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TOPICAL REVIEW

The myth of the immature barrier systems in the developing brain: role in perinatal brain injury

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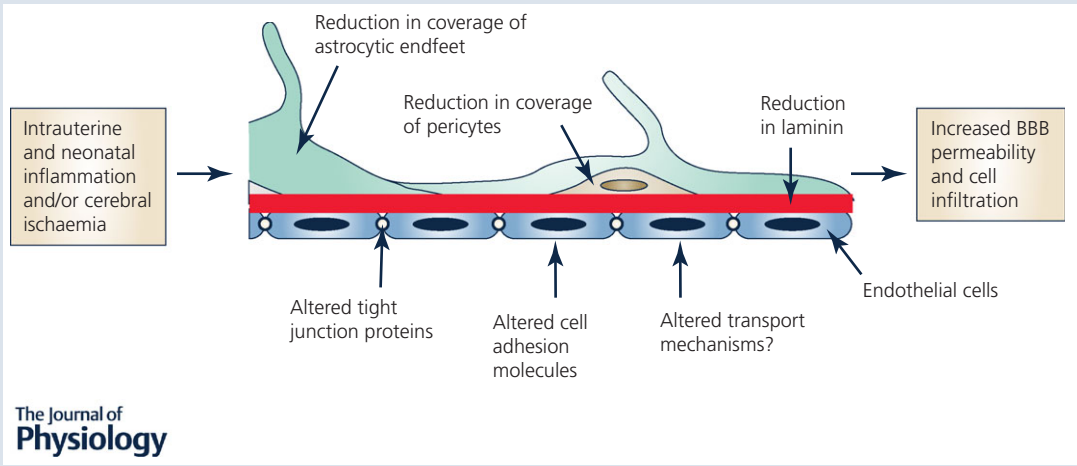

Abstract Central nervous system homeostasis is maintained by cellular barriers that protect the brain from external environmental changes and protect the CNS from harmful molecules and pathogens in the blood. Historically, for many years these barriers were thought of as immature, with limited functions, during brain development. In this review, we will present advances in the understanding of the barrier systems during development and evidence to show that in fact the barriers serve many important neurodevelopmental functions and that fetal and newborn brains are well protected. We will also discuss how ischaemic injury or systemic inflammation may breach the integrity of the barriers in the developing brain.

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Abstract figure legend Perinatal conditions such as intrauterine and neonatal inflammation and cerebral ischemia can result in a disturbed blood brain barrier (BBB), which may contribute to brain injury. Negative effects on the BBB include reduced coverage of astrocytic endfeet and pericytes, reduced expression of laminin, the major basement membrane glycoprotein in blood vessels, altered expression of tight junction proteins between endothelial cells and altered adhesion molecules. Some data also point towards altered transport mechanisms in the BBB. These changes of the BBB can contribute to increased BBB permeability and immune cell infiltration from the blood to the brain.

Central nervous system barriers

Central nervous system (CNS) homeostasis is maintained by cellular barriers that protect the brain from external environmental fluctuations and shield the CNS from harmful molecules and pathogens in the blood. These barrier structures also serve important functions in providing transport of nutrients and other essential molecules to the brain, elimination of brain waste products and have lately also been recognised to provide signalling functions, such as immune sensing, between blood and CNS. A physical interface between the CNS and the peripheral circulation was first described by Paul Ehrlich who noted that dyes injected into the circulation did not stain the brain (Ehrlich, 1885). However, it was Goldmann, at the beginning of the last century, who concluded from experiments similar to those conducted earlier by Ehrlich that there must be a barrier between the brain and peripheral organs (Goldmann, 1909, 1913). For a long time, these barrier systems were considered to be immature and not functional until later in brain development. However, similar experiments to those performed by Goldmann were later performed in embryos from different animal species to demonstrate the existence of the blood-brain barrier (BBB) already early in development (Weed, 1917; Cohen & Davies, 1938; Grazer & Clemente, 1957; Wislocki, 1920). The importance of these findings to fetal human development was further confirmed in the 1950s when it was demonstrated that a barrier to Trypan Blue exists in the human fetus from approximately 12 weeks of gestation (Grontoft, 1954). Later, carefully conducted studies in several different species including humans have further extended our understanding of the barrier systems during fetal development. Thus, there is convincing evidence today to show that the CNS barrier systems develop before birth and are functional in all species examined so far (Møllgård & Saunders, 1975; Ek *et al.* 2003, 2006; Ballabh *et al.* 2005; Kratzer *et al.* 2012). However, it is important to point out that it should not be assumed that the CNS barrier systems are static during development; on the contrary, they appear to change significantly with maturation processes of the brain. There are few studies that have directly examined barrier permeability over the lifespan in the same species, but experiments in sheep demonstrate that influx of an inert amino acid is higher in the fetus compared to the adult (Stonestreet *et al.* 1996). Further increases in the understanding of maturational changes of the barriers based on similar studies using different permeability markers will be important.

