The meninges as barriers and facilitators for the movement of fluid, cells and pathogens related to the rodent and human CNS

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Abstract

Meninges that surround the CNS consist of an outer fibrous sheet of dura mater (pachymeninx) that is also the inner periosteum of the skull. Underlying the dura are the arachnoid and pia mater (leptomeninges) that form the boundaries of the subarachnoid space. In this review we (1) examine the development of leptomeninges and their role as barriers and facilitators in the foetal CNS. There are two separate CSF systems during early foetal life, inner CSF in the ventricles and outer CSF in the subarachnoid space. As the foramina of Magendi and Luschka develop, one continuous CSF system evolves. Due to the lack of arachnoid granulations during foetal life, it is most likely that CSF is eliminated by lymphatic drainage pathways passing through the cribriform plate and nasal submucosa. (2) We then review the fine structure of the adult human and rodent leptomeninges to establish their roles as barriers and facilitators for the movement of fluid, cells and pathogens. Leptomeningeal cells line CSF spaces, including arachnoid granulations and lymphatic drainage pathways, and separate elements of extracellular matrix from the CSF. The leptomeningeal lining facilitates the traffic of inflammatory cells within CSF but also allows attachment of bacteria such as *Neisseria meningitidis* and of tumour cells as CSF metastases. Single layers of leptomeningeal cells extend into the brain closely associated with the walls of arteries so that there are no perivascular spaces around arteries in the cerebral cortex. Perivascular spaces surrounding arteries in the white matter and basal ganglia relate to their two encompassing layers of leptomeninges. (3) Finally we examine the roles of ligands expressed by leptomeningeal cells for the attachment of inflammatory cells, bacteria and tumour cells as understanding these roles may aid the design of therapeutic strategies to manage developmental, autoimmune, infectious and neoplastic diseases relating to the CSF, the leptomeninges and the associated CNS.

Keywords Leptomeninges · CSF · Barriers and facilitators · Developing human and rat brain · Perivascular compartments and spaces · Attachment of *Neisseria meningitidis*, inflammatory cells and tumour cells to leptomeninges · Lymphatic drainage of the CNS

Introduction

The meninges covering the rodent and human CNS are divided primarily into the dura mater or pachymeninx and the arachnoid and pia mater or leptomeninges. Both dura and arachnoid encompass the brain and spinal cord and the arachnoid forms the outer barrier that contains CSF within the subarachnoid space (SAS). The pia is more closely applied to the brain and spinal cord but is separated from the CNS tissue on the surface of the CNS by a subpial space containing blood vessels and varying amounts of collagen.

For decades the meninges had been considered to be no more than a protective shield, but recent evidence suggests that leptomeninges have complex functions as barriers and facilitators for the movement of fluid, solutes and cells at

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the surface of the CNS and of fluid and solutes within the CNS parenchyma. Thus, the SAS functions as an important distribution system. The fundamental pattern of blood supply to the CNS is different from that of most other organs in which a single artery and accompanying veins penetrate the surface at a single point and divide into smaller and smaller branches within the organ. In the CNS, the arteries branch and divide into small muscular arteries and arterioles within the SAS prior to entering the brain and spinal cord, the surface of which is covered by a close network of numerous small vessels that enter and leave the CNS. The outer barriers of the brain function as morphogenetic signalling centres [27, 89], dynamic transport systems [108], a stem cell niche [27] and regulators of immune cell entry into the CNS [93] as summarised by Brøchner et al. [14].

The adult CNS is separated from peripheral tissues by a set of structures and physiological mechanisms that are present at various interfaces between the CNS parenchyma and surrounding fluids. Five main interfaces can be identified in the adult brain: (a) the arachnoid barrier, (b) the blood–brain barrier, (c) the blood–CSF barrier, (d) circumventricular organs (CVO) barrier formed by tight junctions between adjacent tanycytes, (e) the inner interface between the CSF and the brain (glia limitans) in the adult. An additional sixth interface, (f) the interface between the CSF and the embryonic brain is present only in the foetus [62, 83]. In the adult, an arachnoid blood–CSF barrier separates the fenestrated blood vessels in the dura from the CSF in the SAS by means of a cell layer joined by tight junctions (the arachnoid barrier layer) [65]. Since the arachnoid blood–CSF barrier is avascular and provides a relatively small surface area, it is generally considered that it does not contribute significantly to blood–brain exchange [1]. However in light of recent findings of drug transporters and drug-metabolising enzymes in the arachnoid blood–CSF barrier [108] and taking into account that the arachnoid barrier cell layer constitutes a much larger fraction of the brain surface area during early development, it is clear that the arachnoid blood–CSF barrier is an important and changing interface that should not be ignored. It clearly warrants further investigation in the developing and adult brain.

In this review, we first examine the involvement of the leptomeninges in the development of the human and rat brains. We then review the structure of the adult leptomeninges to establish the interrelations between leptomeninges, CSF, blood vessels and the CNS parenchyma. The human CNS is much larger than the rodent CNS and the anatomical arrangements between the leptomeninges, blood vessels and CNS parenchyma are much easier to discern in the human CNS than in rodents. We will therefore define the major structural attributes of the human leptomeninges and compare them with rodent leptomeninges. This is an important aspect of the review as the majority of

experimental studies examining the relationships between inflammatory cells and leptomeninges are performed in mice and how these studies relate directly to the human leptomeninges needs to be clearly defined. Although many investigations of the leptomeninges are performed through the medium of immunocytochemistry, ultrastructural studies form a valuable contribution to our understanding of the exact relationships between the delicate cell layers of leptomeninges and other structures within the human CNS. For this reason, we illustrate the structure of the adult human leptomeninges largely by their ultrastructure.

Barrier functions of the leptomeninges constitute an emerging field of research in both human and rodent CNS and are discussed in this review. However, just as important to the biology and pathology of the CNS is the role of leptomeninges as facilitators for the movement of fluid, solutes and inflammatory cells in relation to the CNS and its diseases. Leptomeninges as facilitators for attachment and movement of cells are also important in infections and tumour metastases in the SAS. We will review the attachment of bacteria to leptomeningeal cells, especially with regard to *Neisseria meningitidis*, and the role that leptomeningeal elements may play in the location and spread of tumour metastases within the SAS. Although in its early stages, the identification of ligands on the surfaces of leptomeningeal cells to which bacteria and tumour cells attach has clear implications for therapies in these and other disorders involving the leptomeninges.

As often occurs in fields undergoing active investigation, misconceptions have arisen and are perpetuated within the literature in relation to the leptomeninges and to the exchange and drainage of fluid associated with the CNS. For example, it has been assumed, perhaps wrongly, that the SAS is lined by collagen, that there are perivascular spaces for the entry of CSF into the brain and for the drainage of interstitial fluid (ISF) from the brain. Furthermore, the concept of the CSF as a sink for the drainage of ISF from the brain may need to be re-examined, particularly as the best estimate is that 85% of ISF from the brain drains to lymph nodes and only 15% of ISF passes into the CSF under normal conditions [94]. These experiments were performed in the rat using radioiodinated serum albumin. Injections into the caudate nucleus and internal capsule resulted in 10–15% of tracer passing into CSF. Injections into the much smaller midbrain resulted in 60–75% efflux of tracer into the CSF which was probably due to direct efflux following injection into the very small midbrain. The probability of direct efflux of tracer into the CSF following injection into the brain through too large a needle in too great a volume was identified by Carare et al. [17].

Meninges in the developing human and rat brains

Features of outer brain barrier and meningeal development in human and rat are summarised in Table 1 [14] (from Brøchner et al. [14]).

Human brain and meninges

In early foetal life, there are two separate CSF systems: inner CSF in the future cerebral ventricles and outer CSF in the future SAS. Following the development of the midline foramen of Magendi and the lateral foramina of Luschka, there is one continuous CSF system. However, during foetal life, CSF is not likely to drain through arachnoid granulations as they are not present prior to 39 weeks gestation [36]. They appear in greater number as children age and are clearly visible by 2 years and then increase further in size and number with increasing age [61]. Thus, it is likely that CSF at least

in the neonatal brain is eliminated by lymphatic drainage pathways passing through the nasal submucosa [61].

