated protein (MAP) that stabilizes microtubules and is the major constituent of the paired helical filaments that give rise to neurofibrillary pathology in AD [14,15]. Tau protein regulates the microtubule (MT) assembly and provides the dynamic stability of tubulin to assemble into MT and regulates the MT spatial organization under physiological condition [13]. Although most of the drug trails today focus on the Aβ peptide in AD, studies on tau protein and its neurobiological function in neurodegenerative disorders provide evidence for development of novel disease-modifying dementia treatments based on prevention of the tau truncation/aggregation, modifying tau hyperphosphorylation and tau-clearance [16].

It is plausible that novel emerging therapeutics find a place in the treatment of other types of dementia, including VaD. In support of this are the clinical trials that have used the currently available AD antidementia drugs in VaD subjects. For example, memantine [17] and acetylcholinesterase inhibitors (donepezil [18]; galantamine [19] and rivastigmine [20]) have been documented to improve both cognitive and behavioral symptoms in VaD [18]. A most recent study on an animal (rat) model of VaD similarly confirmed the beneficial effect donepezil has on the cognitive function, with the neurobiological substrate of this being the enhancement of immature, new-born neurons [21], although acetylcholinesterase inhibitor effects upon angiogenesis cannot be excluded [22]. These studies provide further support for altered cholinergic system in VaD. In addition, acetylcholine is a powerful dilatator of most vascular beds, and any changes in its expression and/or altered muscarinic receptors but not nicotinic receptors [23] result in loss of the ability to dilate specifically cerebral arteries and arterioles [24]. Thus, impaired cholinergic dilatation of blood vessels may play a role not only in the pathophysiology of AD, but also in focal cerebral ischemia. However, the etiological drivers for the altered cholinergic neurotransmission in VaD are poorly understood. We cannot exclude the possibility that they may well be similar to those described in AD, including dendritic, synaptic, and axonal degeneration, neurofibrillary pathology and neuronal cell loss [25]. This also suggests that tau protein may be similarly altered in VaD and also underlie both cognitive and behavioral symptoms in this disease.

**tau protein in dementia**
tau protein is a natively microtubule-associated protein that regulates the assembly and stabilization of microtubules under physiological conditions [26]. Within neurons, tau protein is concentrated in axons in a highly soluble fashion [27]. It plays a role in axonal transport of neuronal organelles and even has a dendritic function in postsynaptic targeting of NMDA receptor substrate [28,29]. There are six distinct isoforms of tau protein identified in the human brain, and they are generated through alternative mRNA splicing from a single microtubule-associated protein tau (MAPT) gene [30,31]. The interaction between tau protein and tubulin is regulated by the repeated tau protein domains encoded by exons 9–12 [32]. A variety of neurodegenerative disorders, including AD, frontotemporal dementia, parkinsonism linked to chromosome 17, corticobasal degeneration and progressive supranuclear palsy (PSP), have characteristic tau pathology, and they are referred to as tauopathies.

In AD, tau protein is posttranslationally modified (e.g., hyperphosphorylated) and forms intraneuronal aggregates [33]. Hyperphosphorylation of tau protein is the most common posttranslational modification occurring in tauopathies [33] and it results in detaching tau protein from microtubules, and destabilizing them, giving rise to reduction of axons, dendrites and synapses [34], degeneration of plasma membrane and subsequently apoptosis [35]. The tau protein hyperphosphorylation alone [36] or resulting in tau truncation [37] leads to the formation of insoluble and filamentous intraneuronal structures, known as paired helical filaments (PHF) [38] (Figure 1). A number of evidence suggests that fibrillar inclusions have a key role in clinical symptoms and pathology in tauopathies. More
Figure 1. Sketch of a neuron, amyloid plaques, neurofibrillary tangles and aggregated paired helical filaments (PHF).

importantly, frontal-temporal dementia and Parkinsonism linked to chromosome 17 mutations within the MAPT gene (FTDP–17) provide further support that tau dysfunction in the absence of amyloid pathology is sufficient to trigger clinical dementia, neurofibrillary pathology, neuronal loss [40] and collagen deposits within the blood vessel walls [41].

Linking vascular pathology to neuronal cytoskeletal changes

A number of clinical, neuroimaging and neuropathological studies has confirmed overlap between clinical risk factors and pathological changes in VaD and AD. Vascular changes are involved in the pathogenesis of both AD and VaD, with nearly half of all older subjects having pronounced vascular pathology in their brain tissue [42]; similarly, large vessel abnormalities, such as carotid artery stenosis and bilateral carotid plaques are common in AD [43]. Furthermore, about 30% of AD cases have some degree of vascular pathology, and a similar proportion of VaD cases have AD pathology, with perivascular Aβ depositions being present in both VaD and AD [43,44]. Meanwhile, there is an increasing evidence supporting the role of Aβ peptide and apolipoproteins in VaD [8,45], and their links to tau protein. Thus, Aβ protein toxicity appears to be tau dependent [29], though the mechanism of this link still remains unclear. Interestingly, a most recent neuroepidemiological study, conducted on a total number of 6025 subjects with a single neurodegenerative diseases, linked coincident cerebrovascular disease with lower neurofibrillary pathology (e.g., Braak stages I-IV), with subjects with sole tau pathology (e.g., frontotemporal lobar dementia) exhibiting substantially lower rate of vascular pathology (<60%) than that seen in AD [46]. These findings bear resemblance to an earlier correlative clinicopathological study that reported dementia during life to be linked to medial temporal lobe neurofibrillary pathology in those with a clinical diagnosis of AD, whereas vascular pathologies, especially microinfarcts, were more common in those with clinical diagnoses of vascular dementia [47]. These findings raise an interesting possibility that vascular pathology may halt the progression of neurofibrillary degeneration in the aging brain. However, the mechanisms behind this remain unknown. One possibility is that vascular pathology influences directly the expression of tau protein in the human brain [48] and thus the pathophysiological mechanisms underlying the development of neurofibrillary pathology, that of phosphorylation [49] and truncation [50] of the tau protein.

