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REVIEW

Lymphatic drainage of the brain and the pathophysiology of neurological disease

Roy O. Weller · Effie Djuanda · Hong-Yeen Yow · Roxana O. Carare

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Abstract There are no conventional lymphatics in the brain but physiological studies have revealed a substantial and immunologically significant lymphatic drainage from brain to cervical lymph nodes. Cerebrospinal fluid drains via the cribriform plate and nasal mucosa to cervical lymph nodes in rats and sheep and to a lesser extent in humans. More significant for a range of human neurological disorders is the lymphatic drainage of interstitial fluid (ISF) and solutes from brain parenchyma along capillary and artery walls. Tracers injected into grey matter, drain out of the brain along basement membranes in the walls of capillaries and cerebral arteries. Lymphatic drainage of antigens from the brain by this route may play a significant role in the immune response in virus infections, experimental autoimmune encephalomyelitis and multiple sclerosis. Neither antigen-presenting cells nor lymphocytes drain to lymph nodes by the perivascular route and this may be a factor in immunological privilege of the brain. Vessel pulsations appear to be the driving force for the lymphatic drainage along artery walls, and as vessels stiffen with age, amyloid peptides deposit in the drainage pathways as cerebral amyloid angiopathy (CAA). Blockage of lymphatic drainage of ISF and solutes from the brain by CAA may result in loss of homeostasis of the neuronal environment that may contribute to neuronal malfunction and dementia. Facilitating perivascular lymphatic drainage of amyloid-β (Aβ) in the elderly may prevent the accumulation of Aβ in the brain, maintain homeostasis and provide a therapeutic strategy to help avert cognitive decline in Alzheimer's disease.

R. O. Weller (✉) · E. Djuanda · H.-Y. Yow · R. O. Carare
Clinical Neurosciences, Southampton General Hospital,
University of Southampton School of Medicine,
LD74, South Lab and Path Block, Southampton SO16 6YD, UK
e-mail: row@soton.ac.uk

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Introduction

The central nervous system (CNS) does not possess defined lymphatic channels that are comparable with lymphatics vessels in organs elsewhere in the body, but this does not mean that the brain is devoid of lymphatic drainage. Both cerebrospinal fluid (CSF) and interstitial fluid (ISF) drain partly or wholly to regional lymph nodes [1, 17, 18]. Lymphatic drainage of the CNS has implications for neuroimmunology and for homeostasis of the neuronal environment in the brain [1].

Cerebrospinal fluid and ISF appear to drain by separate routes from the brain, especially in humans. However, there are interrelationships between the two fluids that become more significant when drainage of CSF or ISF is impaired by disease processes.

Most of our knowledge regarding the physiology and anatomy of CSF and ISF drainage pathways comes from animal experiments [13, 40]. However, there are significant differences between animals and humans. In particular, up to 50% of CSF in most animals drains to lymph nodes [18, 40], whereas the greater proportion of CSF in adult humans appears to drain directly into venous blood through arachnoid villi and granulations [19].

In this review, we examine the separate routes for lymphatic drainage of CSF and ISF from the CNS and explore

the ways in which the two fluids are interrelated. We will discuss the evidence that lymphatic drainage of antigens from the brain plays a role in neuroimmunology and in autoimmune diseases of the CNS. Finally, we will review the role of impaired lymphatic drainage of the brain in the pathogenesis of Alzheimer's disease.

Lymphatic drainage of CSF

Cerebrospinal fluid is produced by the choroid plexuses and flows from the ventricles into the subarachnoid space [40]. Lymphatic drainage accounts for the elimination of approximately 50% of CSF in rats, rabbits and sheep and the rest of the CSF drains either directly back into the blood through arachnoid villi [9, 18] or may be absorbed into the blood vessels of the brain parenchyma [33]. The picture is rather different in adult humans in whom the majority of CSF appears to drain directly into the blood through arachnoid villi and granulations [40] and lymphatic drainage appears to play only a minor role [22] except perhaps in neonates before arachnoid villi develop [33, 40].

Pathways for lymphatic drainage of CSF in experimental animals

Most studies of CSF drainage have been performed in rats, rabbits and sheep using a variety of tracers such as Indian ink [14, 44, 75], ^{125}I-labelled human serum albumin (RISA) [85] and Microfil [40]. In rats, CSF is produced by the choroid plexuses in the cerebral ventricles at a rate of 3–5 μl/min [19] and passes into the subarachnoid space. Tracers injected into the CSF of the cisterna magna flow forwards to the inferior surfaces of the olfactory bulbs and drain into nasal lymphatics and then to deep cervical lymph nodes [44]. Closer examination of this pathway reveals a direct connection between the subarachnoid space and lymphatic channels passing through the cribriform plate of the ethmoid bone alongside olfactory nerves and into the nasal submucosa (Fig. 1) [44]. Dissection of the neck in these animals reveals tracer in the cervical lymph nodes [44]. The cerebral subarachnoid space in the rat is restricted to the basal cisterns and to channels surrounding major arteries. Tracers injected into the CSF over the vertex of the cerebral hemispheres drain along periarterial CSF channels to the circle of Willis and then alongside the ethmoidal artery to the under-surface of the olfactory bulbs [96, 110].

The speed of drainage of CSF via the cribriform plate has been measured in rats by injecting RISA into the lateral ventricles of rats [60]. Average concentrations of RISA were highest in the middle turbinates in the lateral wall of the nose 30 min after injection [60]. Indian ink tracers injected into the CSF of the cisterna magna reach the

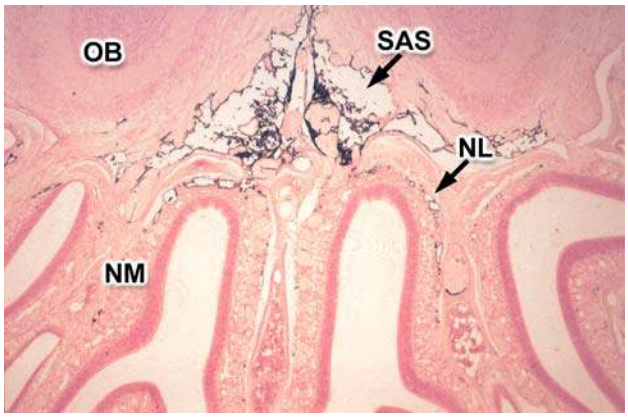

Fig. 1 Drainage of CSF into nasal lymphatics in the rat. Indian ink (*black*) in the CSF of the subarachnoid space (*SAS*) under the olfactory bulbs (*OB*) drains through the cribriform plate into lymphatics (*NL*) in the nasal submucosa (*NM*) (reproduced with permission from Kida et al. [44])

cervical lymph nodes via the nasal mucosa within 30 min and the lumbar lymph nodes via the spinal CSF within 6 h [44]. In large mammals, such as adult sheep, the capacity for nasal lymphatics and arachnoid villi to drain CSF seems to be approximately equal, and drainage along both pathways increases with a rise in intracranial pressure [9]. However, under normal circumstances, drainage of CSF directly into the blood via arachnoid villi is secondary and complimentary to the nasal route [107]. In new born lambs, the nasal pathway is the primary route as arachnoid villi do not develop until later in development [65]. Lymphatic drainage of CSF through the cribriform plate and into the nasal mucosa has been well demonstrated in many animal species including sheep, pigs and monkeys by the injection of Microfil tracers injected into the CSF [41]. Other routes of lymphatic drainage of CSF have been identified in rats especially around cranial and spinal nerves and via dural lymphatics [18, 44] (Fig. 2).

