Pathology of Cerebrospinal Fluid and Interstitial Fluid of the CNS: Significance for Alzheimer Disease, Prion Disorders and Multiple Sclerosis

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Abstract. Extracellular fluid in the central nervous system (CNS) is composed of cerebrospinal fluid (CSF), derived from the choroid plexus, and of interstitial fluid (ISF) in gray and white matter. Investigation of CSF plays a significant role in diagnosis and management of neurological disease and pathologies involving the CSF have important effects on the CNS itself. Hydrocephalus has many causes; clinical effects are due to a mixture of obstruction to CSF flow and damage to periventricular white matter with CSF edema, axonal loss and gliosis. Meningitis and subarachnoid hemorrhage are mainly confined to the subarachnoid space emphasising how this compartment is separated from the CNS by the pia mater and glia limitans; brain damage results from thrombosis of leptomeningeal vessels and infarction of CNS tissue. ISF from white matter appears to drain mainly to CSF, but ISF from gray matter drains along periaxial pathways in CNS and meninges, to lymph nodes in experimental animals, and probably in humans. B-amyloid in Alzheimer disease and prion proteins accumulate in the extracellular spaces of gray matter and in periaxial ISF drainage pathways as cerebral amyloid angiopathy, emphasising the role of periaxial drainage for the elimination of high molecular weight substances from the brain, possibly to regional lymph nodes. Lymphatic drainage of ISF drainage plays a major role in B- and T-lymphocyte mediated immune reactions in the CNS in animals. By analogy with experimental autoimmune encephalomyelitis, lymphatic drainage of brain antigens in ISF from the human CNS may play a key role in the pathogenesis of Multiple Sclerosis.

Key Words: Alzheimer disease; Cerebrospinal fluid; CNS; Interstitial fluid; Meningitis; Multiple sclerosis; Prions; Lacunes.

INTRODUCTION

Extracellular fluid associated with the central nervous system (CNS) is composed of cerebrospinal fluid (CSF) and interstitial fluid (ISF). CSF is distributed through the ventricular system and the subarachnoid space, whereas ISF is present in the intercellular spaces of gray and white matter (1). Pathology of the CSF has been extensively investigated, especially as a diagnostic tool for neurological disease and in relation to the tissue damage that results from hydrocephalus, meningitis and subarachnoid hemorrhage. In contrast, the pathology of ISF has been less thoroughly studied, even though it may have important implications for diseases as diverse as Alzheimer disease, prion disorders and multiple sclerosis. This review starts with a brief account of the historical development of concepts concerning CSF and ISF and then focuses on the pathology of these 2 CNS-associated fluids.

HISTORICAL PERSPECTIVE

Concepts regarding extracellular fluid and its drainage from the human CNS have developed over the last 2,500 years and have largely concentrated upon the structure of the cerebral ventricles and on the production of CSF and its bulk flow through arachnoid villi and granulations. In recent decades, the focus on CSF has been accentuated by the introduction of CT scanning and MRI, both of which allow fluid dynamics of the large volume of CSF to be studied directly. Apart from the investigation of cerebral edema, however, there has been little in vivo investigation of interstitial fluid and its drainage from the human brain.

Herophilus of Calcedon (335–280 BC), working in Alexandria in the 4th century BC, proposed that the cerebral ventricles were the centers of intelligence rather than just a cooling system to chill the ardours of the heart, as taught by Aristotle (2); his anatomical description of the lateral, third and particularly the fourth ventricle were frequently referred to in Galen's (3) book on anatomical procedures. Herophilus believed that the forces responsible for the animal spirit resided in the ventricular system, particularly in the fourth ventricle near the spinal cord and the origins of the spinal nerves. Galen (3), some 400 years later, also accepted such a "pneumatic" role of the ventricles in purifying the vital spirit to produce the animal spirit. It was probably thought that the ventricles contained air rather than fluid. Waste products from this refining process were thence dispersed into the air sinuses or into the nose. Animal spirit passed from the ventricles, down the nerves, to produce all actions of the body (4). Such views were still accepted in the 16th century even at a time when Versalius in 1542 (5) was describing and illustrating the detailed anatomy of the ventricular system.