Imaging studies have reported that Aβ burden in VaD is related to white matter ischemia [51], the latter corresponding to axonal changes as demonstrated in postmortem studies [52]. The widespread axonal damage in VaD likely alters tau protein function or physical status. Furthermore, these changes not only appear to be confined to axons with microtubule structures
but also involve glial cells. It has been previously shown that after focal cerebral ischemia, there is an increase in tau expression in glial cells within the neuropil and in oligodendrocytes [53]. This suggests that tau protein undergoes dephosphorylation and degradation in axons and perikarya as a result of cerebral ischemic injury [53]. Moreover, pathologically modified tau protein exists in microglial cells around the cerebral ischemic focus [54].

The above findings indicate that tau protein alterations may be an early pathological change in response to ischemic mechanisms in certain neurons [55]. Furthermore, the dephosphorylation of tau protein may cause damage to the organelle transport between axons and neurons, and subsequently influence its susceptibility to proteolysis [56]. Nonetheless, following a brain ischemia, accumulation of hyperphosphorylated tau protein occurs in cortical neurons and accompanies apoptosis [57]. Furthermore, ischemic damage alone is associated with neurofibrillary tangle-like tauopathy [58]. Similarly, blood–brain barrier (BBB) dysfunction results in brain parenchymal accumulation of blood (plasma) derived neurotoxic and vasculotoxic molecules (e.g., fibrinogen, thrombin, hemsiderin, plasmin), cerebral hypoxia and reduction in cerebral blood flow. All these vascular-derived insults can precede, initiate and/or contribute to neuronal degeneration, including altered tau processing [59–62]. Transgenic mice expressing human tau besides the well documented neurofibrillary degeneration also show mild BBB disruption [63]. Interestingly, most recent research supports that tau alone can both initiate BBB breakdown and reverse it [64]. The stabilization of the BBB disruption in this transgenic animal model was achieved via depletion of tau protein (Figure 2). All these studies provide a neuropathological explanation for the role of tau protein in dementia after ischemic event.

Cerebrospinal fluid tau protein in VaD

Another piece of evidence for tau protein involvement in VaD comes from cerebrospinal fluid (CSF) biomarker studies in dementia [65] (Table 1; [66–84]). CSF-tau protein is considered as a peripheral biomarker of neurodegenerative changes [85] and dementia progression in several types of dementia [86]. A number of studies have shown a consistent increase in CSF total (t-) and phosphorylated (p-) tau protein levels in subjects with AD (Table 1). In most reports, in VaD, CSF tau protein levels (both t- and p-tau) were somewhat lower than those in AD subjects (Table 1), thus suggestive of these differences to have a potential to discriminate VaD from AD, respectively, of the clinical stage of the disease [69]. However, several studies also demonstrated conflicting evidences [87]. Thus, the use of CSF tau protein in discrimination between AD and VaD remains still controversial in clinical practice.

- t-tau in VaD CSF

Both studies on subjects with VaD, subcortical vascular disease and stroke indicate an increase in CSF t-tau levels, which are significantly higher than those in age-matched control subjects or those with mental health problems (e.g., depression) but substantially lower than in AD (Table 1; [51]). Similarly, CSF samples obtained after stroke contain increased t-tau levels, with a peak after 1 week following the acute stroke, and normalization of the t-tau levels after 3–5 months [88]. Furthermore, the level of t-tau protein appears to reflect the size of the stroke, but not the clinical measures of disability [89].

A recent meta-analysis of CSF tau measures in the Chinese population included 16 studies on VaD, which consisted of heterogeneous vascular dementia syndromes, in the context of ischemic and hemorrhagic strokes, cerebral hypoxic-ischemic events, and senile leukoencephalopathic lesions. Consistently higher CSF t-tau was reported in VaD subjects compared with controls, but still lower in relation to AD [65]. This analysis of CSF did not include phosphorylated tau. All these findings argue that the increase in CSF t-tau in VaD subjects may reflect a degree of neuronal damage following ischemia [88,89], that appears to be normalized in the follow-up period [89], suggesting that the transient upregulation of t-tau is an immediate response to an acute axonal injury. This would be consistent with the previously attributed increase in t-tau in AD as a result of axonal damage [90]. However, in some instances, normal CSF levels of t-tau have also been reported in VaD (Table 1; [39]), and they correspond to white matter changes, occurring in the absence of recent stroke and concomitant AD pathology [70].

- p-tau in VaD CSF

Phosphorylated tau (p-tau) has similarly been investigated in VaD, and attributed to the underlying AD pathology [91]. Similarly to t-tau, the increase in CSF p-tau in VaD does not exceed
Figure 2. Brain ischemic damage and tau protein and Aβ. NFTs: Neurofibrillary tangles; p-tau: Hyperphosphorylated tau.

that found in AD (Table 1). Interestingly, the apoptosis of neurons due to ischemia gives rise to hyperphosphorylated intraneuronal tau protein, which does not appear to be elevated immediately after the stroke, in contrast to CSF t-tau [91]. This finding argues that changes in t-tau happen quickly following a sudden damage to the neuronal and axonal integrity, whereas p-tau elevation legs behind.

