Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Mechanisms of restriction of viral neuroinvasion at the blood–brain barrier

Jonathan J Miner[1] and Michael S Diamond[1,2,3,4]

Abstract

The blood–brain barrier (BBB) consists of highly specialized cells including brain microvascular endothelial cells, astrocytes, microglia, pericytes, and neurons, which act in concert to restrict the entry of pathogens, immune cells, and soluble molecules into the central nervous system (CNS). If pathogens manage to cross the BBB and establish infection within the CNS, the BBB can open in a regulated manner to allow leukocyte transmigration into the CNS so that microbes, infected cells, and debris can be cleared. This review highlights how different inflammatory cytokines or signaling pathways disrupt or enhance BBB integrity in a way that regulates entry of neurotropic viruses into the CNS.

Addresses
[1] Department of Medicine, Washington University School of Medicine, St Louis, MO 63110, USA
[2] Department of Pathology & Immunology, Washington University School of Medicine, St Louis, MO 63110, USA
[3] Department of Molecular Microbiology, Washington University School of Medicine, St Louis, MO 63110, USA
[4] Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, St Louis, MO 63110, USA

Corresponding author: Diamond, Michael S (diamond@borcim.wustl.edu)

Current Opinion in Immunology 2016, **38**:18–23

This review comes from a themed issue on **Innate immunity**

Edited by **Eric Vivier** and **Ruslan Medzhitov**

For a complete overview see the Issue and the Editorial

Available online 16th November 2015

http://dx.doi.org/10.1016/j.coi.2015.10.008

Introduction

Neurotropic viruses that trigger encephalitis are a significant cause of morbidity and mortality globally, resulting in clinical phenotypes that range in severity from mild cognitive impairment and memory loss to permanent central nervous system (CNS) damage and death [1,2]. However, most patients who are infected at peripheral sites with neurotropic viruses never develop evidence of CNS infection [3]. Since neuroinvasion occurs in only a small minority of infected patients, it is thought that host–pathogen interactions and immune system responses in peripheral organs and at the blood–brain barrier (BBB) prevent viruses from gaining access to and establishing infection within the CNS.

Structure of the BBB

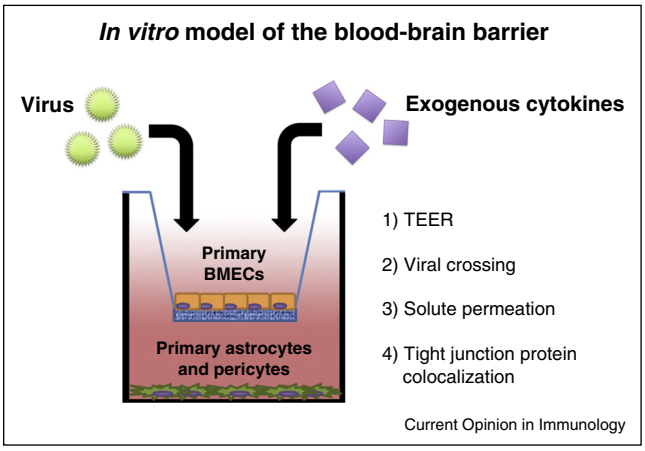
In 1885, Paul Ehrlich noted that dyes administered intravenously into animals failed to enter the CNS, although his initial interpretation was that the dye did not stain the brain because of altered affinity rather than a physical barrier [4]. In 1909, Goldmann theorized the existence of a structural barrier in the brain because intravenously injected trypan blue dye failed to enter the CNS [5]. It was not until the mid-20th century when electron microscopy studies revealed the ultrastructural characteristics that define the BBB [6,7]. Brain microvascular endothelial cells (BMECs) line post-capillary venules and function as the primary structural component of the BBB. BMECs closely associate with the foot processes of astrocytes, which secrete soluble factors that promote tight junctions and barrier integrity, pericytes, which regulate angiogenesis, vessel integrity, and blood flow, and microglia, which release cytokines and matrix metalloproteinases (MMP) in response to pathogen-associated stimuli [8–10]. Cytokines and other inflammatory mediators secreted by these supporting cells regulate tight junctions composition and the opening and closing of the BBB (see below).

The use of *in vitro* models of the BBB in combination with *in vivo* studies in small animals has advanced our understanding of the cellular and molecular mechanisms that prevent viruses from disseminating into the CNS. Many studies that have defined the contributions of cytokines to BMEC permeability have used *in vitro* transwell systems in which BMECs are cultured over supporting astrocytes and/or pericytes (Figure 1). Colocalization of tight and adherens junction proteins is evaluated by immunofluorescence confocal microscopy, and barrier integrity is quantitated by measuring trans-endothelial electrical resistance or transit of virus/solutes across a BMEC monolayer [11,12••]. *In vivo* assessment of BBB integrity primarily relies on the measurement of dye (e.g., fluorescein) or protein (e.g., immunoglobulin) permeation into the CNS [13••].

