Blood Brain Barrier: Structure, Function and Bypass by Microorganisms

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Discovery

- 1885: Paul Ehrlich: intravenous dyes in experimental organisms caused staining of all organs except the brain
- 1913: Edwin Goldmann put forward hypothesis that the cerebral capillaries provide anatomical basis for a physiological barrier between brain and the rest of the body
- 1950s: Electron microscopy demonstrated that the outermost layers of endothelial cells in brain capillaries are fused together

What is the Blood Brain Barrier?

- Structural and functional barrier which impedes and regulates the influx of most compounds from blood to brain
- Formed by brain microvascular endothelial cells (BMEC), astrocyte end feet and pericytes
- Essential for normal function of CNS
- Regulates passage of molecules in and out of brain to maintain neural environment.
- Responsible for metabolic activities such as the metabolism of L-dopa to regulate its concentration in the brain.

Structure of Blood Brain Barrier

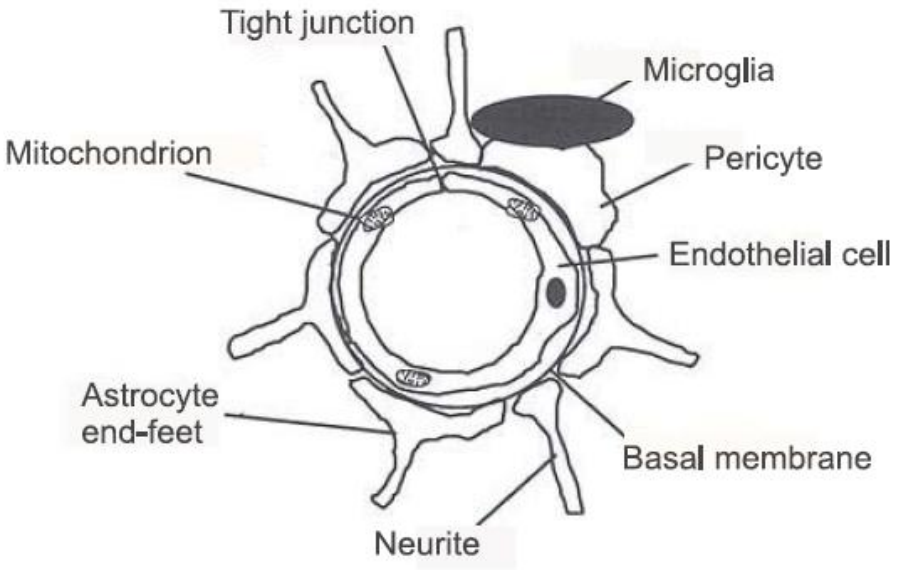


Figure 2: The blood-brain barrier: Morphology of the capillary epithelium in the region of the central nervous system and surrounding tissue.

Source: Bock et al

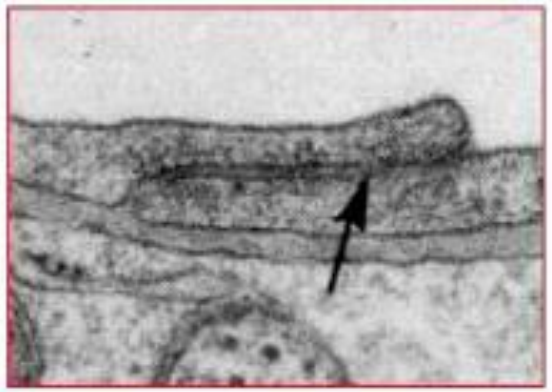
Differences between BMEC and normal endothelial cells

- Structural differences:
  - Absence of fenestrations
  - More extensive tight junctions (TJ)
- Functional differences:
  - Impermeable to most substances
  - Sparse pinocytic vesicular transport
  - Increased expression of transport and carrier proteins: receptor mediated endocytosis
  - No gap junctions, only tight junctions
  - Limited paracellular and transcellular transport