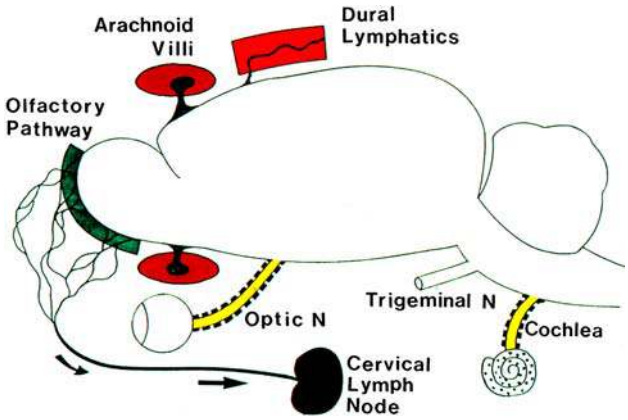

Fig. 2 Diagram showing the routes of drainage of cerebral CSF in the rat (reproduced with permission from Kida et al. [44])

Pathways for lymphatic drainage of CSF in humans

Compared with experimental animals and even large animals such as sheep, the volume of CSF produced in humans is very high. Sheep produce 118 μl/min CSF, whereas humans produce 350 μl/min [19]. Furthermore, the volume of CSF in humans is higher than that in other species with 30 ml in the ventricles and 110 ml in the subarachnoid spaces [7]; CSF is thought to play a major role as a buoyancy fluid for the large human brain [40]. The majority of the CSF in humans appears to drain directly back into the blood via well-developed arachnoid villi and granulations in the walls of major venous sinuses [40], but some CSF drains into the nasal mucosa as shown by Indian ink and Microfil injections postmortem [41, 111]. Villous projections of arachnoid extend through the cribriform plate alongside olfactory nerves into the human nasal submucosa [22] in a similar arrangement to the rat [44]. There are however no data available regarding the quantity of CSF that drains by this route and how much CSF actually reaches lymph nodes in the neck in humans. Small arachnoid villi are present in the nasal mucosa adjacent to veins, but how much CSF drains into the blood by this route is unknown [22]. Occasional reports of intracranial tumours metastasising to cervical lymph nodes [32] suggest that neoplastic cells may pass along this route from CSF to cervical lymph nodes.

Arachnoid villi and granulations for drainage of CSF

Arachnoid villi are small in rats and located mainly dorsal to the olfactory bulbs [44] (Fig. 2); even in sheep, arachnoid villi are not well developed. In humans, arachnoid villi and granulations develop during early childhood forming organised structures in the walls of major venous sinuses [19]. Prolongations of arachnoid mater project through the dural wall of the sinus to form a core of channels separated by collagenous trabeculae at the centre of the arachnoid granulation [45, 87, 92, 95]. Surmounting the core is a cap of arachnoid cells through which channels carry CSF to the venous sinus endothelium [45, 87]. Bulk flow of CSF into the blood appears to occur via large vacuoles that form on the abluminal aspect of the endothelial cells, pass through the cell and expel their contents into the venous blood [86].

In addition to the prominent arachnoid granulations in the superior sagittal sinus, smaller arachnoid villi have been observed in venous sinuses associated with cranial nerves (Kelsey and Weller, unpublished observation) and on almost every thoracic and lumbar spinal nerve root in primates and humans [46, 91].

The major route for drainage of CSF in humans appears to be into venous blood via arachnoid villi and granulations. Although some lymphatic drainage of CSF may occur in humans via nasal mucosa and spinal nerve roots, it is also possible that even with these routes, CSF may pass into the blood through small arachnoid villi [22, 91]. Newborn infants lack prominent arachnoid villi and granulations in the superior sagittal sinus and this has been used as an argument against the drainage of CSF via these structures and in favour of drainage into cerebral capillaries [33, 63]. However, there are no data available regarding the number of arachnoid villi associated with spinal and cranial nerve roots that could be the major route of drainage for CSF in the newborn pending the development of villi and granulations in the major cranial venous sinuses. Alternatively, the nasal route for drainage of CSF [111] may be more important in the newborn than in adults.

Immunological aspects of lymphatic drainage of CSF

The immune reactivity of the CSF compartment including the stroma of the choroid plexus, the meninges and circumventricular organs is similar to that in the periphery [28]; none of these regions has a blood–brain barrier. When soluble antigens are injected into the CSF, they evoke an antibody response in the cervical lymph nodes, which is enhanced compared with antibody responses elsewhere in the body [18]. Myelin basic protein that is used to induce experimental autoimmune encephalomyelitis (EAE) however induces an immunosuppressive response when injected into CSF [18]. There is evidence that lymphocytes traffic from the brain with CSF via the cribriform plate and nasal mucosa to cervical lymph nodes [31].

Lymphatic drainage of interstitial fluid

Interstitial fluid is derived from the blood and from the metabolic activity of the tissue itself [1]. ISF in the CNS is equivalent to ISF in other tissues, although the blood–brain (blood–ISF) barrier normally exerts tight control on the entry of solutes into the CNS in a way that is not seen in most other tissues [1, 5]. In organs other than the CNS and the eye, ISF is the only extracellular fluid outside the vascular system; most organs do not have the equivalent of CSF. Lymphatic drainage of ISF from the CNS differs significantly from lymphatic drainage of most other organs and is less well understood. Here, we will outline the major features of lymphatic drainage of organs other than the brain and then compare them with lymphatic drainage of the CNS.

