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Review Article

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The cerebrovasculature is a multicellular structure with varying rheological and permeability properties. The outer wall of the brain capillary endothelium is enclosed by pericytes and astrocyte end feet, anatomically assembled to guarantee barrier functions. We, here, focus on the pericyte modifications occurring in disease conditions, reviewing evidence supporting the interplay amongst pericytes, the endothelium, and glial cells in health and pathology. Deconstruction and reactivity of pericytes and glial cells around the capillary endothelium occur in response to traumatic brain injury, epilepsy, and neurodegenerative disorders, impacting vascular permeability and participating in neuroinflammation. As this represents a growing field of research, addressing the multicellular reorganization occurring at the outer wall of the blood-brain barrier (BBB) in response to an acute insult or a chronic disease could disclose novel disease mechanisms and therapeutic targets.

The multicellular assembly at the abluminal–cerebrovascular interface

Accumulating evidence advocates for a role of cerebrovascular dysfunction in central nervous system (CNS) diseases, negatively impacting neurovascular coupling and neurophysiology [1-8]. We here focus on capillary blood–brain barrier (BBB) and the abluminal compartmentwhere pericytes and astrocyte end feet coexist to maintain the barrier's properties [9]. We specify that the terms abluminal, perivascular, or outer wall will be generally used to identify cells: (i) residing around the capillary endothelium, (ii) anatomically adhering to the endothelial cells or the basal membrane, (iii) and existing within the perivascular cuff. At the capillary level, pericytes and astrocyte end-feet are structurally intertwined, enveloping the outer endothelial wall (Figure 1, *authors' images*). This unique anatomical assembly of cells is strategic, allowing the communication of pericytes with the endothelial cells and the parenchymal glia (e.g., astrocytes and microglia) during health and disease conditions (Figure 1A, B-B1). Pericytes outline 60–70% of the abluminal endothelial surface, with astrocyte end-feet completing the coverage and overlaying pericytes (Figure 1B2). Pericytes are identified based on their round soma and thin ramifications lining the endothelium (Figure 1A,C). An array of markers (e.g. platelet-derived growth factor receptor β, CD13, desmin etc.), transgene NG2 constructs (Figure 1), and the fluoro-Nissl dye [10] are used to recognize pericytes. The intimate endothelial–pericyte–astrocyte assembly is maintained by the basal lamina and regulated at the cellular level by a machinery of junction proteins, including connexins and N-cadherins (see [2,11-14] for details). Figure 1C-C1 (*authors' unpublished observations*) shows neighboring pericyte-microglial cells *in vivo* in the brain. The significance and the exact molecular cross-talk existing between pericytes and microglial cells in physiological and disease conditions remain to be elucidated.

Understanding the multicellular reactivity and plasticity occurring around the capillary endothelium during CNS diseases is important to develop BBB repair strategies (Figure 2A). It has been proposed