By 5 weeks post-conception (wpc), the neural tube has closed around a fluid-filled neural cavity, initially filled with amniotic fluid but progressively modified and referred to as eCSF (embryonic cerebrospinal fluid) or the inner CSF. Closure of the neural tube occurs before the appearance of the choroid plexuses but also at a time when vascularisation of the brain tissue has barely begun. The first barriers or interfaces for exchange that develop in and around the embryonic brain are: (1) between the brain and inner CSF (eCSF) entrapped following closure of the neural tube; this occurs in humans 26 days post-conception (dpc) and (2) between the brain and loose vascular mesenchyme that is derived from the neural crest and forms the primary meninx at the outer surface of the brain anlage. There is no trace of a SAS at this time, just a primary meninx (meninx primitiva). Even when the SAS starts to develop, the origin of the fluid it contains is unclear. The fluid does not originate from the

Table 1 Features of outer brain barrier and meningeal development (from Brøchner et al. [14])

Weeks post-conception	Feature	Species	References
6th	Appearance of a primary meninx around most parts of the brain	Human	O’Rahilly and Müller [70]
	Amoeboid microglial cells penetrate the brain by crossing the pial basement membrane	Human	Verney et al. [100]
7th	The skeletogenous layer becomes visible	Human	O’Rahilly and Müller [70]
	Differentiation of a leptomeningeal meshwork that is presumed to contain liquid and represent the future SAS	Human	O’Rahilly and Müller [70]
8th	The dural limiting layer is almost complete in hind-brain and midbrain but only present in the area adjacent to the lateral fossa in the forebrain	Human	O’Rahilly and Müller [70]
	The fenestrated sinusoids of the pia–arachnoid become non-fenestrated (E14)	Rat (E14)	Balslev et al. [10]
	Most of the cisternae of the adult are already present	Human	O’Rahilly and Müller [70]
7th–10th	Initial communication between the ventricular and subarachnoid compartments	Human and rat (E17)	Brocklehurst [15], Johansson et al. [49]
11th	Completion of the subpial end feet layer (E16) and claudin-11 positive arachnoid blood–CSF barrier (E18) and thereby appearance of a clearly defined SAS	Rat (E16 and E18)	Balslev et al. [10]
12th–13th	Second wave of microglial cells penetrate the brain via the BBB and inner CSF–brain barrier	Human	Verney et al. [100]
12 (13th–15th)	Radial glial cells begin to transform into astrocytes	Rhesus monkey (E64) and newborn ferret	Schmechel and Rakic [86]; Voigt [101]
25th–28th	Transition from subpial end feet layer to glia limitans	Human	Kadhim et al. [50]

The meninges and outer brain barriers form during embryogenesis and early foetal life. Many factors are involved in this dynamic process and are necessary for adequate development of the brain. O’Rahilly and Müller [70] described the development of the human meninges in 1986 according to Carnegie stages, but have recently revised this staging model [71]. This Table is a summary of important events in the development of the human meninges and the barrier between the outer CSF and the brain approximated to weeks post-conception (wpc). Results in Carnegie stages have been translated according to the revision by O’Rahilly and Müller [71] and from mammals to human according to the work of Clancy et al. [22]

E14 etc. embryonic day 14, SAS subarachnoid space

choroid plexus as this has not yet developed. Even when the choroid plexus has developed, CSF is secreted into a closed ventricular system that has no connection with the SAS. There are probably two separate CSF spaces until 10 wpc in humans and E17 in the rat (Table 1). The outer CSF space is the future SAS and the inner CSF spaces are the future ventricles. At this stage of development, the forebrain is still devoid of vasculature [62].

Vascularisation

In the early stages of vascularisation of the brain, a loose vascular ectomesenchyme, derived from the neural crest,

develops rostral to the mesencephalon and continues caudally along the brain stem and spinal cord as a vascular mesenchyme originating from the paraxial mesoderm. This layer of loose mesenchymal tissue, that surrounds the neural tube and the developing early brain anlage, has been termed the primary meninx [70]. Prior to the development of the dura and the leptomeninges (arachnoid, SAS and pia) an extensive microvascular network of interdigitating small arterioles, capillaries and venules, termed the perineural vascular plexus (PNVP), is established by vasculogenesis at the surface of the neural tissue. Following differentiation of the leptomeninges from 7 to 10 wpc, the PNVP will form

Fig. 1 Immunocytochemistry of the leptomeninges in the developing rat brain at low magnification. **a–d** Sagittal sections from an E18 rat brain at low magnification demonstrating the arachnoid barrier cell layer stained for claudin 11 (**a**, **c**), together with dural border cells and dura stained for AQP1 (**d**). **b** In contrast to the meninges that are immunonegative for AQP4, the astrocyte end feet layer of the entire ventral surface of the brain stem up to the cephalic flexure (CF) is strongly immunopositive for AQP4 (arrowheads). **a** Parasagittal section at the level of the olfactory bulb (OB) depicting the discontinuity ('the olfactory bulb hole') (between arrows) in the arachnoid barrier cell layer indicated by arrowheads. **b–d** Consecutive near-midsagittal sections through the developing pineal gland (PG), third ventricle (3V) and median eminence (ME) and superior sagittal sinus (SSS) in **d**. Note the discontinuities in the barrier cell layer between double arrows corresponding to the pineal gland ('the peripineal slit') and the median eminence ('the peripituitary slit') which is visible at higher magnification in **e**. The caudal part of the peripineal slit can be recognised even at low magnification between the central small and the larger arrow pointing to the pineal gland diverticulum. Higher magnifications of the cisterna magna (CM) and the subarachnoid space (SAS) in relation to the choroid plexus and brain surface are shown in Fig. 2. Note the dense group of oligodendrocyte progenitor cells (OPC) facing the cephalic flexure in **c**. **e**, **f** At higher magnification the meninges and their relationship to the pontine cistern (PC) and the primordium of the pituitary gland, including the posterior lobe (PL), Rathke's pouch (RP) and the anterior lobe (AL) are depicted. The ventral surface of the pons (P) and the basilar artery (BA) are not stained either for claudin-11 or AQP-1. The posterior lobe is separated from the cisterns of the subarachnoid space by a claudin-11-positive arachnoid barrier cell layer which terminates abruptly (small arrows) where the neurohypophysis emerges from the median eminence (ME). Note the strong AQP-1 immunoreactivity of the dural border cells (DBC) in **f**. 3V third ventricle, 4V fourth ventricle, AL anterior lobe, AQP-1 aquaporin-1, AQP-4 aquaporin-4, BA basilar artery, *cln-11* claudin-11, CF cephalic flexure, CM cisterna magna, DBC dural border cells, LV lateral ventricle, ME median eminence, OB olfactory bulb, OPC oligodendrocyte progenitor cells, P pons, PC pontine cistern, PG pineal gland diverticulum, PL posterior lobe, RP Rathke's pouch, SAS subarachnoid space, SSS superior sagittal sinus, TC tentorium cerebelli. **a–d** Same magnification. Scale bar 2000 µm. **e–f** Same magnification. Scale bar 200 µm

a very dense leptomeningeal vascular plexus, the vascular distribution system, in the SAS [62].

Development of barriers in the brain

There is a clear distinction in the pattern of development between blood–brain and blood–CSF barriers and the complex arachnoid barrier. In a recent paper, it was shown by claudin-11 immunolabelling that the interfaces associated with the human arachnoid barrier layer develop over a prolonged period of 5 weeks—from 7 wpc to 12 wpc—before there is a well delineated and, thus well-defined, subarachnoid space [62]. The barrier layer appears first in the posterior cranial fossa from where it extends rostrally towards the olfactory bulbs, initially covering the base of the skull from where it spreads towards the vertex (for a more detailed description, see [62]). This process, which is completed by the 12th wpc in humans corresponds to embryonic day 18

(E18) in the rat (see Table 1) from which a number of new findings will be presented in the next section.

The interface between the outer CSF and the brain restricts diffusion between brain and subarachnoid CSF through an initial layer of radial glial end feet covered by a surface layer of pia and, in the third trimester, by glia limitans covered with a surface layer of pia [14]. Complex barriers at the surface of the brain, particularly during development, are poorly defined [10], but by mid-gestation subarachnoid interfaces are almost fully developed. The SAS is delineated by the arachnoid barrier cell layer adjacent to the dura and the astrocyte end feet layer on the surface of the brain forming two individual interfaces: (1) the arachnoid forming a barrier or interface between the blood vessels in the dura and the outer CSF in the SAS and (2) the interface between the outer CSF and the surface of the brain. In addition there is a third interface within the SAS between the outer CSF and the blood in the pial microvasculature [14]. The vasculature in the SAS is characterised from earliest development by claudin-5 positive tight junctions in endothelial cells that confer its barrier properties as confirmed by the exclusion of plasma proteins, e.g. AFP [62].

Meninges and claudin-11 in the developing rat brain

The tight junction protein, claudin-11, was initially described as the basis for the blood–testes barrier (Sertoli cell tight junctions) and shown to be the same as oligodendrocyte specific protein (OSP) responsible for the tight junctions between oligodendrocytic myelin sheaths and axons [63]. Later, the developing arachnoid barrier cell layer was shown to be immunoreactive for claudin-11 in humans and in rats from E18 [14] and also in the E16 mouse [105]. The arachnoid is thus the barrier between the outer CSF in the SAS and the fenestrated blood vessels in the dura.