There are three main barrier systems surrounding the CNS: the blood-brain barrier (BBB) consisting of cerebral endothelial cells and the surrounding glial limitans and pericytes; the epithelial cells of the choroid plexus that is the barrier between blood and cerebrospinal fluid (CSF), and the arachnoid epithelium in the leptomeninges, which is the outer barrier between dura mater and subarachnoid space and separates the fenestrated dural vessels from the subarachnoid CSF (Nabeshima *et al.* 1975; Rascher & Wolburg, 1997). The unique barrier mechanisms are achieved by tight junction proteins situated on the luminal side and adherens junctions on the basolateral side of these cells, providing tightness of the barrier but also contributing to regulation of metabolism by specific transport mechanisms. In addition, pericytes and astroglial end-feet surround the endothelial cells of the BBB, further contributing to separation of blood components from neural tissue. Further, an intimate contact between perivascular microglia/macrophages and the BBB, particularly around penetrating vessels and venules, serve important immune functions in the CNS.

Central nervous system barriers in development

The meninges and the outer brain barrier form during early fetal life (O'Rahilly & Muller, 1986; Goasdoue *et al.* 2017). In parallel with the formation of the subarachnoid space, the arachnoid barrier cells are joined to each other by numerous tight junctions, which is the structural basis for the arachnoid blood-CSF barrier (Nabeshima *et al.* 1975). By the 11th post conception week in humans and embryonic day 16 (E16) in rats, the subpial end-feet layer is complete and by E18 the tight junction protein claudin-11 is present in the arachnoid blood-CSF barrier (Balslev *et al.* 1997; Brochner *et al.* 2015).

The choroid plexus epithelium develops from the neural tube early in fetal life in close association with the development of the surrounding vasculature

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(Dziegielewska *et al.* 2001). Choroid plexus epithelial cells are post-mitotic and new choroid plexus cells originate from progenitor cells in the dorsal hindbrain neuroectoderm. Sonic hedgehog is believed to be crucial for both choroid plexus vascular growth and signalling to choroid plexus epithelial progenitors (Huang *et al.* 2009; Nielsen & Dymecki, 2010). Secretory, transport and barrier functions are observed in choroid plexus epithelial cells shortly after differentiation (Johansson *et al.* 2005; Liddelow *et al.* 2009; Ek *et al.* 2010) and tight junction, enzymatic and transporter proteins are already present in the embryonic choroid plexus in marsupials (Ek *et al.* 2003), rats (Kratzer *et al.* 2012) and non-human primates (Ek *et al.* 2015*b*).

The maturation of the BBB is closely related to recruitment of endothelial cells and pericytes into the brain (Hellstrom *et al.* 1999; Daneman *et al.* 2010; Armulik *et al.* 2010). This occurs early in fetal life prior to astrocyte generation, as shown in, for example, rats, where the first vessels invade the cerebral cortex starting at E11, whereas astrogliogenesis begins in the cerebral cortex closer to birth (Qian *et al.* 2000). The newly formed vessels immediately restrict transfer of small molecule tracers into the CNS in the opposum (Ek *et al.* 2006), or within a few days in mice, and permeability of BBB endothelial cells is tightly restricted by E15 in mice (Ben-Zvi *et al.* 2014; Sohet *et al.* 2015). The permeability of brain microvasculature to endogenous albumin in newborn mice is not significantly different from that in adult animals under physiological conditions (Vorbrodt & Dobrogowska, 1994). In support of these observations, the tight junction protein ZO-1 starts to be expressed in E15 cerebral vessels in the mouse and on E19 the endothelial tight junction appears completely differentiated (Nico *et al.* 1999). Thus, the current understanding is that astrocytes do not contribute to the early induction of 'tightness' of the BBB (Saunders *et al.* 2016); however, astrocytes do have important modulating effects on multidrug transport proteins in the developing brain (Baello *et al.* 2016).