Viral crossing of the BBB

Many viruses infect the CNS including retroviruses, morbillivirus, picornaviruses, rhabdoviruses, flaviviruses, bunyaviruses, alphaviruses, and coronaviruses, among others [14]. With the exception of rabies virus, most neuroinvasive viruses enter the CNS in a small subset

Figure 1



In vitro model of the BBB. Primary brain microvasulcar endothelial cells (BMECs) are cultured in a transwell above primary astrocytes and/or primary pericytes. Virus and/or exogenous cytokines are added to the upper chamber followed by measurement of trans-endothelial electrical resistance (TEER), virus crossing, and/or permeation of solutes into the lower chamber. BMECs also can be examined by immunofluoresence and confocal microscopy to assess expression and colocalization of tight junction proteins, which regulate permeability across BMEC monolayers.

of infected individuals. The host mechanisms that restrict viruses from crossing into the CNS vary depending on the route of entry of the particular virus.

The pathways by which individual neurotropic viruses enter the CNS have been difficult to demonstrate with precision. Viruses invade the CNS by either directly crossing the BBB or by circumventing the BBB *via* non-hematogenous routes of entry [15]. Pathways of direct viral transit across the BBB include: (1) spread of viruses across BMEC tight junctions due to high levels of viremia and inflammation [12••]; (2) direct infection of BMECs and transport of nascently generated viruses across basolateral membranes [16]; and (3) a 'Trojan horse' pathway in which infected leukocytes in the blood migrate across the BBB to seed the CNS with infectious virus [17,18]. This can occur in the context of inflammation-directed diapedesis or as part of tissue surveillance, which occurs at low levels at baseline. Some viruses can circumvent the BBB entirely by utilizing non-hematogenous routes of entry into the CNS. These mechanisms include: (1) retrograde axonal transport of virions from peripheral nerves into the CNS [19] and (2) infection of the olfactory epithelium followed by transit of virus into the CNS across the cribriform plate and infection of cells in the olfactory bulb [20]. When considered together, these five pathways of entry into the CNS are not mutually exclusive and may vary depending on the immune context or specific virus. It is plausible that more than one pathway may be used by certain viruses. For example, Venezuelan equine encephalitis virus can invade the CNS *via* the cribriform plate, which subsequently triggers a delayed opening of the BBB that allows a second wave of viral neuroinvasion directly across the BBB [20].

Young mice are more susceptible to viral encephalitis than adult mice, an observation that was described nearly 80 years ago [21]. Vulnerability of young mice to neurotropic viruses may occur in part as a result of BBB breakdown during viral infection, which has been demonstrated by intravenous injection of dye and measurement of permeation into the CNS [12••]. This phenomenon is not limited to animal models, since human neonates and children also are more susceptible to many forms of viral encephalitis [1]. Age-dependent effects on viral neuroinvasion can also be seen in the context of viruses that are not classically considered neurotropic. For example, old world alphaviruses including chikungunya virus (CHIKV) typically cause inflammatory arthritis and only rarely cause neurological disease in adults. In the 2006 epidemic of CHIKV on La Reunion Island, there were multiple cases of neuroinvasive CHIKV infection in neonates, which resulted in microcephaly, flaccid paralysis, cerebral palsy, seizure disorders, and even death [22–25]. Some have speculated that young animals have an immature BBB despite the fact that tight junctions and other relevant structural components observed in adult animals are present early in development [26,27]. Young animals may be more vulnerable to viral encephalitis as a result of a combination of host factors, with BBB breakdown being just one of the variables.

Modulators of BBB integrity

Host factors regulate BBB integrity both to prevent pathogen invasion into the CNS and if necessary, to enable leukocyte transmigration after a neuroinvasive infection is established. The balance and type of cytokines and their cumulative effects at the BBB are complex and regulated by multiple signaling pathways and cell types, including BMECs, astrocytes, and pericytes.

Type I and type III interferons

Interferon (IFN)-α/β signaling through the type I IFN receptor (IFNAR1/IFNAR2) activates an antiviral program that up-regulates IFN-stimulated genes (ISGs), which antagonize viral replication in many cell types. Recent studies have shown that activation of viral RNA-sensing pathways in BMECs leads to Rac-1-dependent tight junction formation and enhanced BBB integrity to prevent viruses from transiting across BMEC monolayers [12••]. The discovery that type I IFNs promote BBB integrity may explain, in part, the efficacy of IFN-β in the treatment of multiple sclerosis, an autoimmune disease characterized by BBB breakdown and unchecked