The general morphogenesis of cranial meninges in the rat was described by Angelov and Vasilev [6] at a time when claudin-11 as a marker for arachnoid barrier cells had not yet been identified. More recently it has been demonstrated that the early rat brain (E10–E14) shows no signs of claudin-11 immunoreactivity, but that at E15, weak staining is present in the loosely packed arachnoid barrier cells at the base of the brain and extending into the future tentorium cerebelli [14]. In the following description we summarise some new immunohistochemical findings from the stage in rat brain development (E18) in which a well-defined SAS is first present.

A large number of sagittal sections from the E18 rat were stained for claudin-11 to ensure that there is a complete arachnoid barrier between the outer CSF and the fenestrated blood vessels in the dura, apart from the known openings where the cranial nerves perforate the dura-arachnoid (see Fig. 1A in Ref. [14]). Due to the interest in CSF flow, adjacent sections

were stained for aquaporin-1 and -4 (AQP-1 and AQP-4). Parasagittal sections at the level of the olfactory bulb demonstrate a consistently large disruption in the arachnoid barrier layer overlying the cribriform plate ('the olfactory bulb hole') providing free access for drainage of CSF to the nasal submucosa (Fig. 1a). Even in other midsagittal sections 'the olfactory bulb hole' is still present but now accompanied by discontinuities in the arachnoid barrier corresponding to the median eminence (the peripituitary slit') and the pineal gland ('the peripineal slit') (Fig. 1c). Not only CSF but also blood cells belonging to the immune system could well pass into the SAS at this location.

Dura, including dural border cells, are immunoreactive for AQP-1 (Fig. 1d) but not for AQP-4 which stains the astrocyte end feet layer of the entire ventral surface of the brain stem (Fig. 1b). The arachnoid barrier cell layer covering the posterior lobe of the pituitary is not positive for AQP-1 in contrast to the AQP-1 positive subdural border cells which are not stained for claudin-11 (compare Fig. 1e, f).

At higher magnification, strong claudin-11 immunoreactivity extends from the arachnoid barrier cell layer via arachnoid trabeculae towards the tela choroidea to cover the base of the stroma of the choroid plexus in both fourth and third ventricles (Fig. 2a, c). These findings are important in discussions about the continuity of the SAS and pial tissues with the stroma of the choroid plexus [107] and the so-called functional leak at the tela choroidea between ependyma and choroid plexus epithelium [13]. The claudin-11 positive pial cells within the stroma of the choroid plexus could well act as a narrow barrier layer between the blood and CSF in the SAS.

In addition to the AQP-1 immunoreactivity in dural border cells, choroid plexus epithelium and the external granular layer of the cerebellum, the leptomeningeal vascular plexus in the SAS covering the dorsal brain stem (Fig. 2b) and cortex (Fig. 2d) are clearly positively stained. These pial microvessels are also strongly immunoreactive for claudin-5 (Fig. 2e) in parallel with early human perineural and leptomeningeal blood vessels. Influx transporters—here exemplified by the excitatory amino acid transporter-1 (EAAT-1)—are also present in the arachnoid barrier cell layer (arrowheads in Fig. 2f). Materials and methods are described in Bröchner et al. [14].

Meninges associated with the adult human brain

Dura mater

The cranial dura in humans is approximately 1 mm thick; it is composed of dense fibrous tissue and forms the inner periosteum of the skull. Sheets of dura, the falx cerebri and tentorium cerebelli divide the cranial cavity into three major compartments. In children, the dura adheres firmly to the

sutures of the skull but, as the sutures close in young adults, the dura can be easily stripped from the inside of the skull; with advancing age, the dura adheres more firmly to the inner aspect of the skull. Much of the blood supply for the dura is derived from the middle meningeal branches of the maxillary artery and other arteries derived from the external carotid and from branches of the occipital and vertebral arteries in the posterior fossa. Microscopically the dura consists of densely packed bundles of collagen fibres with interspersed arteries, veins and lymphatics. There is no barrier comparable to the blood–brain barrier associated with blood vessels in the dura. Major venous sinuses draining blood from the brain are enclosed within the cranial dura and dural lymphatics contribute to the drainage of CSF from the SAS [8, 54, 59]. As no direct connections between the brain and dural lymphatics have been established, it is unlikely that fluid and solutes from the brain drain into dural lymphatics.

Arachnoid separates easily from the inner aspect of the dura over most of the surface of the cerebral hemispheres in humans during surgical operations and at post-mortem. The only regions of adherence are where cortical veins join venous sinuses in the dura and where a small number of vascular bundles containing arteries and veins cross the subarachnoid and subdural spaces from the surface of the brain to the dura [11]. Such vascular bundles are mainly in the temporal regions and in the posterior fossa. Arteriovenous malformations in the dura have a significant effect on the vasculature of the brain through these connections and may be associated with cerebral venous thrombosis or intracranial haemorrhage [82].

In the spinal column, the dura forms a tube around the outer layer of arachnoid and, in humans, is separated from the bones of the vertebral column by epidural adipose tissue and venous plexuses. There is a series of dentate ligaments along the length of the human spinal cord that connect the dura to the substantial layer of subpial collagen encompassing the human spinal cord [68]; the dentate ligaments appear to stabilise the cord within the spinal SAS.

Both the cranial and spinal dura in rodents are much thinner than in humans and are often best examined histologically within the intact skull [23]. As in humans, the rodent dura is composed of bundles of collagen fibres and also contains arteries, veins and lymphatics [8, 54, 59].

Leptomeninges

General features of leptomeningeal cells

Leptomeningeal cells show variable ultrastructural and immunocytochemical characteristics depending upon the structures with which they are associated [4]. Intercellular junctions include tight junctions in the barrier layer of arachnoid where it abuts the dura and desmosomes in the

Fig. 2 Immunocytochemistry of the leptomeninges in the developing rat brain at higher magnification. **a–f** Higher magnification of **c**, **d** of Fig. 1a–d and two more consecutive sections stained for claudin-5 (**e**) and EAAT-1 (**f**). The claudin-11 immunoreactivity extends from the arachnoid barrier cell layer via some arachnoid trabecular cells (AT) towards the tela choroidea (TC) to cover the base of the stroma of the choroid plexus in the fourth and third ventricles (4V and 3V). The roof of the fourth ventricle, velum medullare (VM), is not reacting. Dural border cells (DBC), choroid plexus epithelium, external granular layer of the cerebellum (EGL) and the dense leptomeningeal vascular plexus (LVP) covering the dorsal surface of the brain are stained for AQP-1. In **e** the dense leptomeningeal vascular plexus (LVP) in the subarachnoid space facing the dorsal brain sur-

face in a tangential section is strongly stained for claudin-5 (**e**). **f** The immunoreactivity of the arachnoid barrier cell layer stained for influx transporter EAAT-1 (arrowheads). Note the similarity between the claudin-11 staining in **c**, e.g. next to the tela choroidea (TC), with that of the EAAT-1 staining but in contrast to the lack of AQP-1 reactivity in **d** thus supporting the specificity of the immunoreactions. 3 V third ventricle, 4 V fourth ventricle; AQP-1 aquaporin-1, AQP-4 aquaporin-4, AT arachnoid trabecular cells; *cld-5* claudin-5, *cld-11* claudin-11, CM Cisterna magna, DBC dural border cells, EAAT-1 excitatory amino acid transporter-1, PG pineal gland diverticulum, PR pineal recess, SAS subarachnoid space, TC tela choroidea, VM velum medullare. **a–f** Same magnification. Scale bar 500 μ m

body of the arachnoid; desmosomes are also distributed to a lesser extent among leptomeningeal cells in the pia [4]. Most of the intercellular junctions between cells forming the pia, coating the trabeculae, arteries and veins in the SAS are relatively small gap junctions [13]. Immunocytochemistry of leptomeningeal cells in the adult human brain has identified the presence of desmosomes and tight

junctions and of vimentin and epithelial membrane antigen (EMA) in the cytoplasm of leptomeningeal cells.

Distribution of leptomeningeal cells in relation to the CNS

Leptomeningeal cells form substantial sheets such as the human arachnoid and thin, delicate sheets that form the pia on the surface of the CNS and coat trabeculae in the SAS.

