The Gut Immune Barrier and the Blood-Brain Barrier: Are They So Different?

Team: FENAS
Agenda

- Intro
- Gut-Immune Barrier
- Blood-Brain Barrier
- Barrier Regulation
- Junctions
- Disorders
- Barrier Evasion
Introduction

- The body has evolved defense mechanisms against toxins
  - Gut-immune barrier
  - Blood-brain barrier
- In both cases, there exists a physical barrier formed by a cellular layer that tightly regulates the movement of ions, molecules, and cells between two tissue spaces
- Each barrier also contains a different array of immune cells that survey the physical barrier and provide innate and adaptive immunity
Gut Immune Barrier (GIB)

- First line of defense against potentially harmful agents ingested in food
  - GIB Deals with food antigens,
  - Tolerates microbiota
  - Protects against pathogens

- Defects in these functions can lead to intestinal disorders
  - Inflammatory bowel disease
  - Irritable bowel syndrome
  - Food allergy or intolerance
  - Microbial infection
GIB Structure

- Structure includes a mucus layer and a physical barrier
- Physical barrier: gut epithelial cells separate the gut lumen from internal space
  - Also composed of enterochromaffin cells (ECCs), and Paneth cells
- Unlike BBB, the GIB is constantly exposed to microbiota
GIB Mucous Layer

- Physically separates microbiota from epithelial cell barrier
- Mesh of networking fibers made of mucins, glycoproteins, and lipids allowing the passage of molecules within a certain size tolerance
  - Composed of viscous luminal gel and firm layer adhering to epithelium
  - Viscous layer formed by soluble mucins released by goblet cells
  - This layer is exploited by the microorganisms to form a biofilm that allows their growth and prevents their washout with the luminal content, due to intestinal peristalsis
  - Thickness of layer varies along GI tract - depends on microbes
- Serves as a barrier and anchor for sustainable growth of microbiota
Blood-Brain Barrier

- Specialized structure formed by the blood vessels of the central nervous system (CNS).
- Blood vessels tightly restrict the flow of blood-borne ions, molecules, and cells from entering the neural tissue
  - CNS tissue does not regenerate after injury or disease
  - Damage to CNS can lead to paralysis, dementia, and death
- Evasion of the barrier by pathogens and infection of the CNS can lead to potentially fatal neuroinflammatory diseases
  - Meningitis, encephalitis, or focal abscesses
BBB Structure

- Barrier consists of CNS endothelial cells (blue)
  - Separates lumen of blood vessels from bulk of CNS
  - Pericytes are situated on the outer surface of the endothelial tube
  - Surrounded by a basal lamina
  - Astrocytes form intimate contacts with the vessels

- Endothelial cells held together by tight junctions, creating a paracellular barrier
  - Lack of transcytotic vesicles creates transcellular barrier

- Cells have nutrient transporters to move lipophillic molecules and deliver nutrients to CNS

Function Regulated by interactions between endothelial cells and astrocytes, pericytes, and other neural and immune cells regulate the properties of the BBB
The BBB Equivalent of Mucous Layer

● Luminal surface of the endothelial cells is covered by a complex glycocalyx
  ○ Uncertain of specific properties of glycocalyx at the BBB

● Vascular glycocalyx shown to:
  ○ Regulate vascular homeostasis
  ○ Protect vascular wall from shear stress
  ○ Regulate interaction with blood cells

● Specific pathogens including Haemophilus somnus can bind to the glycocalyx and use it to anchor to CNS endothelial cells
Cellular Barriers

- Barriers provide protection from pathogens and control microenvironment of tissue
  - GIB: regulation of movement of nutrients, water as well as salt absorption
  - CNS: maintains precise cellular ion concentrations that neural activity relies on
    - Protects neural tissue from ionic fluctuations in blood
Barrier Differences - BBB and GIB

- BBB and GIB barrier cells differ in origin
  - Gut epithelial cells endodermal in origin
    - Protects organism from external hazard
  - CNS epithelial cells mesodermal in origin
    - Protects organism from hazard within
Paracellular Junctions & Barrier Properties

Gut–brain–endocrine axis co-metabolism

Lifestyle–environment – diet – nutrition

Endogenous host/liver – microbiota – co-metabolism

Stress

Hormones
Pituitary
CRH
ACTH

Hypothalamus

SCFAs

Metabolites

Ghrelin

Leptin

Toxins

Precursors

Neurotransmitters

Psychoactive substances

Cortisol

Adrenal

HPA Axis

Increased disease and infection susceptibility versus health effects
Gut Paracellular Junctions

- Gut epithelial cells are sealed by **tight junctions (TJ)**
  - Formed from protein occludin, JAM, Claudins, cytoplasmic proteins binding TJ to cytoskeleton
- TJ allow the flow of solutes into the body by absorption
- TJ are continuously displaced!
Brain Paracellular Junctions

