The phenomenon of clasmatodendrosis

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1. Introduction

In a recent consensus statement [1] regarding the many names used to describe astrocyte reactions in health and disease, a word of warning emerged specifically regarding clasmatodendrosis: astrocytes may be damaged by cleavage of membrane and cytoskeletal proteins (such as glial fibrillary acidic protein, GFAP), but the phenomenon of clasmatodendrosis could be an artifact without pathophysiological bearing. What is clasmatodendrosis? What is its relationship, indeed there is any relationship, to ongoing pathophysiology in the central nervous system?

Etymologically, clasmatodendrosis derives from the Greek for fragment (klasma), tree (dendron), and condition (-osis). Cajal first used the term in 1913: he observed disintegration of the distal cell processes of astrocytes, along with a fragmentation or beading of proximal processes closer to the astrocyte cell body. In contemporary clinical and experimental reports, clasmatodendrosis has been observed in models of cerebral ischemia and seizures (including status epilepticus), in elderly brains, in white matter disease, in hippocampal models and cell cultures associated with amyloid plaques, in head trauma, toxic exposures, demyelinating diseases, encephalitides and infection-associated encephalopathies, and in the treatment of cancer using immune effector cells. We examine evidence to support a claim that clasmatodendritic astrocyte cell processes overtly bead (truncate) as a morphological sign of ongoing damage premortem. In grey and white matter and often in relationship to vascular lumina, beading becomes apparent with immunohistochemical staining of glial fibrillary acidic protein when specimens are examined at reasonably high magnification, but demonstration of distal astrocytic loss of processes may require additional marker study and imaging. Proposed mechanisms for clasmatodendritic change have examined hypoxic-ischemic, osmotic-demyelinating, and autophagic models. In these models as well as in neuropathological reports, parenchymal swelling, vessel-wall leakage, or disturbed clearance of toxins can occur in association with clasmatodendrosis. Clasmatodendritic features may serve as a marker for gliovascular dysregulation either acutely or chronically. We review correlative evidence for blood-brain barrier (BBB) dysfunction associated with astrocytic structural change, with attention to interactions between endothelial cells, pericytes, and astrocytic endfeet.

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In this review, we examine evidence to support a claim that clasmato-
dendrotic GFAP-positive astrocytic cell processes in cortex or white matter
overtly bead (truncate) as a major morphological sign of ongoing damage
pre mortem. Beading becomes apparent with GFAP immunohistochemical
(IHC) staining examined by light microscopy at reasonably high magni-
fication (Figure 2), but demonstration of distal astrocytic loss of processes
may require additional imaging and marker study, as we will describe.
Since we contend that clasmatodendrotic features may serve as a marker for
gliovascular dysregulation either acutely or chronically, we review
correlative evidence for blood-brain barrier (BBB) dysfunction associated
with astrocytic structural change.

In contemporary clinical and experimental reports, clasmatodendro-
osis has been observed in white matter disease with or without de-
mentia [7, 8, 9, 10, 11], models of interaction between astrocytes and
beta-amyloid plaques [12, 13], in hippocampal models and cell cul-
tures [14, 15, 16, 17], head trauma [18, 19, 20, 21], models of ce-
rebral ischemia [22, 23, 24, 25, 26], models of status epilepticus [27,
28, 29, 30, 31], in toxic exposures [32, 33, 34, 35], demyelinating
diseases [36, 37, 38], in osmotic-induced demyelination and an
inherited condition with cerebral edema [39, 40], in association with
autophagy and/or apoptosis [24, 27, 41, 42, 43, 44], encephalitides
or infection-associated encephalopathies [45, 46, 47, 48], and in
chimeric antigen receptor (CAR) T-cell therapy [49, 50]. Parenchymal
swelling, vessel-wall leakage, or disturbed clearance of toxins
commonly occurs in association with clasmatodendrosis in patholog-
ical and in vivo reports; in cell-culture studies and some seizure or
Alzheimer models, astroglial changes are reported without comment
about non-astrocytic edema [12, 13, 15, 16, 29, 30, 31, 35, 42].
Table 1 summarizes how clasmatodendrosis has been defined, visu-
alized, and characterized in the above contexts.

Figure 1. Autolytic phenomena in the white matter of the brain of a female
adult autopsied two hours after death (Ramón y Cajal 1913, fig. 18, public
domain. [2]). A. Cell with preserved processes. B. Astrocyte with fragmenta-
tions. C, D, E. Astrocyte with disrupted cytoplasmic expansions, but with pres-
ervation of perikaryon. a. capillary. b. disaggregated end feet.

Figure 2. Postmortem neuropathologic evaluation showing prominent clasma-
todendrosis in a patient with a history of acute lymphoblastic leukemia treated
with anti-CD19 CAR T-cell therapy complicated by fulminant cerebral edema.
Beadng and fragmentation of astrocytic processes (highlighted by GFAP
immunostains) were seen in sections of cortex (A, 200x; B, 400x) and were
accentuated around blood vessels (C, 600x).