Fig. 3 Leptomeninges in the adult human brain and spinal cord. **a** Subarachnoid space over the human cerebral hemispheres. The arachnoid encompasses the subarachnoid space (SAS) and trabeculae, composed of collagen coated by leptomeningeal cells, extend across the SAS to join the pia covering the cerebral cortex. Blood vessels (BV) are suspended within the SAS by trabeculae. Resin section stained with toluidine blue. Scale bar 200 μ m. Published with permission from Ref. [4]. **b** Subarachnoid space (SAS) and human spinal leptomeninges. Part of the spinal cord is seen bottom left and the thick layer of dura is at the top and to the right of the figure. The arachnoid has become focally detached from the overlying dura. A delicate, highly perforated dorsolateral ligament (DL) extends from the spinal cord to the arachnoid and incorporates dorsal spinal nerve roots (DR). The pia has a thick underlying layer of subpial collagen. Scale bar 600 μ m. Published with permission from Ref. [68]. **c** Human cerebral subarachnoid space looking down on to the pia. Branches of a histologically proven artery enter the brain. In the upper part of the figure the pia is reflected on to the surface of an artery as it enters the underlying cortex (white arrow). By this arrangement, the subarachnoid space is separated by a layer of pia from the brain and from arterioles in the brain. Towards the bottom of the figure, a branch of the artery is associated with a V-shaped depression in the pia (black arrow) as the artery enters the cortex (see text). Scanning electron micrograph: Scale bar 150 μ m. Published with permission from Ref. [45]

Thin sheets of leptomeningeal cells extend into the CNS parenchyma around penetrating arteries. Leptomeningeal cells also surround collagen bundles in the stroma of the choroid plexus [3] and in the cores of arachnoid granulations [56, 98]. In this section, the structural features and functional roles of the human leptomeninges will be reviewed and related to leptomeninges in rodents.

Arachnoid mater

The arachnoid forms a translucent sheet that encompasses the outer aspects of the cerebral and spinal SAS. In humans, the arachnoid is some 200 μ m thick (Fig. 3a) and the main body of the arachnoid is composed of closely packed leptomeningeal cells joined by desmosomes and devoid of basement membranes [4]. In the outer barrier layer of the arachnoid that abuts on to the dura, the cells are joined by tight junctions that relate to the function of the normal arachnoid as a barrier to the passage of CSF out of the SAS and the entry of solutes and cells into the SAS [4, 65]. Sheet-like and filiform trabeculae with cores of collagen fibres coated by leptomeningeal cells extend across the human SAS to join the pia on the surface of the brain (Fig. 3a). Trabeculae divide the SAS into compartments and also suspend arteries and veins within the SAS [4, 45].

Human spinal leptomeninges form an outer barrier layer of arachnoid that is closely applied to the inner aspects of the dura [68]. Intermediate layers of arachnoid within the spinal SAS are composed of a complex array of perforated sheets, mainly over the dorsal aspects of the spinal cord [68,

103]; these perforated sheets may play a role in regulating the flow of CSF within the spinal SAS. Similar perforated sheets of leptomeninges form the delicate dorsolateral ligaments extending from the surface of the spinal cord to the arachnoid (Fig. 3b) [68, 103].

The SAS is shallower and much less extensive in rodents than in humans [23, 54]. Injection of tracers into the cisterna magna in rats shows that the SAS is most extensive over the base of the rodent brain and extends over the superior-lateral aspects of the cerebral hemispheres mainly as narrow channels adjacent to major branches of the middle cerebral arteries [54]. SAS surrounds the whole circumference of the rodent spinal cord.

Pia mater

In both rodents and humans, the pia is composed of a thin layer of leptomeningeal cells that are joined by gap junctions [13] and occasional desmosomes. For the most part, the pia is only one cell layer thick and is reflected on to arteries and veins as they enter or leave the surface of the CNS (Fig. 3c). By this anatomical arrangement, the subpial spaces and the surface of the CNS are separated from the SAS by a thin layer of pia mater [4, 45, 68]. Arteries enter the surface of the human brain in two ways as shown in Fig. 3c [45]. They may pass obliquely from the SAS to run parallel to the surface of the cortex as pial arteries in the subpial space (see white arrow in Fig. 3c). Alternatively, arteries enter perpendicular to the surface of the brain where they are associated with a V-shaped depression in the pia as shown by the black arrow in Fig. 3c. Pial arteries running parallel to the surface of the cerebral cortex are surrounded by a single layer of pia (Fig. 4a) and are separated from the glia limitans on the surface of the cortex by subpial space. Veins in the subpial space may or may not be surrounded by pia [4, 109]; the vein in Fig. 4b has no sheath of pia. Pial cells can be identified on the surface of vessels by the presence of intercellular gap junctions (Fig. 4a, c); desmosomes that are so prominent in the arachnoid are less frequently seen in the pia. Basement membranes associated with the cells of the pia may be incomplete (Fig. 4c, d). Within the subpial space delicate bundles of collagen fibres are interspersed with occasional inflammatory cells (Fig. 4a).

The pia and the subpial space are similar in structure in rodents and humans (Fig. 4a, b, d) but are more delicate in rodents; the basement membrane associated with the pia is variable in its extent.

Fig. 4 Pia mater and subpial space in the adult human and mouse brain. **a** An artery in the human cortical subpial space. The pia is at the top and a thin layer of leptomenigeal cells derived from the pia (P) completely surrounds the arteriole in the subpial space. Gap junctions (GJ) (see **c**) join adjacent pial cells. Glia limitans (G) is separated from the artery by subpial space. Inflammatory cell (IC). Scale bar 6 μ m. Published with permission from Ref. [109]. **b** A vein in the human cortical subpial space. The pia (P) is at the top of the picture and the thin-walled vein in the subpial space (SPS) has no coating of leptomenigeal cells. The glia limitans (G) is at the bottom of

the picture and bundles of collagen fibres are seen within the subpial space (SPS). Scale bar 2 μ m. Published with permission from Ref. [4]. **c** High magnification of a gap junction (GJ) between cells in the perivascular pia as indicated in **a**. Smooth muscle cell of the artery (SM). Scale bar 200 nm. Published with permission from Ref. [109]. **d** Surface of cerebral cortex from a normal mouse showing pia, glia limitans (G) and a shallow intervening subpial space containing collagen fibres (Coll). A macrophage (M) adheres to the surface of the pia and extends a process between two pial cells (between *arrows). Scale bar 1 μ m

Perivascular compartments and perivascular spaces in the brain

Arteries and veins in the cerebral cortex

There are no perivascular (Virchow–Robin) spaces around arteries in the normal human cerebral cortex (Fig. 5a, b). As arteries enter the surface of the human and rodent brain parenchyma perpendicular to the surface of the cerebral cortex, they carry with them a single-layered sheath of pial cells that intervenes between the outer aspect of the artery and the glia limitans (Fig. 5a, b) [64, 103, 109]. Figure 5a shows an artery in the human cerebral cortex in which the perivascular compartment is composed of compacted layers of smooth muscle cells, basement membrane and pia closely associated with the basement membrane and astrocyte end feet of the glia limitans but there is no actual space [109]. A similar arrangement is seen in arteries in the mouse brain (Fig. 5b) [64]. Perivascular macrophages are embedded in the basement membranes that surround arteries and other blood vessels in the normal brain [37].

There has been much debate for many years regarding the presence of periarterial (Virchow–Robin) spaces around arteries in the cerebral cortex. Much of the confusion has arisen first from artefact in histological and electron microscope preparations due to swelling of perivascular astrocytes thus forming an artefactual space around cortical arteries [81]. Second, there has been misinterpretation of anatomical features. For instance, the subpial space shown in Fig. 4a has been mistaken for an intracortical periarterial space. Similarly, the subarachnoid space has been mistaken for perivascular spaces around cortical arteries. For example, in descriptions of the “glymphatic” system [46], electron micrographs were interpreted as intracortical periarterial spaces but are, in reality, subarachnoid space. In their illustrations [46], the authors show electron micrographs which they claim to show periarteriolar spaces within the brain. There are two layers of leptomeningeal cells with an intervening space in the figure; one layer of leptomeningeal cells is associated with the surface of an artery and the other layer is the pia mater associated with the glia limitans on the surface of the brain. This strongly suggests that the intervening space is subarachnoid space as intracortical arterioles have only one layer of leptomeningeal cells associated with their walls and no periarteriolar space (Fig. 5a) [109]. Furthermore, the entry of tracers from the CSF into the brain is along pial–glial basement membranes as shown by electron microscopy (Fig. 5b) [64] and by high-resolution confocal microscopy (Albargothy, Hawkes and Carare submitted paper) and not along periarteriolar “spaces”.

Veins in the human cerebral cortex usually have only isolated leptomeningeal cells associated with their walls and no complete coating of leptomeningeal cells [109]; small

blebs of perivenous space are seen [109]. Within the subpial spaces, the leptomeningeal layer may be absent around veins (Fig. 4b) [4] but as veins pass into the SAS they acquire a complete layer of leptomeningeal cells [109].