During the subsequent centuries, the various foramina associated with the circulation of the CSF were described as were the arachnoid granulations (Pacchionian bodies). The first good evidence for the formation of CSF by the choroid plexus was the demonstration in the early 20th century that the lateral ventricles became distended with CSF following occlusion of the aqueduct (6) and much less CSF accumulated if the choroid plexus were first removed (7). Welch in 1963 (8) showed that fluid was actually lost from the blood as it passed through the choroid plexus...
vessels and Sisson in 1969 (9) found that CSF production was reduced if the choroidal arteries were constricted.

Quincke in 1872 (10), and Key and Retzius in 1876 (11) showed that dyes injected into the spinal subarachnoid space of human cadavers passed through the arachnoid granulations of Pacchioni into the sagittal venous sinus. Objections to this route of CSF drainage were later raised by Dandy and Blackfan partly because arachnoid granulations were not found in children, nor in many other animals. However, arachnoid villi have been described in many nonhuman species, ranging from very small structures in the rat (12) to the slightly larger structures in sheep and dogs (13); none, however, reaches the size and complexity of arachnoid granulations in man (14, 15, 16).

The prominence of arachnoid villi and granulations and the large volume of CSF draining through these pathways in man (13) drew attention away from the parallel studies of lymphatic drainage of CSF in animals (17). In 1869, Schwalbe (18) demonstrated that tracers injected into the cisterna magna of the rat rapidly appeared in cervical lymph nodes. Some years later, Zwilling (19) showed that tracers injected into the CSF of human infant cadavers drained into the lymphatics of the nasal mucosa. A number of later studies confirmed the drainage of intracranial fluid (20) and spinal CSF (21) to regional lymph nodes in a variety of animal species (22). Furthermore, the pioneering work of Helen Csern, Michael Bradbury and their colleagues (22, 23) emphasised that ISF from the brain also drained to cervical lymph nodes and that such drainage played an important role in immunological reactions within the CNS (22, 24). Some 47% of radio-iodinated serum albumin injected into the caudate nucleus of the rabbit drained to deep cervical lymph by 25 hours and 30% of the label was recovered from deep cervical lymph 6 hours after injection into the rabbit CSF (22). In larger mammals, such as sheep, clearance of I-131-human serum albumin from CSF is almost equally distributed between lymphatic and arachnoid villous pathways (25). Antibodies to antigens injected into the brain or CSF are produced mainly in cervical lymph nodes and removal of these nodes very significantly reduces such antibody production (26, 27). Similarly, cervical lymph nodes play a very significant role in T-cell mediated immune reactions in the brain (24, 28). Despite this evidence, the direct connections between the CNS and the immune system and the role of lymphatic drainage from the CNS in neuroimmunology have, until recently, remained largely unrecognized (29).

From a historical perspective, it seems that the prominence of CSF in man, the ease with which CSF is studied, and the focus of many animal experiments by Weed (30) and later workers on arachnoid villi and granulations have overshadowed the lymphatic drainage of ISF (22). But, as emphasised in this review, ISF and its drainage pathways may be as important, if not more important, than CSF in the pathology of the CNS.

**PATHOLOGY OF THE CEREBROSPINAL FLUID**

CSF *is produced* by choroid plexuses derived from folds of leptomeninges and ependyma extending into the ventricular system of the brain (13, 31). It is estimated that CSF is secreted at 2.1 μl/min in the rat (32) and at a considerably greater rate of 350 μl/min for man (33), and has an important, if not sole, role as a buoyancy fluid for the brain and spinal cord, particularly in man. The blood-CSF barrier is at the choroid plexus epithelium (13) and within the ventricles, CSF is separated from periventricular gray and white matter by ependyma across which CSF may diffuse (13). In the subarachnoid space, CSF is separated from brain tissue by a delicate layer of pia mater (34, 35, 36) and by the underlying glia limitans. The pia mater, in man, is reflected from the surface of the brain and spinal cord on to the blood vessels as they enter or leave the CNS; thus the pia mater separates the subpial and perivascular spaces from the subarachnoid space (35, 37, 38). Experimental studies suggest that there is no significant interchange between the CSF in the subarachnoid space and interstitial fluid in brain or spinal cord tissue (22, 39), but it is uncertain whether this barrier is pia mater (40), the subpial collagen (37, 38), the underlying glia limitans, or a combination of all these structures.