Integrity of BBB

- Tight Junctions
- Adherens Junctions
- Pericytes
- Astrocyte end feet

Tight Junctions between BMEC

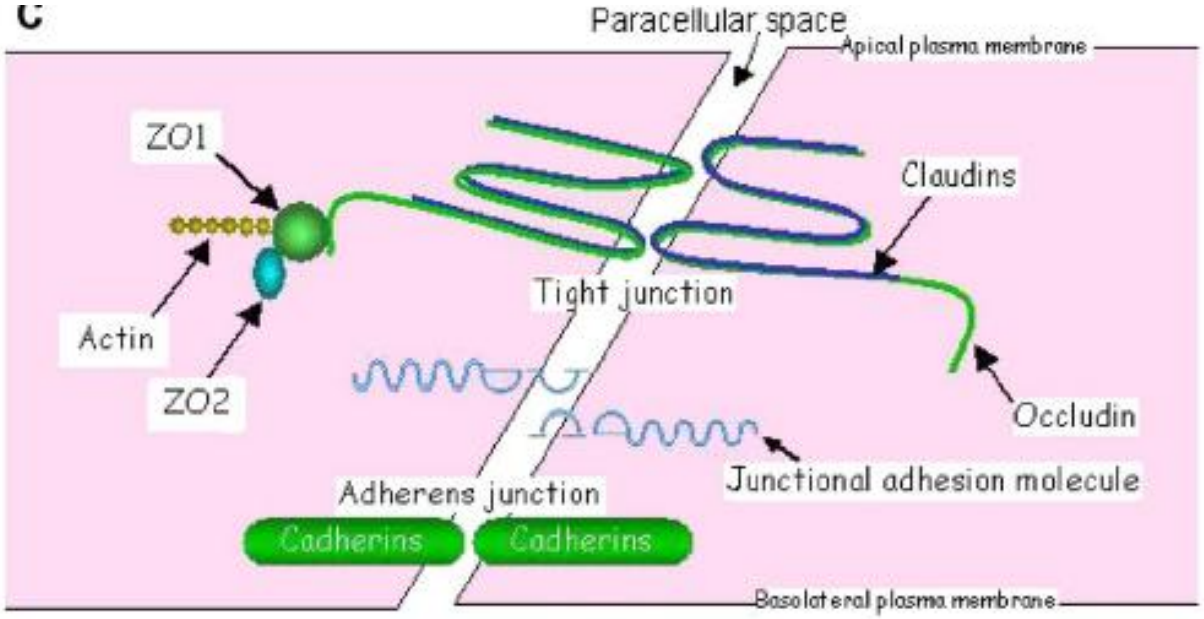


Source: Ballabh et al

- Appear at sites of apparent fusion between outer leaflets of plasma membrane of endothelial cells
- Continuous
- Anastomosing
- Intramenbranous strands or fibrils on P face with complementary groove on E face
- Protein components:
  - Claudin
  - Occludin
  - Junction Adhesion Molecules
  - Accessory proteins

