MINI-SYMPOSIUM: White matter damage in dementia

White Matter Changes in Dementia: Role of Impaired Drainage of Interstitial Fluid
Roy O. Weller1; Cheryl A. Hawkes1; Raj N. Kalaria2; David J. Werring3; Roxana O. Carare1

1 Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.
2 Institute of Neuroscience, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, UK.
3 Stroke Research Group, Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, National Hospital for Neurology and Neurosurgery, London, UK.

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Corresponding author:
Roy O. Weller, MD PhD FRCPath, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Mail Point 806, Southampton SO16 6YD, UK.
(E-mail: row@row@row@soton.ac.uk)

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INTRODUCTION

Leukoaraiosis, seen as cerebral white matter hyperintensities (WMH) on magnetic resonance imaging (MRI) or low attenuation on computed tomographic scanning (CT), is frequent in patients with dementia. However, the causes are unclear and appear to be multiple. It has long been assumed that WMH are due to arteriosclerotic small vessel disease and infarction but another strong candidate is emerging as a result of MRI, clinical and pathological studies. This strong candidate is the failure of elimination of interstitial fluid (ISF) from white matter, especially associated with cerebral amyloid angiopathy (CAA) (92), but also related to other disorders of small cerebral blood vessels such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (16). Pathological studies and detection of dilated perivascular spaces (PVS) by MRI in the white matter in association with evidence of CAA-related intracerebral hemorrhage support a relationship between CAA and impaired drainage of ISF from the white matter. In the wider context, failure of elimination of ISF from the aging brain indicates a failure in disposal of soluble metabolites from the brain with a consequent failure of homoeostasis of the neuronal environment with important implications for the pathogenesis of dementia (16).

In this review, we first set the scene with a description of the clinical and radiological aspects of leukoaraiosis and dilated PVS in relation to intracerebral hemorrhage and lobar microbleeds putatively because of CAA. This is followed by a brief account of the production and elimination of cerebrospinal fluid (CSF) and ISF from the brain and the interrelationships between CSF and ISF. A more detailed account of how ISF and solutes drain from the brain along basement membranes in the walls of cerebral capillaries and arteries in young individuals. Lymphatic drainage of the brain is impaired with age and in association with apolipoprotein E ε4, risk factors for Alzheimer’s disease and cerebral amyloid angiopathy (CAA). Deposition of proteins in the lymphatic drainage pathways in the walls of cerebral arteries with age is recognized as protein elimination failure angiopathy (PEFA), as in CAA and cerebral autosomal dominant arteriopathy and leukoencephalopathy (CADASIL). Facilitating perivascular lymphatic drainage from the aging brain may play a significant role in the prevention of CAA, WMH and Alzheimer’s disease and may enhance the efficacy of immunotherapy for Alzheimer’s disease.

Abstract

White matter abnormalities on magnetic resonance imaging (MRI) are associated with dementia and include white matter hyperintensities (WMH; also termed leukoaraiosis) and visible perivascular spaces (PVS). We review the potential role of impaired drainage of interstitial fluid in the pathogenesis of WMH and PVS. Whereas the volume of extracellular space in the grey matter is tightly controlled, fluid accumulates and expands the extracellular spaces of the white matter in acute hydrocephalus, vasogenic edema and WMH. Although there are no conventional lymphatic vessels in the brain, there is very effective lymphatic drainage for fluid and solutes along restricted pathways in the basement membranes of cerebral capillaries and arteries in young individuals. Lymphatic drainage of the brain is impaired with age and in association with apolipoprotein E ε4, risk factors for Alzheimer’s disease and cerebral amyloid angiopathy (CAA). Deposition of proteins in the lymphatic drainage pathways in the walls of cerebral arteries with age is recognized as protein elimination failure angiopathy (PEFA), as in CAA and cerebral autosomal dominant arteriopathy and leukoencephalopathy (CADASIL). Facilitating perivascular lymphatic drainage from the aging brain may play a significant role in the prevention of CAA, WMH and Alzheimer’s disease and may enhance the efficacy of immunotherapy for Alzheimer’s disease.
role of immune complexes in the impairment of perivascular elimination of Aβ from the brain. Finally, we examine the generic concept of protein elimination failure angiopathy (PEFA) that includes Aβ and other amyloid angiopathies together with prion angiopathies and CADASIL. In this way, we seek to emphasize the wide range of pathologies that are associated with failure of perivascular drainage of ISF and solutes from the brain, particularly in the aging population.

**CLINICAL SIGNIFICANCE OF WMH AND DILATED PVS**

The term leukoaraiosis [from the Greek λευκός (leukos): white; and ἀραιός (araios): rarefied] was coined by Hachinski et al in 1986 (48) to describe changes in the hemispheric cerebral white matter seen on neuroimaging: initially as areas of low attenuation on CT and subsequently as hyperintensities on T2-weighted or Fluid Attenuated Inversion Recovery (FLAIR) MRI. The term was developed to avoid ascribing a particular pathological cause to these increasingly detected imaging findings, which may reflect a range of pathological processes in different populations (eg, in younger patients may be due to leukodystrophy or inflammatory demyelination). Leukoaraiosis is common in patients with cognitive impairment including dementia as well as those with symptomatic cerebrovascular disease (29). In this review, we focus on leukoaraiosis in older individuals, in whom the changes have been presumed to be due to cerebrovascular disease, principally affecting small vessels. Here, we assess to what extent it is possible to correlate fluid levels in white matter on MRI with dilated PVS (Figure 1) and cerebral amyloid angiopathy.

Figure 1. T2-weighted axial image showing MRI-visible PVS in the hemisphere white matter in a patient with CAA-related ICH.