**Drainage of CSF** in man is largely through arachnoid granulations pouting into the venous sinuses and in relation to spinal nerve roots (13). Arachnoid granulations are composed of a central core of collagenous trabeculae and a cap of compacted arachnoid cells (14, 15, 16); CSF appears to percolate along channels within the arachnoid cap towards the endothelial lining of the sinus. Despite the large size of the arachnoid granulations, the area over which the arachnoid cap is in apposition to the endothelium is limited to an area some 300 μm in diameter at the apex of each granulation (15). Experimental studies in monkeys suggest that bulk flow of CSF across the sinus endothelium is by macrovascular transport (41). Following subarachnoid hemorrhage, erythrocytes become enmeshed in the drainage pathways in the granulations (15) but permanent occlusion of arachnoid granulations seems to be a rare event (42).

Arachnoid villi are present in smaller mammals such as the rat but they are very simple in form and protrude into venous sinuses in the region of the olfactory bulbs (12). Tracers injected into the CSF in the rat drain through the cribiform plate, via nasal lymphatics to cervical lymph nodes, and via arachnoid villi into the blood; tracers injected into the spinal CSF drain largely to paravertebral lymph nodes (12, 43). Injection of nanoparticulate contrast medium into either the CSF or the interstitial fluid of the rabbit brain has been shown, by CT scanning, to arrive in the cervical lymph nodes within 4 hours, with
a peak at 12–24 hours, again suggesting that both ISF and CSF drain to the cervical lymph nodes (44).

CSF in the Investigation of CNS Disease

The measurement of CSF pressure and the examination of CSF for cells, changes in chemistry, or for the presence of micro-organisms, has long been a valuable diagnostic tool in neurology (45). Local anesthetic agents, antibiotics and antitumor agents are not infrequently injected into the lumbar CSF for the management of neurological disease. It is beyond the scope of this review to dwell upon the changes in CSF that reflect pathology of the CNS. Investigation of the CSF is probably most reliable in diseases such as meningitis, and subarachnoid hemorrhage, and in detecting primary and metastatic tumor infiltration of the subarachnoid space and leptomeninges, but it is also valuable for the diagnosis and management of diseases of nerve roots such Guillain Barré Syndrome and disorders as wide-ranging as multiple sclerosis, Alzheimer disease and syphilis (46). As will be emphasized in this review, ISF drainage from the human brain appears to be separate from the CSF. How much ISF leaks into CSF is unclear but this would have a bearing on how the results of lumbar CSF investigations are interpreted in relation to pathology deep within CNS tissue.

Hydrocephalus

The many causes of hydrocephalus (47) range from obstruction to CSF flow within the ventricles, foramina, aqueduct and subarachnoid space, to diseases such as Alzheimer disease and other dementias in which ventricular dilatation is associated with loss of brain tissue. In some cases, the clinical picture is due to a mixture of obstruction to CSF flow and tissue destruction. This is seen particularly in intermittently raised pressure hydrocephalus (normal pressure hydrocephalus) in which tissue damage from Alzheimer disease, or cerebrovascular disease may have an additive effect upon the dementia induced by the hydrocephalus (48). Determining the relative proportions of destruction and obstruction in the generation of the clinical picture, presents a significant therapeutic challenge (48, 49, 50).

Despite the wide range of obstructive causes of hydrocephalus, the pattern of brain damage resulting from the rise in intraventricular pressure is similar in hydrocephalus from multiple aetiologies. In acute hydrocephalus, periventricular edema due to insudation of CSF from the ventricles into the periventricular white matter is characteristically seen in CT scans (51) and MRI (52) and histologically, both in human brains (53) and in experimental animals (54, 55). Typically, in acute hydrocephalus, the white matter is severely affected but the gray matter is relatively well preserved (Figure 1). Rupture of the ependymal lining of the ventricles may accompany the flow of CSF into the periventricular white matter (54).

The CSF edema itself may be associated with reduced cerebral blood flow (56), functional neurological disorders (52), reduced myelination (57), axonal degeneration, reactive astrogliosis, and gliosis (52, 53). Both the functional effects and the white matter edema can be reversed by inserting a CSF shunt and early treatment in this way may prevent progressive axonal loss and gliosis in the white matter in hydrocephalus (52, 53).