Lymphatic drainage of ISF and immune cells from organs other than the brain

Organs such as the lungs, gut and skin have well-defined lymphatic drainage channels distinct from arteries and

veins [105]. Tissue fluid that does not return to the blood drains along thin-walled lymphatics lined by endothelium to regional lymph nodes. Lymphocytes, macrophages, reticulum cells, tissue debris, tumour cells, bacteria and soluble antigens also drain along lymphatics to lymph nodes. Lymphatic drainage is driven by filtration pressure, contraction of adjacent muscles and pulsations of neighbouring arteries [105]. The soluble antigens, lymphocytes and antigen-presenting cells that pass along lymphatics to lymph nodes constitute the afferent arm of the adaptive immune system. Antibodies formed in the lymph nodes and the effector lymphocytes of the immune system pass into efferent lymph, and circulate through the blood to their target tissues [28].

Perivascular drainage of ISF and solutes from the brain in experimental animals

Early work on ISF drainage from the brain employed the injections of soluble radioactive tracers, horseradish peroxidase, Evans blue and particulate Indian ink into the caudate putamen and other sites in the brains of rabbits and rats [10, 14, 85]. These studies showed that tracers were associated with the walls of cerebral arteries and that they drained to lymph nodes on the same side of the neck as the injection.

Injection of fluorescent tracers ranging from 3 kDa fixable dextran to 40 kDa ovalbumin has revealed the ISF drainage pathways within vessel walls [13]. Within 5 min of injection into the caudate putamen of a mouse brain, fluorescent tracers spread diffusely in the brain parenchyma and are located in capillary and artery walls (Fig. 3). Confocal microscopy and immunocytochemistry showed specific colocalisation of 3- and 40-kDa tracers with laminin in the basement membranes surrounding capillaries and in the basement membranes between smooth muscle cells in the tunica media of arteries. Some uptake of tracer by vascular smooth muscle cells was also observed. By 30 min after injection, the fluorescent tracers were no longer present in the brain parenchyma or in vascular basement membranes. However, a small amount of tracer was located in perivascular macrophages on the outer aspects of artery walls in brain parenchyma and leptomeninges [13].

Probably due to the small volumes injected, fluorescent tracers could not be traced beyond the leptomeningeal arteries on the surface of the brain [13]. Previous studies, however, have shown that radioactive tracers and horseradish peroxidase injected into the brain pass along the adventitia of the larger intracranial arteries and to lymph nodes in the neck [85]. Tracers were not present in the wall of the internal carotid artery in the neck suggesting that they had drained to lymph nodes at the base of the skull [85]. ISF drains from brain to cervical lymph nodes at

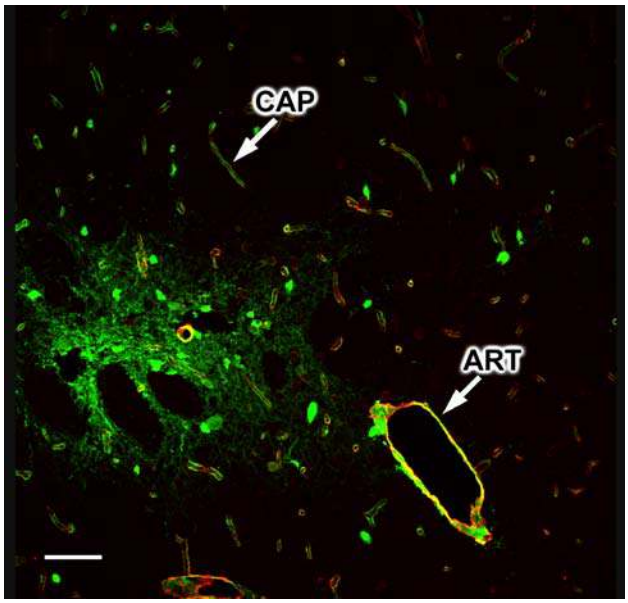

Fig. 3 Perivascular lymphatic drainage of ISF from the mouse brain. Fluorescent ovalbumin (*green*) has spread diffusely in the grey matter and is in the walls of capillaries (*CAP*) and arteries (*ART*) within 5 min of injection. Yellow colouration indicates colocalisation of laminin staining (*red*) and ovalbumin (*green*) in the basement membranes of the walls of capillaries and arteries. Confocal microscopy. Immunocytochemistry for laminin. *Bar* 50 μm (reproduced with permission from Carare et al. [13])

0.11–0.29 μl/g brain/min, which is comparable to the rate of lymphatic drainage in the rest of the body [85]. As little as 10–15% ISF appears to pass into the CSF [85]; this suggests that the perivascular route for drainage of ISF from the brain is largely separate from CSF.

Perivascular drainage of ISF and solutes from the human brain

Injecting tracers into the human brain to investigate lymphatic drainage of ISF and solutes is not feasible, but there is a natural tracer in humans in the form of amyloid-β (Aβ) that outlines the perivascular lymphatic drainage pathways of the human brain [97, 100]. Aβ is derived from the transmembrane protein amyloid precursor protein (APP) by enzymic cleavage. APP is some 700 amino acids long and cleavage by a series of secretase results in Aβ peptides of varying length [89, 90]. Two Aβ peptides in particular Aβ 1–40 and Aβ 1–42 (40 and 42 amino acids long, respectively) are present in the brain in soluble form and aggregate as insoluble amyloid fibrils to form plaques in brain tissue. Aβ also deposits in blood vessel walls as cerebral amyloid angiopathy (CAA) [72] (Fig. 4).

In the early stages of CAA, Aβ is deposited in basement membranes of capillary and artery walls in exactly the same distribution as fluorescent tracers injected into mouse

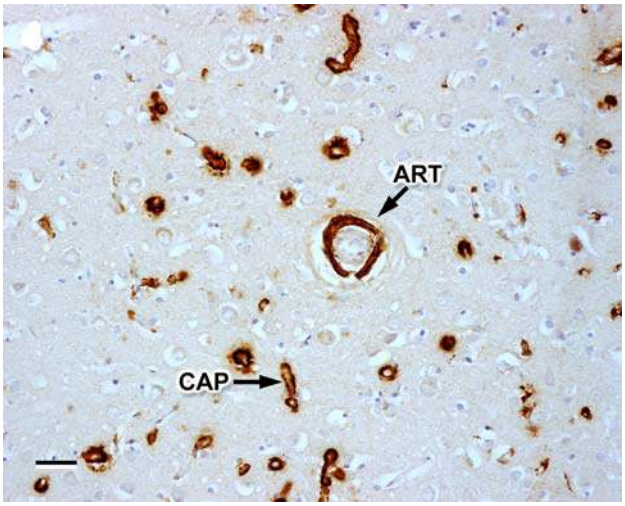

Fig. 4 Cerebral amyloid angiopathy (*CAA*) in Alzheimer's disease. Transverse section of a cortical artery and its capillary bed showing deposits of Aβ in the walls of an artery (*ART*) and its capillaries (*CAP*). Immunoperoxidase for Aβ: *Bar* 30 μm