Periarterial basement membranes as routes for bulk flow of fluid and solutes into and out of the brain

Studies using high-resolution confocal microscopy and electron microscopy suggest that basement membranes in the walls of cerebral capillaries and arteries are major pathways for the bulk flow of fluid and solutes into and out of the brain [18, 64]. Tracers injected into the cisterna magna enter the brain from the CSF along pial–glial basement membranes on the outer aspects of cerebral arteries [64] (Albargothy, Hawkes and Carare submitted paper) (Fig. 5a), and leave the brain along basement membranes within the walls of capillaries and arterioles (Albargothy, Hawkes and Carare submitted paper). Intracerebral injections of fluorescent tracers suggest that interstitial fluid and solutes drain rapidly out of the brain along basement membranes in the walls of capillaries and along basement membranes surrounding smooth muscle cells in the tunica media of cerebral arterioles and arteries [7, 16–18] [the Intramural Peri-Arterial Drainage (IPAD) pathway]; IPAD is impaired with age in mice [40]. Similarly, accumulation of amyloid β (A β) in basement membranes of capillaries and arteries in human cerebral amyloid angiopathy (CAA) suggests that IPAD pathways are also impaired with age and Alzheimer’s disease (for review see [18]). A β initially accumulates in the lamina densa at the junction between adjacent smooth muscle cell basement membranes and at later stages completely separates the adjacent, fused basement membranes [52, 106]. The molecular mechanisms involved in bulk flow of fluid and solutes along basement membranes in the walls of cerebral capillaries and arteries have yet to be fully characterised.

Leptomeningeal cells surrounding arteries and veins in the cerebral white matter and basal ganglia

As they pass from the cerebral cortex into the subcortical white matter, arteries tend to be surrounded by two layers of leptomeningeal cells (Fig. 5c), although one layer may be incomplete [26]. Veins, on the other hand, usually have only a single layer of leptomeninges [26]. In this way arteries in the white matter differ from those in the cortex and the arrangement of leptomeningeal coats may be related to the capacity of the perivascular spaces around white matter arteries to dilate [79, 104]. A similar arrangement of two coats of leptomeningeal cells is seen around arteries in the basal ganglia that may also exhibit dilatation of perivascular spaces (Fig. 5d) [75, 80].

Fig. 5 Periarterial leptomeninges in the CNS. **a** An artery within the human cerebral cortex. There is no perivascular (Virchow–Robin) space around the artery as compacted layers of cells and basement membrane leave no space. A layer of pia (P) is closely apposed to the glia limitans (G) on one side and the smooth muscle (SM) coat of the tunica media of the artery on the other side. The lumen of the artery is surrounded by endothelium (End). Scale bar 1.3 μ m. Published with permission from Ref. [109]. **b** Distribution in the brain of 15-nm nanoparticles injected into the CSF. Groups of 15-nm nanoparticles (Np) are seen in the pial–glial basement membrane of a cortical artery 5 min after injection into the CSF of the cisterna magna in a mouse. Layers of the artery wall can be identified moving outwards from the lumen lined by endothelium (End), smooth muscle cells (SM). A layer of pia is separated from the glia limitans (GL) of the brain parenchyma by a layer of pial–glial basement membrane (BM). The group of nanoparticles (np) in the pial–glial basement membrane indicates the pathway for the entry of CSF into the brain following the convective influx/“glymphatic” pathway. There is artefactual disruption of the pial–glial basement membrane associated with the nanoparticles as is frequently seen related to mineral deposits [3].

Scale bar 500 nm. Published with permission from Ref. [64]. **c** An artery in the white matter of a 24-month-old dog brain. The lumen is surrounded by endothelium and smooth muscle cells (SM). There are two layers of leptomeninges (L1 (coloured blue) and L2 (yellow) surrounding the vessel wall and separating the smooth muscle coat of the artery from the glia limitans of the brain parenchyma. In this normal brain there is a narrow perivascular space between the layers of leptomeninges that may have the potential to expand as in human patients with cerebral amyloid angiopathy. Small bundles of collagen fibres (Coll) are present between the layers of leptomeninges. The methods are described in [26]; this unpublished image is from the same study. Scale bar 1 μ m. **d** Dilated perivascular space in the basal ganglia of a 72-year-old human brain. A scanning electron micrograph shows an artery lined by endothelium (E). Transmission electron microscopy confirmed the presence of two layers of leptomeninges (L1 coating the artery wall and L2 either side of the dilated periarterial space). The outer layer of leptomeninges is partly separated from the basement membrane (BM) of the glia limitans. Scale bar 330 μ m. Published with permission from Ref. [75]

Dilatation of perivascular spaces in cerebral white matter and basal ganglia

Although there are no perivascular spaces around arteries in the cerebral cortex, spaces may be present around arteries in the cerebral white matter [19, 79, 104], basal ganglia [75] and midbrain and they may become very large [80]. The capacity for periarterial spaces to dilate in the white matter and basal ganglia appears to correlate with the arrangement of the leptomeninges around those vessels. There is one layer of leptomeningeal cells around arteries in the cerebral cortex but there tends to be two layers of leptomeninges surrounding arteries in the white matter and basal ganglia [26, 75, 109].

Dilated perivascular spaces around arteries in the human cerebral white matter can be detected by MRI and by histology and have been associated with CAA and consequent failure of drainage of interstitial fluid [19, 79, 104]. It appears that CAA impedes the drainage of interstitial fluid from cerebral white matter and results in dilatation of periarterial spaces, but only in the white matter and not in the grey matter of the cerebral cortex [19, 79, 104].

Dilatation of perivascular spaces in the basal ganglia is related to age and small vessel disease and can be detected by MRI [38] and by histology [75]. Two layers of leptomeninges surround arteries in the basal ganglia and dilated perivascular spaces are encompassed on each side by a single layer of leptomeninges as seen by scanning electron microscopy (Fig. 5d) and confirmed by transmission electron microscopy [75].

Leptomeninges as barriers and facilitators in the CNS

The most prominent barrier function performed by the leptomeninges is the arachnoid that surrounds the outer aspect of the CNS; it is impermeable to CSF and thus contains the CSF within the SAS and separates the CSF from blood vessels in the dura that have no blood–brain barrier or blood CSF barrier. Leptomeningeal cells also perform other barrier functions particularly as intervening cell layers between collagen fibres and the CSF. Inflammatory cells appear to readily penetrate the thin layers of leptomeningeal cells such as the pia but not the thicker arachnoid barrier layer. Although veins in the SAS are coated by a layer of leptomeningeal cells, the main barrier to entry of inflammatory cells from the blood into the CSF in the SAS is the vascular endothelium lining veins in the SAS and subpial spaces [30, 31]. The arachnoid barrier, besides containing the CSF within the SAS, also plays an important role in preventing activated immune cells invading the CSF from dural blood vessels.

Leptomeningeal cells also facilitate the circulation of CSF and the attachment of inflammatory cells, tumour cells and the bacteria within the SAS. There is a complete lining of leptomeningeal cells around the SAS; furthermore leptomeningeal cells cover collagen bundles in the stroma of the choroid plexus, line the pathways for drainage of CSF into the blood through arachnoid granulations and line the pathways for lymphatic drainage of CSF into nasal lymphatics. In addition, extensions of leptomeningeal cells into the CNS along the outer walls of arteries facilitate entry of CSF into the CNS.

Barriers

Arachnoid mater

In humans, the main body of the arachnoid is composed of closely packed leptomeningeal cells joined by desmosomes [4]. However, the outer barrier layer of the arachnoid that abuts on to the dura is composed of cells joined by tight junctions that prevent the escape of CSF from the SAS [4]. Arachnoid also acts as a barrier to the spread of inflammatory cells from the SAS in cases of leptomeningitis and resists the spread of pus from subdural infections into the SAS.

Leptomeningeal cells separate collagen fibres from the CSF in the subarachnoid space

The SAS is surrounded by connective tissue but leptomeningeal cells separate collagen fibres from the CSF. In this way, leptomeningeal cells form a smooth surface that separates cells circulating in the CSF from underlying tissue elements; this is similar to the way in which vascular endothelial cells form a surface for the smooth transport of blood. As discussed below, leptomeningeal cells also form a surface for the attachment of inflammatory cells, tumour cells and bacteria within the subarachnoid space. The inner layer of the arachnoid facing the CSF has a thin underlying layer of collagen that extends into trabeculae that cross the SAS to join the pia or suspend subarachnoid blood vessels within the SAS [4, 45]. A layer of leptomeningeal cells separates the collagen in the arachnoid, the subarachnoid trabeculae and in the subpial compartment from the CSF (Fig. 6a). The layers of leptomeningeal cells performing this barrier function are often only one cell thick and joined by gap junctions or desmosomes [4, 13].

The principle of leptomeningeal cells separating collagen from fluid applies not only in the SAS but is also seen in the stroma of the choroid plexus and in cores of arachnoid granulations.

Choroid plexus

Bundles of collagen are present in the stroma of the choroid plexus but they are surrounded by leptomeningeal cells (Fig. 6b) [3]. In older humans, spheres of collagen fibres, produced and surrounded by leptomeningeal cells, may become calcified to form calcospherites in the stroma of the choroid plexus [3]. The anatomical arrangement suggests that fluid, solutes and inflammatory cells passing out of the blood vessels into the stroma and trafficking towards the choroid plexus epithelium, the site of the blood–CSF barrier, are separated from collagen bundles by leptomeningeal cells in a similar way to the separation of collagen from CSF in the SAS.