Meningitis

Meningitis results from infection by viruses, bacteria, fungi or protozoa (58). In the acute stages of bacterial meningitis, polymorphonuclear leukocytes adhere to the endothelia of medium size and small veins in the subarachnoid space and in the subpial spaces and then pass through the vein walls into the CSF. Both polymorphonuclear leukocytes and the monocytes and macrophages which arrive later are capable of traversing the pia mater on the surface of the brain (34) and also of penetrating the leptomeningeal coating of arteries to enter perivascular spaces (35). For the most part, inflammation and invasion by hematogenous cells in acute meningitis is confined to the subarachnoid and subpial spaces. Inflammatory cells less commonly penetrate the glia limitans on the surface of the brain where inflammation is usually limited to microglia activation; this emphasizes the separation of the subarachnoid space from the brain. More severe involvement of the brain surface occurs in chronic meningitis, due to tuberculosis or fungal infections, in which the granulomatous or inflammatory process spreads to involve the glia limitans and the underlying tissue (58). In the majority of cases of meningitis, underlying brain damage is due to the ischemia and infarction which accompanies thrombosis and occlusion of blood vessels on the surface of the brain. Fibrosis which follows resolution of meningitis is thought to be responsible for obliteration of the subarachnoid space and for postmeningitic hydrocephalus. It is, however, difficult to define exactly how much fibrosis needs to occur in the subarachnoid space before CSF flow is impeded.

Both primary neuroectodermal and metastatic tumor cells invade the cerebrospinal fluid and the subarachnoid space, but they differ in their ability to penetrate the brain surface. In some carcinomas, cells remain floating free within the CSF and there is little involvement of CNS tissue but, in other tumors, particularly in malignant melanoma and leukemias, there may be extensive penetration of tumor cells into the surface of the CNS and along perivascular spaces deep into the parenchyma. Neuroectodermal tumors invade the CSF from the brain; they may seed to and invade distant parts of the brain and spinal cord.

Subarachnoid Hemorrhage

Rupture of saccular aneurysms with subarachnoid hemorrhage and intracerebral hemorrhage still carries a
Fig. 1. Periventricular white matter edema in hydrocephalus. Coronal sections of the heads of 2 mice with autosomal recessive hereditary hydrocephalus. (a) In the early stages, with moderate ventricular dilatation, there is edema of the cerebral white matter. At a later stage, when the head is greatly enlarged (b) the edema is severe, the white matter is largely destroyed and there are cystic spaces. The central gray matter and cortex, on the other hand, are not edematous and are relatively well preserved. (a) Hematoxylin and cosin (b) PTAH.

Fig. 2. Interstitial fluid (ISF) drainage pathways in the rat. Tracers injected into the ISF gray matter in the rat brain drain along periartrial pathways within the brain and enter channels alongside the middle cerebral artery (MCA), pass to the circle of Willis, then alongside the ethmoidal artery to the olfactory bulbs and drain with CSF to cervical lymph nodes (12, 63). Reproduced by kind permission of Dr S. Kida.

Fig. 3. Role of cervical lymph nodes in T-cell mediated immunity in the brain. A histogram showing a statistically significant increase in the number of EAE lesions in the brain following a cryolesion, and the 40% reduction of such enhancement following lymphadenectomy: results are compared with EAE-only. In sham operated animals, lymph nodes are exposed but not removed. Coronal sections of cerebral hemispheres at the level of the optic chiasm were assessed (28).
high mortality and morbidity (59). In those patients who survive the initial bleed, there are the subsequent complications of vasospasm and cerebral infarction. Later, hydrocephalus may supervene and this is thought to be due to fibrosis of the meninges and obliteration of the subarachnoid space. The circulatory changes that result in cerebral infarction following subarachnoid hemorrhage are thought to be due to the effect of blood and blood products on arteries within the subarachnoid space. Despite the spread of fresh blood through the subarachnoid space following subarachnoid hemorrhage, erythrocytes do not penetrate into the brain and, in particular, they do not pass into the cortical perivascular spaces (35). Thus, the pia mater forms a barrier which separates the subarachnoid space from the perivascular spaces and subpial spaces (40) especially for particles such as erythrocytes. Although the permeability characteristics of the pia mater are not fully understood, failure of tracers and pharmacological agents to significantly penetrate CNS tissue from the subarachnoid space (22, 39) does suggest that the subarachnoid compartment is largely separate from underlying brain and spinal cord. Thus, much of the effect of subarachnoid hemorrhage on cerebral blood flow may be due to responses in leptomeningeal vessels rather than vessels within brain tissue.