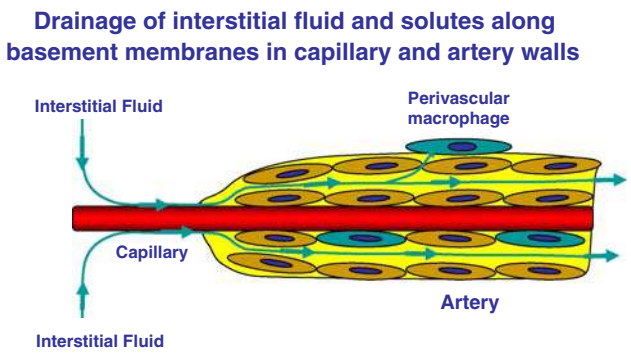

Fig. 5 A summary diagram showing how interstitial fluid and solutes (*green line*) drain out of the brain along basement membranes in capillary walls, and along basement membranes between smooth muscle cells in the tunica media of arteries. Some smooth muscle cells (*brown*) in the tunica media and perivascular macrophages on the outer aspect of the artery wall take up soluble tracer [13] (these cells are coloured *green*)

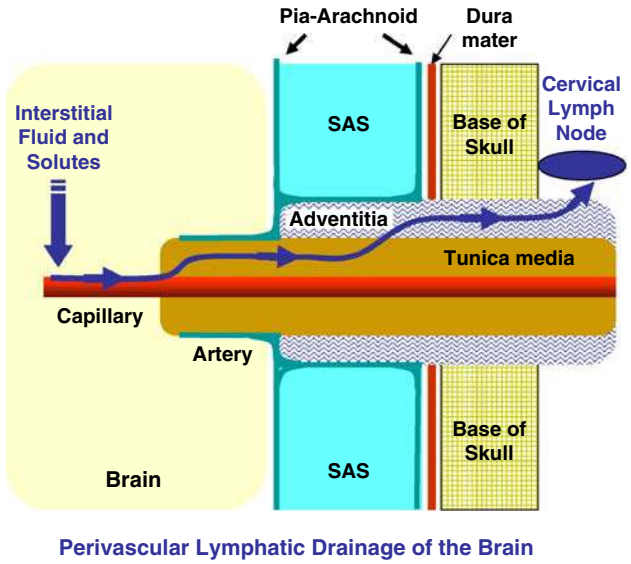

Fig. 6 Diagram of the proposed route for lymphatic drainage of the brain. Interstitial fluid and solutes drain from the brain parenchyma into the basement membranes of capillaries and then along the basement membranes between smooth muscle cells in the tunica media of arteries. ISF and solutes then enter the adventitia around leptomeningeal arteries and continue through the base of the skull along the carotid artery (and probably the vertebral artery) to cervical lymph nodes. A layer of pia-arachnoid separates the adventitia of the leptomeningeal arteries from the CSF in the subarachnoid space (*SAS*)

brains [13, 69, 100]. This suggests that Aβ acts as a natural tracer for the perivascular drainage pathways for ISF and solutes from the human brain [100]. Transgenic mice that produce human Aβ but only in the brain [12, 37] develop CAA indicating that Aβ in the vessel walls in CAA is derived from the brain.

Biochemical analyses of the distribution of Aβ in human cerebral vessel walls support the hypothesis derived from the animal studies that solutes drain along the walls of cerebral arteries to cervical lymph nodes. Although Aβ is present in the walls of middle cerebral and basilar arteries at the base of the brain, no Aβ is detectable in the walls of the carotid arteries in the neck [77]. This suggests that Aβ leaves the wall of the carotid artery at the base of the skull in humans to drain to the cervical lymph nodes that are closely associated with the internal carotid artery at the base of the skull (Kelsey and Weller, unpublished observation). This drainage pathway would be similar to the route drainage of tracers to cervical lymph nodes in experimental animals [85] (Fig. 6). The pathways for perivascular drainage of ISF and solutes along the walls, capillaries and arteries are summarised in Figs. 5 and 6.

Motive force for perivascular lymphatic drainage

The rapid clearance of tracer injected into brain parenchyma in live animals and a lack of clearance in dead animals suggest that there is a defined vital motive force, such as arterial pulsations, involved in the lymphatic drainage of ISF and solutes from the brain [13]. Theoretical models indicate that the motive force is related to the pulse wave travelling along arteries [74]. Following each pulse wave is a contrary (or reflection) wave travelling in the reverse direction and it is the contrary wave that may drive the perivascular lymphatic drainage out of the brain [74]. Drainage of ISF and solutes along artery walls in the reverse direction to the flow of blood would require the driving force of the contrary (reflection) wave and a valve-like action to prevent back-flow during the passage of the anterograde pulse wave [74]. Conformational changes in the vascular basement membranes during expansion and recoil of the

vessel wall may be one mechanism for the valve-like action. The model also suggests that the reduction in amplitude of the pulse wave that occurs with stiffening of arteries with age and arteriosclerosis would reduce the amplitude of the contrary wave and thus impede or slow the periarterial lymphatic drainage of fluid and solutes from the brain [74].

There is also some evidence that innervation of blood vessels in the brain plays a role in perivascular drainage of ISF and solutes. Cholinergic deafferentation in rabbits resulted in a significant increase in Aβ in cortex and artery walls [3, 4]. This suggests that an intact innervation is required for efficient perivascular drainage of solutes such as Aβ from the brain.

Relationships between CSF and interstitial fluid in the brain

The pathways for the drainage of CSF and ISF appear to be distinct from one another particularly in humans. CSF drains to lymph nodes via nasal lymphatics and directly into the blood via arachnoid villi and granulations, whereas ISF and solutes drain to cervical lymph nodes along the walls of capillaries and arteries. However, there is an interrelationship between the CSF and ISF.

Cerebrospinal fluid passes from the ventricles into the white matter, where it may be absorbed into the blood or drain with ISF along blood vessel walls. Early experimental studies showed that trypan blue did not enter brain tissue when injected into the blood due to the presence of the blood–brain barrier [29]; however, when it was injected into the CSF in the ventricles, it spread into the brain [30]. Similarly, tracers injected into the ventricles are taken up by perivascular macrophages [6] suggesting that CSF may permeate into perivascular spaces. In the acute stages of hydrocephalus, due to impaired drainage of CSF from the ventricles, CSF passes into the periventricular white matter causing interstitial oedema [93, 99, 101–103]. However, the grey matter of the cerebral cortex and basal ganglia does not develop interstitial oedema [21, 93, 99, 101–103], possibly due to the efficient perivascular drainage of ISF from grey matter along perivascular pathways [13]. There is an upregulation of aquaporin-4 in hydrocephalus suggesting that CSF is absorbed into the blood from the oedematous periventricular white matter [54].