2. Clasmatodendrosis as an astrocytic reaction in vitro and
in vivo

Andriezen [51] drew first attention to morbid structural alteration of
glia in the human brain, and he observed different prominent glial
populations in grey and white matter–protoplasmic glia in the former and
stellate fiber cells in the latter. He described that with brain injury or
disease, protoplasmic glia extend a surfeit of fibrils in their immediate
| First Author | Reference [reference number in brackets] | Definition of clasmatodendrosis | Staining | Microscopy used | Location | Magnification | Context |
|--------------|------------------------------------------|----------------------------------|----------|----------------|----------|---------------|---------|
| Cone 1928   | [6]                                      | fragmentation of cell expansions with little or no swelling | gold sublimate | light | not specified | 2000x | not specified |
| Friede 1961 | [52]                                     | not formally defined; described as shortened and swollen processes with tendency to disintegrate, forming small corpuscles | Hortega silver carbonate | light | white matter | 260x | post-mortem rats |
| Kraig 1990  | [53]                                     | not defined. Described as isolation of distal processes and disintegration of branches | horseradish peroxidase. Astrocytes confirmed by high membrane potential and absence of injury spontaneous discharges via single-barrel microelectrode intracellular recordings | light | not specified | not specified, 50 micrometer scale bar | acidosis and ischemia in rats |
| Rafalowska 1992 | [38]                              | fragmentation of perivascular glial fibers | peroxidase-antiperoxidase method to visualize GFAP | light | white matter | not available | young and senile multiple sclerosis plaques |
| Cavanagh 1993 | [35]                               | loss of glial filaments, hydropic swelling of astrocyte perikaryal cytoplasm and nearby processes | GFAP IHC | light, electron microscopy (EM) | inferior colliculus, but at high dose exposure to toxin (100 mg/kg/day), all areas of brain and spinal cord exhibited changes | x35 | mice and rats exposed to neurotoxin, alpha-chlorohydrin |
| Tomimoto 1996 | [26]                               | swelling of astroglia, decreased GFAP immunoreactivity, intracytoplasmic vacuolization, beading | Klüver-Barrera stain of white matter, anti-fibrinogen, anti-HLA-DR, GFAP IHC, anti-immuno-globulin, anti-amyloid precursor protein (all IHC) | light | tissue blocks anterior caudate including cingulate, superior, middle, and inferior frontal gyri | not specified, 50 micrometer scale bars | ischemic cerebro-vascular disease, Alzheimer's disease, 6 controls |
| Tomimoto 1997 | [10]                               | swelling, vacuolation of white matter astroglia; disintegrated and beaded processes; condensed nuclear chromatin; large, membrane-bound osmiophilic cytoplasmic inclusions corresponding to lipophilic granules visualized by light microscopy | IgG, IgM, fibrinogen, C3, Clq, C3d, vimentin, alpha-B crystallin, ApoE, laminin, leukocyte common antigen, anti-HLA-DR; double labeling with GFAP (all IHC) | light; EM | deep and periventricular white matter | not specified, 50 micrometer scale bars | human post-mortem in cerebro-vascular and Alzheimer's diseases |
| Hulse 2001  | [15]                                     | loss of distal processes, formation of filling bodies | gold sublimate, GFAP-GFP | differential interference contrast; brightfield | hippocampal organ tissue culture, neocortical polygonal astrocytes in primary culture | 20x and 50x objectives used, 100 micrometer scale bars | simulated ischemia using ischemic Ringer's solution |
| Sahlar 2002 | [9]                                      | cytoplasmic swelling and vacuolation with beading of their dendrites | H&E, GFAP immune-staining | light | periventricular white matter | x40-100 | case report of mixed dementia |
| Simpson 2007 | [11]                                | swollen, vacuolated cell bodies. Disintegrated processes | double-labeled GFAP and Cluster of differentiation 68 (CD68) IHC, fibrinogen IHC | brightfield | periventricular and subcortical white matter | x20 and x40 objectives used but not specified if for clasmatodendrosis. 100 micrometer scale bars | older adults |
| Gelot 2009  | [25]                                     | loss of distal processes (short rigid and broken and/or beaded), chromatin condensation | vimentin IHC, GFAP IHC, some double labeled TUNEL and GFAP IHC | Light; fluorescence | cerebral cortex and different depths of white matter | x20 and x40 objectives used for cell counts, but not specified if used for judging clasmatodendrosis. 20–50 micrometer scale bars | rats, neonates |
| Kim 2011   | [28]                                     | swelling and vacuolization of somata; disintegrated/beaded processes | GFAP, GFAP IHC | Confocal microscopy (CONFOC) | hippocampus | not specified, 30 micrometer scale bar | status epilepticus in rats |
| Qin 2010   | [41]                                     | not mentioned, but they do mention fragmentation of astrocyte “indicative of dying cells” | GFAP, GFAP IHC, LAMP-1, microtubule associated protein 1A/1B-light chain | Light; fluorescence; transmission EM | possibly white matter (not specified in text) | 200–400x, 50 micrometer scale bars | focal ischemia, glucose and oxygen deprivation in rats |