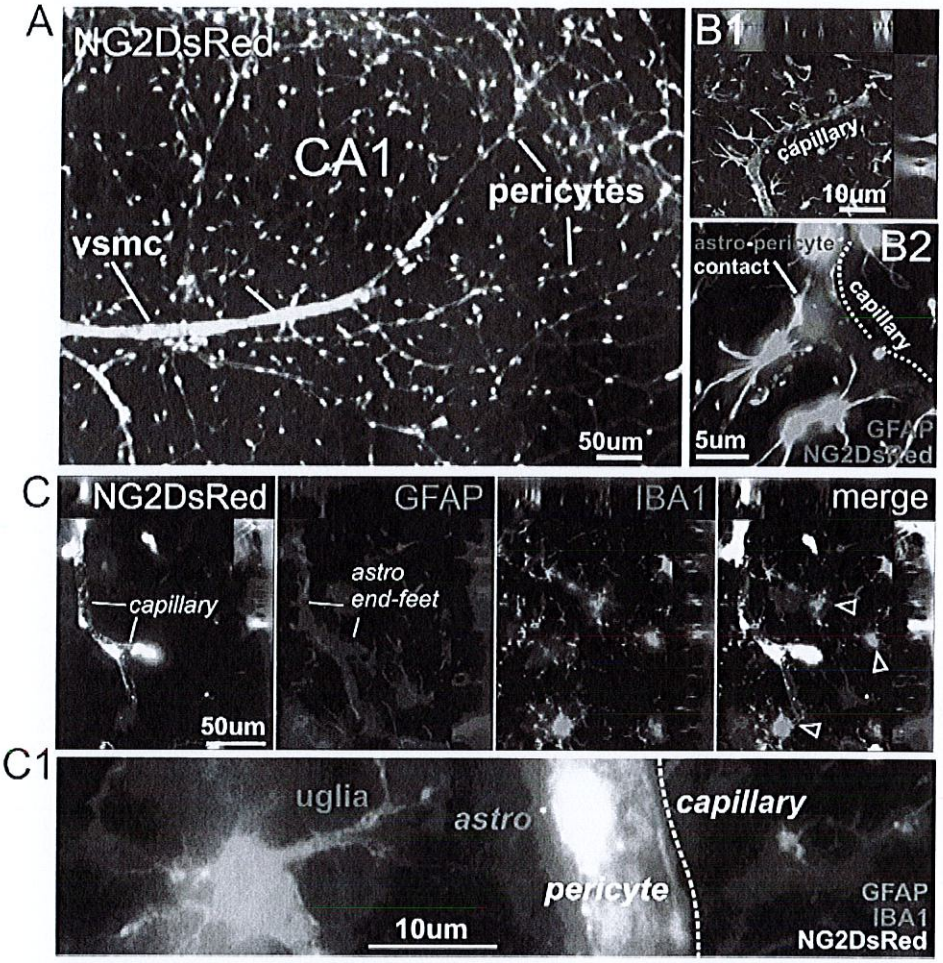

Figure 1. Pericyte–glia structures at the capillary BBB

(**A**) Pericytes are emerging players in cerebrovascular biology. Pericytes (NG2DsRed) envelope the abluminal side of arterioles and capillaries, while smooth muscle cells control the tone of larger vessels (e.g. arteries). (**B1**,**B2**) Astrocyte end-feet and pericytes are in direct contact, defining capillary properties and integrity. (**C**,**C1**) The pericyte–astrocyte–microglial complex. Three cell types are anatomically positioned within a 10–20 micrometers distance one from another and at the outer endothelial wall. Microglial cells are parenchymal (*arrowheads*) and proximal to the capillaries, possibly playing distinct roles during physiological and pathological conditions (*authors' previously unpublished images*).

that sealing a leaky BBB may decrease the accumulation of blood-derived products (Figure 2A) into the brain parenchyma, rectifying the neurovascular system toward a physiological interstitial homeostasis. The latter could improve the efficacy of neuronal drugs (Figure 2B) [4,6,15]. In general, capillary damage follows a sterile injury (e.g. status epileptics, non-penetrating trauma, and vascular accident), develops during disease progression or lingers during chronic pathological stages [16,17]. The inflammatory response accompanying CNS pathologies promotes an aberrant neuro-vascular remodeling [18,19], formation of scar tissue [20,21], and neuronal dysfunction [22]. In case of the pericyte–endothelium interface, the platelet-derived growth factor receptor signaling (PDGFRβ/PDGF-BB), transforming growth factor β (TGF-β), and the Notch pathway have been proposed as potential targets to modulate BBB stability and, perhaps, inflammation [2]. Early targeting of BBB damage and neuroinflammatory process may be disease modifying, whereas the importance of controlling inflammation may be different when chronic pathological stages are established (Figure 2B).