Claudin

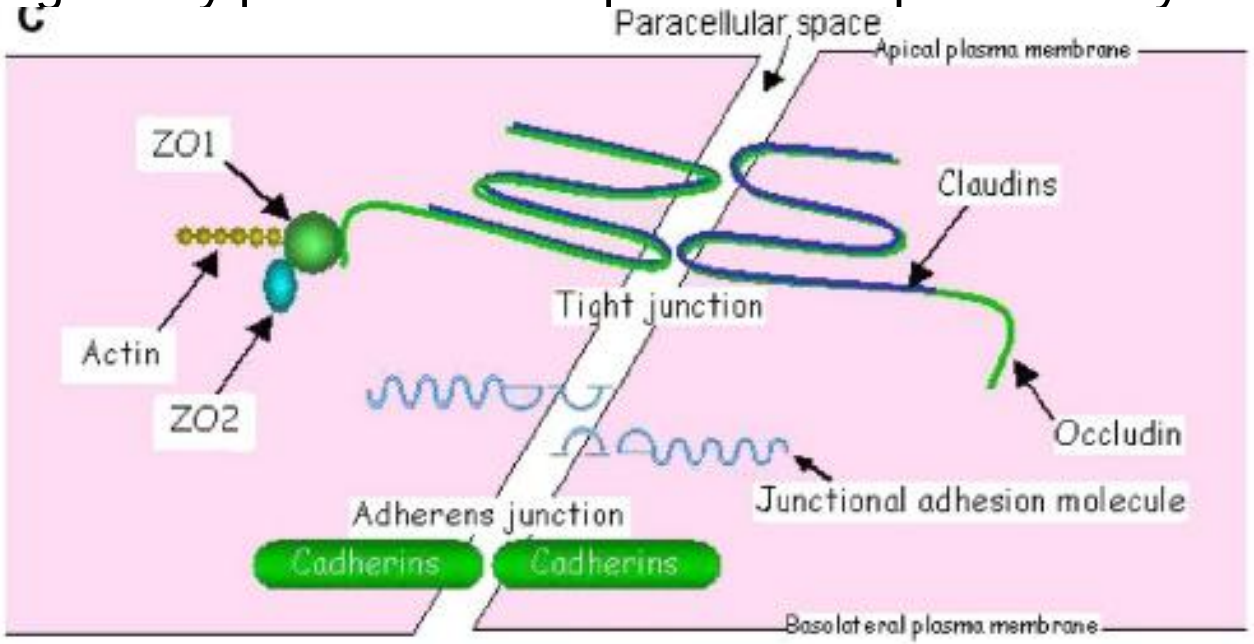
- 22kDa phosphoprotein
- 4 transmembrane domains
- localized in TJ strands



Source: Ballabh et al

Occludin

- 65kDa phosphoprotein,
- 1° structure very different from claudin
- Regulatory proteins: alters paracellular permeability.



Source: Ballabh et al

Barrier Function of Occludin and Claudin

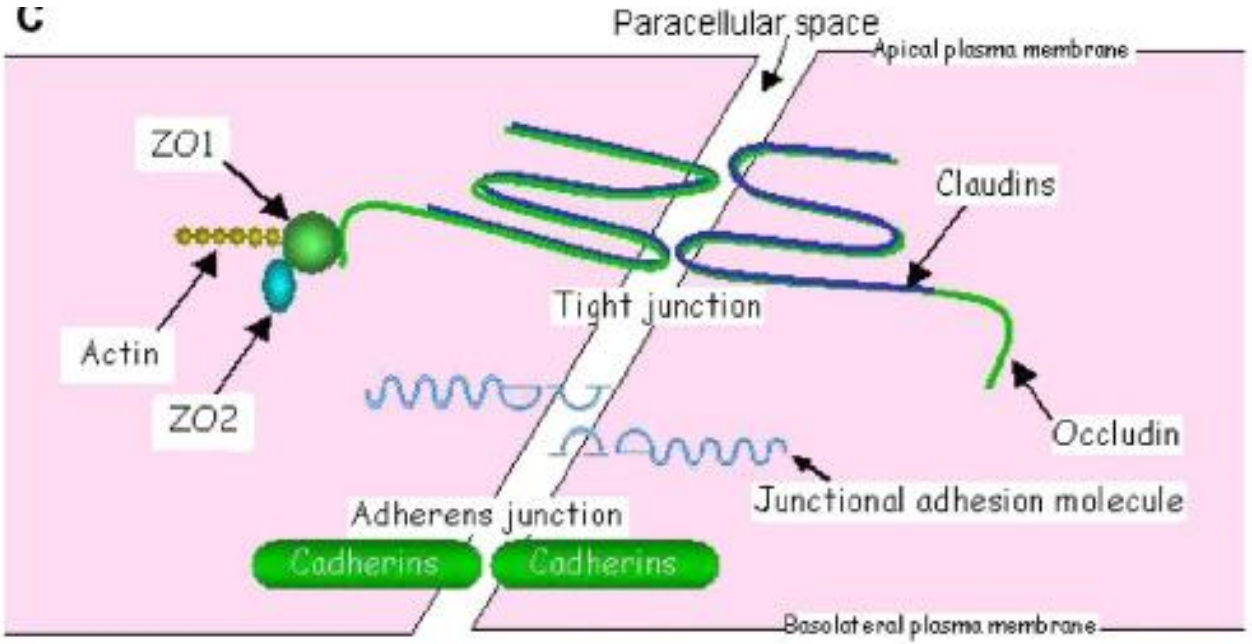
- Assemble into heteropolymers and form intramembranous strands which contain channels allowing selective diffusion of ions and hydrophilic molecules.