(continued on next page)
| First Author | Reference [reference number in brackets] | Definition of clasmatodendrosis | Staining | Microscopy used | Location | Magnification | Context |
|--------------|-----------------------------------------|---------------------------------|----------|----------------|----------|---------------|---------|
| Ryu 2011     | [42]                                    | swelling and vacuolization of somata, disintegrated and beaded processes | GFAP IHC. Pyridoxyl 5′-prime phosphate phosphatase (PLPP)/chronophotin (CIN), LAMP-1, LC3-II stained, but not used to define clasmatodendrosis. | CONFOC | hippocampus | not specified. 25–50 micrometer scale bars | status epilepticus in rats |
| Ryu 2011     | [44]                                    | irreversible astroglial degenerative change, swelling and vacuolization of cell bodies; short, blunt, beaded, and disintegrated and beaded processes; GFAP tangles in the cytoplasm and nuclear dissolution | GFAP, Beclin-1, LAMP-1, LC3-II staining and p65/RelA Ser529 phosphorylation (subset of NFκBβ) positive, but not used to define clasmatodendrosis. | fluorescence | hippocampus | 40x objective used for cell count, but unclear if used for judging clasmatodendrosis. 5–10 micrometer scale bars | status epilepticus in rats |
| Misu 2013    | [36]                                    | cytoplasmic swelling and vacuolation, beading and dissolution of their processes and nuclear alterations resembling apoptosis | GFAP IHC. Compared with AQP4, AQP1, IgG (all IHC) which all showed granular internalization | light | Medulla | x1100 | neumyelitis optica |
| Sakai 2013   | [18]                                    | beading and fragmentation astrocytic processes, cytoplasmic swelling and vacuolation of somata | GFAP, GFAP IHC, ubiquitin and lysine 48-linked polyubiquitin chains (K48), K48 IHC. Double immunostaining with GFAP and p62-K48 | Light; CONFOC laser | cerebral cortex | not specified. 10–20 micrometer scale bars | head trauma in humans |
| Nara 2015    | [47]                                    | disruption of astrocytic projections | H&E | light | cerebrum, not otherwise specified | x400 | Cytokine storm-derived influenza associated encephalo-pathy |
| Chen 2016    | [8]                                     | morphology of irreversibly injured astrocytes; cytoplasmic swelling, somatic vacuolation, beading and fragmentation of dendritic processes | GFAP and ALDH1L1 IHC | brightfield | white matter | not specified. 10–20 micrometer scale bars | aging, post-stroke dementia |
| Daschil 2016 | [12]                                    | loss of distal processes; isolated fluorescent bodies | GFAP-GFP | three-dimensional CONFOC | cerebral cortex | 63x objective. 5–40 micrometer scale bars | mouse model of Alzheimer’s |
| Ko 2016      | [31]                                    | round, edematous soma; short, blunt processes; loss of distal processes | GFAP, lysosome associated membrane protein 1 (LAMP-1) for lysosomal vacuolization | Immunofluorescence | hippocampus | not specified. 3.75–30 micrometer scale bars | status epilepticus in rats |
| Lana 2016    | [17]                                    | beading and disintegration of distal cell processes; cytoplasmic vacuolization and swelling | GFAP | CONFOC laser scanning | hippocampus | 63x objective. 10 micrometer scale bars | aged rats |
| Mercatelli   | 2016 [14]                               | beading and disintegration of astrocyte projections | GFAP | three-dimensional CONFOC | hippocampus | 40x and 63x objectives. 90 micrometer scale bars | rats given lipopoly-saccharide (LPS) with induction of amyloid deposition |
| Nishiyama 2016 | [37]                                 | not specified. mentions findings of shrinkage of processes and spherical change in soma | GFAP, AQP4 IHC | Light and fluorescence | astrocytes in culture (GC-2565, Lonza Japan, Tokyo, Japan) | not specified. 20–100 μm scale bars | human astrocyte culture; complement-dependent and complement-independent astrocytopathy may operate in NMO |
| Wang 2016    | [48]                                    | beading of astrocytic processes | H&E; cleaved caspase 3, AQP4, GFAP (all IHC) | light | Brainstem | not specified. Scale bars not provided for images of clasmatodendrosis | post-mortem hand-foot-mouth disease |
| Canchi 2017  | [20]                                    | voids in cytoplasm, rounded somata, disintegrating processes | GFAP | CONFOC laser | forebrain | 63x objective. 25 micrometer scale bars | blast TBI in rats |
| Eltony 2017  | [33]                                    | loss of processes, hypertrophy of soma | PTAH | light | optic nerve | x1000 | |