Transport systems in barriers during development

A range of transport systems at the blood-CNS interfaces help deliver nutrients into the brain but also keep out potentially toxic compounds, including many drugs. We still have only limited understanding of how these transport systems change during development. Not surprisingly many of the nutrient transport systems are highly active in the newborn, with amino acid transport into the brain being higher in newborn compared to adults (Cornford *et al.* 1983; Cornford & Cornford, 1986). For efflux mechanisms, the transporters belonging to the ABC (ATP-binding cassette protein) -B/-C/-G families are considered the most important at the brain barriers in relation to xenobiotic access to the brain. These transporters can work on their own to remove molecules but also act in conjugation with phase II metabolism, which first tags compounds (by glutationation, sulfonation or glucuronidation), which then makes them more available for ABC protein-mediated elimination. Phase II metabolic enzymes have been shown to be concentrated in CNS barrier interfaces and activity of glutathione-S-transferase (GST) family, the enzymes facilitating glutationation, have been shown to be higher in the fetal choroid plexus compared to adult (Ghersi-Egea *et al.* 1994; Ghersi-Egea *et al.* 2006). These findings are supported by a high variety and expression level particularly within the GST family of enzymes in the fetal baboon choroid plexus (Ek *et al.* 2015*c*). ABCB1 (p-gp) present on the cerebrovasculature in the adult can play a pivotal role in keeping drugs out of the brain and a striking example is Loperamid, an μ-opioid-receptor agonist, that lacks central action because of ABCB1-mediated brain efflux. The complement of these ABC-transporters is different at the BBB and blood-CSF barrier and also changes with development. Both ABCB1 and ABCC4 (MRP4) have been shown to increase during development at the BBB, while ABCG2 (BCRP) is abundant on vasculature early on, whereas at the blood-CSF barrier ABCG2 decreases during development (Gazzin *et al.* 2008; Ek *et al.* 2010). Other transporters that are considered important in relation to xenobiotic transport at the brain barriers are the SLCO (solute carrier organic anion), SLC15 (solute carrier family 15), SLC22 and SLC47, which have wide substrate specificity for many drugs including epileptics, antibiotics, immunomodulators, hormones among others. In general, expression studies have shown rather complex changes (both increases and decreases) with development within these families, discussed in detail elsewhere (Kratzer *et al.* 2013; Liddelow *et al.* 2013). Notably, in the non-human primate there was a consistent increase within the SLCO family with development at the brain-CSF barrier (Ek *et al.* 2015*c*); these proteins mainly transport hydrophobic organic anions such as thyroid hormone, bilirubin and a diverse range of drugs. However, functional studies of many of these transport systems at the BBB are lacking, making translation of expression and localisation data to function at the blood-brain interfaces mostly speculative. It is also important to consider that these transporters may play a role in elimination of waste brain products. Understanding these systems in development is clinically important in order to minimise the risk of adverse drug reactions, since these transport systems are saturable and care has to be taken when several drugs are given that overlap in transporter specificity. Overall these data suggest specialisation of these barrier transport systems to specific stages of development, probably reflecting changing brain requirements in relation to brain maturation processes, nutrient and detoxification needs.

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BBB integrity after perinatal hypoxia-ischaemia

A number of studies in rodents have indicated that BBB integrity is disrupted following neonatal hypoxia-ischaemia (HI) with extravasation of large endogenous plasma borne molecules such as IgG or albumin (Svedin *et al.* 2007; Tu *et al.* 2011; Yang *et al.* 2013), as well as an increase in permeability of smaller injected molecules (Ferrari *et al.* 2010*a*; Ek *et al.* 2015*a*). In a neonatal mouse HI model, our recent study demonstrated increased BBB permeability to a small molecule (sucrose, 342 Da), peaking at 6 h after the insult followed by complete normalization by 3 days, although changes were modest (Ek *et al.* 2015*a*). In a rat HI model Ferrari and colleagues reported a much higher magnitude of BBB permeability, as well as a longer time for normalization, with the BBB open for up to 7 days after insult (Ferrari *et al.* 2010*b*). The differences in the timing of the increased permeability in these two latter studies may be due to injury severity in different models; the latter study reported BBB changes in the contralateral hemisphere as well. Our study showed that brain pathology was closely related to reductions in cerebral blood flow during the HI as well as the areas with compromised BBB (Ek *et al.* 2015*a*).