Arachnoid granulations

The SAS is continuous with the core of each arachnoid granulation such that CSF flows into a network of channels in the core of the granulation (Fig. 6c) [56, 98]. Delicate trabeculae of collagen fibres form the walls of the channels but the trabeculae are coated by leptomeningeal cells that separate the collagen from the CSF within the channels. Each granulation has a cap of compacted leptomeningeal cells through which channels pass to carry CSF to the venous endothelium [55, 56, 98]. CSF then appears to pass by macrovacuolar bulk flow through the venous endothelium into the blood [97]. By this arrangement, CSF is separated from collagenous elements in the arachnoid granulations by a coating of leptomeningeal cells.

Facilitators

Layers of leptomeningeal cells lining the SAS, the stroma of the choroid plexus, drainage channels in arachnoid granulations and the channels that drain CSF into nasal lymphatics [54] facilitate the production, circulation and drainage of CSF. They also facilitate the movement of inflammatory cells within the SAS. The leptomeningeal cells of the pia offer little resistance to the passage of inflammatory cells [45, 57] (Fig. 4d) although they do block the passage of erythrocytes and particulate matter [45]. Similarly, the leptomeningeal cells coating veins in the SAS appear to offer little resistance to the passage of T lymphocytes, polymorphonuclear leukocytes and monocytes entering the SAS from the blood [85, 102]. The major barrier to the passage of inflammatory cells from CSF into the CNS appears to be the glia limitans [31].

Most of the research into traffic of inflammatory cells into the CSF and CNS has been on experimental autoimmune encephalomyelitis (EAE) as an animal model of multiple sclerosis. Experiments in EAE suggest three potential routes for the entry of encephalitogenic T cells into the CNS, either

directly from the blood or via the CSF. Migration of T cells from the blood into the CNS parenchyma in inflammation is by a two-step process [30, 31]. Step 1: T cells in the blood with activated $\alpha 4\beta 1$ -integrins (VLA4) on their surfaces engage VCAM-1 on endothelial cells and migrate into the perivascular spaces around postcapillary venules. Step 2: from the perivenular spaces, T cells penetrate the basement membrane and astrocyte processes of the glia limitans to enter the CNS parenchyma; this step is facilitated by the expression of matrix metalloproteinases (MMPs) produced by macrophages [30]. Encephalitogenic T cells also migrate across the walls of leptomeningeal veins directly into the CSF in the SAS [85]. Another route of entry of T cells into the CSF appears to be through the choroid plexus and blood CSF barrier into the ventricles [78].

T lymphocytes in the spinal SAS in EAE attach to the leptomeninges through steps reminiscent of vascular adhesion in the CNS blood vessels involving integrins VLA-4 and LFA-1 on lymphocyte cell surfaces binding to their respective ligands; the integrins appear to be activated by chemokines produced by resident macrophages [85]. Chemokine signalling via CCR5/CXCR3 and antigenic stimulation of T cells in contact with leptomeningeal macrophages enforces their adhesiveness to the leptomeninges. T cells that become detached from leptomeninges and float free in the CSF display significantly lower activation levels compared to T cells attached to the leptomeninges and from the parenchyma of the CNS [85]. This suggests that attachment to the leptomeninges induces more activation in the T cells. Invasion of T cells into CNS tissue from the CSF would entail crossing the glia limitans which is the main barrier on the surface of the CNS to the passage of inflammatory cells into the CNS parenchyma [30].

Arteries and leptomeningeal cells as facilitators for the entry of CSF into the CNS

Layers of leptomeningeal cells that accompany arteries as they enter the surface of the CNS appear to facilitate the entry of CSF into CNS tissue. When tracers are injected into the CSF, they enter the brain along the pial–glial basement membranes on the outer aspects of cerebral arteries (Figs. 5b, 6a) [64]. This suggests that leptomeninges play a facilitating role in fluid balance between CSF and interstitial fluid within the CNS. Convective flow of CSF and interstitial fluid through the brain parenchyma driven by aquaporin 4 as suggested by the “glymphatic” hypothesis [46] has been strongly contested [2, 91]. Furthermore, the suggestion that tracers entering the brain from the CSF are eliminated along perivenous pathways in the “glymphatic” system [46] has also been strongly disputed by Albargothy, Hawkes and Carare (submitted paper) who have shown that such tracers

Fig. 6 Leptomeninges as barriers and facilitators for the movement of fluid and cells. **a** The subarachnoid space (SAS), leptomeninges and the surface of the brain. Veins, arteries and lymphatics (VAL) in the dura are separated from the arachnoid by the arachnoid barrier layer (ABL) that appears to prevent the escape of CSF from the SAS and the entry of solutes from the “leaky” dural blood vessels into the CSF. Collagen fibres (blue layers) are separated from CSF in the SAS by layers of arachnoid and pial cells (green). Collagen bundles in the cores of trabeculae (Tb) that cross the subarachnoid space are also separated from CSF by leptomeningeal cells (green). Lymphocytes (L) and monocyte/macrophages (M) enter the SAS from the blood via the walls of veins and adhere to leptomeningeal cells and cross the pia. Similarly, tumour cells (TuC) and bacteria (*Neisseria meningitidis* (Nm), derived from the blood, adhere to leptomeningeal cells lining the SAS. Periarterial compartments facilitate entry of CSF into the brain along pial–glial basement membranes on the outside of the artery (green arrow). Interstitial fluid (ISF) from the brain drains along basement membranes in the walls of capillaries and in the tunica media of cerebral arteries that form the Intramural Peri-Arterial Drainage (IPAD) pathway as indicated by the yellow arrow.

The glia limitans (purple) forms a barrier to the entry of inflammatory cells into brain tissue. **b** Choroid plexus. Leptomeningeal cells (green) cover collagen bundles and collagen whorls within the stroma of the choroid plexus. Fluid and solutes entering the CSF from the choroid plexus are derived from blood vessels (Bv) in the stroma but the blood CSF barrier is located in the choroid plexus epithelium. **c** Arachnoid granulation. CSF passes from the subarachnoid space (SAS) into channels in the core of the granulation. The channels are formed by trabeculae that have a core of collagen (blue) covered by leptomeningeal cells (green). CSF then flows into channels within the meningeal cap to reach the venous endothelium and pass into the venous sinus (VS). In this way, the CSF is separated from collagen bundles by leptomeningeal cells as it drains through the arachnoid granulation. Arachnoid: AM. **d** Lymphatic drainage of CSF through the cribriform plate. Channels lined by leptomeningeal cells (green) pass from the subarachnoid space (SAS) inferior to the olfactory bulbs, through the cribriform plate alongside olfactory nerves (ON) into the nasal mucosa. These channels develop during foetal life and form a major pathway for drainage of CSF to lymph nodes. Arachnoid: AM

are eliminated from the brain along basement membranes in the walls of capillaries and arteries—IPAD (Fig. 6a).

Leptomeningeal cells facilitate lymphatic drainage of CSF via the cribriform plate and nasal submucosa [54] in both foetal and adult human and rodent brains (Figs. 1c, 6d). Channels lined by leptomeningeal cells form in the subarachnoid space under the olfactory bulbs and pass through the cribriform plate alongside olfactory nerves into the nasal submucosa [54]. This system for the drainage of CSF appears to develop before the appearance of arachnoid villi and granulations.

Tumour cell invasion of subarachnoid spaces and adherence to leptomeninges

Three major categories of tumours cause *Meningeosis neoplastica*: namely carcinomas, lymphomas and primary tumours of the CNS, particularly medulloblastomas [28, 77]. However, little is known about the mechanisms by which tumour cells enter the SAS or are maintained within the CSF.

Tumour cells frequently accumulate in regions in which there is reduced circulation of CSF, such as the basal cisterns, cauda equina and hippocampal fissures. Medulloblastomas have received considerable attention as most deaths from this disease are due to leptomeningeal metastases [77]. Four major molecular subgroups of medulloblastoma have been recognised: wingless (WNT), sonic hedgehog (SHH), group 3 and group 4 [77] but whole genome landscapes have revealed additional new molecular subtypes [69]. It is predicted that molecular characterisation will lead to more targeted therapies for leptomeningeal metastases of medulloblastoma [67]. In vitro and in vivo data suggest that expression of $\alpha 9$ and $\beta 1$ integrins, in combination with tenascin in the extracellular matrix, mediate the adhesion of medulloblastoma cells to the leptomeninges [33]. Blockade of $\alpha 9$ and $\beta 1$ integrin binding by antibodies reduces tumour cell survival which suggests that adhesion of medulloblastoma cells to the meninges is necessary for the survival and proliferation of tumour metastases in the SAS [33].