Leukoaraiosis can be defined as white matter abnormality (low density on CT, hyperintensity on T2-weighted or FLAIR MRI), which may be patchy or confluent. These white matter appearances on MRI have variously been termed white matter abnormalities, white matter changes and WMH; a recent consensus group recommended the term “white matter hyperintensities of presumed vascular origin” (111). The term leukoaraiosis nevertheless has the advantage of being applicable to both CT and MRI. Here, we will mainly focus on imaging changes seen on MRI, which will be referred to as “white matter hyperintensities” (WMH).

**White Matter Hyperintensities**

WMH are commonly detected on MRI in various populations: in the general population, the prevalence is between about 10% and 20% in the seventh decade of life and nearly 100% in the ninth decade. WMH are more common and extensive in patients with cardiovascular risk factors and symptomatic cerebrovascular disease (69). WMH appear to be of clinical relevance because of a strong relationship with vascular risk factors and cognitive impairment in cross-sectional studies. WMH also contribute to gait disturbance and falls (9). There is now also a strong evidence that WMH are important for clinical prognosis: a systematic review of 958 published prospective studies found strong evidence of an increased risk of stroke, cognitive decline (especially in the executive function and processing speed domains), dementia and death (29). WMH were significantly associated with an increased risk of stroke, both in the general population and in high-risk populations with a history of stroke or vascular disease, even after adjusting for the potential confounding effect of vascular risk factors known to be associated with WMH (eg, hypertension) (29). WMH were associated with an increased risk of dementia in the general population but not in high-risk populations (29). The mechanisms underlying this association with future cognitive risk may include direct damage to white matter subcortical networks or aggravation of neurodegenerative processes including AD. CT-defined leukoaraiosis has been associated with an increased risk of intracerebral bleeding in patients with stroke treated with high intensity oral anticoagulation with warfarin (43).

Finally, WMH may be a useful biomarker or “intermediate phenotype,” which could help to identify new genetic or environmental risk factors and potentially function as a surrogate end point in clinical trials (111).

The pathological substrates of WMH include tissue rarefaction associated with loss of myelin and axons, enlarged (dilated) PVS (see: Pathology of Dilated PVS) and mild gliosis. These lesions are located in deep hemispheric white matter, typically sparing the subcortical U-fibers, and are often seen in association with vessels affected by small vessel disease including arteriosclerosis. However, the pathological basis of WMH is heterogeneous (44) as the basis of increased signal on T2-weighted and FLAIR MRI is simply an increase in free tissue water content, which is not specific for any particular pathological process. Furthermore, the degree of pathological heterogeneity appears to reduce as the severity of MRI-defined lesions increases from small punctate lesions to confluent WMH; the latter almost always have an ischemic appearance with myelin loss, gliosis and small areas of infarction (36).
Pathophysiological mechanisms underlying WMH

Despite their high prevalence and clear clinical relevance, the pathophysiological mechanisms underlying WMH remain uncertain. One hypothesis is that damage to small medullary perforating vessels, which supply the white matter, causes chronic hypoperfusion, with associated disruption of the blood–brain barrier, leading to both ischemic injury and chronic leakage of potentially toxic plasma proteins into the white matter. However, which comes first (ischemia or blood–brain barrier dysfunction) remains controversial. Evidence supporting an ischemic mechanism of WMH comes from the observation that some forms of hypoxic-ischemic injury cause selective white matter injury (Figure 2). The anatomy of the blood supply to cerebral white matter is also consistent with a vulnerability of deep white matter regions to reduced perfusion and ischemic injury. Moreover, cerebral blood flow is reduced in areas of WMH in patients with small vessel disease (83).

An alternative hypothesis is that WMH (known to be associated with increased tissue water content) are due to alterations in CSF flow. Normal pressure hydrocephalus is known to be associated with WMH and experimental hydrocephalus causes changes in the white matter that can be reversed by shunting (78). However, it is also possible that WMH causes ex vacuo dilatation of the ventricular system suggesting a possible reverse causation. It is suggested that the increased accumulation of CSF in the ventricles raises interstitial pressure in the periventricular parenchyma and causes ischemia of the white matter (93). The potential contribution of impaired ISF drainage to WMH outside the context of hydrocephalus remains even more uncertain.

Dilated Perivascular Spaces

Dilated PVS seen on MRI (Figure 1) have recently emerged as a new MRI marker of small vessel disease, as they are associated with other MRI features of small vessel disease, including lacunes and WMH (30). Dilated PVS have particular interest in the study of ISF dynamics as they are closely linked to the established clearance pathways for ISF and solutes along basement membranes (15, 16) and are enlarged in patients with conditions known to impair ISF drainage, including AD with associated CAA (92). There is no detectable PVS around the arteries in the cerebral cortex (127) whereas PVS may dilate in the white matter and are thought to indicate failure of drainage of fluid and solutes from the white matter (92) (see Pathology of dilated PVS section).

Recent data in cohorts with intracerebral hemorrhage (ICH) (another end result of small vessel disease, but in this case causing fragility) indicate that the anatomical distribution of MRI-visible dilated PVS is linked to the underlying small vessel pathological process: in patients with lobar ICH attributed to probable CAA, severe dilatation of PVS was noted in the cerebral hemispheric white matter, while in other ICH cases (including deep ICH attributed to arteriosclerosis-related rupture of deep basal ganglia perforating small vessels) severe dilatation of PVS was found in the basal ganglia (Figure 2) (21). A similar topographical association of hemispheric white matter PVS with radiologically defined CAA was also noted in a memory clinic cohort, supporting this hypothesis (72). The clinical relevance of dilated PVS is less well established than that of WMH, with no clear association shown with cognitive function (54) and no available prospective data on the risk of future stroke or cognitive impairment.