Soluble tracers injected into the subarachnoid space of experimental animals pass along perivascular spaces into the brain and spinal cord [71], but the passage of particulate matter and erythrocytes into the brain from the subarachnoid space is blocked by the pia matter [39]. It was thought that the CSF in the subarachnoid space connected directly with the subpial space and the brain. However, ultrastructural studies have shown that the pia mater separates the subarachnoid space from the underlying brain and CSF from the ISF [2, 39, 48, 95, 109]. The pia mater is a very thin layer of cells [2, 26] and may be fully permeable to fluid and small molecules. Although it forms a barrier to particulate matter and erythrocytes, the pia mater does not appear to prevent the migration of macrophages [47]. This suggests that the compacted astrocyte processes of the glia limitans on the surface of the cerebral cortex [2, 94] and the subpial collagen on the surface of the human spinal cord [61] are the main barrier to the passage of inflammatory cells from the subarachnoid space into the CNS rather than the pia mater [94].

Less is known about the proportion of ISF that drains into the CSF. Gadolinium injected into the cerebral cortex of normal young rats spreads into the white matter and enters the ventricles, but this movement of fluid is impaired in hydrocephalus [79]. The presence of deposits of Aβ in the surface layers of cerebral cortex in Alzheimer's disease suggests that solutes may drain through the surface layers of the brain into the CSF and that Aβ becomes entrapped in the glia limitans [97, 100]. As solutes and ISF drain from the brain along the walls of vessels in the subarachnoid space (Fig. 6), they are separated from the CSF by layers of smooth muscle cells, adventitia and by a thin coating of pia-arachnoid. To what extent these layers block the passage of solutes and ISF into the CSF in humans is not known, neither is proportion of ISF and solutes from the brain that reach the draining lymph nodes. In rats, 10–15% of ISF drains into the CSF [85], and it is possible that a similar degree of leakage occurs in humans. Despite this uncertainty, levels of brain-derived proteins in the CSF are used as biomarkers for dementias such as Alzheimer's disease [27], Creutzfeldt Jacob disease [80] and Parkinsonian disorders [16].

Lymphatic drainage and the pathophysiology of neurological disease

Perivascular lymphatic drainage of the CNS plays a fundamental role in neuroimmunological reactions and in regulating the balance of ISF and solutes within the parenchyma of the CNS. Physiological drainage of antigens from the brain to regional lymph nodes appears to play a role in neuroimmunological responses in autoimmune disease [67, 88], as discussed below. Similar mechanisms may operate in infections of the CNS.

The balance of ISF and solutes in the CNS may be disturbed in a variety of neurological diseases either by the generation of excess ISF or by failure of the drainage pathways (Table 1). In vasogenic oedema [25] associated with damage to the CNS, the volume of fluid and proteins may overload the perivascular drainage system, contributing to the problems associated with resolution of the oedema in the CNS. Similarly, infusion of CSF into periventricular

Table 1 A range of neurological disorders associated with the physiology and pathology of lymphatic drainage of the nervous system

Category	Neurological disorder
Neuroimmunology (lymphatic drainage of antigens from the brain)	Infections Autoimmune disorders: multiple sclerosis, EAE
Vasogenic oedema: disturbed Interstitial fluid balance	Associated with infarction, trauma, infections, tumours and metabolic disorders
CSF oedema: disturbed interstitial fluid balance	Infusion of CSF into periventricular brain tissue in acute hydrocephalus
Neurodegenerative disorders: deposition of amyloid in perivascular drainage pathways and impaired drainage of fluid and solutes.	Cerebral Amyloid Angiopathy due to deposition of Aβ, cystatin, prion protein and other amyloids in perivascular drainage pathways (*types of PEFA*). Association with dementias and intracerebral haemorrhage
CADASIL	Failure of elimination of protein produced within artery walls (*PEFA*)
Immune complex arteritis	Accumulation of immune complexes in artery walls (*PEFA*)
Peripheral nervous system	Amyloid neuropathy with accumulation of amyloid in artery walls (*PEFA*)

EAE experimental autoimmune encephalomyelitis, *CSF* cerebrospinal fluid, *CADASIL* cerebral autosomal dominant arteropathy with subcortical infarcts and leukoencephalopathy, *PEFA* protein elimination failure arteriopathy

brain tissue in acute hydrocephalus appears to overwhelm the ISF drainage system particularly in the white matter [93]. Disturbance of perivascular drainage of fluid and solutes in the pathogenesis of CAA, Alzheimer's disease, Creutzfeldt–Jacob disease and other dementias will be discussed below as part of the complex of protein elimination failure arteriopathies (PEFA) that may also include cerebral autosomal dominant arteropathy with subcortical infarcts and leukoencephalopathy (CADASIL) [100]. It is possible that immune complex inflammatory arteritides [25] also fall into the PEFA group with deposition of immune complexes in the ISF drainage pathways in artery walls. Peripheral nerves also appear to rely upon perivascular drainage for the elimination of fluid and solutes as illustrated by the occurrence of amyloid angiopathy particularly involving transthyretin [70, 100].

Neuroimmunological disorders

The full significance of lymphatic drainage of the brain for neuroimmunological reactions has not been fully elucidated. Many studies in the past have concentrated on the entry of lymphocytes into the brain [5, 23] and only a few have investigated the role of lymphatic drainage and lymph nodes in the genesis of neuroimmunological reactions.

Antibody production

The formation of antibodies in cervical lymph nodes following injection of antigen into the brain parenchyma or CSF is well established [18, 28]. Furthermore, myelin antigens in EAE and in multiple sclerosis (MS) [20, 24] and axonal antigens following focal brain damage [58] have been detected in cervical lymph nodes. Such antigens may be involved in cell-mediated immunity and antibody production [20, 24] or have a tolerogenic effect [58]. Removal of cervical lymph nodes markedly reduces antibody formation [35].

T lymphocyte-mediated immune reactions in the brain

Many types of EAE depend upon T lymphocyte-mediated immune reactions in the brain and similar mechanisms appear to play a significant role in the pathogenesis of MS [5, 23]. Entry of encephalitogenic T lymphoblasts into the CNS depends upon specific α4β1-integrins on the surface of T cells and the vascular adhesion molecule (VCAM) on the endothelial cell together with a number of other adhesion molecules [23]. Antibodies that bind to α4-integrins has been used to inhibit the entry of T lymphocytes into the CNS in MS, but this treatment has also been associated with the development of progressive multifocal leukoencephalopathy suggesting that defences against virus infections are also impaired [82]. If targeting of the brain by encephalitogenic T lymphocytes depends upon the expression of α4β1-integrins on the surface of T cells [23], where do the lymphocytes acquire their integrin label?

Lymphatic drainage of immune cells from the brain—does it occur?