Leptomeninges as barriers and facilitators of bacterial meningitis

Leptomenigitis is an acute, compartmentalised intracranial inflammatory response centred on the leptomeninges and can be caused by a plethora of different bacteria that can gain access to the SAS [20]. *Neisseria meningitidis* (meningococcus) is a well-studied exemplar for understanding the interactions between bacterial ligands and receptor molecules on blood vessel endothelium and the leptomeninges. In the pathogenesis of meningococcal meningitis, the bacteria interact with several physical cellular barriers

including the mucosal epithelium of the nasopharynx, the blood compartment, endothelial cells of blood vessels that separate the blood from the CSF-filled SAS, and the leptomeninges. Meningococci express a variety of surface ligands that mediate their attachment to human cells and enable meningococci to cross these cell barriers, by inter- and/or intracellular routes.

Meningococcal bacteraemia

Bacteraemia is a pre-requisite for the entry of bacteria into the CSF and the development of meningitis. The fact that meningitis commonly presents without fulminant sepsis also suggests that bacteraemia is asymptomatic. Meningococci have evolved strategies to avoid innate immune defence mechanisms and they express several factors that are important for survival in the blood. Both the capsule polysaccharide (CPS) and lipooligosaccharide (LOS) exhibit characteristic antigenic variation and molecular mimicry that enable meningococci to avoid antibody deposition, complement-mediated killing and phagocytosis. For example, the $\alpha(2-8)$ *N*-acetyl-neuraminic acid linked homopolymer of serogroup B CPS is structurally identical to a modification of mammalian neural cell adhesion molecule (N-CAM) [95]. Sialylation of LOS enables meningococci to mimic host cell surfaces that express sialic acid [60] and to interact with myeloid cell SIGLECs (sialic acid-binding, immunoglobulin-like lectins). Phosphoethanolamine modification of LOS can increase adherence to human cells and also protects meningococci from neutrophil extracellular trap (NET)-bound cathepsin G [58]. Natural release of outer membranes (OM) and expression of the ZnuD surface protein also enable meningococci to escape NET interactions. Other surface-exposed proteins important for bacterial survival include TspB (T/B cell-stimulating protein B), TbpA/TbpB (Transferrin-binding proteins), LbpA/LbpB (Lactoferrin-binding proteins), HpuA/HpuB/HmbR (Haemoglobin-binding receptors), Ig-binding protease and NHBA (*Neisseria* Heparin Binding Protein). Meningococci also avoid complement killing through expression of Porin A that binds the complement inhibitor C4 bp protein [47], and of fHbp (factor H binding protein), NspA (Neisserial surface protein A) and PorinB3, which all bind human factor H [35, 88].

Attachment and penetration of vascular endothelium

Meningococci show a tropism for microvasculature endothelial cells and bacterial invasion of the SAS requires meningococci to breach the blood–CSF barrier (BCSFB), located either at the choroid plexus or in the veins within the SAS. Post-mortem studies of meningococcal meningitis patients demonstrated the adherence of bacteria to endothelial cells of choroid plexus capillaries, but no bacteria were found

inside choroidal epithelial cells or penetrating the tight junctions [76]. Furthermore, leptomeningitis is centred within the SAS and ventriculitis is usually a late complication, indicating that unchecked bacterial proliferation in the SAS eventually spreads to other CSF-filled compartments. Thus, meningococci probably do not traverse the BCSFB at the choroid plexus and are likely to enter the CSF via veins in the SAS, with the blood vessel endothelium as the first barrier.

Meningococcal adherence to the endothelial cell barrier and the mechanism of traversal has been extensively studied [24]. The Type 4 pilus (Tfp) adhesin promotes the initial binding event between meningococci and endothelial cells, but only in microvessels where blood flow shear stresses are low. Expression of the Tfp-associated protein PilX stimulates bacterial aggregation and spreading across the cell surface [41]. Adherence occurs through the interaction between Tfp and the CD147 (extracellular matrix (ECM) MMP inducer, EMMPRIN or Basigin)– β 2-adrenergic receptor (β 2AR) complex, with the Tfp subunit PilE protein and associated PilV protein interacting directly with the extracellular N-terminal domain of the β 2-adrenergic receptor. Activation initiates a signalling pathway that leads to the formation of ‘cortical plaques’ of cytoplasmic ezrin and ezrin-binding and actin microfilament-binding proteins. Signalling leads to relocation of vascular endothelial cadherin from endothelial cell junctions at the sites of bacterial adherence, and also induces the production of MMP8 that promotes cleavage of the tight junction protein occludin [87]. Other Tfp-associated proteins that may be involved in cell interactions include the tip-associated PilC1/C2 and a phosphorylcholine modification of Tfp that interacts with Platelet Activating Factor receptor [48]. Adherence of meningococcal also stimulates endothelial cells to release cytokines, chemokines and reactive oxygen and nitrogen species [21], that contribute to cell stress and degradation of intercellular junctions. Thus, crossing the endothelial barrier is primarily by the intercellular route. An intracellular route of invasion is possible, though considered to be secondary: retraction of Tfp draws meningococci towards the endothelial cell plasmalemma and concomitant down-regulation of CPS expression exposes OM adhesins and invasins that can interact with endothelial cell receptors to promote internalisation of bacteria. These include the well-characterised Opa (colony opacity-associated), Opc (5C), NadA (Neisserial adhesin A) proteins and the OmpA-like sequence of RmpM (Reduction modifiable protein M) (Table 2).

Penetration of leptomeningeal layers

After crossing the vessel endothelium, meningococci will encounter a thin layer of connective tissue and then the leptomeningeal coating that encloses the vein within the

CSF-filled SAS. The mechanism by which meningococci penetrate this leptomeningeal coating is unclear, but the fact that meningeal cells do not internalise meningococci suggests intercellular penetration, perhaps as a consequence of degradation of desmosomes and other junctions by endothelial cell metabolites. It is possible that the ErbB family receptor protein EGFR on leptomeningeal cells (Table 2) is activated by meningococcal infection. In endothelial cells, EGFR, Erb2 and Erb4 are activated in response to meningococcal infection and involved in the internalisation of bacteria [90]. However, activation of EGFR on the leptomeninges could be involved in weakening intercellular junctions to allow transmigration of meningococci, mimicking a process that has been described for transmigration of *N. gonorrhoeae* across polarised epithelial cells [29].

The ability of meningococci to weaken endothelial and leptomeningeal intercellular junctions is a trafficking mechanism shared with T lymphocytes, B cells, PMNL and other myeloid cells for their entry into the CNS, and includes activation of MMP proteins [30]. However, the nature of the ligands used by trafficking immune cells and their targeted receptors appear to be distinct from those used by meningococci. Interestingly, Tfp-mediated adherence of *Neisseriae* to the uropod of polarised PMNL suggests a hypothesis that the bacteria can hitchhike on PMNL both to hide from their phagocytic activity and to enable dissemination through cell barriers [92].

Adherence of meningococci to leptomeningeal cells

The CPS and Tfp phenotype of *N. meningitidis*, which is normally isolated from blood and the CSF, shows a specific predilection for binding to leptomeninges but not to cortical brain tissue (Fig. 7a, b) [39]. Meningococci adhere similarly to the surface of cultured tumour cells derived from benign meningiomas arising from the leptomeninges (Fig. 7c). Meningioma cells share many structural features with normal leptomeningeal cells [32]; they are characterised by the presence of desmosomes, and, except in certain sites in the leptomeninges, tight junctions are absent. Both types of cell express the cytoplasmic intermediate filament proteins nestin Type VI, cytokeratin and vimentin Type II and the microtubule-associated protein doublecortin and both secrete Type IV collagen, Type III procollagen, tenascin and fibronectin ECM proteins. Meningioma cells provide an in vitro model for studying meningitis and all the major meningeal pathogens can adhere to these cells (Fig. 7c) [5, 9, 34, 39], although the nature of the bacterial ligands and meningeal cell receptors has not been explored extensively.