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| First Author | Reference [reference number in brackets] | Definition of clasmatodendrosis | Staining | Microscopy used | Location | Magnification | Context |
|--------------|------------------------------------------|---------------------------------|----------|----------------|----------|--------------|---------|
| Hase 2017[24] | damaged astrocytes with enlarged somata, loss of processes | GFAP IHC, GFAP-AQP4 double immune-fluorescent staining (AQP4 not used to define clasmatodendrosis) | brightfield; CONFOC | corpus callosum | brightfield: 20x objective; CONFOC: not specified. 10 micrometer scale bars | chronic sildenafil exposure in rats |
| Hayashi 2017 [34] | not specified | GFAP IHC | light | occipital white matter | not specified. 20 micrometer scale bars | single case autopsy |
| Kim 2017 [27] | swollen vacuolized cell bodies; disintegrated/beaded processes | GFAP | fluorescence | hippocampus | not specified. 25–50 micrometer scale bars | status epilepticus in rats |
| Miller 2017 [21] | beading, dissolution of astrocytic processes | GFAP to define clasmatodendrosis. Cells double-labeled with GFAP against propium iodide (PI), glial glutamate transporter-1 (GLT-1), and Annexin V | CONFOC laser scanning | hippocampus | not specified. 20 micrometer scale bars | blast TBI in rats |
| Shimoda 2017 [40] | irreversible, “necrobiotic” change of astrocytes; swelling and vacuolization of soma; disintegration/beading of the processes | GFAP IHC | light | periventricular and cerebellar white matter | not specified. 20–100 micrometer scale bars | ataxia-telangiectasia |
| Zhang 2017 [32] | irreversible astroglial degenerative change; swelling and vacuolization of soma; disintegrated and beaded processes | GFAP IHC | light | cerebral cortex | not specified. 50–500 micrometer scale bars | Methamphetamine use in humans |
| Hase 2018 [7] | cytoplasmic swelling and somatic vacuolation; beading and fragmentation of dendritic processes | GFAP and AQP4 double-staining | fluorescence | white matter | not specified. 10 micrometer scale bars | dementia in humans |
| Kim 2018 [43] | astroglial autophagy, swollen, vacuolized soma and disintegrated/beaded processes | GFAP, LAMP-1, TUNEL, HSPB1 | fluorescence | hippocampus | not specified. 25 micrometer scale bars used | status epilepticus in mice |
| Torre 2018 [49] | beading of glial fibrillary acidic protein consistent with astrocyte injury | GFAP IHC | light | superficial cortex | 400x and 1000x | ICANS in one patient |
| Bouchat 2019 [39] | “distal extensions removal,” beading, and irreversible astrocyte injury. Autophagyosis | GFAP | automated fluorescence; EM | thalamus | not specified. 1 micrometer scale bars for fluorescence; 200 nm scale bars used for electron micrographs | osmotic demyelination in mice |
| Tachibana 2019 [45] | disintegration of distal processes, somatic swelling and vacuolation | GFAP and GFAP IHC to define clasmatodendrosis. AQP4, TUNEL, MAP2, SMI31, synaptophysin tested | CONFOC laser scanning microscopy; transmission EM | cerebellum, thalamus, splenium, corpus callosum | not specified. 50 micrometer scale bars for confocal; 0.2–1 micrometer scale bars for electron | influenza-associated encephalopathy |
| Early 2020 [19] | beading, diminishment of astrocyte projections; vacuolization and swelling of cytoplasm | GFAP, vimentin, S100beta, immune-fluorescent co-labeling, AQP4 | CONFOC | hippocampus | not specified. 25–100 micrometer scale bars | traumatic brain injury in mice |
| Lee 2020 [30] | astroglial autophagy; vacuolization, disintegrated/beaded processes | double-labeled GFAP and HSP25, GFAP and LAMP1, and GFAP and p-focal adhesion kinase (FAK)-Y397 | fluorescence | hippocampus | not specified. 6.25 micrometer scale bars | status epilepticus in mice; kainic acid model |
| Stevenson 2020 [22] | loss of processes; swollen, round soma | GFAP IHC | brightfield | hippocampus | 20x objective. 10 and 50 micrometer scale bars | carotid stenosis in mice |
vicinity, many directed towards vessels, as the glial cell body eventually assumes a ghost-like, empty appearance.

In a further characterization of astrocytic reaction in time, now with particular attention to clasmoadenrosis, Frieden and Houten [52] examined rat astrocytes in hippocampal cell cultures under conditions of abolished oxidative metabolism. Anaerobic glycolysis in astrocytes can persist for a while after anoxic insult, depending on local carbohydrate reserves, with acidity rising as a function of accumulating lactic acid. With ongoing anaerobic glycolysis, astrocyte cell processes shortened, swelled, and tended to disintegrate or to form corpuses, consistent with clasmoadenrotic beading. When the glycolytic pathway was blocked experimentally, clasmoadenrosis was “eliminated.”

As measured by pH-sensitive microelectrodes inserted into astrocytes in live rats, an intracellular increase in acidity under anoxic and hyper-glycemic conditions has been documented [53]. The authors also noted increases in astrocytic surface membrane resistance related, they argued, to progressive loss of astrocytic arbors (reduced overall astrocytic cell surface area, as occurs in clasmoadenrosis), reduced potassium conductance due to acid sequestration within astrocytes, and reduced coupling via gap junctions between neighboring astrocytes, perhaps especially at the level of astrocytic endfeet at their interface with cerebral vessels. Corroborating the in vivo report, reduction in the length of astrocytic cytoplasmic processes, with distal ends showing the earliest change, occurred ~15 min after reduction in pH combined with mitochondrial inhibition in a hippocampal cell culture [15]. Temporal and spatial changes in morphology were determined both by GFAP immunostaining (thinning, then shortening or frank truncation of astrocytic process length visualized by way of three-dimensional optics) and by transfection of cultured astrocytes with green fluorescent protein (GFP). The latter allowed the authors to visualize by a different means the progressive reduction in process diameter over minutes, followed by a disruption and loss of process fluorescence distally.