In a fetal sheep model of HI injury, a careful examination of BBB blood-to-brain transfer of ^{125}I-radiolabelled IL-1β showed increased penetration after ischaemia, suggesting loss of BBB integrity (Sadowska *et al.* 2015). Also, chronic fetal hypoxia induced by single umbilical artery ligation resulted in BBB pathology, including reduction in cellular components of the neurovascular unit, such as reduced pericyte coverage, and astrocytic end-feet were observed in association with increased albumin extravasation (Castillo-Melendez *et al.* 2015). Moreover, quantitative studies in sheep have shown an increase in the unidirectional transfer rate constant of aminoisobutyric acid (an inert amino acid) after transient bilateral carotid artery occlusion up until at least 48 h following occlusion, with a peak increase at 4 h following occlusion (Chen *et al.* 2012). This correlates quite well with peak BBB changes in the neonatal HI mice studies outlined above (Ek *et al.* 2015*a*), suggesting that BBB function starts recovering reasonably fast after hypoxia-ischaemia.

Clinical studies show that albumin CSF/blood ratios are elevated in human babies following an episode of HI, indicating compromised barrier integrity, and the severity of asphyxia is positively correlated to higher ratios (Kumar *et al.* 2008; Aly *et al.* 2009). Clinical samples within the CNS are most often restricted to CSF and care should be taken in interpreting these changes exclusively as loss of BBB integrity, as changes in CSF turnover rates could also produce changes in CSF/blood ratios. It is notable that these two studies report vastly different albumin ratios in controls, as well as changes in ratios for babies diagnosed with hypoxic-ischaemic encephalopathy, which could affect results.

As indicated above, there are a great number of experiments that point to BBB integrity changes following HI in both rodents and larger animal species, but the mechanisms underlying BBB opening are often uncertain. We found that the opening of the BBB following neonatal HI in mice was associated with lower levels of the proteins ZO-1 and occludin, whereas gene expression of BBB proteins was increased, with the latter maybe being a part of the response to restore BBB integrity (Ek *et al.* 2015*a*). Sheep experiments have shown decreases in occludin, claudin-5 and ZO-1/2 at 4 h after cerebral ischaemia, with occludin and claudin-5 already returning to normal levels at 24 h (Chen *et al.* 2012). However, at this point it still remains unclear whether these BBB protein changes contribute to functional changes. Recent studies indicated that vascular damage occurs early after HI and is initiated by nitric oxide (NO) stress, with inhibition of neuronal NO synthase leading to reduced vascular and neuronal damage, although whether this treatment preserved BBB integrity was not investigated (Hsu & Kanoski, 2014). Importantly, this study underscores the vasculature as a promising therapeutic target for HI-related brain injury.

BBB integrity after perinatal focal arterial stroke

Following a 3-h transient middle cerebral artery occlusion (tMCAO) in adult and postnatal (P7) rats, comparison of BBB leakage of Evans Blue or fluorescent intravascular tracers 70 kDa dextran or TRITC-albumin at 24 h after reperfusion showed significantly lower extravasation of these molecules and a strikingly better preserved BBB integrity in injured neonatal brains (Fernandez-Lopez *et al.* 2012). Gd-DTPA-enhanced T1W imaging performed 24 h after reperfusion in P7 rats consistently showed only negligible contrast enhancement in injured regions, within 10% (Fernandez-Lopez *et al.* 2012). Alexa-647-albumin extravasation was also low in P9 mice subjected to 3-h tMCAO in animals with unperturbed microglial cells (Fernandez-Lopez *et al.* 2016). Low neutrophil infiltration and the presence of microglial cells protected from BBB leakage in injured regions, as was evident from increased albumin leakage under conditions of increased neutrophil infiltration (Fernandez-Lopez *et al.* 2012) or after pharmacological deletion of microglial cells following tMCAO in neonatal rodents (Fernandez-Lopez *et al.* 2016). In contrast to tMCAO, permanent MCAO in P7 rats resulted in rapid BBB disruption and leukocyte extravasation (Benjelloun *et al.* 1999), suggesting that persistent lack of cerebral microcirculation contributes to the BBB collapse. Two studies that utilized the tMCAO model in P10 rats demonstrated increased BBB permeability during a sub-chronic injury phase, 72 h