The type 4 pilus (Tfp) is the primary ligand that mediates adherence of meningococci to leptomeningeal cells [39], and Tfp-mediated adherence is not influenced by CPS expression, the antigenic class of pilus expressed (Class I or Class

Table 2 Profile of known and potential surface receptor molecules on human leptomeningeal cells and meningioma cells

Molecule	Receptor function and meningococcal ligand recognised
Laminin receptor 1 (LamR; also known as RPSA)–galectin 3 (GAL3) complex)	Binds PorA and PilQ [73] and NhhA (<i>Neisseria</i> hia/hsf homologue; Msf, meningococcal surface fibril) [84]
Heparan sulphate proteoglycans (HSPG)	Binds NhhA [84], NHBA (<i>Neisseria</i> Heparin Binding Antigen) [99], Opa and Opc proteins [42]
Integrins ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 1$, $\beta 4$)	Expressed to varying degrees in meningioma/meningeal cells. Bind Opa proteins (which also recognise CEACAM (carcinoembryonic antigen-related cell adhesion molecule) family receptor proteins) and Opc protein [42]. $\beta 1$ -integrin binds NadA (<i>Neisseria</i> adhesin A (autotransporter TAA adhesin–invasin) [66]
MARCO (Class A Mo scavenger receptor (SR-A) and macrophage receptor with a collagenous structure)	Expressed on (rodent) meningeal cells. Binds to various meningococcal proteins (NMB0278, DsbA; NMB0346, NMB0667, NMB1513, hypothetical; NMB1220, Stomatin/Mec-2 family protein; NMB1567, Macrophage infectivity potentiator) [74]
CD46 (membrane co-factor protein)	Expressed on all cell types except erythrocytes. Putative receptor for Tfp [51]
TLR2	<i>Toll</i> -like receptor 2: pathogen recognition receptor (PRR) for Gram-positive lipoteichoic acid and other lipoproteins and meningococcal PorB [96]
TLR4	<i>Toll</i> -like receptor 4: PRR for LOS endotoxin
Epidermal growth factor receptor (EGFR)	Transmembrane protein with intrinsic tyrosine kinase. Microbial ligand-binding to EGFR potentially activates cell signalling, phosphorylates protein kinases, and rearranges cytoskeletal proteins, thus facilitating pathogen uptake
HLA-DR	Do phagocytic arachnoid cells act as antigen-presenting cells?
Interleukin-1R	Binds Interleukin 1 cytokine
N-CAM	Surface glycoprotein CD56; mimicked by serogroup B CPS
Fc γ receptors	Potential phagocytosis-related functions; increased by GM-CSF production
Bone morphogenetic protein (BMP) 4 and 7 receptors	Expressed on leptomeninges—BMPs influence vascular development in this tissue
CD44	Multi-structural glycoprotein with functions in cell proliferation, differentiation, migration, angiogenesis, signalling pathways for cell survival, presentation of innate molecules and proteases to receptors
Desmosomal desmoplakin	Protein component of desmosomes, which are meningeal cell intercellular junctions. Desmosomes function in cell–cell adhesion and for the attachment of intermediate filaments (IF)
G protein-coupled melatonin receptor Mel1a	Receptor for melatonin that may play role in early neural development
YKL-40	Heparin- and chitin-binding glycoprotein, participates in inflammatory states
β -Type platelet-derived growth factor (PDGF) receptor	PDGF stimulates tumourigenesis in arachnoid cells
CXCR4 and CXCR7 chemokine receptors	Involved in tumour cell proliferation and survival
Endothelin receptors	Role in tumour growth
Epithelial membrane antigen	Marker of meningioma; also known as mucin glycoprotein CD227, MUC1, episialin. Normally acts as a barrier to apical cell surfaces
Fibroblast growth factor receptor-1 (FGFR-1)	Associated with meningioma cell growth and tumourigenesis
Mesothelin	Function unknown, possibly a tumour marker
Prolactin receptor (PRLR)	Detected in meningiomas; proliferative effects
Sex hormone receptors (progesterone, oestrogen, androgen, aromatase)	May play a role in the tumorigenesis of meningiomas
Somatostatin receptor (hsst) 2a	Binds somatostatin hormone; diagnostic marker for meningioma
TGF- β receptors	Receptor loss related to malignancy
Histones	Intracellular proteins that can associate with App (Adhesion and penetration autotransporter protein) and MspA/Aus1 (meningococcal serine protease A/autotransporter serine protease 1) [53]

Fig. 7 Interactions of pathogenic *Neisseria meningitidis* with human leptomeninges. **a** Control cryostat section of human brain stained by immunocytochemistry for vimentin. Arachnoid (Ar) with underlying sulcus and subarachnoid space (SAS) containing CSF that separates cerebral cortex on either side. Blood vessel (BV) in the sulcus. **b** The same section as in **a** infected with *N. meningitidis* (green) that adhere to the leptomeningeal cells in the arachnoid, coating trabeculae and in the pia and coating blood vessel wall within the subarachnoid space

(SAS) of the sulcus. Cortex is labelled on one side of the sulcus and pia is indicated by an arrow on the other side of the sulcus. *N. meningitidis* do not adhere to the cortical brain tissue. **c** Adherence of *N. meningitidis* (yellow–green) to meningeal meningioma cells (red) in culture. **a, b** Fluorescence microscopy scale bar 300 µm. **c** Confocal microscopy: scale bar 20 µm **a–c** Published with permission from Ref. [39]

II) or by variable expression of PilC1 protein. However, variation within Class I pilin that modifies pilin structure and/or the ability to form bundled pili, does influence meningococcal adherence to leptomeningeal cells, as similarly observed with epithelial and endothelial cells. For capsulated bacteria, expression of Opc protein is not involved in mediating adherence, whereas expression of Opa protein does increase the association of capsulated meningococci that express low-adhesive pili, but do not influence the association of high-adhesive piliated bacteria [39]. Expression of TspA (T cell-stimulating protein A) has been reported to contribute to the adhesion of meningococci to meningioma cells [72]. Only one other protein, the Adhesin Complex Protein, has been reported to bind directly to cell surfaces and mediate adherence of capsulated meningococci to meningeal cells [44]. A comprehensive classification of the leptomeningeal surface receptors directly binding meningococcal ligands is lacking, but can be generated hypothetically from what is known currently of bacterial ligand interactions with other cell types and the nature of receptors identified on leptomeninges and meningioma cells (Table 2).

The OM of CPS-null meningococci has been shown to interact intimately with the meningioma cell plasmalemma, inducing significant cell surface activation and membrane ruffling, that are not observed following the binding of capsulated meningococci [39]. It is likely that exposed OM

adhesin–invasins such as the Opa and Opc proteins mediate these interactions, which occur also on endothelial and epithelial cells. However, the meningeal cell receptors involved have not been confirmed. Despite these membrane rearrangements, meningococci do not invade meningioma cells and classical membrane-bound intracellular vacuoles containing bacteria are not observed [39]. Since bacteria and the inflammatory response are contained within the SAS, with little involvement of the underlying brain, this suggests that an intact pia is a considerable barrier to penetration of meningococci into the brain. Nonetheless, late-stage and fatal meningitis is characterised by considerable death of leptomeningeal cells, thus presenting bacteria with the opportunity to interact with collagen underlying the pia and with astrocytes of the glia limitans superficialis and to potentially activate microglia.

Leptomeningitis

After entry into the CSF, leptomeningitis is established as a consequence of bacterial growth without immune restriction in the SAS and the direct interactions of meningococci with microvascular endothelial cells, the leptomeninges (with little involvement of the dura) and sentinel macrophages. LOS is the major component of meningococci that induces leptomeningitis [12], via binding to CD14-*Toll*-like receptor

(TLR)4-MD2 on endothelial cells, macrophages and infiltrating PMNLs. Such interactions activate cell signalling pathways to induce the inflammatory response. By contrast, cytokine release by meningeal cells can occur independently of LOS-CD14/TLR4/MD2 interactions or the involvement of other host accessory proteins such as HSP70, HSP90 α , CXCR4 or GDF5 [43]. Leptomeningitis is an ‘inflammatory soup’ of host response molecules [21] to invading meningococci and infection is characterised by the rapid infiltration of PMNLs. Neutrophils with intracellular bacteria have been observed in the CSF of patients, suggesting that this organism can survive and replicate within these cells [25]. Infiltrating PMNL augment the inflammatory response, and leptomeningitis serves to limit bacterial spread but at the expense of the host, leading to gross cell and tissue necrosis within the SAS. Untreated meningococcal leptomeningitis is invariably fatal and for survivors, permanent neurological sequelae are common.

Conclusions

This review has emphasised the key roles that leptomeningeal cells, in their many and diverse forms, play as barriers and facilitators for the movement of fluid, solutes, cells and bacteria in relation to the CNS. In foetal development the establishment of the arachnoid barrier complements the formation of the blood–brain barrier thus isolating the CSF and developing CNS from peripheral tissues that lack vascular barriers. Such isolation appears to be essential for ensuring appropriate morphogenic signalling within the CNS which is concentration-dependent and in which the arachnoid may play a part. The arachnoid barrier is retained in the adult brain and is prominent in containing CSF within the subarachnoid space and isolating the CSF from the dura. Leptomeninges also play many other subtle roles as summarised in Fig. 6, ranging from separation of connective tissue from the CSF to lining channels for the movement of CSF within the brain and drainage of CSF via arachnoid granulations and lymphatic pathways. Within the cranial and spinal SAS, leptomeninges appear to regulate patterns of flow for CSF and are sites for attachment of inflammatory cells which may facilitate their immunological and anti-microbial activities. Bacteria and tumour cells enter the CSF and attach to leptomeninges by mechanisms that may involve molecular mimicry and hijacking some of the attachment mechanisms used by inflammatory cells. Understanding such mechanisms has a potential role for the future management of leptomeningitis, autoimmune disorders of the CNS and tumour metastases in the CSF.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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