In permanent middle cerebral artery occlusion in rats and a parallel in-vitro study of cultured astrocytes undergoing oxygen and glucose deprivation, structural changes occurred in both settings. Shortening and fragmentation of astrocytic cell processes occurred. But astrocytic cytoplasm also contained numerous multimembrane vesicles described as typical for autophagosomes, which eventually fused with lysosomes in the cytoplasm [41]. Autophagosomal markers (see Table 1 for specific molecules) increased and levels of cytoprotective B-cell-lymphoma 2 protein (Bcl-2) decreased. The changes were mitigated, but not entirely reversed, by pharmacological inhibition of autophagy.

In a rat model of status epilepticus, an interesting mechanism for clasmoadenrosis specifically associated with autophagy has been advanced [44], although a number of autophagic or apoptotic pathways have been studied [24, 27, 43]. Tumor necrosis factor alpha (TNF-alpha), expressed at low levels in normal brain, is upregulated in status epilepticus, and the increase is associated with selective phosphorylation of a particular subunit of nuclear factor-kappa-B (NFkappaB) in astrocytes. The specific phosphorylation and ensuing autophagic (vacular/cytomplasmic) and clasmoadenrotic changes were reversed by neutralization of TNF-alpha.

A general autophagic mechanism for clasmoadenrosis mediated by TNF-alpha remains an open issue, however, in light of an autopsy series examining influenza-associated encephalopathy, a condition thought to be mediated by inflammatory cytokines such as interleukin 6 (IL-6) and TNF-alpha rather than by viral invasion into brain [45]. Compared to control brain, clasmoadenrosis, characterized as GFAP-positive bead strings (and other marker positivity, see Table 1), was present in all examined regions of influenza-encephalopathy brains. Aquaporin 4 (AQP4) distributions differed between control and disease groups. Intense AQP4 staining occurred at astrocytic perivascular endfoot in control brains. AQP4 staining in the area of GFAP-positive bead strings was observed along with a decrease in AQP4 staining in the vicinity of blood vessels in encephalopathy brains. In addition, autophagic markers in astrocytes were notably absent, and electron microscopy revealed neither autophagosomes nor vacuolization. The authors surmised that autophagy may not be a mechanism for clasmoadenrotic formation in this virus-associated encephalopathy. All the brains in the encephalopathic group were heavy and exhibited diffuse edema (mean brain weight at autopsy was 1,318 g in encephalopathic brains compared to 1,023 g in the control group; mean ages for the two groups were 4.5 years and 5.8 years, respectively).

The significance of AQP4 redistribution associated with diffuse brain edema merits consideration of a relevant model.

3. Clasmoadenrosis and the BBB in vivo: the case of osmotic demyelination

Clasmoadenrosis figures prominently in the timing of glial changes associated with osmotic demyelination in mice. Building on in vitro observations that astrocytic GFAP expression downregulates after exposure to pro-inflammatory cytokines, particularly interleukin-1-beta (IL-1beta) [54], Nicaise et al. [55] found that astrocytes of demyelination-prone regions underwent profound astrocytic endfoot change and fragmentation of astrocyte processes, with swelling of perinuclear areas, associated with loss of astrocyte cell markers such as AQP4 and aldehyde dehydrogenase 1 family, member L1 (ALDH1L1). Loss of AQP4 immunoreactivity happened prior to demyelination in susceptible regions and before GFAP down-regulation. Scalisi et al. [56] have also observed “massive” breach of the BBB on the order of 48 h after correction of hypotension and after oligodendrocyte and astrocyte loss. The above studies hint that BBB leakiness could be secondary to astrocyte reaction: also at 48 h post-correction, brain endothelial cell changes reached their apogee at those places where astrocyte endfeet were most disrupted.

In the aggregate, the above observations regarding morphological change in astrocytes and a single model of BBB leakiness suggest that clasmoadenrosis could be characterized by either, but how do we clarify how morphological change leads to variable BBB permeability? What locales might be most vulnerable to edema associated with clasmoadenrotic beading in grey and white matter?

4. Astrocytes, their endfeet, and vascular lumina

Astrocytic processes interface with vessels in ways that have been examined with increasing sophistication. Ultrastructural differences between vessels located at the pia-lined cortical surface and those downstream in the arteriovenous axis have been examined using single-cell RNA sequencing [57]. The transcriptome as reported in the rat could be highly evolutionarily conserved, and certain observations are relevant to the distal astrocytic process in humans. In comparison to step-like, discrete transitions in gene expression from one type of smooth muscle cell to another, endothelial cells lining vascular lumina exhibit gradual, not punctuated, changes in gene expression from arterial to capillary to venous endothelium. At a lumen, the distal astrocytic process widens into an endfoot to form, along with other astrocytic endfeet or the ends of a single endfoot, a continuous sheath that possesses water channels (AQP4) and potassium channels (ATP-dependent inwardly rectifying potassium channel 4.1, Kir4.1) facing an abluminal glycoprotein- and collagen-rich matrix [58, 59, 60]. An endothelial cell (or endothelial cells) line/s the lumen; abluminal pericytes (without smooth muscle cells, at the capillary level) loosely embed themselves in the matrix underneath the astrocytic endfoot [57, 58].