In organs such as gut, lung and skin, memory T cells circulate between tissues and regional lymph nodes and integrins on the surface of the lymphocytes play a role in organ-specific

circulation of lymphocytes [81] (Fig. 7a). Whether lymphocytes circulate from the brain to regional lymph nodes in a similar way to other organs is not clear. Lymphocytes in perivenous spaces and in inflammatory lesions in the brain parenchyma are removed by apoptosis [50] and most of the evidence suggests that lymphocytes do not migrate from brain to regional lymph nodes in significant numbers [28]. Naïve lymphocytes do enter the normal brain, but in general they are few in number especially when compared with other organs such as the lung or gut [28]. Some traffic of lymphocytes from the CSF occurs via the cribriform plate and nasal lymphatics [31] and dendritic cells that are injected into the CSF migrate to regional lymph nodes [36]. Dendritic cells have been identified in the human brain associated with perivenous inflammatory exudates in MS [34]. However, experiments in which dendritic cells were injected into the brain parenchyma suggest that they may not migrate to lymph nodes from brain tissue [36].

The basement membranes of capillaries and arteries that are the route for lymphatic drainage of fluid and solutes from the brain [13] appear to be too narrow for the passage of lymphocytes. To explore whether a perivascular pathway for the migration of lymphocytes from the brain does exist, particles 0.02 and 1.0 μm in diameter were injected into grey matter of the brain in mice [13]. The particles tracked along the outside of arteries creating a space between the artery wall and the surrounding brain, but they did not move following the injection even when the tissue was inflamed [13]. Other experiments have shown that perivascular macrophages ingest injected particles of Indian ink in perivascular spaces and they remain in situ for 2 years or more [110]. Unless particles leak directly into the CSF during the injection, they do not reach the cervical lymph nodes [13, 110]. The results of these experiments suggest that there is no perivascular pathway for lymphocytes and macrophages to migrate out of the brain in the same way as fluid and solutes (Fig. 7b).

Reduction of autoimmune reactions in the brain by removal of cervical lymph nodes

T lymphocyte-mediated immune reactions in the brain are reduced following removal of cervical lymph nodes [67]. A model of EAE was developed in the Lewis rat in which cerebral inflammation was very significantly enhanced by a cryolesion on the surface of one cerebral hemisphere [68]. This model was then used to test the hypothesis that regional lymph nodes played a role in cerebral EAE. A cryolesion on the surface of one cerebral hemisphere 7 days into the incubation period for EAE in Lewis rats resulted in a fivefold enhancement of cerebral EAE [68] possibly due to the release of chemokines [84] and proinflammatory cytokines [83]. If the cervical lymph nodes were removed at the same time as the cryolesion, inflammation in the brain was reduced by 50% compared with the sham-operated animals [67].

The results of the lymphadenectomy experiments in cryolesion-EAE suggest that the cervical lymph nodes may be a major source of lymphocytes targeting the brain. It seems that specificity for the brain is bestowed on lymphocytes within the cervical lymph nodes as a result of soluble antigens draining from the brain to the lymph nodes rather than by the transfer of antigen-presenting cells or lymphocytes. This conclusion is supported by the observation that lymphocytes isolated from lymph nodes (cervical and other nodes) at the height of cerebral inflammation in cryolesion-EAE results in adoptive transfer of cerebral EAE [49].

Immunological privilege

If an allograft is implanted into the brain, rejection is delayed when compared to grafts in other organs. This phenomenon has given rise to the concept of immunological privilege of the brain [28]. However, if a similar allograft is placed in the skin, the brain graft is rapidly rejected, which suggests that rejection from the brain relies upon immunisation of peripheral tissues.

How does immunological privilege fit into what we know about the relationship between the brain and the regional lymph nodes? No antigen normally enters the brain unless it passes through peripheral tissues; even entry through the nose entails contact with the nasal mucosa. Although immune cells do not traffic from CNS to regional lymph nodes (Fig. 7c), drainage of soluble antigens appears to be sufficient to stimulate a T cell-mediated reaction in the brain. It is probable that antigen-specific T lymphocytes circulate from nodes in other parts of the body to cervical lymph nodes where they receive an "address" to target the brain [49] (Fig. 7d).

Multiple sclerosis

The lack of evidence for migration of antigen-presenting cells from the parenchyma of the CNS to regional lymph nodes in the neck or lumbar region [36] suggests that the pathogenesis of MS as a T lymphocyte-mediated autoimmune disease depends upon drainage of soluble antigen from the CNS to regional lymph nodes. One possibility in MS is that antigen-specific activation of T lymphocytes to CNS proteins occurs in the periphery due to molecular mimicry by a virus or other agent [51]. The activated T cells may then migrate to cervical or lumbar lymph nodes in which antigen draining from the brain or spinal cord is presented. Antigen-specific T cells primed with the appropriate integrins [23] may then target the CNS to initiate an autoimmune reaction [49].