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following tMCAO (Wang *et al.* 2007; Dzietko *et al.* 2011). The affected region was larger in spontaneously hypertensive pups (Wang *et al.* 2007) than in normotensive pups of the same age (Dzietko *et al.* 2011).

While the phenomenon of a better-preserved BBB integrity in neonatal arterial stroke has been established, the underlying mechanisms are poorly understood. Comparative endothelial transcriptome data obtained in adult and neonatal rats subjected to tMCAO have provided some mechanistic insight into age differences in BBB susceptibility to stroke. It appeared that, strikingly, the patterns of up- and down-regulated endothelial genes in injured regions are largely non-overlapping between the two age groups (Fernandez-Lopez *et al.* 2012). Transcript levels of several adhesion molecules and extracellular matrix (ECM) components, including E-selectin and P-selectin, were differentially affected by injury in immature and adult brain. Gene expression of Mmp-9 was significantly up-regulated in injured adults and, while high transcript levels of collagen type IV α1 (Col4a1) and Col4a2 remained unaltered in neonates, a significant increase of these two genes was evident in injured adult rats. Interestingly, transcripts of angiogenic regulators Vegfr-2 and Angpt2 were increased after stroke in adults but not in neonates (Fernandez-Lopez *et al.* 2012). Furthermore, comparisons of protein expression of occludin, caludin-5 and ZO-1 between adult and neonatal rats after tMCAO showed better preserved expression in neonates than in adults (Fernandez-Lopez *et al.* 2012). Endothelial-ECM interaction via β1 integrins regulates the expression of claudin-5 and BBB tightness whereas other ECM proteins, like galectin-3, mediate integrin-induced stabilization of focal adhesions and activate cytokine receptors to enhance actions of growth factors (Goetz *et al.* 2008). Laminin degradation occurs after focal stroke in adults and causes detachment of astrocytic end-feet, disrupts BBB and induces intracranial haemorrhage (Fukuda *et al.* 2004), while in neonates expression of this ECM protein is not reduced acutely (Fernandez-Lopez *et al.* 2012). The role of other ECM proteins in injured neonates is less studied, but opposite effects of galectin-3 in adult stroke and hypoxia-ischaemia (HI) have been demonstrated (Doverhag *et al.* 2010; Lalancette-Hebert *et al.* 2012). Together, these data suggest that intrinsic developmental differences in basement membrane and ECM formation may contribute to a better-preserved BBB integrity after acute neonatal arterial stroke.

It is not known at the moment whether the distinct responses of the neonatal BBB to tMCAO are due to the model (i.e. the lack/presence of hypoxia), severity of the ischaemic episode, the extent of reperfusion, and animal species and strain. Direct comparisons of BBB dysfunction in HI and focal stroke models in neonatal rodents are lacking. Further, since both male and female rodents were used in experiments, the role of sex in the observed effects is unclear. It is notable in this respect that hypoxia in piglets has shown resistance to BBB opening, indicating species-specific damage responses (Stonestreet *et al.* 1992). The effects of ischaemic injury on the many transport systems present on the BBB and BCB still remain largely unknown. Further, data on the extent and the timing of angiogenesis after neonatal stroke and HI are limited. A few available studies that looked at endothelial proliferation 72 h to 2 weeks after tMCAO have demonstrated increased numbers of BrdU-positive endothelial cells starting in only the second week post stroke (Shimotake *et al.* 2010; Dzietko *et al.* 2013; Fernandez-Lopez *et al.* 2013). In contrast to neonatal rodents, ovine fetuses exposed to bilateral carotid artery occlusion show neovascularisation starting between 48 and 72 h after artery obstruction, as well as an increase in the proliferation marker Ki67 in endothelial cells around this time (Virgintino *et al.* 2014). Whether these dissimilarities are due to different models and animal species, developmental stages or severity of injury remains unclear.