Dating to tracer leakage assays in the 1960’s, tight junctions, where leaflets of endothelial cell membranes appose each other, have been understood as a sine qua non of the BBB [61]. Tight junctions, among other barriers, restrict the entry of hydrophilic molecules into brain parenchyma. Brain endothelial cells themselves exhibit less transcellular transport compared to peripheral endothelium, and their endocytosis and transcytosis are highly selective processes [62, 63]. Tight junctions restrict movement of small ions such that transendothelial electrical resistance (TEER) is orders of magnitude greater across brain endothelium compared to endothelial TEER elsewhere in the body (1,000 Ω·cm²).
5. Astrocytes, pericytes, endothelial cells

Just beyond the distal NVU, cells in brain parenchyma may interact with endothelial cells, for example, after vascular injury [66], but we concentrate on three cell populations.

In development, the interaction between astrocytes, pericytes, and endothelial cells is constitutive not only of a barrier, but also of a lumen. Co-culture studies indicate that astrocytes contribute to the orientation of endothelial cells and pericytes into capillary-like structures [67]. Astrocyte precursor cells mitogenically induce the development of endothelial cells, and endothelial cells drive, by way of a cytokine (leukemia inhibitory factor), the differentiation of astrocyte precursor cells into astrocytes [68]. Endothelial cells secrete vascular endothelial growth factor (VEGF) and insulin growth factor 1 (IGF-1), both considered important in developmental neurovascular patterning [69]: VEGF disrupts tight junctions [70], but knockout mice without endothelial expression of IGF-1 receptors do not exhibit changes in BBB permeability in life [71].

The synthesis of a factor in development may or may not impact barrier function in the long term. Yet, in an important in vivo study, pericyte-deficient mouse mutants showed robust increases in BBB water permeability by endothelial transcytosis (the permeability can be reversed by the tyrosine kinase inhibitor imatinib), and pericyte-deficient mice exhibited abnormal channel polarization at the astrocytic endfoot, with redistribution of AQP4 away from the ablumen [72]. The loss of channel polarization at the endfoot or frank loss of the endfoot has implications that we detail below in two clinical contexts associated with clasmatodendrosis.

In maturity, endothelial cells secrete platelet-derived growth factor BB (PDGF-BB), the receptor for which is located on pericytes (a paracrine interaction important for pericyte recruitment); in turn, pericytes express major facilitator superfamily domain-containing protein 2a (MFSD2a) critical to endothelial cell-wall integrity [69]. Pericytes and astrocytes secrete angiopoietin-1 (Ang-1) that acts on a tyrosine kinase/immunoglobulin-like receptor-2 (TIE-2) located on the endothelial cell membrane. Ang-1 at TIE-2 contributes to BBB impermeability [73]. Astrocytes secrete apolipoprotein E 2 and 3 (APOE2 and APOE3), glial cell line-derived neurotrophic factor (GDNF), and fibroblast growth factor-2 (FGF-2), all imputed, based on cell culture studies (and in vivo in the case of APOE3, the most abundant APOE isoform), to induce and maintain the BBB [74]. Astrocytes also secrete Src-suppressed C-kinesin substrate (SSECKS, also known as A-kine anchor protein-12) that transcriptionally regulates synthesis of tight junctional protein complexes in the endothelial cells [69]. Many other molecular mechanisms of BBB control have been described [65, 74].

Among paracrine signals that contribute to BBB leakiness, three are of note: endothelin-1 (ET-1), tumor necrosis factor-alpha (TNF-alpha, a cytokine known to induce ET-1 synthesis by endothelial cells), and interleukin-1-beta (IL-1-beta). In a cell-culture study that illustrates the nuances of interaction between endothelial cells and astrocytes [75], ET-1 mRNA expression in endothelial cells increased in the presence of TNF-alpha; both TNF-alpha and ET-1 mediated increased leakiness, but only in the presence of astrocytes; TNF-alpha induced astrocyte synthesis of IL-1-beta, as mediated by ET-1; and IL-1-beta increased BBB leak. Ultrastructural changes in cells were not reported.

6. Clasmatodendrosis in small-vessel disease and immune-effector-cell associated toxicity

In a neuropathological investigation of sub-populations of persons with manifest small-vessel disease on neuroimaging (but with varying degrees of cognitive impairment by clinical rating scales and clinical histories) [8], double immunofluorescent staining for GFAP and AQP4 revealed overall reduced distal astrocytic cell processes, a morphology Alzheimer and Cajal had observed. In addition, in that study, AQP4 stain was displaced, aggregating at the edges of swollen GFAP-positive cells, in counterpoint to the even, though dotted staining for AQP4 along normal astrocytic endfoot. Punctate staining likely represents normal local clustering of AQP4 in orthogonal arrays of particles at the interface with the abluminal glycoprotein and collagen matrix in the distal NVU. The pathological findings suggest that disruption of normal arrays, with loss of the polarized location of AQP4 at the astrocytic endfoot, could be a marker for gliovascular dysfunction. Further, capillaries in clasmatodendritic areas were denuded of AQP4 immunoreactive astrocytic endfoot, a finding also found in neuropathologic sampling from a simian model for ischemic encephalopathy reported in the same paper. Loss of antibody staining to alkaline dehydrogenase 1 family, member L1 (ALDH1L1), a cytoplasmic marker, corroborated cytoplasmic disintegration of astrocytes [8]. Loss or disorganization of astrocytic AQP4 would have effects not only on local BBB permeability, but also on the efficient clearance of toxins (tau in head trauma and in tau-associated neurodegenerations, beta-amyloid, others) from the perivascular space and from the parenchymal interstitium via a “lymphatic” pathway unique to the brain and dependent on a relatively intact distal NVU in the arterio-venous capillary bed [76, 77, 78].