If dilated PVS in the hemispheric white matter are due to impaired drainage of ISF then they should logically be associated with markers of severe leptomeningeal and cortical arteriolar amyloid deposition in CAA. Indeed, a recent study showed that dilated PVS in hemispheric white matter were strongly linked to one such marker of severe superficial CAA, namely cortical superficial siderosis (because of recurrent subarachnoid bleeding and hemosiderin deposition from fragile, amyloid-laden superficial vessels) (22). Another study used an amyloid-binding Positron Emission Tomography (PET) ligand and showed that increased amyloid binding was associated with the severity of WMH in patients with CAA (but not AD), supporting the hypothesis that vascular amyloid may aggravate or cause white matter injury in CAA (47). However, ischemic injury to white matter associated with CAA in small vessels perforating the cerebral cortex is also possible (45) as an alternative mechanism to impaired ISF drainage. An effect of amyloid on the pathogenesis of WMH is also supported by the finding that in AD, CAA, mild cognitive impairment and white matter changes correlate with serum levels of Aβ1–40 peptide (the main constituent of vascular amyloid deposits in CAA) (46).

PHYSIOLOGY AND PATHOLOGY OF CSF AND ISF OF THE BRAIN

CSF and ISF are the major extracellular fluids associated with the central nervous system (CNS). The total volume of CSF in humans...
is 140 mL with 30 mL in the ventricles and 110 mL within the cerebral and spinal subarachnoid spaces; ISF has an estimated total volume of 280 mL in the CNS (6).

**Production and drainage of CSF**

CSF is produced by the choroid plexuses with a probable contribution from brain ISF (59). Following circulation through the ventricular system, CSF enters the subarachnoid space and passes directly into the blood through arachnoid granulations and villi. An estimated 50% of CSF drains to cervical lymph nodes (60) in smaller mammals along channels that pass from the subarachnoid space through the cribriform plate of the ethmoid bone to join nasal lymphatics (66). This pathway appears to be a major pathway in small mammals and is also present in humans (60, 66, 116).

**Pathology of CSF**

The major pathologies of the CSF are leptomeningitis, infiltration by neoplastic cells (carcinomatous/neoplastic meningitis), and more important in the context of this paper, hydrocephalus because of impaired drainage of CSF. In the acute stages of hydrocephalus, interstitial edema of the periventricular white matter may be observed (112) which suggests that there is restricted capacity for ISF drainage from the white matter that cannot cope with the excess of CSF in hydrocephalus. This may be a further indication of the limited capacity of ISF drainage from the white matter that is present in children with hydrocephalus and may be a factor in the pathogenesis of leukoaraiosis in aging adults.

**Lymphatic drainage of Interstitial Fluid**

ISF is generated from the blood and from metabolic activity within the brain tissue at an estimated rate of 0.1–0.3 μL/minute/g in the rat (1). In the 1980s, Helen Cserr and her group showed that radiolabeled human serum albumin, injected into ISF of the rat brain, drained rapidly to cervical lymph nodes on the same side of the neck (103). The drainage pathway was associated with the walls of the arteries within the brain, leptomeningeal arteries and the upper part of the internal carotid artery in the neck. Absence of radiolabeled human serum albumin in the lower part of the internal carotid suggested that most of the tracer had drained to the cervical lymph nodes. The speed of perivascular drainage was comparable with lymphatic drainage of other organs in the body and only 15% of tracer leaked from the ISF drainage pathways in artery walls into the CSF (103).

In more recent investigations of the ISF drainage pathways, soluble lysine-fixable fluorescein-labeled 3 kDa dextran and Fluorescein Isothiocyanate (FITC)-labeled ovalbumin (40 kDa) were injected separately into the grey matter of the corpus striatum in mouse brains. The distribution of the tracers was defined at 5 minutes, 30 minutes, 3 h and 24 h after injections. By 5 minutes, dextran had spread diffusely in the brain parenchyma and was within the basement membranes of capillary and artery walls (Figure 3) (15). At 30 minutes, dextran had largely disappeared from brain parenchyma and from the walls of capillaries and arteries. At 3 h, dextran was present in a punctate form within perivascular macrophages associated with capillaries and arteries (15). Further analysis by confocal microscopy following intracerebral injection of fluorescent dextrans and fluorescent soluble Aβ revealed that tracers in the extracellular spaces of the brain parenchyma rapidly enter bulk flow pathways in basement membranes in the walls of cerebral capillaries. From there, tracers drain into basement membranes of smooth muscle cells within the tunica media of cerebral arteries (Figure 3) (15, 114). These experiments defined the 100–150 nm thick basement membranes as the route for lymphatic drainage of ISF and soluble metabolites from the brain. The findings are highly relevant to the pathology of human neurodegenerative disease as Aβ is deposited in this pathway in CAA (Figure 4) (16) and relevant to neuroimmunology as antigens may drain from the brain to regional lymph nodes in the neck as part of the induction of neuroimmunological reactions (68, 118).

![Figure 3. Perivascular drainage. ISF and solutes, including Aβ, diffuse through the extracellular spaces of the brain and then drain out of the brain along basement membranes (BM) in the walls of capillaries and arteries. The drainage pathway is along the BM colored green: that is capillary endothelial BM and the BM of smooth muscle cells (SMC) in the mid-zone of the tunica media (TM) of the artery (BM2). In the artery wall, BM on the outer aspect (BM1) and on the endothelial aspect (BM3) is not part of the drainage pathway. With reference to Figure 8, immune complexes (*IC*) are trapped within the BM2 pathway and obstruct perivascular drainage. ENDO, endothelium.](image-url)
Relationship between CSF and ISF