Previous studies found increased CSF p-tau to be more characteristic for subjects with cognitive disturbances attributed to AD than other forms of dementia, including VaD, Dementia with Lewy Body, Parkinson’s Disease Dementia or cognitively intact population [68,78–80]. However, elevated CSF p-tau (phosphorylated at Thr181; p-tau181) is also present in VaD (Table 1; [78]). One large-scale, multicenter study reported elevated CSF p-tau199 to discriminate AD from other forms of dementia, including VaD [92]. Moreover, p-tau181 has been most consistently reported to be similarly increased in both pure VaD and AD [69]. In the latter study, most of the VaD cases had also chronic leukoencephalopathy and multiple infarcts rather than single cortical infarct. Although CSF p-tau181 failed to discriminate between different types of dementia, it, however, predicted a more rapid cognitive decline, irrespective of the clinical dementia diagnosis [78]. The reported high CSF p-tau level, irrespective of the phosphorylation site, in both VaD and AD might be explained by the similar neurodegenerative and vascular pathological features in VaD and AD, which also
Table 1. Summary of most relevant cerebrospinal fluid tau studies in vascular dementia.

<table>
<thead>
<tr>
<th>Study</th>
<th>tau-measures</th>
<th>Technique</th>
<th>No. of subjects</th>
<th>No. of controls</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VaD vs AD</strong></td>
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<tr>
<td>Arai et al. 1998 [66]</td>
<td>√</td>
<td>–</td>
<td>ELISA</td>
<td>29 CVD (21 demented and 8 non-demented), 69 AD, 21 VaD</td>
<td>CSF t-tau was significantly decreased in VaD than AD (p &lt; 0.0001). However, CSF t-tau in VaD was not significantly different from nondemented CVD and control subjects.</td>
</tr>
<tr>
<td>de Jong et al. 2006 [67]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>61 AD, 25 VaD,</td>
<td>t-tau was significantly increased in AD compared to VaD and control group (p &lt; 0.001). However, VaD and control participants had similar levels of t-tau. p-tau 181 was significantly increased in AD compared to VaD and control participants (p &lt; 0.001). No significant differences between VaD and control participants were reported.</td>
</tr>
<tr>
<td>Schonknecht et al. 2003 [68]</td>
<td>√</td>
<td>–</td>
<td>ELISA</td>
<td>88 AD, 23 VaD, 25 major depression</td>
<td>t-tau was significantly increased in VaD compared to controls and subjects with major depression, but significantly lower than in AD (p &lt; 0.005)</td>
</tr>
<tr>
<td>Stefani et al. 2005 [69]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>20 VaD, 66 AD (31 AD+WMC; 35 AD)</td>
<td>Increased t-tau in AD and VaD compared to controls (p &lt; 0.001). However, VaD subjects had similar level t-tau with AD (p = 0.5). Increase in p-tau was similarly noted in both VaD and AD subjects (p &lt; 0.005). VaD subjects had lower levels of p-tau than their AD counterparts (p &lt; 0.05)</td>
</tr>
<tr>
<td>Wallin and Sjögren, 2001 [70]</td>
<td>√</td>
<td>–</td>
<td>ELISA</td>
<td>25 subjects with SWD</td>
<td>Although CSF t-tau was increased in the SWD cases, this increase was not statistically significant. However, the CSF tau measures had specificity of 85% and 36% sensitivity for SWD.</td>
</tr>
<tr>
<td>Hjalmarsson et al. 2014 [71]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>20 patients studied after AIS onset</td>
<td>Patients with AIS had significantly higher levels of neurofilament (NFL), t-tau, myelin basic protein (MBP) and inflammatory markers such as YKL–40, and glial fibrillary acidic protein (GFAP) compared with controls. t-tau, MBP, GFAP, and YKL–40 correlated with clinical stroke severity, whereas NFL correlated with severity of white matter lesions.</td>
</tr>
<tr>
<td>Roseels et al. 2014 [72]</td>
<td>√</td>
<td>–</td>
<td>ELISA</td>
<td>20 AD and 20 VaD</td>
<td>Novel anti-tau protein antibody that recognizes tau protein oligomers shows similar distribution of tau protein as that of t-tau; the tau protein oligomers in the CSF are significantly increased in VaD subjects compared to that of controls (p &lt; 0.05), and slightly, but not significantly, lower than those of AD subjects.</td>
</tr>
<tr>
<td>Liquori et al. 2015 [73]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>145 AD (mild 67 and moderate–severe 78) and 44 VaD</td>
<td>Significant increases of CSF lactate concentration were observed in AD group compared to control and VaD, which had a negative correlation with the CSF t-tau and p-tau levels in corresponding groups.</td>
</tr>
<tr>
<td>Hermann et al. 2014 [74]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>27 AD; 59 CSVD with cognitive impairment 33 with CSVD and intact cognition</td>
<td>After stratification of cognitive impaired patients (n = 59) two groups were identified: 32 patients with normal beta-amyloid 42/40 ratio (&gt;0.975) who had impaired blood–brain barrier function (e.g., elevated albumin ratio), representing pure AD and AD+CSVD, and the other group (VaD) consisting of subjects with normal beta-amyloid ratio. The CSVD subjects, irrespectively of their cognitive functioning, had similar levels of both t-tau and p-tau Thr181, and substantially lower than that observed for participants with pure AD and AD+CSVD.</td>
</tr>
</tbody>
</table>

p-tau alone is used when studies did not report the site of tau protein phosphorylation.
AD: Alzheimer’s disease; AIS: Acute ischemic stroke; ALS: Amyotrophic lateral sclerosis; CJD: Creutzfeldt-Jakob disease; CSVD: Cerebral small vessel disease; CVD: Cerebrovascular disease; DLB: Dementia with Lewy bodies; FTLD: Frontotemporal lobar degeneration; HCONT: Healthy controls; NCONT: Neurological controls; SWD: Subcortical white matter dementia; VaD: Vascular dementia; WMC: White matter changes.
Table 1. Summary of most relevant cerebrospinal fluid tau studies in vascular dementia (cont.).