Not surprisingly, endothelial cells and pericytes themselves exhibit changes in small-vessel disease [79], but the specific mechanisms by which clasmatodendrosis occurs as a consequence of disturbed signaling between the three cell populations remains to be explored fully. Nevertheless, there are clues pointing to at least part of a mechanism, based on experience in a very different clinical context associated with clasmatodendrosis pathologically.

Among biomarkers of endothelial activation in disease, angiopoietins have been studied in infections for some time [80], but, relevant to clasmatodendrosis, we can study the role of two angiopoietins in chimeric antigen receptor (CAR) T-cell immunotherapy for refractory malignancies. We have chosen images (Figure 2) to illustrate clasmatodendrotic beading specifically from our experience with CAR T-cell therapy.

Earlier we described pericytic and astrocytic Ang-1 and its interaction with the endothelial-cell receptor TIE-2. Now we introduce the significance of angiopoietin-2 (Ang-2), which is released from a specialized organelle (the Weibel-Palade body) within endothelial cells [81]. Endothelial Ang-2 binds to endothelial TIE-2; in the presence of Ang-1, Ang-2 is an antagonist at TIE-2 [82]. The ratio of the two ligands at TIE-2 has contrary downstream effects [73, 82].

In the setting of cytokine release in CAR T-cell therapy and other pro-inflammatory “storms,” [83] endothelial Weibel-Palade bodies exocytose Ang-2 along with the principal cargo of Weibel-Palade bodies, von Willebrand factor (VWF). The two angiopoietins and VWF are measurable in serum, although, as a cautionary point, biomarkers may only partially reflect the complexity of NVU signaling. In a prospective study in pediatric traumatic brain injury, serum increases in Ang-2 were associated with poorer coma scores, as one would expect, but Ang-2 increases were also associated with decreases in ET-1 (a paracrine signal associated with increased BBB permeability, see above), though none of the correlations between biomarkers reached statistical significance in that study [84].

The CAR T-cell therapy experience suggests that Ang-2 expression may, however, be a practical index of gliovascular dysregulation [82]. Ang-2 expression and the ratio Ang-2/Ang-1 are both low in quiescent, normal, mature vessels. The Ang-2/Ang-1 ratio increased by day 7 after infusion of CAR T cells; serum VWF also increased in that interval—the latter observation consistent with Ang-2 release from endothelial Weibel-Palade bodies. Clinical severity of neurotoxicity tracked with rises in Ang-2 and the Ang-2/Ang-1 ratio; Ang-1 levels did not differ between persons with lesser or no neurotoxicity versus those with severe neurotoxicity and accompanying radiographic evidence for BBB leak. In brief, endothelial Ang-2 to astrocytic and pericytic Ang-1 ratio is telling: low is normal and anti-leak; higher is pro-leak.
The complexity of the cytokine release syndrome (CRS) that typically precedes, but may temporally overlap with immune effector-cell associated neurotoxicity syndrome (ICANS) cannot be done justice in this brief review, but it is important to note that many signals at critical points in CRS evolution are already synthesized by (and have known effects on) endothelial cells, pericytes, and/or astrocytes at the gliovascular interface. Further, CAR T cells in current use are known to produce many of the same cytokines or paracines synthesized at the distal NVU, though additional CAR T-cell in vitro modification to change a cell’s synthetic profile is already being studied [85].

As we have reviewed, some of the critical signals include (the associated effect on BBB permeability follows in parentheses): Ang-1 (less leaky), Ang-2 (leaky), IL-1-beta (leaky), IL-6 (leaky), TNF-alpha (leaky), VEGF (leaky). Complicating the overall picture are conversions that occur among cytokines [83]—for example, from IL-6 to interleukin 17 (IL-17)—elevations of both are associated with ICANS [86]; in addition, interventions targeting, for example, IL-6 (e.g., tocilizumab, an IL-6 inhibitor) may be associated with transiently associated with increases in circulating IL-6 [87]. Nevertheless, an awareness of key signals at the distal NVU has become necessary for both the hospitalist and neurohospitalist in the immunological treatment of cancer. Attention to the short list provided above has practical value in contemplating common therapies to mitigate BBB permeability.

7. NVU salvage, possibly to impede clasmatodendrosis

Treating ICANS mandates vigilance for the possibility of a rapid decline in sensorium, over minutes or hours, associated with radiographic loss of grey-white matter boundaries, effacement of cisterns indicative of intracranial edema, and other worrisome signs. The mainstay of treatment is dexamethasone.

Neuropathologic examination of patients with severe ICANS [49, 50] demonstrates alterations that are in keeping with impaired BBB permeability. Postmortem analysis of one patient who died from fulminant cerebral edema after treatment with CAR T cells [49] showed perivascular fluid extravasation (highlighted by positive fibrin and factor VIII A staining), aberrant glucose transporter-1 staining (GLUT 1, a marker for endothelial integrity), and beading and fragmentation of the astrocytotic processes. Beading and fragmentation were prominent around vessels. In the days preceding death in such cases, the effort to treat vasogenic edema may influence post-mortem observations. For example, reductions in intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells may relate to the aggressive use of dexamethasone before a patient’s demise [49].