Integrity of the brain barriers and perinatal inflammation

The brain is under constant immune surveillance by both blood-borne immune cells in leptomeningeal and perivascular spaces and by resident microglia (Prinz & Priller, 2017). Further, the epithelial barrier in the choroid plexus has been suggested to function as an 'educational gate' for leukocytes under normal conditions (Shechter *et al.* 2013). However, under physiological steady-state conditions, the neural parenchyma is protected from peripheral immune cells by the barrier systems (Prinz & Priller, 2017). Systemic infections can change this situation dramatically, making the vasculature inflamed and breaching BBB integrity, and leading to excessive inflammation in the brain due to trafficking of peripheral immune cells into the parenchymal tissue, induction of pro-inflammatory mediators in the vasculature, and/or activation of microglia (Obermeier *et al.* 2013). Further, systemic inflammation changes barrier transport systems (Coisne & Engelhardt, 2011). For example, administration of the TLR-3 ligand, polyinosinic:polycytidylic acid (PolyI:C) to pregnant mice (E15.5) followed by a P-gp substrate ([^{3}H]digoxin), resulted in increased accumulation of the substrate in the fetal brain (Bloise *et al.* 2017).

Bacterial infections in the newborn that give rise to meningitis clearly involve an opening of the BBB, at least partly due to the microbial-induced systemic inflammation (Kim, 2008; Barichello *et al.* 2013). However, it is unclear to what extent systemic inflammation by itself affects the integrity of the BBB. Importantly, infection can produce long-lasting effects on the barrier function. Newborn rats given five intraperitoneal injections of

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the endotoxin (lipopolysaccharide, LPS) at postnatal days 0, 2, 4, 6, and 8 demonstrated variable effects in BBB opening. When brain/plasma sucrose concentration ratios were measured as an index of BBB permeability, there was no significant change in BBB permeability at either P9 or P20 (Stolp *et al.* 2011), but when permeability was assessed at adult age, significantly higher brain/plasma sucrose concentration ratios were observed following early postnatal LPS treatment (Stolp *et al.* 2005). Similarly, while the combination of LPS and HI induced significant changes in BBB integrity in P12 rats, there was little acute evidence of neutrophil infiltration into the brain or increased albumin leakage after a single injection of LPS (Brochu *et al.* 2011). In the fetal sheep, extravasation of plasma albumin has been demonstrated in the cerebellum after placental inflammation induced by LPS administration into the uterine artery of pregnant sheep at 134–136 days gestation (Hutton *et al.* 2007). Following low dose LPS (0.1 μg kg^{-1}) to late gestation fetal sheep, intraparenchymal albumin was also found around cerebral blood vessels, indicating increased BBB permeability (Yan *et al.* 2004). Whether these differences in BBB response to systemic inflammation are dependent on stages of development, or are specific to species or subject to variable sensitivity to the different inflammatory substances is unknown. Interestingly, it was recently shown that hypoxia promotes bacterial entry to the brain, suggesting synergy between different insults that should be considered in future studies (Zarate *et al.* 2017).