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<th>No. of controls</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kester et al. 2014 [75]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>547 AD; 30 VaD</td>
<td>Presence of microbleeds was linked to lower CSF Aβ42 (p = 0.003) in both AD and VaD subjects, whereas in control subjects there were higher levels of CSF t-tau (p = 0.03). White matter hyperintensity was associated with lower Aβ42 in control and VaD participants (p = 0.002), whereas lacunes with higher Aβ42 in VaD (p = 0.07) and lower t-tau in AD participants (p = 0.05). None of the vascular pathology influenced the CSF levels of p-tau 181. These effects were mostly attributable to APOE ε4 allele.</td>
</tr>
<tr>
<td>Krishnan and Rani, 2014 [76]</td>
<td>√</td>
<td>-</td>
<td>ELISA</td>
<td>30 AD; 35 VaD</td>
<td>Plasma Aβ42 level were significantly elevated in both AD and VaD subjects compared to controls (p &lt; 0.001). In contrast, CSF t-tau and tau-to-amyloid ratio were significantly lower in both AD and VaD subjects compared to controls (p &lt; 0.001), with the VaD participants having significantly higher values in both measurements compared to the AD subjects. The receiver operating characteristic (ROC) curve-derived cut-off values of &lt;3.5 for tau-to-Aβ ratio and &lt;520 pg/ml for t-tau had 67–72% sensitivity and specificity for differentiating AD from VaD and 90% for AD from controls.</td>
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<tr>
<td>VaD vs mixed and cerebrovascular dementias</td>
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<tr>
<td>Paraskevas et al. 2009 [77]</td>
<td>√</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>92 AD, 23 VaD, 17 Mixed</td>
<td>CSF t-tau was slightly, but significantly (p &lt; 0.05) increased in VaD compared to control; t-tau levels were highest in mixed group and followed by AD (p &lt; 0.0001), p-tau 181 was significantly decreased in VaD compared to control, mixed and AD groups (MIXed&gt;AD&gt;control&gt;VaD; p = 0.0021)</td>
</tr>
<tr>
<td>Ravaglia et al. 2008 [78]</td>
<td>–</td>
<td>p-tau 181</td>
<td>ELISA</td>
<td>51 AD, 13 pure VaD, 6 possible VaD</td>
<td>CSF p-tau discriminated patients with dementia from control group (p = 0.0001, 100% specificity). However, p-tau was not useful in differentiating distinct forms of dementia.</td>
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<tr>
<td>VaD vs other forms of dementia</td>
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<tr>
<td>Blennow et al. 1995 [79]</td>
<td>√</td>
<td>p-tau</td>
<td>ELISA</td>
<td>17 VaD, 44 AD, 11 FTLD, 15 PD, 10 Depression</td>
<td>CSF t-tau was significantly higher in AD (p &lt; 0.0001), VaD (p &lt; 0.0001) and FTLD (p &lt; 0.05) compared to control subjects. However, AD and VaD subjects had similar levels of CSF t-tau. CSF p-tau was significantly higher in AD (p &lt; 0.0001) VaD (p &lt; 0.01) and FTLD (p &lt; 0.01), than in controls. CSF p-tau in AD was significantly higher than VaD (p &lt; 0.005), FTLD (p &lt; 0.05), PD (p &lt; 0.001) and subjects with depression (p &lt; 0.0001). Moreover, in MIX group CSF t-tau was significantly increased as compared with NCONT (p &lt; 0.005) and HCONT (p &lt; 0.0001).</td>
</tr>
<tr>
<td>Andreasen et al. 1998 [40]</td>
<td>√</td>
<td>–</td>
<td>ELISA</td>
<td>21 VaD, 43 AD, 11 MIX(AD/VaD), 18 NCONT, 18 HCONT, 9 ALS</td>
<td>CSF t-tau was significantly increased in AD subjects compared to ALS (p &lt; 0.002), NCONT (p &lt; 0.0001) and HCONT (p &lt; 0.0001), whereas there was no significant difference between AD and VaD. CSF t-tau was also significantly higher in VaD than NCONT (p &lt; 0.005) and HCONT (p &lt; 0.0001). Moreover, in MIX group CSF t-tau was significantly increased as compared with NCONT (p &lt; 0.005) and HCONT (p &lt; 0.0001).</td>
</tr>
<tr>
<td>Herbert et al. 2013 [81]</td>
<td>√</td>
<td>p-tau</td>
<td>ELISA</td>
<td>14 DLB, 64 AD, 15 VaD, 26 FTLD</td>
<td>CSF t-tau levels in AD were significantly increased compared to DLB and VaD (p &lt; 0.01)CSF p-tau levels were also significantly increased in AD compared to other groups (p &lt; 0.001), but no significant difference between VaD, DLB or FTD groups.</td>
</tr>
</tbody>
</table>

p-tau alone is used when studies did not report the site of tau protein phosphorylation.