PATHOLOGY OF INTERSTITIAL FLUID

White Matter

Interstitial fluid in the CNS is derived from the blood (22) and behaves differently in the white matter and gray matter; this is reflected in their different pathologies. In the white matter, ISF is increased in vasogenic edema around tumors and other types of damage in the brain and spinal cord (60). Nerve fibres in the white matter are widely separated by the edema fluid which appears to drain directly through the ependyma into the ventricular CSF, possibly reflecting a major route for the normal drainage of ISF from white matter (61).

Gray Matter

The extracellular space in gray matter is very tightly controlled (1, 22) and is not expanded to the same extent as in the white matter by vasogenic edema (60) or by the CSF edema of hydrocephalus (52, 53, 54, 55) (Fig. 1). The major pathological significance of ISF and its drainage pathways lies in (a) the accumulation of insoluble β-amyloid (Aβ) and prion proteins in extracellular spaces and in perivascular ISF channels in Alzheimer and Prion diseases; (b) the formation of perivascular lacunae in the basal ganglia; (c) the immunological reactions of the CNS associated with viral infections, and multiple sclerosis.

Experimental studies are the main source of data regarding the drainage of ISF from gray matter of the CNS, but these data are gradually being confirmed in man. Bulk flow of fluid requires spaces of large caliber; the resistance of the narrow intercellular clefts in gray matter (1) is too high to accommodate appreciable bulk flow of ISF (22). Thus, the main channels for bulk flow of ISF in gray matter are the periartrial spaces, as first suggested by Hiss (62) over a century ago and emphasised by Weed (30) in the 1920s. Tracers injected into gray matter of the rat and rabbit cerebrum flow along spaces around arteries within the brain (12, 22, 63), enter perivascular spaces surrounding leptomeningeal arteries on the surface of the brain (63, 64, 65) and drain to cervical lymph nodes (12) (Fig. 2). The clearance of high and low molecular weight tracers from the brain by this pathway is consistent with bulk flow but inconsistent with diffusion (66). Flow rates for the clearance of large molecules from ISF have been estimated at between 0.18 and 0.29 µl/g brain/minute for different regions of the rat brain (64). It is estimated that some 10% of fluid draining from the rat brain is ISF (22) and 50% or more of ISF and CSF from the rat brain drains to cervical lymph nodes (22). High molecular weight substances, including proteins, injected into brain and CSF can be recovered from deep cervical lymph at higher concentrations than from blood plasma (65, 67). Small molecular weight substances
of 5 kDa or less, however, drain into the blood, possibly through small arachnoid villi or into blood vessels of the nasal mucosa, and present only in low concentrations in the lymph (67). The role of lymphatic drainage of antigens from the brain in B-cell (22) and T-cell mediated immune reactions (28) (Fig. 3) will be discussed later in this review.

A summary of ISF drainage pathways from the rat brain is presented in Flow Chart A.

In the human cerebral cortex, periarterial spaces homologous with ISF drainage pathways in the rat brain are encompassed on their outer aspects by a layer of pia mater and on the inner aspects by collagen of the arterial adventitia (68). As in the rat brain, periarterial spaces contain perivascular cells (69) which are the resident histiocytes in the drainage pathways; these cells are activated in the dilated perivascular spaces in edematous peritumoral brain (70) in association with the drainage of edema fluid along such pathways. Periarterial ISF drainage channels in the cerebral cortex are continuous with periarterial channels in the leptomeninges (36, 68). Such channels are difficult to detect in normal brain as they are collapsed but they are seen in their expanded state when the underlying cortex is edematous or when inflammatory cells have invaded the periarterial channels (35).

With increasing caliber of vessel, periarterial compartments around cerebral arteries contain more and more substantial amounts of collagen. This collagen-rich perivascular compartment can be traced through the base of the skull, alongside the internal carotid artery to the neck, thus forming a potential channel for the drainage of interstitial fluid from the brain to deep cervical lymph nodes in man (Djuanda, Kelsey and Weller 1998—in preparation). A scheme for the drainage of ISF from the human brain, especially from gray matter, is presented in Figure 4 and in Flow Chart B.