- Breakdown of BBB in tissue surrounding brain tumors occurs with concomitant loss of 55kDa occludin expression

Junction Adhesion Molecules:

- 40kDa
- Integral membrane protein, single transmembrane region
- Belongs to immunoglobulin superfamily
- Localizes at tight junctions
- Involved in cell-to-cell adhesion and monocyte transmigration through BBB
- Regulates paracellular permeability and leukocyte migration
- Also found on circulating leukocytes, platelets and lymphoid organs.

BMEC intercellular space



Source: Ballabh et al

Barrier function of JAM

- **Homotypic** binding between JAM molecules on adjacent endothelial cells acts as a barrier for circulating leukocytes
- **Heterotypic** binding of endothelial JAM to leukocyte JAM might guide transmigration of leukocytes across interendothelial junctions
- So factors that decrease leukocyte migration must either strengthen homotypic interactions or weaken heterotypic interactions.

Cytoplasmic accessory proteins

- (ZO-1, ZO-2, ZO-3, cingulin etc)
  - These link membrane proteins to actin
  - maintenance of structural and functional integrity of endothelium
  - crosslink transmembrane proteins.
- Membrane associated guanylate kinase-like proteins (MAGUKS)
  - subunits function as protein binding molecules
  - role in organization the plasma membrane

Adherens Junction

- Complex between membrane protein cadherin and intermediary proteins called catenins
- Cadherin-catenin complex joins to actin cytoskeleton
- Form adhesive contacts between cells.
- Assemble via homophilic interactions between extracellular domains of calcium ion dependent cadherins on surface of adjacent cells

Pericytes:

- Cells of microvessels including capillaries, venules, and arterioles that wrap around endothelial cells.
- Provide structural support and vasodynamic capacity to microvasculature.
- Role in structural stability of vessel wall
- Endothelial cells associated with pericytes are more resistance to apoptosis than isolated endothelial cells
  - Indicates role of PC in structural integrity and genesis of the BBB
- Phagocytic activity

Astrocyte end feet

- Star shaped glial cells
- Provides biochemical support for BMEC
- Influence of morphogenesis and organization of vessel wall
- Factors released by astrocytes involved in postnatal maturation of BBB
- Direct contact between endothelial cells and astrocytes necessary to generate BBB (Rubin et al, 1991)
- Co-regulate function by the secretion of soluble cytokines such as (LIF, leukemia inhibiting factor), Ca^{2+} dependent signals by intracellular IP-3 and gap junction dependent pathways, and second messenger pathways involving extracellular diffusion of purinergic messenger.