AD: Alzheimer’s disease; AIS: Acute ischemic stroke; ALS: Amyotrophic lateral sclerosis; CJD: Creutzfeldt-Jakob disease; CSVD: Cerebral small vessel disease; CVD: Cerebrovascular disease; DLB: Dementia with Lewy bodies; FTLD: Frontotemporal lobar degeneration; HCONT: Healthy controls; NCONT: Neurological controls; SWD: Subcortical white matter dementia; VaD: Vascular dementia; WMC: White matter changes.
share a number of genetic and pathophysiological similarities [93].

- Does CSF tau differentiate VaD from other dementia subtypes?
The potential value of CSF tau in differentiating VaD from AD and other dementia subtypes still remains controversial. The heterogeneity of VaD needs to be taken into account when interpreting the CSF findings. Although elevated CSF tau in VaD group may reflect a direct acute cerebral ischemic injury, this needs to be interpreted cautiously, taking into account the temporal relationship between the onset of ischemic event(s) and the CSF examination. Thus, CSF t-tau levels in acute cerebral infarction are transiently upregulated after 2–3 weeks following an ischemic event, and are normalized few months later [66]. Another study showed that the elevation of t-tau in the CSF happens within the first 1–2 days of an acute ischemic stroke and similarly falls back to baseline levels after 3–5 months of the stroke [89]. Although the role of p-tau in differentiating VaD from AD is disputable, a combination strategy measuring CSF biomarkers including tau (both t- and p-tau), Aβ1–42 and inflammatory cytokines (e.g., IL–6 and TNF) may improve the sensitivity and specificity of these biomarkers to diagnose and differentiate VaD from other types of dementia [94], predict extent of cognitive deterioration [66], and even be used to monitor treatment outcomes.

Table 1. Summary of most relevant cerebrospinal fluid tau studies in vascular dementia (cont.).

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<tbody>
<tr>
<td>Schoonenboom et al. 2012 [82]</td>
<td>√ p-tau 181</td>
<td>ELISA</td>
<td>52 DLB, 512 AD, 34 VaD, 16 CBD, 144 FTLD, 20 PSP, 6 CJD</td>
<td>135, 275 SMC</td>
<td>In VaD, CSF t-tau levels were similar to those seen in control subjects (p&gt;0.05), but substantially lower than those in AD (p&lt;0.05). In contrast the VaD subjects had the; lowest CSF p-tau levels, significantly lower than control and AD subjects (p &lt; 0.05)</td>
</tr>
<tr>
<td>Herbert et al. 2014 [83]</td>
<td>√ p-tau 181</td>
<td>ELISA</td>
<td>14 DLB; 64 AD; 15 VaD; 26 FTLD</td>
<td>-</td>
<td>All groups had similar levels of CSF t-tau and p-tau Thr181. The combination of Aβ42, t-tau, and p-tau yielded a sensitivity of 61.9% and a specificity of 91.7% for the discrimination between DLB and AD, but could not discriminate between DLB and VaD or FTLD. The addition of 3-methoxy-4-hydrophenylethyleneglycol to the Aβ42, t-tau, and p-tau improves the discrimination of DLB from AD (65.1% sensitivity and 100% specificity), but did not distinguish DLB or VaD from other forms of dementia</td>
</tr>
<tr>
<td>Amadoro et al. 2014 [84]</td>
<td>√ p-tau 181</td>
<td>ELISA western blot</td>
<td>26 AD, 6 MIX, 9 VaD, and 22 other forms of dementia, (e.g., PDD, PSP, FTLD, MSA, CBD, ALS, CJD etc.)</td>
<td>14</td>
<td>t-tau and p-tau (Thr181) and N-terminal end tau measured. No specific reference for VaD or mixed dementia. However, t-tau and p-tau CSF measures in the other dementia forms were with intermediate values between control and AD subjects. Subjects with AD and other forms of dementia had similar elevated levels of amino-terminal end of tau protein in comparison to their control counterparts (p &lt; 0.0001)</td>
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p-tau alone is used when studies did not report the site of tau protein phosphorylation.

AD: Alzheimer’s disease; AIS: Acute ischemic stroke; ALS: Amyotrophic lateral sclerosis; CJD: Creutzfeldt-Jakob disease; CSVD: Cerebral small vessel disease; CVD: Cerebrovascular disease; DLB: Dementia with Lewy bodies; FTLD: Frontotemporal lobar degeneration; HCONT: Healthy controls; NCONT: Neurological controls; SWD: Subcortical white matter dementia; VaD: Vascular dementia; WMC: White matter changes.
Hyperglycemia & neurovascular changes

Changes to the vascular endothelium are consistently found in the presence of hyperglycemia [98], a characteristic feature of one of the major risk factors for dementia, diabetes. However, the neurophysiological mechanisms by which diabetes influences cognitive functioning are poorly understood. Rodents with streptozotocin-induced Type 1 diabetes exhibit various extent of cognitive deficits and this is accompanied by exacerbation of Aβ deposits, especially in a highly vascularized area of the hippocampus, where amyloid deposits are found surrounding but not within the blood vessels [99]. These Type 1 diabetic mice also have an increase in hyperphosphorylated tau at Ser396, whereas their total tau protein remains unaltered [99]. These findings confirm that diabetes can induce endogeneous amyloid and tau pathology that is also accompanied by changes in the cerebral vasculature. Furthermore, they provide further support between the link between diabetes, tau pathogenesis and amyloid deposits, although they fail to address the specific link in relation to the development of brain vascular pathology in diabetes-induced VaD.