The brain is directly related to the CSF on its ventricular and pial surfaces. CSF appears to play a major role as a buoyancy fluid for the brain. Although there is a well-characterized barrier between the blood and the brain, tracers injected into the CSF diffuse into the surface of the brain and spinal cord as shown by Goldman in 1913 (42). Injection studies by Rennels et al. in the 1980s (89) showed that horseradish peroxidase injected into the cisterna magna diffused from the subarachnoid space into the brain alongside arteries in the cerebral hemispheres to reach capillary level. Fluid entry appeared to be driven by vascular pulsations and was termed convective tracer influx (89). Entry of tracers from the CSF into ISF was confirmed by the injection of fluorescent tracers that revealed an entry pathway from the CSF into the brain along extravascular compartments between cerebral arteries and brain parenchyma (57, 58). Entry of low molecular weight tracers from the paravascular pathway into the brain parenchyma is mediated by aquaporin-4 associated with astrocytes in the brain (57). Because of its association with astrocytes and the eventual drainage of tracers to cervical lymph nodes, the name of the system has been changed from the original terms of convective tracer influx to the “glymphatic system.” The relationship between the glymphatic system and the periarterial basement membrane drainage pathway described in Figure 3 (15) for elimination of solutes from the extracellular space of the brain is unclear. However, it seems that the glymphatic system is a slow equilibration between CSF and ISF. On the other hand, drainage of ISF and solutes along basement membranes in the walls of the arteries is a much more rapid response system that may play a signaling role between solutes in the ISF and smooth muscle cells in the walls of the arteries.

PATHOLOGY OF PERIVASCULAR DRAINAGE OF ISF AND SOLUTES FROM THE BRAIN

The pathology of perivascular drainage of ISF and solutes from the brain relates to two major areas: first, neuroimmunology (68) and second, the failure of ISF drainage in hydrocephalus and perhaps more significantly in the aging brain, CAA and AD (116).

Antigens drain from the brain to regional lymph nodes in the neck with ISF along basement membranes in the walls of capillaries and arteries (15, 103). However, this pathway is too narrow to allow the migration of antigen-presenting cells from the brain to cervical lymph nodes (15, 16). The absence of such effective migration of antigen-presenting cells may be a major factor in the relative immunological privilege of the brain (18, 118). Evidence for a significant role for cervical lymph nodes in neuroimmunological reactions in the brain comes from experiments whereby the severity of experimental autoimmune encephalomyelitis (EAE) is reduced by cervical lymphadenectomy (87, 108). Antigen-presenting cells do appear to migrate from the CSF to cervical lymph nodes via nasal lymphatics and appear to play a role in the induction of immunological tolerance (68).

Deposition of amyloid in perivascular drainage pathways in CAA

CAA is a major pathological manifestation of impaired ISF drainage (16). The presence of CAA in transgenic mice that produce human Aβ solely in the brain is taken as firm evidence that CAA is due to the failure of drainage of Aβ from the brain (53). A number of amyloidogenic proteins are deposited in the basement membranes in the walls of cerebral arteries, the most common of
which is Aβ associated with AD (90). Other amyloids such as cystatin, transthyretin and certain forms of prion protein and the Notch-3 associated protein in CADASIL are also deposited in cerebral vessel walls (16). These disorders have been grouped under the collective term PEFA (16). One of the major pathological effects of impairment of perivascular drainage of ISF and solutes from the brain with age and CAA appears to be loss of homoeostasis of the extracellular environment in the brain, so that metabolites such as soluble Aβ and probably other metabolites accumulate within brain tissue in AD (71, 76). Such a change in the extracellular environment of neurons may result in oxidative stress and ultimately in dementia.

The distribution of Aβ in the walls of cerebral capillaries and arteries in the early stages of CAA mirrors exactly the pathways by which ISF and solutes drain from the brain (16) (Figures 3 and 4). Electron microscope studies indicate that Aβ is initially deposited as fibrils in the central portion (lamina densa) of basement membranes surrounding smooth muscle cells (120), although at the time the authors suggested that the Aβ was derived from vascular myocytes rather than from the brain. One of the major questions about CAA is exactly why amyloid is deposited in basement membranes in the walls of cerebral capillaries and arteries. What interferes with the perivascular drainage of soluble Aβ that causes it to precipitate as fibrillar amyloid in vascular basement membranes?

Age and possession of the ε4 allele of APOE4 are major risk factors for CAA and for AD and the next section of this review shows how both these factors impede the drainage of ISF and solutes along perivascular pathways.

**Impairment of perivascular drainage of ISF and solutes with age and in the presence of apoE4**

Age and possession of APOE4 allele are the strongest risk factors for the development of sporadic AD and CAA. Experiments described here show that age, CAA and possession of APOE4 are associated with impaired perivascular drainage of ISF and solutes, including Aβ.

Perivascular drainage of Aβ was assessed by confocal microscopy following intracerebral injection of 0.5 μL fluorescent dextran or soluble human Aβ40 over a period of 2 minutes into the hippocampus in three groups of animals. First: To test the effects of age on perivascular drainage, intracerebral injections of fluorescent dextran were performed on young and aged wild-type C57B1/6 mice, at 3, 7 and 22 months of age (49). Second: To assess the effect of CAA on perivascular drainage, fluorescent dextran was injected into the brains of Tg2576 transgenic mice with CAA (49). Third: To test the effects of APOE4 on perivascular drainage, intracerebral injection of soluble Aβ40 was performed in targeted replacement (TRE) mice carrying the human APOE ε3 or APOE ε4 allele (50).

The results of these studies showed that perivascular drainage was significantly (P < 0.001–P < 0.05) impaired in capillaries and arteries:

(i) in 22-month-old wild-type mice compared with 3- and 7-month-old animals [furthermore, age-related changes in the levels of laminin, fibronectin and perlecain in vascular basement membranes were observed in these animals (49)] and

(ii) in 7- and 22-month-old Tg2576 mice with CAA (49).

