Review

Viral disruption of the blood–brain barrier

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The blood–brain barrier (BBB) provides significant protection against microbial invasion of the brain. However, the BBB is not impenetrable, and mechanisms by which viruses breach it are becoming clearer. *In vivo* and *in vitro* model systems are enabling identification of host and viral factors contributing to breakdown of the unique BBB tight junctions. Key mechanisms of tight junction damage from inside and outside cells are disruption of the actin cytoskeleton and matrix metalloproteinase activity, respectively. Viral proteins acting in BBB disruption are described for HIV-1, currently the most studied encephalitic virus; other viruses are also discussed.

Virus entry to the brain

Viral encephalitis is a potentially deadly sequela of viral infection for which there are few treatment options. It is frequently associated with blood-brain barrier (BBB; see Glossary) disruption, enabling entry of virus, inflammatory cells, and deleterious molecules into the brain parenchyma. Members of at least 11 virus families, including DNA viruses, retroviruses, and RNA viruses, cause encephalitis with significant morbidity and mortality [1]. There are a variety of means by which viruses enter the brain, primarily via neuronal transport or by crossing of one of several barriers to the central nervous system (CNS), including the BBB or the blood–cerebrospinal fluid barrier (choroid plexus). Several recent reviews covered viral entry via axonal transport and the resulting neuronal damage [2–4]. This review will focus on CNS entry mechanisms used by viruses that breach the BBB. Cell culture and animal studies of viral encephalitis have recently progressed through approaches used in studies of pathogenic conditions of the CNS such as ischemic stroke and multiple sclerosis. We first present background information on the structure, function, and disruption of the BBB, followed by a discussion of specific mechanisms by which viruses breach the BBB.

Components of the BBB

The BBB is a physical, metabolic, and transport barrier between the peripheral circulation and the CNS [5]. The function of the barrier is contributed by features specific to brain microvascular endothelial cells, which form the walls of brain capillaries, and the interactions of these cells with other components of the neurovascular unit (NVU), especially astrocyte endfeet and extracellular matrix [6]. The NVU also includes pericytes, microglia, and neurons (Figure 1). Brain endothelial cells form extremely tight cell–cell junctions that are distinct from tight junctions of endothelia and epithelia elsewhere in the body, reflecting brain endothelial cell morphology, biochemistry, and interactions with other cells of the NVU [5–7]. Brain endothelial cells lack fenestrations, have high numbers of mitochondria, are very thin, and have a low rate of pinocytosis, characteristics that relate to their specialized function. For example, high mitochondrial content in the NVU relative to other tissues is likely important to provide the energy required to maintain the structure and function of the BBB. Endothelial cells and associated pericytes are ensheathed by an endothelial cell basal lamina (vascular

Glossary

Adherens junctions: protein complexes located at cell–cell contacts in endothelium and epithelium. Adherens junctions are essential for the formation of tight junctions and are anchored on actin cytoskeletons.
Astrocytes: cells with long processes ('star' shaped) that comprise of the majority of neuroglial cells in the brain and spinal cord. Astrocytes are important for development and/or maintenance of BBB characteristics.
Blood–brain barrier (BBB): the interface between the brain and peripheral circulation. It is composed of specialized capillaries and adjoining cells that function to strictly regulate the entry into the brain of substances from the peripheral circulation.
Central nervous system (CNS): the part of the nervous system that contains the brain and the spinal cord. Responsible for the control and coordination of the entire body.
Claudins: a family of small transmembrane proteins important for tight junction formation.
Endothelial cells: cells forming the main structural component of blood vessels.
Junctional adhesion molecules (JAMs): members of the immunoglobulin family involved in cell–cell adhesion.
Lymphocytes: a subset of white blood cells of the immune system that includes T cells, B cells and NK cells.
Matrix metalloproteinases (MMPs): zinc-dependent endopeptidases that are involved in a variety of processes including tissue repair, angiogenesis, cell division, apoptosis and host immunity. These proteins can be soluble, matrix-bound or cell-associated.
Microglia: resident macrophages of the brain and spinal cord. They are the initial and main host immune system responders in the CNS.
Neurovascular unit (NVU): an association of endothelium, extracellular matrix, astrocytes, pericytes, microglia, and neurons that contributes structurally and functionally to permeability of the microvasculature.
Occludin: transmembrane tight junction protein.
Paracellular transport: transport of substances between or around cells.
Reactive oxygen and nitrogen species (ROS, RNS): highly reactive molecules or free radicals that contain oxygen or nitrogen, respectively. These chemicals can mediate cellular damage by attacking biological molecules.
Tight junctions: protein complexes that include transmembrane and cytoplasmic proteins. Tight junctions are located in intercellular clefts and form close associations that restrict the passage of molecules.
Tissue inhibitors of MMP (TIMPs): natural regulators of MMPs.
Transcellular transport: transport of substances through a cell.
Zona (or zonula) occludens (ZO) proteins: a family of intracellular scaffolding proteins important for the structural integrity of intercellular tight junctions.