Transgenic tau animal models & VaD disease mechanisms

Transgenic (Tg) animal models similarly can aid the understanding of VaD disease mechanisms and provide a further research platform to investigate the overlap of VaD with other forms of dementia, including AD. One such study indicates that overexpression of tau protein (TgP301L tau mice) may reduce the volume of ischemic infarcts and reduce the neuronal apoptosis [100]. Furthermore, this study also reported that different tau phosphorylation sites may be altered differently in ischemia/hypoxia, and they can appear as early as 24 h posttrauma. Thus, phosphorylation at Ser199/Ser202 and the phosphorylated independent tau sequence 210–230 of human tau protein remain unaltered, whereas Thr231 phosphorylation is significantly decreased in the wild strain postischemia [100]. This study was done on young animals that did not harbor any neurofibrillary pathology at the time of the ischemic insult and were sacrificed 24 h post the insult. It, thus, remains undetermined how these biochemical and neuropathological changes would have progressed long-term postinsult, and influence the neurofibrillary pathology progression poststroke.

Another way of addressing the tau protein in relation to vascularly mediated mechanisms in VaD is via the use of adeno-associated virus (AVV) vector-mediated expression of mutant P301L tau protein (AVV-tauP301L), injected in the left hippocampus. This novel model for tau-mediated hippocampal neurodegeneration results in early apical dendrites damage in CA1 pyramidal hippocampal neurons, and is accompanied by loss of dendritic spines, damage to the brain–blood barrier integrity, constriction of brain capillaries that are surrounded by swollen astrocytes with extensions contacting degenerated dendrites and axons [101]. These data provide further evidence to link tau neurodegeneration with inflammation and vascular changes in the brain tissue as a result of the initial neuronal injury due to tau protein overexpression.

Triple transgenic mouse model & vasculature changes

The triple transgenic mouse model of Alzheimer’s disease [3xTg-AD; harboring PS1 (M146V), APP (Swedish mutation; double mutation at codons 670 and 671 of APP 770 transcript in exon 16), and tau(P301L) transgenes] which is characterized by both Aβ and tau protein rapid brain accumulation, can be, particularly, useful to address the interphase between the neurodegenerative changes characteristic of both aging and AD and those of VaD. This 3xTg AD model has reduced cerebrovascular volume (27%; initially evident in the hippocampus and later on spreading to other brain areas, including cortex [102,103]. The reduced cerebrovascular volume precedes the onset of AD pathology by at least 6 months, and is not
Figure 3. tau protein and synaptophysin in control, VaD and AD subjects. (Top) Total tau protein was measured with mAb 7/51, that recognizes a microtubule binding region of the tau protein molecule. (Middle) Phosphorylated tau protein measured with pAb Ser262 antibody that recognizes phosphorylation site at Ser262 of the tau protein molecule. (Bottom) Synaptophysin was measured with mAb EP10 (a gift from Professor W Honer, University of British Columbia, Canada). All values on y-axes represent percentage Relative Values (RV%). *p < 0.05. **p < 0.01. ***p < 0.0001.

AD: Alzheimer’s disease; C: Control group; VaD: Vascular dementia.

Figure based on data from [45].
associated with reduction in microvessel density [103]. Since the microvessel lumen is reduced as a result of the thickening of the blood vessel wall [102], this 3xTg-AD animal model may also serve as a model of cerebral hypoperfusion. Interestingly, the reduction in cerebrovascular volume is not characteristic for the APP/PS1 transgene [103,104], thus suggesting an interesting role for tau protein in neurovascular pathology in AD. It is of interest to speculate that tau protein may similarly play such a role in non-tauopathies, such as VaD. In support of the latter is a study by Head et al. [105] who described a negative correlation between hyperphosphorylation of tau protein and decrease in cerebrovascular volume in Caveolin–1 knockout mice. This proved further support for the role of tau protein in cerebrovascular integrity and function.

- **Acute & chronic hypoperfusion in transgenic animal models of AD**

  Acute cerebral ischemic damage in 3xTg-AD model resulted in elevated total tau accompanied by enhanced phosphorylation of the amyloid precursor protein (APP) but had not effect on the Aβ level at 3–months posthypoperfusion [106]. Similarly, transient reduction of blood volume (oligemic hypoperfusion), produces acute, significant and long lasting impact on APP and tau protein in this animal AD model, increasing Aβ levels via enhancing β-secretase protein expression. In addition it also leads to significant changes in tau protein expression: a decrease in total tau levels and an increase specifically in phosphorylated tau protein at Ser212 and Thr214 [106], tau epitopes associated with paired helical filaments in AD. Vasoconstriction on its own, used to mimic small lacunar infarcts, similarly leads to an upregulation of tau protein and APP, even in very young animals (5 months old App23 mice [107]).

  Chronic cerebral hypoperfusion induced by obstructing right common carotid artery in Tg 2576 APPs(swe) AD mice produces aggravation of cognitive decline in this animal model of VaD, which is not related to neuronal and axonal loss, or amyloid deposits [108]. The metabolic deficit as documented on FDG-PET scans is suggestive of severe synaptic loss and MAP–2 depletion, as a result of accompanying dendritic loss. This *per sé* does not exclude the possibility that tau protein may also be affected, but this was not addressed in the study.