Regions of brain not enclosed by BBB

- Circumventricular organs
  - area postrema,
  - median eminence,
  - neurohypophysis,
  - pineal gland,
  - subfornical organ and
  - lamina terminalis

  These are regions which need to respond to factors present in systemic circulation

Circumventricular organ functions:

- Pineal gland - secretes melatonin and is associated with circadian rhythms
- Subfornical organ - regulates body fluids, fluid and electrolyte imbalance
- Organum vasculosum of the lamina terminalis – detects peptides
- Choroid Plexus
- Area Postrema - the “vomiting centre” of the brain
- Median eminence - regulates the anterior pituitary through the release of neurohormones
- Neurohypophysis - detects levels of oxytocin and ADH in the blood

Normal BBB transport

- Diffusion
- Facilitated transport by carrier systems
- Receptor mediated endocytosis
- Paracellular transfer more common than transcellular transfer

Diffusion

- Phospholipid bilayer
- Movement of substances down diffusion gradient
- Transfer of lipophilic substances
  - alcohol, nicotine, oxygen, carbon dioxide

Facilitated transport

- Carrier systems
  - particular essential amino acids, glucose, these are extremely specific
    - transport D-glucose only,
    - large neutral amino acids which act as precursors for neurotransmitters,
    - only which the brain cannot make,
    - glycine: it can block the transmission of nerve signals, hence special carrier which ensures that glycine can be removed from brain
- Receptor mediated endocytosis
  - Leptin, insulin, overlaps with carrier systems

Factors which cause increase in BBB during pathophysiology

- **Factors produced by astrocytes**
  - **Glutamate,**
  - **Aspartate**
  - **Taurine**
  - **ATP**
  - **Endothelin-1**
  - **NO**
  - **MIP-2**
  - **Tumor necrosis factor alpha TNF-α**
  - **Interleukin beta IL-β**
- **Paracrine signals secreted by endothelium cells or nerve terminals of neurons running close to blood vessels**
  - **Bradykin**
  - **5HT**
  - **Histamine**
  - **Thrombin**
  - **UTP**
  - **UMP**
  - **Substance P**
  - **Qionolonic acid**
  - **Platelet activating factor**
- **Free radicals**

E. Coli model

- Requirements for BBB translocation and successful infection
  - High degree of bacteremia
  - *E Coli* invading BMEC
  - Rearrangements of actin cytosleleton
  - Traversal of BBB as live bacteria

The Consensus

The basis for microbial host interactions contributing to bacterial invasion of human BMEC and relevant signaling mechanisms has not been fully elucidated

Transfer of microbes across BBB:

- **Physical damage of BBB**
- **Ligand receptor interactions followed by host cell actin cytoskeletal rearrangements**
- **Transcellular transport while maintaining integrity of BMEC**

Physcial damage of BBB

- microhemmorage or necrosis of surrounding tissue
- mechanical obstruction of microvessels by parasitized red blood cells (PRBC), platelets or leukocytes in cerebral malaria,
- overproduction of cytokines Borrelia *bugdorferi* : fibrinolytic system linked by activation cascade may lead to focal and transient degradation of tight junction proteins.

Ligand receptor interactions followed by host cell actin cytoskeletal rearrangements

- *E.Coli* binding to BMEC type I fimbriae, outer membrane protein A, Ibe proteins, cytotoxic necrotizing factor 1 (CNF 1)
- S. *pneumoniae* cell wall phosphorylcholine and BMEC platelet activating factor receptor

Microbe-specific interaction with BBB

- Bacteria
  - bind to BMEC, invade BMEC, induce actin cytoskeletal rearrangement, traverse BBB as live bacteria
- Mycobacteria
  - unclear, although DNA microarray results show that gene expression profile of M. tuberculosis associated with human BMEC showed at least 33 genes that were 8X or more upregelated and 147 genes that were 8X or more down-regulated.
- Spirochetes and Fungi
  - largely unknown, poorly understood: they are able to bind, be internalized and traverse human BMEC without obvious change in integrity of BMEC (*Borrelia burgdorferi*, *C neoformans*, *C. albicans*.

Conclusion

- Knowledge of the morphology and physiology of the blood brain barrier has come a long way, but there are many questions that are still unanswered

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