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Keywords: blood–brain barrier; viral encephalitis; tight junctions; matrix metalloproteinases.

 0966-842X/$ – see front matter http://dx.doi.org/10.1016/j.tim.2012.03.009

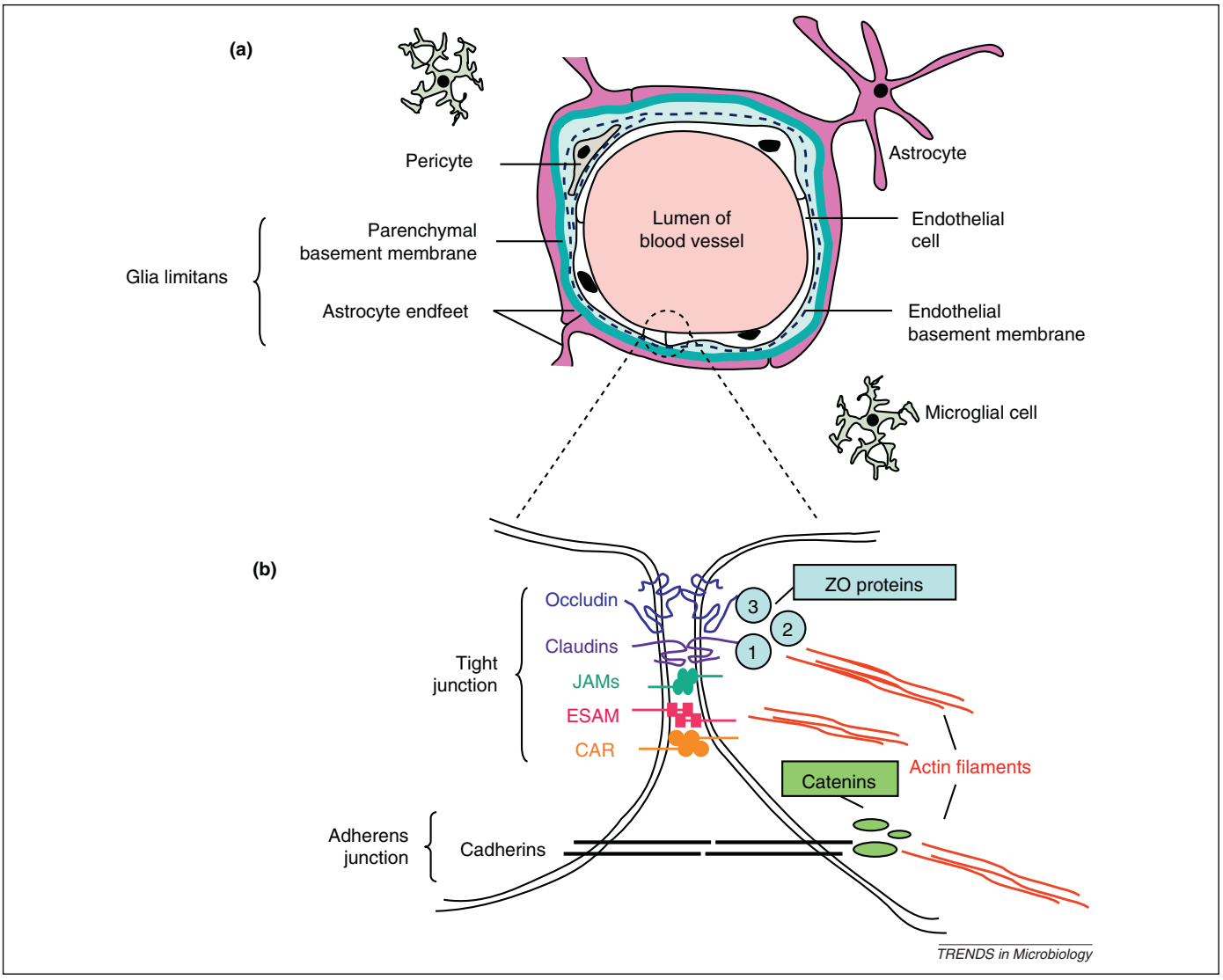

Figure 1. The neurovascular unit and junctions between endothelial cells. **(a)** Cross-section of a brain microvessel, showing cells of the neurovascular unit. Neurons and their contacts with astrocytes are not shown. Cells in the lumen (bloodstream) include red blood cells, lymphocytes, monocytes, and neutrophils (not shown). Note the two basement membranes; the space between them is known as the perivascular space. **(b)** Enlargement of an endothelial cell–cell junction. Major molecules of the tight junction and adherens junction are shown; no order of the tight junction proteins within the tight junction is implied by the figure. Abbreviations: JAMs, junctional adhesion molecules; ESAM, endothelial cell-selective adhesion molecule; CAR, Coxsackie and adenovirus receptor; ZO, zona occludens. The figure is based on figures in [7,95].

basement membrane). The composition of the endothelial basement membrane is distinct from that of a second basal lamina, the parenchymal basal lamina. This parenchymal basal lamina together with the astrocyte endfeet is termed the glia limitans. The perivascular space has been compared to a castle moat between the vascular basement membrane (outer castle wall) and the glia limitans (inner wall) [8]. Leukocytes accumulate in this cerebrospinal fluid-filled perivascular space (moat) where immune surveillance occurs. When leukocytes