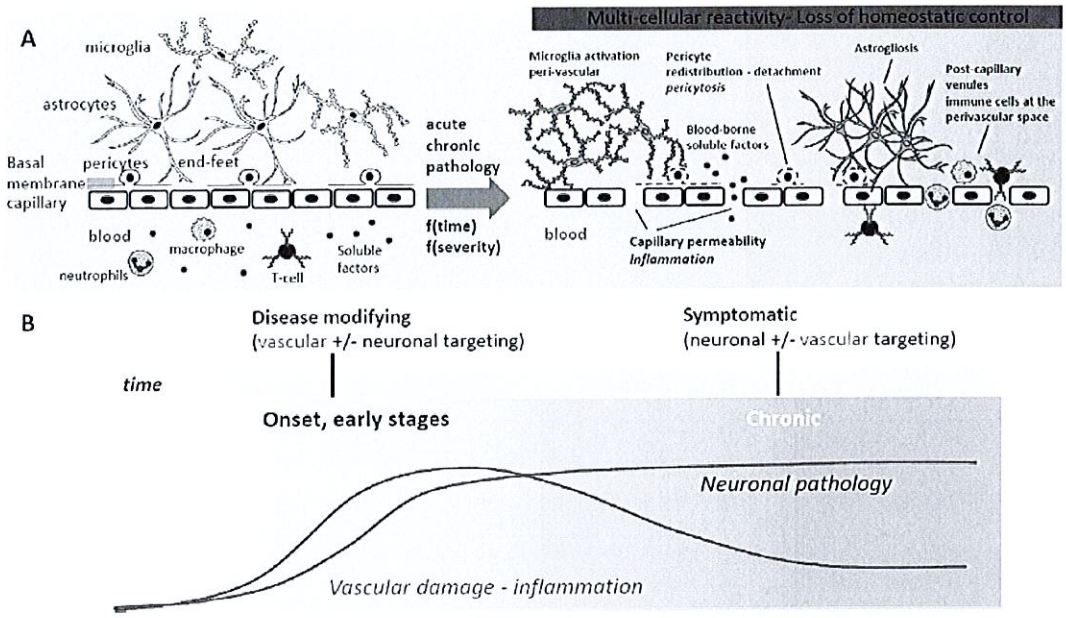

Figure 2. Multicellular deconstruction at the cerebrovascular interface, potential impact of neurovascular targeting, and modulation of neuroinflammation.

(**A**) Cell redistribution around the BBB endothelium during disease conditions. Under physiological conditions (left), astrocytes and pericytes contribute to capillary stability and interendothelial tightness. The stringent permeability at the BBB is crucial for the segregation between the peripheral circulation (blood) and the CNS. Following an acute event (traumatic brain injury, stroke, status epilepticus) or chronic disease (epilepsy, Alzheimer's disease), a multicellular disarray and reactivity occur (as a function of time and severity of the pathology) around the capillary endothelium and at the post-capillary venules. A number of immune molecules can activate pericytes that, in turn, can acquire an inflammatory phenotype (see Table 1). (**B**) Neurovascular targeting during disease progression. We hypothesize a vascular-inflammatory mechanism of disease during the early-mid stages of disease, promoting neuronal pathology. During chronic stages, the vascular inflammatory component may stabilize and neuronal dysfunction could become the main target. We hypothesize anti-inflammatory treatment (+/– accompanied with neuronal drugs) to be disease modifying at early-mid stages when inflammation and cerebrovascular damage unfold and develop.

Cell reactivity at the abluminal compartment: an overview

Pericytes display immune properties, responding to or contributing to inflammation *in vivo* [23-29] and *in vitro* (Table 1). Pericytes participate in both innate and adaptive immunity processes *in vivo* [23,24]. A significant number of *in vitro* studies point to the capacity of pericytes to act as immune cells. However, the ability of pericytes to maintain their properties *in vitro* remains uncertain. Thus, culture conditions (e.g. presence of serum proteins and growth factors) may have an impact on pericyte phenotype, introducing a bias and, perhaps, mimicking BBB damage and the access of serum components into the brain. *In vivo* studies are therefore relevant, especially if the aim is to compare the changes occurring amongst health and disease conditions. *In vivo* studies have shown pericyte modifications and reactivity in CNS pathologies associated with inflammatory changes, including ischemic stroke [25,26], Alzheimer's disease (AD) [15,27], status epilepticus [28], and traumatic brain injury [29]. Similar considerations apply to peripheral CNS pathologies such as spinal cord injury [30].