The role of the choroid plexus has recently evolved as a research topic in health and disease (Marques *et al.* 2017). We investigated the expression of efflux and detoxification proteins in the choroid plexus (MRP1/Abcc1 and glutathione-S-transferase) after administration of LPS, HI or the combination of LPS and HI in neonatal mice (D'Angelo *et al.* 2013). Despite both HI and LPS-HI causing significant parenchymal injury, there was no evidence of cell damage in the choroid plexus and only LPS-HI resulted in a small increase in MRP1 mRNA expression. However, there was a significant down-regulation of the endogenous Nrf2 anti-oxidant system. Further, LPS prevented the endogenous anti-oxidant response following HI, suggesting the possibility that peripheral inflammation may contribute to increased vulnerability of the brain via oxidative mechanisms at the blood-CSF barrier interface. We found that administration of a synthetic mimic of gram-positive infection (toll-like receptor 2 agonist Pam3CSK4, PAM) increased infiltration of leukocytes, mainly neutrophils and monocytes, to the CSF and brain (Mottahedin *et al.* 2017*a*). Although PAM and LPS induced a similar degree of peripheral inflammatory responses, PAM provoked a distinct brain chemokine response and increased permeability, in particular, of the blood-CSF barrier. These results do not support the hypothesis that innate immune activation, in general, induces immune cell infiltration via the choroid plexus. Instead, our results indicate a specific TLR2-mediated mechanism of CNS inflammation and leukocyte invasion into the neonatal brain. This specific interaction between peripheral and central immune responses appears to occur to a large extent via the blood-CSF barrier. This may be important for neurological outcomes, as we have also found that systemic activation of TLR2 suppresses mitochondrial respiration and exacerbates hypoxic-ischaemic injury in the developing brain (Mottahedin *et al.* 2017*b*). Importantly, hypothermia was effective in reducing brain injury in the model of combined PAM-HI in neonatal rats (Falck *et al.* 2017). Further, previous studies have shown that blocking lymphocyte trafficking with FTY720 (fingolimod) prevents inflammation-sensitized HI brain injury in newborn rats, mitigates the influx of leukocytes through the choroid plexus and subsequently leads to attenuated BBB damage and better preservation of growth and white matter functions (Yang *et al.* 2014).

Collectively, the studies described suggest that endotoxin-induced intrauterine inflammation can breach the BBB but increased permeability is less often seen in neonatal rodent studies. On the contrary, experimental induction of gram-positive systemic infections has profound effects on leukocyte infiltration into the brain via the choroid plexus. Thus, it will be of great importance to understand the underlying mechanisms of different bacterial infections on different barrier systems.

Conclusion

It is now clear that the blood-brain and blood-CSF barriers are present and functional in early embryonic development. Tightness of the barriers is evident soon after the appearance of tight junctions and adherence proteins between endothelial or epithelial cells, which occur in parallel with recruitment of endothelial cells and pericytes into the brain. However, it is important to point out that CNS barrier systems are not static during development; on the contrary, they change significantly with maturation processes of the brain. In particular, transport systems are dynamically regulated, which probably reflect the different demands of the developing brain. Astrocytes are believed to play very important modulating roles in these processes during development.

Understanding of barrier functions in the developing brain has significantly moved forward in the last couple of decades, but there are experimental considerations that could affect interpretation. Studies on changes in BBB permeability have to a large extent been performed in rodents, which have an agyric brain that develops primarily after birth. Consequently, rodent experiments on barrier maturation may have less relevance to larger vertebrate species, including humans. Further,

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there are limitations with regard to methods used to study permeability changes. Data based solely on extravasation of blood-borne proteins, such as albumin, are somewhat precarious and should be cautiously interpreted, in particular when assessing extent and temporal changes. Applying exogenous compounds, especially in conjunction with live brain imaging, offer the possibility of more accurately assessing BBB permeability and temporal changes, which is advantageous both for the validity and biological relevance of data. However, care should also be exercised when interpreting experiments based on concentration ratios of exogenous markers between blood and brain, particularly when long circulation times of markers are employed, as parameters other than BBB permeability could influence results. It is also important to increase knowledge on transport systems to better understand how these develop with maturation and may affect neuropathological outcomes in disease models. Although bacterial systemic infections have been shown to breach the barriers, underlying mechanisms still remain to a large extent unclear and systemic inflammation by itself does not necessary increase BBB permeability. Further, new evidence suggests that some bacteria-induced inflammation strongly modulates the blood-CSF barrier across the choroid plexus, allowing leukocyte trafficking into the CSF and the brain parenchyma. These findings raise the question of whether diverse bacteria impair/modulate distinct barrier systems during development. Introducing pharmacological agents into the brain is considered one of the major hurdles in successful brain therapeutics. Our increasing knowledge of the role of barrier systems in brain development and disease suggests that the next generation of pharmacological treatments for perinatal brain injury should target these structures.

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Additional information

Competing interests

The authors do not have any competing interests.

Author contributions

All authors contributed to the conception of the manuscript, participated in drafting and critically revising the work for important intellectual content. All authors have approved the final version of the manuscript, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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