are presented with their cognate antigens they are activated and cross the glia limitans into the brain parenchyma. Brain endothelial cells have active transporters expressed on their apical and basal surfaces that exclude potentially detrimental molecules or enable passage of essential nutrients such as glucose and amino acids into the brain parenchyma. Intact brain endothelium *in vivo* is characterized by a very high transendothelial electrical resistance (TEER) due to its complex tight junctions, which result in both the effective block of passage of macromolecules and restricted diffusion of ions and polar solutes. The consequence of these features of brain endothelial cells is a restrictive barrier with controlled entry of plasma components.

Tight junctions in the brain

The complexes holding brain endothelial cells together are adherens junctions and tight junctions (Figure 1) [5]. The adherens junctions, composed of transmembrane cadherin proteins linked to the cell cytoskeleton by catenins, provide structural support and are important for the development of tight junctions. Tight junction complexes consist of both integral transmembrane proteins and peripheral membrane proteins. The integral transmembrane tight junction proteins include occludin, claudins, junctional adhesion molecules (JAMs), endothelial cell-selective adhesion molecule (ESAM), and the Coxsackie and adenovirus receptor (CAR) [5,9]. Occludin and claudins have external loops that mediate intercellular adhesion by interaction with occludin and claudins of neighboring cells [6]. The tight junction proteins that span the gap between cells can be altered in