Pericytes are required for neutrophil trafficking across the endothelium *in vivo* [31]. Due to their position at the outer endothelial wall, pericytes can react to immune stimuli coming from brain resident glial cells or from circulating leukocytes accessing the perivascular space of post-capillary venules during disease conditions [32-35]. Amongst the immune challenges impacting pericytes we here report (see Table 1):

Pericytes and cytokines

(i) *TNFα*: upon TNFα stimulation pericytes release metalloproteinases (MMP-9), impacting BBB integrity [36-38].
(ii) *IL-1β*: human pericytes exposed to IL-1β overexpress adhesion molecules (intercellular adhesion molecule-1 (ICAM-1)), interleukin-8, monocyte chemoattractant protein-1 (MCP-1), and IL-1β itself [39,40]. TNF-α, IL-1β,

Table 1 Evidence supporting a role of pericytes in neurovascular inflammation

Stimuli	Pericyte response	References
LPS	CXCL10, CCL20, CXCL8, CXCL1, IL-6, CCL2, CXCL2, CXCL3, CCL3, CCL4	[23]
IL-1β	Neutrophils chemo-attraction, IL-1β, IL-8, attenuate COX-2 and SOD-2, ICAM-1, αSMA	[164,39,41]
TNF-α	Pericyte migration, microglial activation, neutrophils chemo-attraction, IL-1β, MMP-9	[42,164,165,37]
IFN-γ	PDGFRβ, HLA expression, MHC II, IP-10, CD68	[56,166,23]
TGF-β1	Anti-inflammatory phenotype, CX3CL1, down-regulation of CD36, CD47, CD68, NOX4, COX-2, MMP-2	[42,23,167]
LPS, TNF-α, IL-1β, IFNγ, and IL-6	iNOS	[168]
LPS, TNF-α, IL-1β	NFκB translocation	[23,169]
TNF-α, IL-1β/TGF-β1	IL-6	[42,43]
Ros production	Stellate morphology	[44]
O_2 deprivation	Reduction in CD13, αSMA, PDGFRβ shedding, and enhanced microglia markers (IBA1, MHC II, CD11b, CD68)	[170]

Abbreviations: aSMA: alpha smooth muscle actin; CCL: chemokine (C-C motif)ligand; COX-2, cyclooxygenase-2; CXCL: chemokine (C-X-C motif) ligand; HLA: human leukocyte antigen; ICAM-1: intercellular adhesion molecule-1; IBA: ionized calcium binding adaptor molecule 1; IFNγ; interferon γ; IL-6: interleukin 6; iNOS: inducible nitric oxide synthase; IP-10: interferon gamma-induced protein 10; LPS: lipopolysaccharide; MCH: major histocompatibility complex; MMP: metalloproteinase; NFkB: nuclear factor kappa; ROS: reactive oxygen species; SOD: superoxide dismutase; TNFa: tumor necrosis factor alpha;

and interferon γ (IFNγ) promote inducible nitric oxide synthase (iNOS) in porcine and rat pericytes [40-42]. (iii) *IFNγ* and*TGF-β1*: IFNγ induces human leukocyte antigen (HLA) expression, the latter blocked by TGF-β1 [43]. Human pericytes react to TGF-β1modifying the expression of a panel of immune-related genes, e.g. increasing NOX4, cyclooxygenase-2 (COX-2), IL-6, and MMP-2 or decreasing IL-8, CX3CL1, MCP1, and vascular adhesion molecule-1 (VCAM-1) expression. TGF-β1 impacts the expression of chemokines and adhesion molecules in pericytes, possibly influencing leukocyte trafficking [43].

Pericytes and immune cells

(i) Pericytes are involved in lymphocyte and neutrophil transmigration across the brain endothelium through IFNγ or TNFα stimulation. The latter factors up-regulate ICAM-1, VCAM-1, and MHC I [35,44]. Macrophage-like properties have been attributed to pericytes, e.g. pinocytosis and phagocytosis of latex beads [43]. A number of macrophage-like markers have been reported in pericytes during inflammation, including MHC-II, CD11b, CD36, CD68, or CD163 [23,40]. Human pericytes display phagocytic traits in response to LPS, TNF-α, or IL-1β stimulation, producing macrophage-like molecules such as MCP-1, IL-8, or metalloproteases [43]. *In vitro*, mouse pericytes display features of multipotent stem cells [45]. Finally, the cross-talk between T cells and pericytes has been suggested to favor the induction of allogeneic $CD25^{high}/FoxP3^{+}$ regulatory CD4 cells. [46].