(iii) In addition, Aβ40 aggregated in periarterial drainage pathways in APOE ε4 mice, but not in APOE ε3 or wild-type mice (Figure 5) (50). The number of Aβ deposits was significantly higher in the hippocampi of APOE ε4 mice than in the APOE ε3 mice, at both 3 and 16 months of age, indicating that clearance of Aβ from the brain was disrupted in APOE ε4 mice (50).

In summary, the results of these studies suggest that age, possession of APOE ε4 and the presence of CAA all result in impaired perivascular drainage of solutes, including Aβ, from the brain. This raises the question as to exactly why these factors interfere with perivascular drainage and whether these data apply directly to humans.

**Age changes in arteries and distribution of Aβ in plaques and CAA**

Aged changes in human cerebral arteries are well recognized, with generalized intimal thickening and fibrosis of the tunica media in arteriosclerosis (115). Theoretical and experimental studies suggest that the motive force for perivascular drainage of ISF and solutes is derived from vascular pulsations (2, 98). As arteries age, they become stiffer (115) which would imply that the amplitude of vascular pulsations is reduced. Such a reduction in pulsations may reduce the motive force for perivascular drainage and the consequent slowing of drainage may allow Aβ fibril formation to occur within the basement membranes of the perivascular pathways, particularly as the nature of the basement membrane proteins change with age. Deposition of Aβ in basement membranes in CAA appears to further impede perivascular drainage (49).

ApoE colocalizes with Aβ within plaques in the brain parenchyma and in CAA (106). It is thought that ApoE is a transport molecule for Aβ. Our experiments suggest that Aβ is not transported along perivascular pathways as efficiently in the presence of apoE4 compared to apoE3 (50). The exact reasons for this are not clear but may be related to a difference in association between the isoforms of APOE and basement membrane proteins or an effect of APOE genotype on composition of basement membranes.

There are regional differences in the deposition of Aβ as plaques and CAA in the brains of aging and AD patients (105). CAA is observed predominantly in the parietal and occipital cerebral cortex, less so in the basal ganglia and thalamus and rarely in vessels of the brain stem. Experiments in which Aβ was injected into different areas of aged mouse brains showed that perivascular drainage from the hippocampus became less efficient with age, whereas there was little change in perivascular drainage from the thalamus with age (51). These results suggest that there is a differential effect of age on perivascular drainage in different regions of the brain and this may have some bearing on the distribution of CAA and the deposition of amyloid as plaques in the brain in AD.

**Biochemical and morphological changes in blood vessels of the grey matter that contribute to impaired drainage of ISF**

Although the mechanisms underlying disrupted perivascular drainage in the aged and ApoE4 brain have not been fully
elucidated, multiple lines of evidence support a role for morphological and biochemical changes in blood vessel walls in the pathogenesis of CAA. In vitro, aggregation of Aβ is inhibited and preformed fibrils are destabilized in the presence of the basement membrane proteins laminin, nidogens and collagen IV. By contrast, incubation of Aβ with heparan sulphate proteoglycans, such as agrin and perlecan, accelerate its aggregation. In mice, brain regions that are vulnerable to CAA show age-related decreases in laminin, collagen IV and nidogen 2 and increased levels of fibronectin and perlecan (51). Decreased amounts of collagen IV have been reported in small diameter vessels (<50 μm) in the grey matter in AD, compared with age-matched controls (23). Conversely, the levels of heparan sulphate proteoglycans were increased in AD brains (99). These data suggest that age-related changes in the expression of individual basement membrane proteins may contribute to a pro-amyloidogenic environment within the vessel wall.

Less is known about the effect of ApoE genotype on the biochemistry of cerebral blood vessels, but a significant reduction in the surface area of agrin immunoreactivity has been reported in the brains of AD patients homozygous for the APOE ε4 allele (94). Changes in the levels of collagen IV between 3 and 16 months are greater in TRE4 than in TRE3 mice (50), suggesting that APOE genotype may also influence the degree to which expression of basement membrane proteins changes over the lifespan.

Age-related changes in the morphology of grey matter vessels include splitting, duplication and thickening of the basement membrane, particularly in areas of the brain that are susceptible to AD pathology and CAA (51, 126). The contribution of basement membrane abnormalities to the pathogenesis of CAA is suggested by the observation that an increase in thickness of basement membranes precedes endogenous CAA development in transforming growth factor-β (TGF-β) transgenic mice (121). Increased rigidity in the artery walls, elongation and tortuosity, as well as a reduction in smooth muscle cells are also observed in the aging brain (63). This reduction in vascular elasticity and contractile force may diminish the amplitude of the arterial pulse and slow the driving force for perivascular clearance of ISF and Aβ, leading to its accumulation as CAA.

Pathology of dilated PVS

Dilatation of PVS occurs in the white matter of the cerebral hemispheres (Figure 1), in the midbrain and in the grey matter of the basal ganglia. In some cases, large giant lacunae form in these areas of the brain (95); in most cases, however, the dilatation is more moderate. PVS, which is the potential space between the outer aspect of an artery wall and the brain parenchyma, can be expanded experimentally by the intracerebral injection of Indian ink particles (128) or fluorescent polystyrene beads (fluorospheres) (15). However, the PVS does not appear to function as a fluid drainage pathway in the same way as the basement membrane pathways within the artery walls themselves. Injection of pharmacological preparations into the brain to expand PVS (intrastratal convection-enhanced delivery) has been developed to create a reservoir for slow release of drugs for the treatment of neurological disorders (5).