Cerebral Amyloid Angiopathy in Alzheimer Disease and Prion Disorders

Alzheimer disease is characterised by neurofibrillary tangles of hyperphosphorylated tau (71) and by the presence of senile plaques of β-amyloid (Aβ) in the extracellular spaces, particularly in the cerebral cortex (72). Insoluble prion protein also accumulates in the extracellular spaces of gray matter in Creutzfeldt-Jakob disease and its familial and iatrogenic variants (73). Cerebral amyloid angiopathy occurs in both Alzheimer disease (74, 75) and in prion disorders (73) involving intracortical and leptomeningeal arteri. β-amyloid angiopathy has been extensively studied; it affects not only arteries in patients with Alzheimer disease but also in aged, nondemented individuals and is associated with an increased risk of intracerebral hemorrhage (74, 75). Recent work suggests that cerebral amyloid angiopathy is due to the deposition of Aβ in ISF drainage pathways (76) (Fig. 5). A pool of Aβ is present in normal cerebral cortex (76) and Aβ can be detected biochemically in the walls of large intracranial arteries, such as the middle cerebral and basilar arteries, even in young individuals (77) but not in the walls of extracranial vessels. The pattern of Aβ deposition in cerebral amyloid angiopathy, involving, as it does, arteries much more severely than veins and small arteries more severely than large arteries, strongly indicates that β-amyloid draining from brain tissue becomes entrapped within ISF drainage pathways. Early small deposits of β-amyloid are in the adventitial regions of leptomeningeal arteries in the position of the putative ISF drainage pathways (Fig. 5) (76). A major component in cerebral amyloid angiopathy of cortical vessels is the more insoluble longer form Aβ 1-42 (78); this suggests that the insoluble form of Aβ 1-42 is deposited first in the ISF drainage pathways and then entraps the more soluble, 1-40, form of Aβ (76).

There are 3 aspects of cerebral amyloid angiopathy which may be significant for Alzheimer disease. First, there is damage to artery walls by the deposition of amyloid in the media; Aβ appears to extend into the media from the perivascular ISF drainage pathways in the ad-
ventitia, with consequent destruction of smooth muscle cells (76, 78, 79); such changes result in microaneurysms and intracerebral hemorrhage (75, 76). Deposition of Aβ in artery walls may have a significant effect on the function of microvessels in the cerebral cortex and the regulation of cerebral blood flow (78, 79), and may be related to the increase in cortical infarcts in patients with cerebral amyloid angiopathy. Atherosclerosis is increased in vessels affected by Aβ deposition (74) but it is unclear, as yet, whether atherosclerosis of cerebral vessel walls inhibits the drainage of Aβ along perivascular pathways. Second, the recognition that amyloid is eliminated from the brain by ISF drainage pathways may provide therapeutic opportunities for increasing the elimination of amyloid from the brain. As in Alzheimer disease in general, apolipoprotein E4(ApoE4) is a risk factor for cerebral amyloid angiopathy (75). Whether, through its binding site to Aβ (72), ApoE plays a role in the transport of Aβ in ISF drainage pathways remains to be determined. Third, Aβ may prove to be useful as a natural tracer to define the physiology and anatomy of interstitial fluid drainage from the human brain (Fig. 5). As yet, it is uncertain how much ISF from the human brain drains to cervical lymph nodes and how much leaks into the CSF in the subarachnoid space (Fig. 4). The perivascular spaces of meningeal arteries are encompassed by only a thin layer of leptomeninges (36, 68) and defects in this layer may allow proteins to leak into CSF at the subarachnoid space. It is perhaps significant that levels of soluble amyloid in CSF fall in Alzheimer disease (80) but insoluble Aβ increases in amount, particularly in the presence of cerebral amyloid angiopathy (81). The relationship between periarterial drainage of Aβ and its presence in the CSF still requires clarification.