Astrocyte–pericyte communication

Astrocyte end-feet and pericytes are anatomically connected [2,47,48] and their cross-talk occur during development and inflammation [49,50]. During angiogenesis, astrocytes and pericytes participate in the deposition of the basal lamina [51-53]. Astrocytes were hypothesized to induce the synthesis of fibronectin by pericytes [54]. Astrocytes and pericytes are involved in the developmental localization and polarization of ATP-binding cassette transporters on endothelial cells [48]. However, the pericyte–astrocyte interplay occurring in the healthy and pathological adult brain remains largely understudied.

Cerebrovascular cell modifications and disassembly during CNS pathology

Addressing the cellular changes occurring around the capillary endothelial wall is important to outline the mechanisms of cerebrovascular damage in disease conditions. We here provide evidence for endothelial, pericytes, and glial cell changes occurring in traumatic brain injury (TBI), epilepsy, Alzheimer's disease (AD), and ageing.

Acute and long-term cerebrovascular cell changes occurring post-TBI

TBI is not a transient event but rather a disease process [55] as TBI patients present long-lasting neurological consequences [56-60]. TBI pathophysiology includes [61]: (i) a primary injury resulting from a mechanical impact causing damage to the neurovascular structures; (ii) a secondary injury with formation of edema, decreased cerebral perfusion, increased glutamate levels, excitotoxicity, and BBB dysfunction that can last from days to years [5,61-68]. Intracerebral hemorrhage and microbleeding can increase intracranial pressure (ICP) and the likelihood of vasogenic edema, while the *heme* group entering the brain can promote oxidative stress and inflammation [69].

Perivascular cells undergo pathological changes and reactivity shortly post TBI. Pericytes migrate away from the endothelial wall after brain trauma [70]. The molecular mechanisms underscoring pericyte–endothelial pathology post TBI are, however, not completely understood. PDGFRβ has been proposed to be involved in tissue or cell remodeling after brain injury, including a biphasic pericyte loss and reactivity pattern [25,29]. Interestingly, treatment with a CO-releasing molecule (CORM)-3 was shown to reduce pericyte death after TBI with beneficial effects on neurological deficits [71]. Changes in cell-to-cell communication at the cerebrovasculature also involve astrocytes, with increased MMP secretion acting on the endothelial tight junctions. The expression of MMP post TBI [72,73]triggers the degradation of the extracellular matrix and weakens barrier structures [74-77].

Infiltration of blood-borne molecules, such as albumin, draws water into the brain parenchyma across the capillary wall, favoring post-TBI epilepsy or neurodegenerative processes [78-83]. However, a structural BBB opening is not always necessary for the permeability to be increased, as transcytosis mechanisms could take the relay [84-86]. For instance, increased Caveolin-1 (Cav-1) expression has been reported in animal models of brain injuries [81,84,87,88]. Caveolins not only contribute to transport functions but also influence the lipid raft structures and regulate the activity of the endothelial nitric oxide synthase (eNOS) [89,90]. Cav-1 stabilizes tight junctions [91] and regulates the activity of the drug-efflux p-glycoprotein (P-gP) [92], as demonstrated by the increase in Cav-1 and P-gP expression 1 week post TBI in rats [81].

TBI also results in long-lasting pathophysiological brain changes [93]. BBB permeability is increased in the majority of mild-TBI subjects that experience a seizure or develop epilepsy after the injury [94]. Multifocal and perivascular IgG staining was found in the gray matter of TBI subjects deceased between 1 and 47 years after the initial injury [66]. Long-term BBB changes were also found in animal models of TBI. IgG staining was visualized in the corpus callosum 3 months after the injury [95]. Increased expression of perlecan and fibronectin and decreased capillary diameter were reported [5,64]. Moreover, the levels of Cav-1 were increased in cortical vessels 2 months post injury [96,97]. Interestingly, increased amyloid depositions were found at the cerebral vesselssuggesting a failing vascular mechanism of interstitial waste clearance. Decreased P-gP expression was proposed as a contributing mechanism [64]. A better understanding of the cerebrovascular cell changes occurring long-term after TBI is important to improve disease management and pharmacology.