There is evidence that dilatation of PVS in the white matter is associated with CAA (92). Figure 6 shows how...
White Matter Changes and Impaired Drainage of ISF

Figure 6. Proposed mechanism for CAA-induced dilatation of perivascular spaces in the white matter. Branches of the leptomeningeal arteries penetrate the cortex (often without branching) to supply the subcortical white matter. CAA in the walls of the leptomeningeal arteries (yellow banding) obstructs the drainage of interstitial fluid and solutes resulting in retention of fluid in dilated perivascular spaces around arteries in the white matter.

WMH AND CAA IN Aβ IMMUNOTHERAPY TRIALS FOR AD

Aβ immunotherapy impairs the elimination of solutes from the brain

Trials of immunotherapy for AD in humans were introduced after successful studies in human amyloid precursor protein (hAPP) transgenic mice showed that insoluble Aβ plaques were removed from the brain following immunization with Aβ42 (97). However, despite the clearance of plaques in patients with AD (39, 73, 79), active immunization seemed to increase, rather than decrease, the amount of arterial CAA both in transgenic animals and in humans (79, 119). It appears that Aβ can be solubilized from the plaques but becomes trapped in the perivascular drainage pathway (85, 117), manifesting as an increase in the severity of CAA. The dynamics of the events resulting in increased CAA following immunotherapy are not clear but it could reflect either increasing flow of Aβ out of the brain in the perivascular drainage pathway or impaired drainage and elimination of soluble Aβ as it becomes impeded by aggregated Aβ and possibly by immune complexes. In addition, human Aβ immunotherapy trials, both active (8, 41) and passive (64, 65), encountered side effects comprising focal abnormalities in the cerebral white matter on imaging, interpreted as amyloid-related imaging abnormalities, a lymphocyte response in the CSF and variable deterioration in neurological function (102). The pathophysiology underlying these side effects is as yet unclear; they may be due partly to the impaired drainage of ISF associated with the increase severity of CAA but may also be due to the effects of immune complexes in relation to the cerebral vasculature.

In CAA, Aβ is seen initially in the basement membranes (Figure 4) and then occupies the whole thickness of the walls of arteries and capillaries, suggesting that insoluble Aβ is deposited in the ISF drainage pathway (16, 18, 24, 40).

Active immunization of humans and mice with Aβ results in decreased plaque load that correlates with the anti-Aβ titre in serum, but the exact mechanism by which antibodies remove Aβ is not clear (10). It seems likely that anti-Aβ IgG enters the brain and may form Aβ immune complexes, interfering with vascular function and impairing the perivascular drainage of Aβ. Immune complexes formed in the brain parenchyma generate a robust and long-lasting inflammatory response, characterized by increased expression of the microglia markers CD11b, CD68 and FcRII/III, but this feature is absent in the immunized Fcγ receptor-null mice (104).

Immune complexes in the walls of arteries disrupt perivascular drainage of ISF and solutes

We tested the hypothesis that immune complexes disrupt the perivascular drainage of solutes along the walls of arteries in normal brains but not in brains of mice lacking Fcγ receptor. The schedule for the experiments is summarized in Figure 7. Using the presence of complement C3, IgG and ovalbumin as markers for immune complex formation (101), we demonstrated that by 24 h, immune complexes are localized to the basement membranes of branches of the leptomeningeal arteries on the surface of the brain pass through the cerebral cortex, often without branching, to supply the underlying white matter (33). Perivascular drainage of fluid and solutes from the white matter seems to be impaired by CAA in the parent leptomeningeal or cortical arteries with consequent dilatation of PVS in the white matter (92) (Figure 6). Dilatation of PVS in the cortical grey matter is rarely seen.

The other major site for dilated PVS is the grey matter of the basal ganglia. This is evident in several diseases involving small cerebral arteries. The structure of arteries in the basal ganglia differs from arteries in the cerebral cortex (88, 127). As arteries enter the surface of the cerebral cortex, the pia mater is reflected from the surface of the brain on to the vessels in the subarachnoid space, thus separating the subarachnoid space from the cerebral cortex (55, 113, 127). There is no PVS around cortical arteries in the normal brain as layers of smooth muscle, basement membrane and one layer of leptomeninges are all compressed together (127). In the basal ganglia, however, there are two layers of leptomeninges surrounding the artery with a PVS between the layers (88). This anatomical arrangement may explain why there is no expansion of PVS in the cortex, whereas perivascular lacunae are frequently seen in the basal ganglia (95, 113).
the cerebral vasculature (17). This response is slower than the one seen systemically, where immune complexes form within 10 minutes (70).

Normally, when solutes similar in molecular weight to Aβ are injected in the grey matter, they diffuse through the extracellular spaces of the brain and drain along the basement membranes of capillaries and arteries within 5 minutes (15). In the animals in which immune complexes had been allowed only 5 minutes to form, there was no change in the pattern of distribution of soluble dextran of 3 kDa at 5 minutes after its injection. At this time, immune complexes were not detected in the cerebrovascular basement membranes, permitting drainage of solutes in a physiological manner.

At 24 h after the formation of immune complexes, confocal microscopy revealed immune complexes in arterial basement membranes (Figures 3 and 8). Quantitative assessment of perivascular drainage in artery walls demonstrated that the drainage of solutes from the brain was significantly impaired by the presence of immune complexes in perivascular drainage pathways (17).

Normally, by 24 h after its injection, dextran has been cleared from the extracellular spaces and is present only in perivascular macrophages around arteries in the ipsilateral hemisphere (15). When left in situ for 24 h in mice with previously formed immune complexes, dextran is taken up by macrophages in the ipsilateral parenchyma, around veins, and by meningeal macrophages. This suggests that, in the presence of inflammation induced by immune complexes, solutes may become sequestered by inflammatory cells and not cleared from the brain parenchyma.