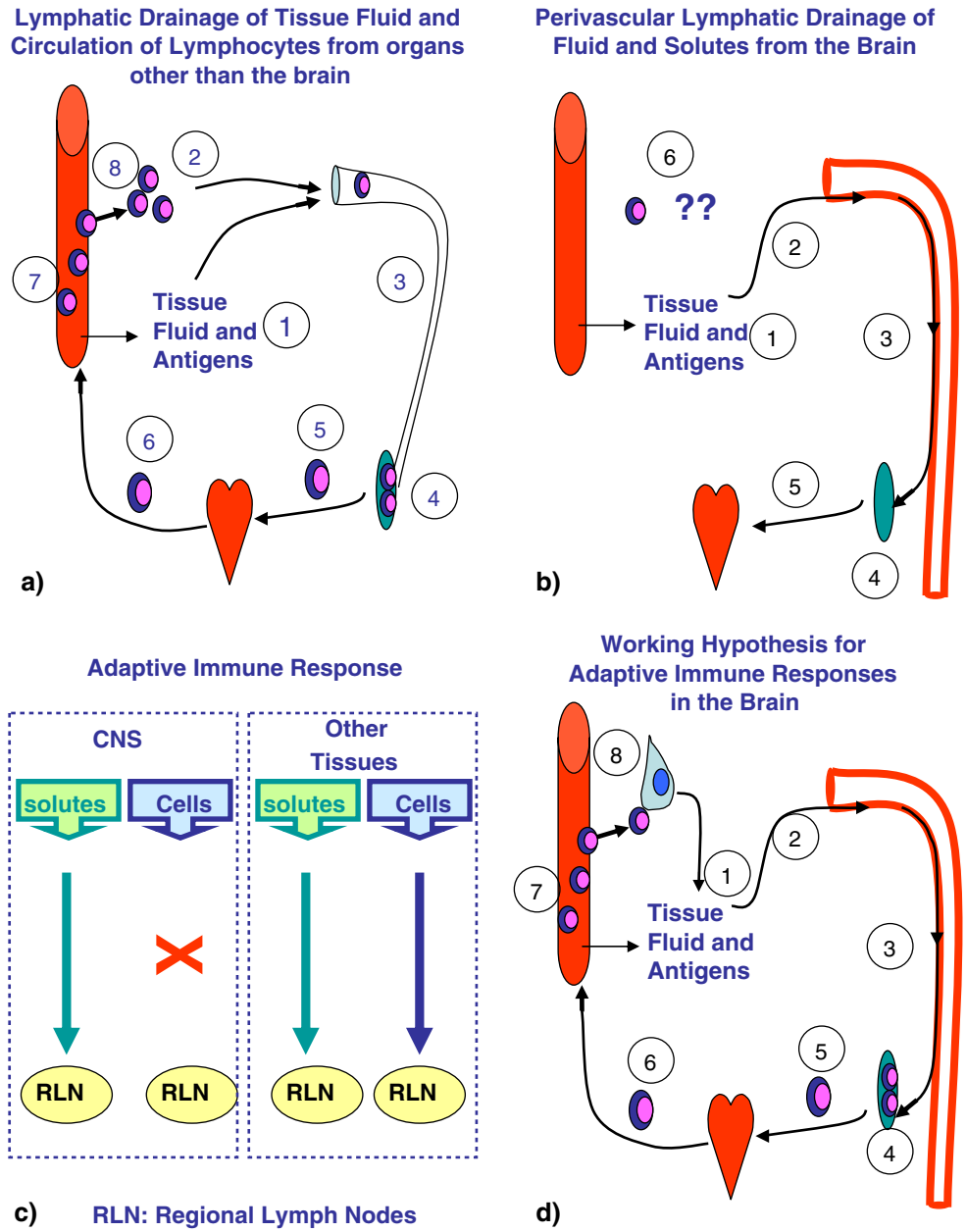

Fig. 7 Immunological privilege and the relationships between lymphatic drainage from the brain and from other organs. **a** Lymphatic drainage of tissue fluid and circulation of lymphocytes from organs other than the brain. Tissue fluid, antigens (*1*), lymphocytes and other immune cells (*2*) drain from tissues along lymphatic vessels (*3*) to regional lymph nodes (*4*). Antibodies and antigen-specific lymphocytes drain into the blood (*5*) and are distributed to their target organs (*6*), which they enter via postcapillary venules (*7*) and encounter their target antigens (*8*). **b** Perivascular lymphatic drainage of fluid and solutes from the brain. Interstitial fluid and solutes in the extracellular spaces of the brain (*1*) spread diffusely through the brain parenchyma (*2*), and then drain out of the brain along the walls of capillaries and cerebral arteries (*3*) to cervical lymph nodes (*4*). Antibodies produced within the lymph nodes pass into the blood (*5*). Lymphocytes and antigen-presenting cells (*6*) do not appear to traffic from the brain to lymph nodes. **c** Adaptive immune responses in the brain differ from those in other organs. It appears that only solutes drain from brain to regional lymph nodes (RLN), whereas, soluble antigens, lymphocytes and antigen-presenting cells all drain or traffic from other tissues to regional lymph nodes. The absence of immune cell trafficking to lymph nodes may be a major factor in immune privilege in the brain. **d** Working hypothesis for adaptive immune responses in the brain. Interstitial fluid and soluble antigens (*1*) diffuse through the extracellular spaces (*2*), enter basement membranes of capillaries and drain along the basement membranes and adventitia of cerebral arteries (*3*) to lymph nodes in the neck (*4*). Antigen is presented to antigen-specific lymphocytes from other lymph nodes, which then receive an integrin "address" for the brain and pass into efferent lymph (*5*). Brain-directed lymphocytes circulate to the brain (*6*), bind to VCAM and other molecules expressed on cerebral endothelia (*7*), and pass into the brain to seek their target antigens (*8*)

Failure of elimination of Aβ from the brain in Alzheimer's disease

The pathology of Alzheimer's disease is characterised by the intraneuronal accumulation of tau protein and by the deposition of insoluble Aβ in the extracellular compartments of the brain [52]. Aβ is also deposited in the perivascular lymphatic drainage pathways in the walls of capillaries and arteries as CAA [100] (Fig. 4).

While excessive or aberrant production of Aβ in the brain may be a feature of the small number of familial cases of Alzheimer's disease [76], failure of elimination of Aβ from the ageing brain appears to be a major factor in the pathogenesis of the more common sporadic Alzheimer's disease [100]. Aβ is produced in the brain and most other tissues by the cleavage of APP [76]. However, the brain is the only organ in the body that has serious problems with elimination of Aβ with advancing age. This failure appears to be partly due to the peculiarities of the perivascular lymphatic drainage pathways of the brain.

Several mechanisms have been identified for the elimination of Aβ from the brain [100] (Fig. 8). They include degradation of Aβ by neprilysin and insulin-degrading enzyme in brain parenchyma and in artery walls [56], and absorption into the blood involving low-density lipoprotein receptor-related protein (LRP)-1 and P-glycoprotein [100].

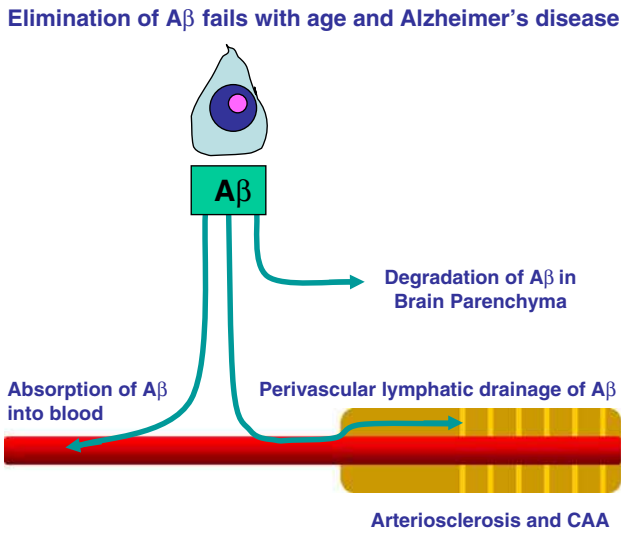

Fig. 8 Failure of elimination of Aβ from the brain with age and Alzheimer's disease. Aβ produced by neurons and other cells in the brain is degraded in brain parenchyma by neprilysin, insulin-degrading enzyme and by other pathways. Absorption of Aβ into the blood involves low-density lipoprotein receptor-related protein (*LRP*)-1 and P-glycoprotein. These mechanisms fail with age. Perivascular lymphatic drainage of Aβ also fails as stiffening of cerebral arteries with age and arteriosclerosis results in cerebral amyloid angiopathy (*CAA*). The consequent loss of homeostasis in the brain may be a major factor in the failure of neuronal function and cognitive decline in Alzheimer's disease

These mechanisms for the elimination of Aβ all appear to fail with age [56, 100].