Epilepsy as a cerebrovascular dysfunction: an update

The evidence of cerebrovascular mechanisms of epilepsy is overwhelming and has been previously addressed [8,98-100]. A neurovascular approach to epilepsy has disclosed a number of entry points to decipher disease mechanisms. Neurovascular coupling is the key in the epileptic brain as ictal-to-interictal neuronal transitions are synchronized to metabolic changes contingent on oxygenation and regional blood flow. The latter depends on the cellular interplay occurring in the cerebrovascular compartment. Status epilepticus (SE) promotes pericytes and astrocytes activation at the endothelium, negatively impacting the barrier's properties and possibly reiterating the epileptic pathology [101]. NG2DsRed pericyte plasticity or ectopic coverage was reported after SE in an experimental model [28,102]. Recent evidence has shown that hypoperfusion and hypoxia occurring after focal seizures are responsible for cell damage, behavioral changes, or comorbidities [100]. From a mechanistic standpoint, inhibition of COX-2 and L-type calcium channels was sufficient to prevent postictal cerebrovascular changes. The latter findings are remarkable as cerebrovascular mechanisms of seizures may overlap, at least in part, with those reported in brain ischemia. The latter could allow a transdisciplinary use of diagnostic imaging to monitor cerebrovascular perfusion, oxygenation, and reactivity [8,98]. A number of reports have suggested the utility of radiological biomarkers of BBB damage as diagnostic or prognostic means in epilepsy [103,104]. The functional involvement of perivascular cells, in particular pericytes and smooth muscle cells, remains to be determined as they could control ictal perfusion changes.

Cerebrovascular cell changes and inflammation in AD

Amyloid plaques, extracellular aggregates of fibrillary amyloid-β (Aβ) protein, and neurofibrillary tangles are traits of AD [105-107]. Accumulating clinical and experimental evidence demonstrate a role of cerebrovascular dysfunction

and inflammation in AD, supporting the hypothesis of sporadic AD as a neurovascular disease [107-114]. Disease conditions bearing an important cerebrovascular component (e.g. diabetes mellitus, thrombotic episodes, high fibrinogen concentrations, or atherosclerosis) are risk factors for AD [115-117]. Interestingly, measuring cerebral blood flow has been proposed as a diagnostic tool to identify preclinical AD stages [114,118]. A recent study [119] indicates early cerebrovascular abnormalities in AD, advocating for a biomarker of disease progression.

Pericytes detachment and ectopic coverage of the outer cerebrovascular wall occur during AD progression [2,120]. Pericyte pathology impacts capillary integrity, potentially facilitating the accumulation of toxic molecules in the brain parenchyma and subsequent neurodegenerative changes [27,121-124]. Available evidence points to modifications in the pericyte–endothelial PDGFRβ/PDGF-BB signaling as a mechanism of vascular damage. *In vivo* studies have shown that an impaired PDGFRβ signaling is associated with pericyte damage and BBB dysfunction, facilitating neurodegenerative changes. Reduced Aβ clearance, resulting from BBB breakdown, was proposed as a contributing mechanism of AD progression. See [2,120] for details.

Microglial cells are involved in the inflammatory response occurring in the AD brain (hippocampus, Figure 3A-A1) [15,125,126]. Figure 3B-B1 shows an overview of the hippocampal microglial cell changes in human AD. Clusters of amoeboid microglia and Aβ are common (Figure 3C-C1). Our previously unpublished data also indicate accumulation of IgG around the capillaries (Figure 3D), suggesting BBB permeability. In experimental AD, microglial cells accumulate around the capillaries (*unpublished observations* in Figure 3E and [27]). Although unfrequent, instances of Aβ accumulation at the capillaries can be found (Figure 3E1). Interestingly, the role of microglial cells in AD remains controversial. Initially considered as a pathophysiological component associated with a negative outcome, recent evidence has instead proposed a more beneficial effect, possibly through amyloid plaque digestion [125,127-129]. Remarkably, a recent report showed no changes in microglia proliferation during AD progression in a cohort of human brains as compared with available control [130]. The latter findings are not immediately comparable with genetic models of AD where incremental glial inflammation is reported [27,33].