At 7 days following the formation of immune complexes, the pattern of drainage of soluble dextran was remarkably different. A striking feature was the presence of dextran in the cellular infiltrates around veins. This pattern of distribution suggests that the dextran tracer is diverted from the extracellular space toward the larger perivascular spaces around veins where it is taken up by activated macrophages. We did not observe cuffs of dextran around capillaries or arteries. There was a trend for the number of capillaries and arteries with dextran associated with their basement membranes to decrease in the animals in which immune complexes had been left for 7 days, but this did not reach statistical significance. These results suggest that even in the presence of inflammatory cells associated with the process of immune complex formation and the taking up of dextran by macrophages, perivascular drainage had returned to normal by 7 days.

In animals without the Fcγ receptor, perivascular drainage was normal, with dextran tracer draining along the basement membranes of capillaries and arteries, with very few veins involved. This is consistent with the absence of inflammatory cells recruited around veins in immunized Fcγ-deficient mice (104).

There are no reports of immune complexes forming in the brains of humans immunized against Aβ. However, it is possible that Aβ solubilized from plaques may encounter Aβ-specific antibodies, generated in the process of immunotherapy, in the walls of cerebral blood vessels. We know that there are high levels of complement in transgenic mice that harbor Aβ mutations and that complement colocalizes with the microvascular Aβ (35). Increased levels of C3 were also found to be associated with increased hemorrhages following experimental immunotherapy (26, 52, 86, 109), suggesting that immune complex formation may explain these side effects. It has been reported that, following immunotherapy with Aβ in mouse models, small amounts of anti-Aβ antibodies enter the brain and form immune complexes that are cleared from the brain.
White Matter Changes and Impaired Drainage of ISF

Weller demonstrate that deletion of Fcγ receptors present ubiquitously in the endothelia (28). The risk of intracerebral hemorrhage may increase if immune complexes are formed in the basement membranes of capillaries and arteries affected by CAA. The vessel wall is already structurally weak from the deposition of basement membranes, such as increased collagen in arteries that form the aging and deposition of Aβ, it may be sufficient to cope with the drainage of the Aβ solubilized from plaques, without rupturing the vascular wall.

It remains unclear whether immune complexes are formed in the extracellular spaces of the brain and then follow the perivascular drainage route, or whether they form in situ within the arterial basement membranes, or indeed whether immune complexes are formed at all after immunization with Aβ. Our results suggest that the absence of Fcγ receptors restores the ability of normal perivascular clearance of solutes, supporting recent studies that demonstrate that deletion of Fcγ receptors prevents the cognitive impairment associated with AD (37).

Future prophylactic and therapeutic strategies in aging and CAA will need to take into account the possibility that immune complexes may interfere with perivascular drainage in patients treated with Aβ immunotherapy.

PEFA IN CAA AND CADASIL

As discussed earlier, we have previously proposed reasons why both CAA and CADASIL are categorized as PEFA disorders (16). Although different types of PEFA involve failure of removal of specific proteins, they share several risk factors, pathological processes and a final common pathway, which also typify them as angiopathies. CAAs are characterized by the vascular deposition of different proteins such as Aβ, cystatin C and transthyretin (91). In prion disease, insoluble prion protein accumulates mainly in the brain parenchyma but the perivascular clearance of anchorless prion protein induces PEFA-CAA (16). The most common type of PEFA-CAA is associated with the deposition of Aβ, which increases in old age and is observed in over 90% of subjects with AD. All CAAs including the Aβ type, however, foremost entail the parenchymal production or impaired metabolism of specific protein(s), which aggregate to accumulate in predominantly cortical areas within the brain. Several processes including uptake by macrophages or microglia are engaged in the clearance of insoluble and degraded proteins from the cerebrum. However, the perivascular lymphatic drainage pathways are an important route for the bulk removal of degraded proteins and solutes along with the ISF. With increasing age and likely greater volumes of solutes and degraded proteins, this unique perivascular system gradually loses its efficiency (49, 116). Walls of cerebral arteries exhibiting arteriosclerosis also stiffen with age and lose their elasticity over time. As a consequence of such weakening, arteries fail to revert to their original form following the pulse wave, leaving them dilated and with a stretched internal elastic lamina (116). Age-related change within vascular basement membranes, such as increased collagen in arteries that form the perivascular pathways (14), is another factor that contributes to the inflexibility of the vessel wall and favors deposition of insoluble proteins and aggregates such as Aβ (7, 107).

CADASIL is characterized by widespread arteriosclerosis, multiple lacunar infarcts and microinfaracts within subcortical grey and the deep white matter (123). There is an exclusive and widespread distribution of the granular osmiophilic material (GOM) in the walls of leptomeningeal arteries and cerebral arterioles. In moderately advanced cases, an abundance of GOM is readily detected as electron-dense masses sited at intervals around vascular smooth muscle cells and along capillaries. The particulate rather amorphous, 0.2–2 μm-sized Periodic acid-Schiff (PAS) positive granular deposits accumulate in arteries (Figure 9) but may also be found in capillary basement membranes, frequently associated with pericytes (34). Analogous to PEFA-CAA, the loss of arterial smooth
muscle cells in CADASIL reduces vascular tone and amplitude of pulsations for the propagation of perivascular lymphatic drainage and results in further accumulation of other insoluble proteins along with GOM. These deposits predominantly contain NOTCH3 fragments but may accrue specific extracellular matrix proteins produced by vascular smooth muscle cells and capillaries (123). The significance of the localization of GOM in the vasculature in terms of the pathogenesis of CADASIL is still unclear. It is likely that Extracellular Domain of the NOTCH3 Receptor (N3ECD)-containing GOM is secreted by the vascular smooth muscle and endothelial cells, possibly to remove potentially cytotoxic, aggregation-prone mutant N3ECD, but its deposition and failure of subsequent removal from the vessel walls incorporates CADASIL within the spectrum of PEFA.