Drainage of Aβ from the brain along perivascular lymphatic pathways also fails with age in humans and in transgenic mice with mutations in the APP gene [37, 100] (Fig. 8). The positive correlation of severe CAA with dementia [15] suggests that blockage of lymphatic drainage of ISF and solutes may play a major role in the pathogenesis of Alzheimer's disease. However, there are still questions to answer: (1) Why do the lymphatic drainage pathways of the brain become blocked in the elderly? (2) What are the effects of the blockage and how does this relate to Alzheimer's disease?

Theoretical data indicate that arterial pulsations and their contrary (reflection) waves are the motive force that drives the perivascular drainage of ISF and solutes (including soluble Aβ) from the brain [74]. With advancing age, artery walls stiffen due to fibrosis of their walls (arteriosclerosis) [59], which may reduce the amplitude of pulsations and the motive force driving perivascular lymphatic drainage [74] and as a result, Aβ precipitates as fibrils of insoluble amyloid in the basement membranes of capillary and artery walls (CAA) [69]. The exact position of the amyloid fibrils in the lamina densa of basement membranes between the smooth muscle cells has been very well shown by electron microscopy [106], although at that time, it was thought that the Aβ was formed by smooth muscle cells in CAA rather than being derived from the brain [12].

Because of their position in the basement membranes of capillary and artery walls in CAA, amyloid deposits almost certainly block the lymphatic drainage pathways. Deposition of Aβ further stiffens the artery walls leading to increasing impedance of solute drainage from the brain. The effects of this are seen in the high levels of soluble Aβ in the brain in Alzheimer's disease that correlate more closely with dementia than the number of plaques of insoluble Aβ [53, 55]. A further indication of blockage of lymphatic drainage in the elderly in Alzheimer's disease is the correlation of fluid accumulation in the subcortical white matter (leukoaraiosis) and the severity of CAA [73]. Another recognised complication of deposition of Aβ in the walls of cerebral arteries in CAA is the loss of vascular smooth muscle cells, weakening of artery walls and intracerebral haemorrhage [98, 100, 108].

The role of Aβ in Alzheimer's disease

Little is known about the role of Aβ in normal brain function. Aβ is present in small amounts in normal brain [97] and its presence in cerebral arteries in young human individuals [78] probably reflects the lymphatic drainage of Aβ from normal brain. Soluble Aβ in the brain is increased in

patients who are recovering from head injury or subarachnoid haemorrhage but not in those who are deteriorating, suggesting that raised levels of Aβ reflect neuronal activity [11].

Insoluble plaques of Aβ are a prominent feature in the pathology of Alzheimer's disease [52]. However, PET imaging has shown that, as a group, patients with mild cognitive impairment have a similar amount of insoluble Aβ in their brains to patients with Alzheimer's disease [43]. Furthermore, removal of Aβ plaques from the brain by Aβ immunotherapy does not prevent progressive neurodegeneration in Alzheimer's disease [38]. Other studies indicate that a high level of soluble Aβ in the brain correlates more closely with cognitive decline in Alzheimer's disease than does a high plaque load of insoluble Aβ [53, 55]. Removal of Aβ plaques from the brain by Aβ immunotherapy increases the level of soluble Aβ in the brain [66] and results in persistence of CAA or an increase in the severity of CAA [62, 66, 104].

The results of the studies reviewed above suggest that Aβ plays a major role in the pathogenesis of Alzheimer's disease. Aβ blocks the perivascular lymphatic drainage pathways in the walls of cerebral capillaries and especially in cerebral arteries (CAA). This results in loss of neuronal homeostasis with the accumulation of soluble Aβ and probably other metabolites in the brain. Insoluble Aβ deposits in brain tissue also interfere with the diffusion of ISF through extracellular spaces in the brain [57]. High levels of soluble Aβ in the brain may be a factor in the formation of the toxic forms of soluble Aβ that result in synaptic loss and neuronal injury [90]. It is not clear how the proposed role of Aβ relates to the accumulation of hyperphosphorylated tau protein in neurons in the pathogenesis of Alzheimer's disease.

Protein elimination failure arteriopathy (PEFA)

Lymphatic drainage along basement membranes of capillary and artery walls appears, at first sight, to be unique to the brain. However, analysis of a number of human diseases suggests that similar perivascular drainage pathways exist in organs other than the brain. Furthermore, peptides and proteins other than Aβ are deposited in artery walls both in the brain and in other organs giving rise to different types of PEFA.

Aβ is the commonest peptide involved in CAA, but other amyloids deposited in the walls of cerebral arteries include cystatin C, gesolin, prion protein, ABri and ADan [72]. In ABri CAA, arteries in both brain and spinal cord are involved. Outside the CNS, peripheral nerves most commonly develop amyloid angiopathy [70]. Peripheral nerves, like the CNS, do not seem to possess conventional lymphatics. Deposits of transthyretin and other amyloids in the endoneurium and vessel walls [8, 70] are similar in distribution to amyloid in the brain, and this suggests that lymphatic drainage of fluid and solutes from peripheral nerves may be along artery walls.

Protein deposition in artery walls in organs other than brain is uncommon, but it does occur. Cystatin amyloid deposits have been described in the walls of arteries in the pancreas [64]. In CADASIL, protein is deposited in the walls of arteries in several organs including skin, kidney and brain, although the major effects of CADASIL are usually in the brain [42]. These examples suggest that perivascular transport of solutes occurs along systemic arteries in a similar way to lymphatic drainage in the CNS.

Conclusions and implications for therapy of neurological disease

We have reviewed the pathways for lymphatic drainage of CSF and ISF and solutes from the brain. While CSF drains to lymph nodes mainly via lymphatics in the nasal mucosa, lymphatic drainage of ISF and solutes from the brain is along perivascular routes and is separate from the drainage of CSF. Lymphatic drainage of antigens may play a significant role in the pathogenesis of MS and further understanding of the afferent lymphatic pathways may lead to the development of therapeutic strategies for this disease. Failure of lymphatic drainage of the brain appears to be a significant factor in the pathogenesis of Alzheimer's disease and other dementias. Therapeutic strategies that facilitate the elimination of Aβ along the walls of ageing arteries may help to prevent CAA, reduce the incidence of CAA-related haemorrhage and retard cognitive decline in Alzheimer's disease.

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