Charactization of dexamethasone’s mechanism of action underscores signaling at the distal NVU that either results from or contributes to clasmatodendrosis. Dexamethasone regulates the expression of VEGF, Ang-1, and Ang-2 by way of action at glucocorticoid receptors on astrocytes, pericytes, and endothelial cells. In a cell culture preparation with steroid concentrations comparable to those observed in the treatment of brain tumor-associated edema [70], dexamethasone decreased VEGF and increased Ang-1 secreted by astrocytes and pericytes; Ang-1 is not synthesized in endothelial cells, although pericytes did elaborate Ang-1 in the study. Dexamethasone had no significant effect on endothelial cell synthesis of VEGF or Ang-2. Drug-induced changes were attenuated in the presence of an antagonist to the glucocorticoid receptor. The effect of glucocorticoids may be more complex, since steroids are known to influence the activity of factors (e.g., nuclear factor kappaB, NFkappaB) known to influence post-mortem observations. For example, NFkappaB may be associated with increases in circulating IL-6 [87]. Nevertheless, an awareness of key signals at the distal NVU has become necessary for both the hospitalist and neurohospitalist in the immunological treatment of cancer. Attention to the short list provided above has practical value in contemplating common therapies to mitigate BBB permeability.

Table 2. Summary of Proposed Mechanisms in Clasmatodendrosis (see text for full discussion; references in brackets).

| Experimental Model or Clinical Context | Observations | Cytokine or paracrine mediators | Reversibility by what means? |
|--------------------------------------|--------------|-------------------------------|-----------------------------|
| Hippocampal cell culture, abolished oxidative metabolism [52] | ∆pH as a function of lactic acid | – | blockade of glycolysis associated with elimination of clasmatodendrosis |
| Rats in vivo, anoxia, hyperglycemia [53] | ∆potassium conductance across astrocytic membrane due to acid sequestration in astrocytes | – | – |
| Neuropathologic sampling of small-vessel disease [5] | demed AQP4 staining of astrocytic endfeet, ALDH1L1 | | – |
| Permanent middle cerebral artery occlusion, rats [41] | appearance of autophagosomes in astrocytes; LAMP-1 immunoreactivity | ↑LC3-II, ↑Beclin-1 (both autophagosome markers); ↑cytoprotective Bcl-2 | pharmacologic inhibition of autophagy (partial response) |
| Status epilepticus (rats) [44] | selective phosphorylation of NFkappaB | ↑TNF-alpha | neutralization of TNF-alpha |
| Influenza-associated encephalopathy [55] | ∆AQP4 staining of astrocytes in the vicinity of blood vessels | ↑IL-6, ↑TNF-alpha | – |
| Ommotic demyelination (rats) [56] | ∆AQP4 and ∆ALDH1L1 staining | ↑IL-1beta | – |
| Pericyte-deficient mice [72] | redistribution of AQP4 away from abluminal astrocytic endfeet | ↑PDGF-BB | tyrosine kinase inhibition (imatinib) |
| Astrocyte and endothelial cell culture [75] | model for ↓BBB permeability | ↑ET-1, ↑TNF-alpha, ↑IL-1beta | – |
| CAR T-cell immune therapy [82, 83, 86, 87] | signs and symptoms of ICANS | ↑Ang2/Ang1 ratio, ↑IL-1beta, ↑IL-6, ↑IL-17, ↑TNF-alpha, ↑VEGF | ↑IL-6 inhibition (tocilizumab), ↑IL-1beta to IL-6 conversion (anakinra), dexamethasone (multiple mechanisms of effect, see text) |
in future trials: if the distal NVU is already breached in some way, as one would expect early in the progression to clasmatodendrosis, does our notion of drug delivery to brain across the BBB change?

In other conditions associated with clasmatodendrosis, interventions will vary depending on the presumed mechanisms of NVU dysfunction—for example, primary and secondary stroke prevention in small-vessel and other cerebrovascular diseases, treatment of infections, etc. It is interesting to consider, however, that permeability changes that either are the result of clasmatodendrosis or which predispose to that change might share common mechanisms. Table 2 and Figure 3 provide summaries of proposed mechanisms addressed in this review.

8. Conclusion

Although clasmatodendrosis could be autolytic and artifactual as Cajal suggested in 1913, data are actively accumulating that invite questions about how the GFAP beading in clasmatodendrosis transpires mechanistically and what consequences ensue from the morphological change. Whether clasmatodendrosis is a response to extracellular (paracrine or cytokine) or intracellular cues (induction of autophagic or other genetic mechanisms associated with expression of proteins normally quiescent in astrocytes, such as apolipoprotein E, APOE [45]) or both extra- and intracellular cues remains to be elucidated. A relationship between clasmatodendrosis, astrocytic endfoot changes, and loss of polarity of AQP4 in astrocytes does not allow one to conclude that the astrocytic reaction starts a cascade resulting in increased BBB permeability. It is more likely that interactions especially between endothelial cells and astrocytes, but not excluding pericytes, need to be further elucidated to characterize how the NVU as a whole dysfunctions, especially in capillary beds. The work will be pertinent to further pathophysiological understanding of many disease states now associated with clasmatodendrosis.

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Additional information

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