Microglial cells can induce astrocyte activation *in vivo* and *in vitro* [131]. The resulting astrocyte phenotype is dependent on the pathological insult. Briefly, astrocytes can be classified as A1 and A2, implicated respectively in neuronal damage and repair. Neurodegenerative disorders are associated with damaging A1 astrocytes [131]. The impact of a microglia-mediated immune response in AD development was recently highlighted in association with TREM2 variants, a surface receptor that modulates the microglia phenotype [132-134]. Genetic screening of a large cohort of patients linked rare variants of microglial genes, including TREM2, to AD [134]. Finally, a link between the ApoE4 isoform, an AD risk factor, and BBB integrity was demonstrated [135,136]. ApoE4 does not maintain low levels of cyclophilin-A (Cyc-A), a cytokine that induces BBB damage. Thus, under physiological conditions, astrocytes release ApoE isoform 3 inhibiting Cyc-A via LRP-1 receptors, while the presence of ApoE4 leads to the failure of this control mechanism [135,137,138]. Although controversial, the involvement of astrocytes in AD may also encompass a mechanism of interstitial clearance [123,139].

Cerebral amyloid angiopathy and the perivascular cells

Cerebral amyloid angiopathy (CAA) is characterized by the deposition of Aβ primarily on cortical and leptomeningeal arteries [140-143]. As larger brain vessels are involved in the regulation of the cerebrospinal or interstitial fluid circulation, CAA could impact amyloid clearance mechanisms [144], e.g. via the lymphatic system [123,145]. Recent evidence supports a role for the *clusterin* gene in AD and CAA pathophysiology [146]. When clusterin is absentAβ clearance by perivascular drainage becomes predominant, resulting in less parenchymal plaques but exacerbating CAA [146]. During the formation of vascular amyloidosis, the glio-vascular unit is compromised with a reported physical separation of the astrocytic end-feet from the endothelium. Although astrocytes may continue releasing vasoactive substances, the vascular amyloid determines vessel stiffness [145]. Progression of capillary pathology and CAA have also been found in the 5xFAD mouse model of AD [27]. In the latter case, amyloid accumulation at the parenchymal capillaries is sporadic (example in Figure 2E1) while amyloid proteins are positioned along larger vascular structures, further supporting a pathological link between CAA and AD [147].

The cerebrovasculature during ageing

Cerebrovascular modifications are reported during ageing. Doppler studies [148,149] and PET imaging [150] have shown a reduction in cerebral blood flow in elderly subjects [151]. Reduced microvessel density has been reported with ageing in the cortex [152,153] and the hippocampus [154-156]. Microvascular rarefaction may result from age-dependent apoptosis, as observed in animal models [157]where oxidative stress and chronic inflammation could