  In another model of chronic cerebral hypoperfusion exhibiting cerebral amyloid angiopathy (CAA) [Tg-SwDI mice, that express APP harboring tandem Dutch (E22Q) and Iowa (D23N) amyloid β mutations [109] bred onto a nitric oxide synthase 2 gene knockout background], the cerebral Aβ deposition occurs along cerebral capillaries and is accompanied by perivascular neuroinflammation, accumulation of phosphorylated tau protein and neuronal loss [110,111]. The accumulation of phosphorylated tau protein (Ser202/Thr205) appears to be restricted to perivascular cortical neurons and activated microglia in thalamus tightly associated with capillary amyloid deposits [111]. This pattern of phosphorylated tau localization is highly reminiscent of that seen in the human CAA Type 1 brain.

  Experimental studies, irrespectively whether conducted on disease models of AD, diabetes, or vascular diseases, link tau protein with the neurovascular changes. Furthermore, tau protein undergoes posttranslational changes, for example, hyperphosphorylation similar to that seen in AD as a result of ischemia. However, the extent of phosphorylation of tau protein varies. Nevertheless, it is undoubtful that drugs that decrease the extent of tau protein phosphorylation, as well as APP phosphorylation and protein kinase C activation [e.g., potassium 2-(1-hydroxypentyl)-benzoate] also have an impact on improving spatial learning and memory deficits in both transgenic APP and PS1 [112] as well as in chronic cerebral hypoperfused animal models [113]. We need now further studies that would provide firmer evidence for the exact role of tau protein in relation to altered inflammation, amyloid processing and vascular changes.

**Conclusions**

The role of tau protein in ischemic injury and VaD is poorly understood. Except for the clinical biochemical studies on tau protein measurements in the CSF, the evidence for altered tau protein metabolism at a molecular level is still lacking. The negligible amount of neurofibrillary pathology that occurs in VaD in most instances is similar to that found in age-matched control subjects [8], thus, arguing that tau protein may not be contributing to the dementia syndrome in VaD subjects. This is in contrast to the neuroradiological evidence of white matter ischemia in VaD [51] that corresponds to extensive axonal loss in postmortem studies [52]. Interestingly, the
latter findings correspond well to the biochemical evidence from CSF studies of consistently increased t-tau protein in subjects with clinical symptoms of dementia and various extent of vascular pathology. This provides further evidence for widespread axonal damage occurring in VaD [90]. Since tau protein is predominantly enriched in axons [13], it is not surprising that the focal white matter changes in VaD would also result in changes in tau protein, as it was recently confirmed in a CSF study on subcortical white matter changes [70].

Neurobiochemical and neuroimaging studies argue that in VaD there is a widespread axonal damage. The latter may be a result of the ischemic changes triggering posttranslational modification to tau [106], or the white matter rarefication and microinfarction [114]. In this, the neurofibrillary pathology appears not to play a major role, unless there is a concomitant AD pathology documented [80]. However, the obvious link between vascular pathology and altered tau protein function is still missing. Interestingly, novel rodent models in transgenic mice genetically engineered to express AD-related changes in tau protein, with coincident activation of macroautophagy and ubiquitin-proteosome pathways [106]. In both these studies, there was a specific increase in tau protein phosphorylated at Ser212 and Thr214, tau epitopes associated with paired helical filaments in AD. The observed pattern of decreased total tau, altered phosphorylated tau and increased Aβ persisted for several weeks after the transient period of cerebral hypoperfusion injury [106]. The findings from these studies display similarities with those in human subjects, where transient up-regulation of CSF t-tau occurs within days following an ischemic injury and is normalized in the following several months [66]. However, we have to bear in mind that although translational dementia research needs to be based on valid animal models to help alleviate some of the unmet needs of dementia sufferers (e.g., diagnosis, disease progression and treatment), none of the currently available models recapitulates all aspects of any of the known dementia syndromes, including VaD. Furthermore, the well-researched amyloid AD animal models proved not to be good in prediction of the drug efficacy in human subjects (reviewed in [115]), thus confirming that difficulties in extrapolating conclusions from animal to human conditions. More neurobiochemical studies on tau protein in VaD subjects are, therefore, needed to determine whether there is a role of this microtubule associated protein in generating cognitive impairment in VaD, and if so, to address the underlying neurobiological mechanisms leading to the altered tau protein processing. If indeed tau protein plays a role in vascular type dementia, this may trigger more vigorous development of much awaited novel therapeutics to combat VaD.

Future perspective
Recently, we reported selective loss of both total tau loss and phosphorylated tau at Ser262 in the temporal lobe in VaD subjects (Figure 3A & B; [48]). This loss occurred in the absence of overt synaptic loss (Figure 3C) and was associated neither with the extent of underlying neurofibrillary and amyloid pathology, nor the age at death. These findings argue that selective tau protein loss in the temporal lobe in VaD subjects may be a result of distal neuropathological changes, for example, deep micro- and macroinfarcts leading to white-matter tracts damage that have been described to be characteristic for the temporal lobe in VaD [116]. However, we cannot exclude the possibility that the temporal lobe per se may be more susceptible to tau protein oligomerization, and more profound inhibition of the anterograde fast axonal transport [117], thus resulting in impaired movement of synaptic vesicles, axonemal precursors and mitochondria, all known to be altered in VaD [118]. It is interesting to note that in an animal model, multiple infarcts were shown to lead to a delayed neuronal death confined predominantly to the temporal lobe [119], suggesting that the vascular changes may result in an ongoing neuropathological process, and the extent of the tau protein loss may be associated also with the time-lapse between the cerebrovascular incident and the lethal outcome.