In CADASIL, vascular smooth muscle cells carrying copies of the mutated NOTCH3 receptor appear affected by age-related physiological changes and there is lack of normal replacement of cells such that continued cell-to-cell signaling is impaired causing ultimate breakdown in the integrity of the vascular wall. These changes impact on function of vascular smooth muscle cells leading to abnormal arterial tone or contractibility (31, 61). The lack of muscular tone in turn would reduce the motive force for perivascular drainage of solutes in the walls of long-perforating arteries downstream to the white matter. Although such aberrations occur in key targeted arterial segments defined by the cerebrovascular architecture (77) they presumably spread along the length of the vessel and extend to other domains within the microvascular network (81). Focal vascular insufficiency in end arteries likely makes the poorly perfused regions such as the white matter susceptible to infarcts.

The small deposits of GOM may also adhere to microvascular walls, even inertly, but their accumulation in perivascular drainage routes suggests that perivascular drainage of fluid and solutes is impaired in CADASIL. One of the consequences of this could be the characteristic enlargement of PVS (20). The dilated PVS result from an even greater burden and insufficiency in the drainage of ISF and degraded protein products into the lymphatic system (116). However, such enlargement could also be attributed to the loss of white matter in tandem with the increased tortuosity and kinking of normally perforating vessels with focal loss of cellular elements within artery walls.

White matter changes are particularly prominent in CADASIL. This is because GOM is widespread even along white matter end arteries and capillaries whereas in Aβ CAA, Aβ deposition is largely restricted to cortical regions and the white matter changes may result from effects of deposits within the cortex that affect perfusion of the deep white matter as a consequence.

**White matter damage and PEFA**

Diffuse and focal white matter changes are a surrogate of small vessel diseases of the brain and common to both CADASIL and CAA (Figures 1 and 10). They manifest as cerebral WMH on T2-weighted MRI or leukoaraiosis as a decreased signal on CT. There is some controversy as to whether deep or periventricular T2-weighted MRI or leukoaraiosis as a decreased signal on CT. There is some controversy as to whether deep or periventricular

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**Figure 10.** WMH in CADASIL. FLAIR MRI scan shows the extent of confluent white matter changes in a 56-year-old CADASIL subject carrying the p.Arg182Cys mutation.
White matter infarcts in CADASIL

Recent imaging studies in CADASIL examined the spatial relationship between the development of incident lacunes or small infarcts and WMH in relation to the vascular anatomy to reveal the mechanistic links between the two lesion types. Remarkably, the majority (>90%) of lacunes develop at the edge of WMH rather within or outside and they also develop proximal to a WMH along the course of perforating vessels supplying the respective brain region (32). These findings suggest that the mechanisms of lacunes and WMH are intimately connected but they identify the edge of WMH as a predilection site for lacunes. As discussed earlier, it is possible that white matter changes in CAA may arise because of several mechanisms; it is plausible that microinfarcts and border zone infarcts associated with CAA (80) are progenitors of changes in the deep white matter.

Impaired blood flow and cellular mechanisms in CADASIL

In CADASIL, the widespread white matter axonal changes, particularly in the frontal lobe, may arise from differential stenosis and sclerosis of arterioles (25), possibly affecting certain axon bundles connecting to targets in the subcortical structures, specifically degeneration of thalamocortical pathways causing cortical atrophy (62). Lesions within the white matter also include spongiosis, that is vacuolation (122). Consistent with this finding, increased volumes of PVS were related to white matter myelin protein changes (122), indicating that reduction in white matter volume is possibly one factor that causes dilated PVS.

The cellular mechanisms involved in loss of myelin and abnormalities in axons in CADASIL are yet to be determined. Lack of integrity of artery walls and endothelia is likely to reduce both blood flow and volume in affected frontotemporal white matter (centrum semiovale) and subcortical grey matter structures and affect the hemodynamic reserve by decreasing the vasodilatory response. As supported by previous studies (20), this indicates that blood flow and volume are reduced in demented CADASIL subjects compared with those with no cognitive impairment. Endothelial cell abnormalities and angiopathic changes may also contribute to white matter damage and blood–brain barrier disruption can cause osmotic demyelination. Disruption of the blood–brain barrier would also result in increased permeability of the vessel wall and leakage of adverse factors, such as macrophages, lymphocytes and complement (123). Neuronal apoptosis has been described predominantly in neocortical layers III and V as a crucial mechanism for cell death in CADASIL (110). It is unclear whether this occurs secondary to the cerebral hypoperfusion, particularly in the white matter, but it is likely to contribute to cortical atrophy and to affect frontal lobe cognitive functions (20).

Another factor that could possibly impact the white matter changes in both CADASIL and CAA is the occlusion of veins and venules by collagenous thickening of the vessel walls. Venous collagenogenesis increases with age and it has been demonstrated that perivenous collagenogenesis increases in concert with leukoaraiosis (11). The presence of apoptotic cells in white matter adjacent to areas of leukoaraiosis suggests that such lesions are dynamic, with progressive cell loss and expansion (11). In turn, vascular stenosis caused by collagenogenesis may induce chronic ischemia or edema in the deep white matter leading to capillary loss and more widespread effects on the brain (12, 13).

CONCLUSIONS

In this review, we have examined the clinical and pathological evidence for the failure of elimination of ISF and solutes from the brain as a significant cause of pathology, particularly in the aging brain. This failure is reflected in the PEFA that extends from the common amyloid angiopathies to the less common CADASIL. The failure of ISF drainage, particularly from the aging brain and compounded by amyloid angiopathy, is a significant factor in the pathogenesis of dementia. Development of therapeutic strategies that facilitate the drainage of ISF and soluble metabolites from the aging brain would be a fruitful path to follow for the prevention and treatment of dementia.

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