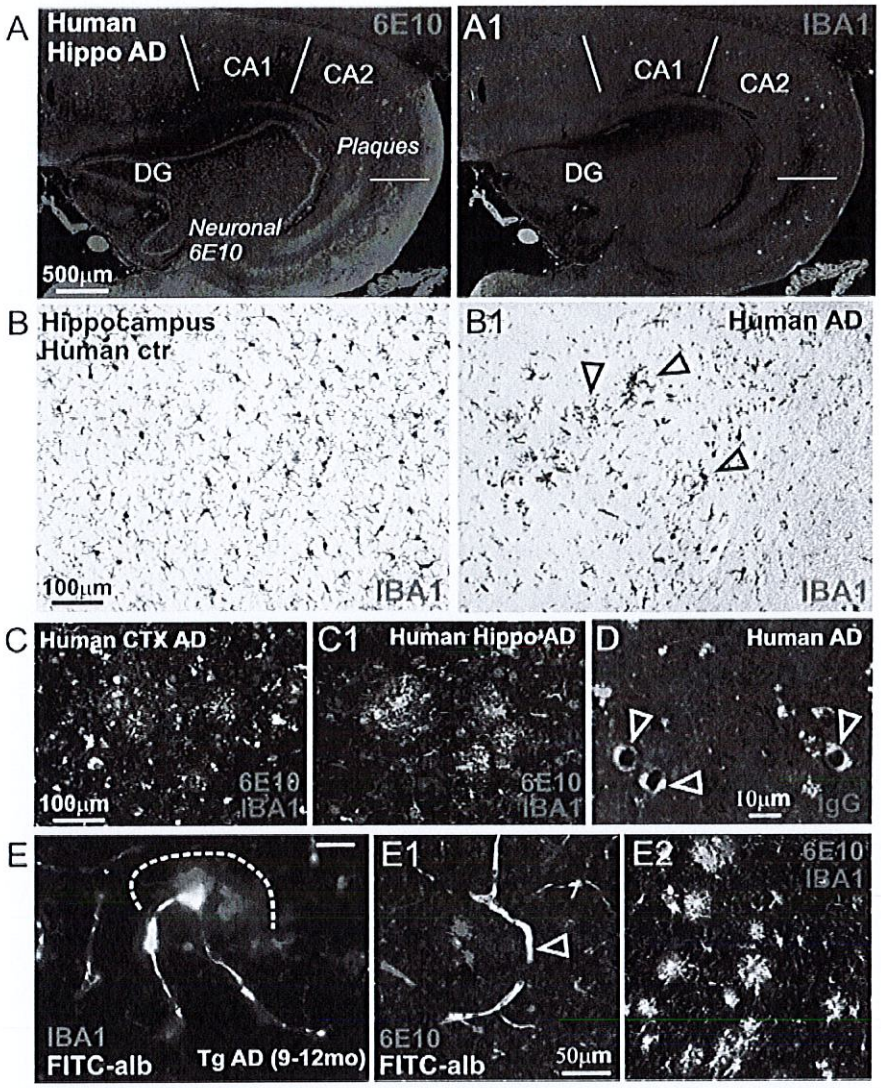

Figure 3. Examples of neuroinflammation and cerebrovascular changes in human and experimental AD

(**A**,**A1**) Montage of human hippocampi to show plaques and microglial distribution in AD. (**B**) Uniformly distributed microglial cells in control human tissue. (**B1**) Post-mortem AD brain shows aggregates (periplaque or perivascular; *arrows*) of IBA1 microglial cells. (**C**,**C1**) Examples of plaque–microglial aggregates in human AD (cortex and hippocampus). (**D**) IgG perivascular accumulation is a sign of BBB damage. (**E**,**E1**) Perivascular pathology (microglial clustering, dotted line in (E); perivascular β amyloid, arrows in (E1)) is observed in an experimental model of AD . (**E2**) Topographic correspondence between microglia and $6E10^{+}$ amyloid in the parenchyma (*authors' unpublished images and observations*).

be contributing factors. Additional studies have shown an increase in vessel tortuosity of penetrating arterioles [156,158,159] and abnormal BBB tight junctions expression at the capillaries in white matter lesions [160].

A recent study [161] indicated the occurrence of BBB damage in the hippocampal CA1 and dentate gyrus regions during ageing. From a cellular standpoint, a link between cognitive decline and pericyte pathology during ageing has been proposed [114,121]. In particular, $PDGFR\beta^{+/-}$ mice displayed accumulation of perivascular fibrin with age as compared with wild-type animals. The age-dependent pericyte-vascular damage preceded neuronal pathophysiology [121]. As pericytes control capillary diameter their loss, or modification, could impact neurovascular coupling [122,162,163]. The cerebrovascular pathological signs observed during ageing could represent a factor aggravating other pathologies, e.g. AD progression or the outcome of stroke accidents.

Future directions

Despite of the accumulating evidence, it remains unclear how pericytes communicate with astrocytes and microglial cells *in vivo* and, amongst these cells, who plays a major role in initiating, or coordinating, a pathological cerebrovascular inflammatory response. It remains to be defined whether genetic modifications occurring at the perivascular cells may constitute clinically significant risk factors for neuropathophysiology. If fully elucidated, the players governing the abluminal cerebrovascular assembly in health and disease may be exploited to develop new therapeutic approaches to treat CNS diseases.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AD, Alzheimer's disease; Aβ, amyloid-β; BBB, blood–brain barrier; CAA, cerebral amyloid angiopathy; Cav-1, Caveolin-1; CNS, central nervous system; COX-2, cyclooxygenase-2; Cyc-A, cyclophilin-A; ICAM-1, intercellular adhesion molecule-1; IFNγ, interferon γ; MCP-1, monocyte chemoattractant protein-1; MMP, metalloproteinase; PDGFRβ, platelet-derived growth factor receptor β; P-gP, p-glycoprotein; SE, status epilepticus; TBI, traumatic brain injury; TGFβ, transforming growth factor β; VCAM-1, vascular adhesion molecule-1.

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