**Perivascular Lacunae**

Lacunae form around vessels in the basal ganglia; they vary in size from a few hundred micra to 1 mm or more (82). Lacunae are rare in the cerebral cortex and this may be due to differences in structure of the perivascular spaces (Fig. 6). In the cortex, periarterial spaces are lined on the inner aspect by collagen of the adventitia and are encompassed by a layer of pia mater (68), whereas in the basal ganglia, periarterial spaces lie between 2 distinct layers of pia mater and are unencumbered by collagen (83). The difference in structure of perivascular spaces in the cortex and basal ganglia may account for the formation of lacunae in the basal ganglia (83) and also for the relative lack of involvement of arteries in the basal ganglia in cerebral amyloid angiopathy (75). In cortical vessels, amyloid may become entrapped in the collagen and glycosaminoglycans in the adventitia of the vessel, whereas perivascular spaces lined on both aspects by leptomeningeal cells may facilitate the drainage of Aβ from the basal ganglia.

**Fig. 6.** The structure of periarterial spaces differs in basal ganglia and cerebral cortex. (a) An artery in the basal ganglia has coatings of leptomeninges closely associated with the vessel wall (L1), and also an outer coating (L2); the perivascular ISF drainage pathway (PVS) lies between the leptomeningeal layers L1 and L2. Perivascular lacunae probably develop by dilatation of the periarterial ISF drainage pathways. (b) In the cerebral cortex, the perivascular space (PVS) for drainage of ISF lies between the outer single layer of leptomeninges (L1) and the collagenous adventitia of the artery. Aβ and prion proteins accumulate within this space in cerebral amyloid angiopathy. SAS: Subarachnoid space; SPS: subpial space on the surface of the brain. Figure used with the permission of the Journal of Anatomy. See reference 83.

**Neuroimmunology and Multiple Sclerosis**

The increasing evidence for direct drainage of ISF from the CNS to regional lymph nodes has obvious implications for the immunology of viral diseases and multiple sclerosis. Some 50% of ISF and CSF from the rat brain drains along channels which pass through the cribriform plate to nasal lymphatics and cervical lymph nodes in the rat (12, 22) (Fig. 2; Flow Chart A). In man, ISF in perivascular spaces seems to be more separate from the CSF than in the rat (Fig. 4; Flow Chart B), but the exact route of ISF drainage to regional lymph nodes is still under investigation. Arachnoid channels pass through the cribriform plate in man to the nasal mucosa (84) (Djuanda, Kelsey and Weller 1998—in preparation), as in the rat, but, the nasal route may not be the most significant pathway for lymphatic drainage of intracranial fluid in man. The major conduit for ISF and CSF to the cribriform plate region in the rat is along the perivascular space of the ethmoidal artery; this is a major branch of the anterior cerebral artery in the rat (63) but it is no longer present in man. Recent studies suggest that lymphatic drainage of the human brain is most likely to occur along periarterial spaces surrounding the carotid and vertebral arteries (Djuanda, Kelsey and Weller 1998—in preparation) (Fig. 4).
B and T lymphocyte mediated immune reactions in the CNS and their dependence upon lymphatic drainage of antigen have been well documented in the rat (22, 24). Injection of soluble antigen, such as human serum albumin, into the central gray matter of the rat brain results in antibody formation in the cervical lymph nodes and removal of these nodes reduces antibody production 10-fold (26, 27). This suggests that cervical lymph nodes play a key role in B-cell mediated immune reactions in the brain.

Involvement of cervical lymph nodes in T-cell mediated immunity of the CNS has been illustrated in a series of experiments using a cryolesion model of experimental autoimmune encephalomyelitis (EAE). Acute, active, EAE is induced by the inoculation of myelin basic protein or homogenised guinea pig spinal cord into the foot pads of Lewis rats. This results in lymphocyte infiltration and microglial activation, mainly in the spinal cord (85), and animals develop hind limb paresis which reaches a peak at about 12 days after inoculation and subsides some 8 days later. A brain wound, in the form of a cryolesion on 1 cerebral hemisphere 8 days post inoculation of antigen, produces a 5-fold enhancement of EAE in the cerebral hemispheres (85). When the cervical lymph nodes are removed at the same time as the cryolesion, the enhancement of EAE in the cerebral hemispheres is reduced by 40% (88) (Fig. 3). These experiments suggest that cervical lymph nodes play a key role in the enhancement of cerebral EAE following focal brain injury. It is probable that antigens from the brain wound drain to cervical lymph nodes and, in the presence of circulating activated T lymphocytes directed against CNS antigens, induce T-cells to target the brain. This is emphasised in experiments in which lymphocytes from animals with cryolesion-EAE were injected into naive recipients and resulted in a 3-fold increase in EAE lesions in the brain, when compared with animals injected with lymphocytes from EAE-only animals (86).