Similar loss of tau protein has been described in mutant knock-out MAPT gene mice. In this rodent tau model, the tau protein loss resulted in reduced brain volumes in the absence of overt histological changes to brain structure and organization, including cortical or hippocampal lamination [120]. This may correspond to the reduced temporal lobe and hippocampal formation volumes in VaD or post-stroke dementia subjects [121,122]. In addition, there is a genetic link between the MAPT gene and VaD [123] that has not been investigated fully. Thus, MAPT...
Single nucleotide polymorphism rs1467967 has been linked to VaD, with the G allele in particular, doubling the risk for developing VaD [124]. Interestingly, the G allele is also associated with lower CSF t-tau levels [124]. Since the G allele is common in both Caucasian and Chinese populations (57% and 84%, respectively) [123,124] it is intriguing to speculate that it may well be over-represented in VaD and thus linked to the down-regulation of temporal lobe tau protein in VaD. Further studies are now needed to explore the effect of vascular pathology upon the tau protein metabolism in both aging and dementia, and address in depth the mechanisms underlying the temporal lobe t-tau protein loss in VaD.

Moreover, the reviewed neurofibrillary tangle-like tauopathy after an ischemic cerebral damage has provided a potential neuropathological basis for the progression of dementia in postischemic brains [58]. Meanwhile, tau protein also demonstrates the potential as a therapeutic target in ischemic stroke [125] and a diagnostic tool to differentiate distinct types of dementia [126]. However, more explorations are still needed to determine how specific vascular pathological changes influence tau protein metabolism in both aging and dementia and vice versa, and address in depth the mechanisms underlying the increase in CSF tau, the neuroradiological evidence of axonal changes and the neurobiochemical loss and role of posttranslational modifications (e.g., hyperphosphorylation) of tau protein in VaD.

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EXECUTIVE SUMMARY

Vascular dementia
- Prevalence rates of vascular dementia double every 5 years, and range from 1.5% (70–75 years) to 15% in those over the age of 80 years.
- The highest proportion of VaD is in older Asian population, accounting for 38% of all dementia cases.
- There is a paucity of therapeutic advances available for subjects with VaD.

tau protein in ischemic injury & vascular dementia
- Animal studies indicate tau protein alterations may be an early pathological change in response to ischemic mechanisms in the cerebral neurons.
- CSF measures of total tau are consistently increased in VaD subjects, providing further evidence for the widespread axonal damage in VaD.

Conclusions
- tau protein role is poorly investigated in VaD.
- Further studies are needed to explore the effect of vascular pathology upon tau protein metabolism in both aging and dementia.
- The link between MAPT polymorphism rs1467967 and VaD needs further exploration.
- Since vascular incidents may halt the tau protein processing to neurofibrillary pathology, identifying the molecular mechanisms of tau protein changes in VaD has a potential for further development of novel anti-dementia treatments.
References

Papers of special note have been highlighted either as:
• of interest or •• of considerable interest


**Reviews prevalence and incidence data for dementia reported in the international literature over 10 years period.**


**Compares risk factors and neuropathology between Alzheimer’s disease (AD) and vascular dementia (VaD).**


•• Reports that brain Aβ accumulates increasingly with age in VaD subjects more so than in elderly without cerebrovascular disease.


**Provides evidence of a causal connection between stroke or CVD and AD.**


**Reviews emerging therapeutic strategies aimed at treating the underlying causes of tau pathology in tauopathies and AD.**


**Demonstrates that paired helical filaments (PHFs) consist of two structurally distinct parts: an external fuzzy region and a pronase-resistant core structure, both containing distinct tau protein epitopes.**


•• Describes isolation of tau protein fragment tightly bound to the PHF core.


•• Describes the microtubule binding domain of tau protein, containing three 18 amino acid repeated sequences, while the amino-terminal half of the protein does not bind.


•• Reports that tau protein is neuron specific and restricted to axons.


•• Reports the sequences of isoforms of human tau protein isoforms, with multiple tau protein isoforms being incorporated into neurofibrillary tangles in AD.


**Describes the microtubule binding domain of tau protein, containing three 18 amino acid repeated sequences, while the amino-terminal half of the protein does not bind.**
tau protein, ischemic injury & vascular dementia

**Review**


Reports changes in the CSF profile between AD, VaD and acute ischemia, with total tau CSF levels being useful in differentiating VaD from stroke subjects.


• A neuropathological study exploring relevance of cerebrovascular disease, vascular pathology and vascular risk factors in dementia – reports that when cerebrovascular disease present, patients with AD have relatively lower burdens of NFTs.


• Correlative clinico-neuropathological study reporting that vascular pathologies, especially microinfaracts, are more common in those with clinical diagnoses of VaD, but not other dementia subtypes.


• Reports that changes in microvascular or microstructural tissue integrity due to ischemic injury in older age may modify tau protein metabolism or phosphorylation, and thus have effects on the burden of neurofibrillary pathology.


• Provides the amino acid sequence and immunological data that A68, although indistinguishable from tau protein, is phosphorylated.


Identifies the minimal protease resistant tau unit of the core PHFs.


• tau protein is dephosphorylated and/or degraded in axons and some neuronal perikarya in response to focal cerebral ischaemia.


• Examines mechanisms of blood–brain barrier dysfunction as a result of vascular changes and highlights therapeutic opportunities relating to neurovascular deficits.

Bell RD, Winkler EA, Singh I et al. Apolipoprotein E controls cerebrovascular


Microsphere embolism in brain microvessels leads to strong Aβ accumulation in brain parenchyma, with hyperphosphorylated tau proteins increased in neurons surrounding regions of Aβ accumulation.


•• Transient global ischemic insult in aged 3xTg-AD mice does not increase the levels of Aβ, but results in increase in insoluble total tau protein 3-months post-injury.