Immune privilege is a concept that arose from the observation that allografted tissue in the brain survives for relatively long periods. This was thought to be due to the absence of lymphatic drainage of the CNS and the presence of a blood-brain barrier. The results of experimental studies outlined in this review suggest that the concept of immune privilege requires re-evaluation (22). Some 50% of intracranial fluid in the rat is eliminated by lymphatic drainage and similar pathways exist in man. Furthermore, T and B lymphocytes can be induced to target the brain (24, 86, 87); this possibly involves the selective expression of LFA-1/Mac-1 and a4-integrins on lymphocytes and of ICAM-1 and VCAM-1 on CNS vessel endothelium (88). Immune privilege, therefore, appears to be a state of tolerance between the CNS and the immune system, which under certain circumstances may be modified (22). There are good examples of how this tolerance breaks down in B-cell and T-cell mediated immunity. Antigen injected into the rat brain may excite little inflammation, but when the animal is challenged peripherally there is trafficking of B-cells across the blood-brain barrier and retention of B-cells at the site of antigen deposition (87). A similar effect is seen in cryolesion-EAE in which release of antigens into the lymphatic system following peripheral immunisation with CNS proteins results in targeting of the brain by T-lymphocytes (28, 85, 86).

Multiple Sclerosis (MS) is considered to be an autoimmune disease of the CNS, and the results of animal experiments showing T- and B-lymphocyte targeting of the brain in autoimmune disease (28, 85, 86, 87) have clear implications for MS. The exact mechanisms for initiation and relapse of multiple sclerosis plaques are unclear but, by analogy with cryolesion EAE (24), drainage of brain antigens to regional lymph nodes in man, in the presence of circulating brain directed activated lymphocytes, could result in lymphocyte targeting of the CNS in MS. The epidemiological distribution of MS suggests that an environmental agent may result in molecular mimicry by which activated T-cells are directed against self antigens (89). Such molecular mimicry may occur when pathogens express a stretch of protein that is related in sequence or structure to a particular component in the CNS. Damage to the brain or spinal cord in the presence of such brain directed, activated lymphocytes may result in targeting of the CNS by those activated cells as in cryolesion EAE (28, 86). Focal damage to the CNS could be a result of small emboli or minor degrees of trauma and this may result in the drainage of antigen to regional lymph nodes. It is known from MRI studies that small foci of brain damage occur within the white matter of the human brain, even in young middle age, and it is possible that similar lesions may also occur earlier in life. Antigens draining from focal CNS lesions to regional lymph nodes in the presence of circulating T- and B-cells directed against brain antigens could play a role in the initiation and relapse of multiple sclerosis lesions.

Conclusions

This review has emphasised the phylogenetic development of extracellular fluid drainage from the CNS. In small animals like the rat and rabbit, ISF and CSF merge and a significant proportion of the fluid drains to regional lymph nodes in the neck and para-aortic regions. In man, the bulk of choroid plexus-derived CSF drains through ventricles and subarachnoid space to blood via arachnoid villi and granulations; pathways of ISF drainage along periarterial spaces show a degree of separation from CSF and ISF may drain to regional lymph nodes. Further study of the physiology and pathology of ISF drainage pathways in man may be of increasing value in the investigation and therapy of disorders as varied as Alzheimer disease, prion disorders, and multiple sclerosis.
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REFERENCES

30. Weiss LH. The absorption of cerebrospinal fluid into the venous system. Am J Anat 1923;191-21
34. Krahn V. The pia mater at the site of entry of blood vessels into the central nervous system. Anat Embryol 1982;164:257-63
41. Tripathi BJ, Tripathi RC. Vascular transcellular channels as a drainage pathway for cerebrospinal fluid. J Physiol (Lond) 1974;239:195-206


78. Roher AE, β-amyloid (1-42) is a major component of cerebrovascular amyloid deposits: Implications for the pathology of Alzheimer’s disease. Proc Natl Acad Sci, USA 1993;90:10836–40