- Endothelial cells are coupled by TJ
- Analogous molecular composition of BBB with GIB
  - Made up of Occludin, JAM, claudins, etc
- Claudin 5 is critical for the BBB in mice
  - Displayed selective leakage in KO mice as well as calcification of the CNS.
- TJ in BBB and GIB identical
  - Claudings may determine the permeability of
  - The respective barriers
GIB & BBB Paracellular Similarities

- TJ forming both barriers display similar molecular similarities
  - Claudin proteins may determine the permeability of each barrier, respectively.
- Downregulation/redistribution of TJ
  - in GIB:
    - Irritable bowel syndrome (IBS)
    - Inflammatory bowel disease (IBD)
  - BBB:
    - Stroke
    - Multiple Sclerosis (MS)
Transcellular Barriers in GIB

- Properties of the GIB differ according to the specific section within the intestine, however, properties are conserved throughout whole intestine.
- Small intestine absorbs nutrients
  - Via invaginations of the mucosa that form villi (increasing surface area)
  - Apical surface of small intestine forms brush border
    - Consisting of microvilli
    - Further increasing surface area
    - Specialized for nutrient absorption
- Large intestine absorbs water & electrolytes
  - Absorbs complex molecules with the help of the GM!
Transcellular properties in BBB

- **CNS endothelial cells** contain fewer transcytotic vesicles than endothelial cells in other tissues, thus limiting the transcellular passage of hydrophilic molecules and ions from the blood to the brain
  - Express many efflux transporters
    - Diffuse from blood to brain via endothelial lipid membranes & back to the blood
  - Express variety of transporters to deliver specific nutrients to the brain,
    - Glucose
    - Amino acids
    - Vitamins
  - Exclude potential toxins from penetrating the brain!
Transcellular Properties in GIB & BBB

- The combination of paracellular TJ and Transcellular transport properties allow the cell layers to tightly regulate the movement of molecules and ions between the two tissue spaces for both barriers.
Regulation of Barrier Properties of BBB

- Regulation of barrier properties is regulated by the interactions with other cells.
  - Astrocytes play a critical role in the BBB
    - As well as astrocyte src kinase, Angiotensin 2
- Angiogenesis occurs the same way in the brain as in other places in the body
  - Formation of leaky vessels that are then induced to become the BBB
    - If astrocytes present
      - Been disproved, however...
- Neural stem cell derived Wnt-Beta-catenin signaling required for CNS angiogenesis!
  - WNT induces BBB-specific transporter expression and tight junction formation
Regulation of Barrier Properties in GIB

- Enteric glial cells are morphologically similar to astrocytes
  - Regulate barrier permeability
  - Release soluble factors controlling paracellular permeability via TJ
    - S-nitroglutathione

- Enterochromaffic cells present in the gut
  - Most abundant neuroendocrine cell in gut
  - Increase in concentration when infections is present

- Luminal stimuli induce secretion of serotonin
  - Targets neighboring epithelial cells, neuronal afferents in lamina propia, immune cells
  - Activation leads to gut contractility and motility
Immune Barrier

- Regulates movement of immune cells
- Immunity at entry sites
- Both barriers (GIB & BBB) have immune cells underneath physical barrier
- Different adaptive immune responses between the two tissues (GIB & BBB)
GIB vs BBB

GIB
- Intraepithelial lymphocytes & dendritic cells (DCs)
- Lamina propria: plasma cells, DCs, macrophages, NK cells & mast cells

BBB
- Perivascular macrophages & mast cells
- CNS: mainly microglial cells
Regulation of Immune Cell Migration

- Cell migration between two tissue spaces
- Both barriers allow limited passage of immune cells for different reasons
- Immune Privilege
- Massive infiltration of immune cells is seen in pathological conditions
# Antigens vs Antibodies

<table>
<thead>
<tr>
<th></th>
<th>Antigen</th>
<th>Antibody</th>
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<tbody>
<tr>
<td><strong>Overview</strong></td>
<td>Substance that can induce an immune response</td>
<td>Proteins that recognize and bind to antigens</td>
</tr>
<tr>
<td><strong>Molecule type</strong></td>
<td>Usually proteins, may also be polysaccharides, lipids or nucleic acids</td>
<td>Proteins</td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td>Within the body or externally</td>
<td>Within the body</td>
</tr>
<tr>
<td><strong>Specific binding site</strong></td>
<td>Epitope</td>
<td>Paratope</td>
</tr>
<tr>
<td><strong>Image</strong></td>
<td><img src="image1.png" alt="Antigen Image" /></td>
<td><img src="image2.png" alt="Antibody Image" /></td>
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BBB Migration

- Almost a complete lack of lymphocytes
- Pathogens can mimic immune cells to cross over
- Migration can occur by rolling adhesion, tight adhesion, vesicular transport (or between endothelial cells)
BBB Migration (cont.)
GIB Migration

- GIB does not keep out adaptive immune cells like the BBB
- T regulatory cells, IgA-secreting plasma cells, T-cells & Th17 cells preserve gut homeostasis (all adaptive immune cells)
- Gut resident immune cells can facilitate in epithelial repair, while also retaining their microbicidal activity
Wee Little Intro

- Looking at Small Intestine
- Note*- Invaders that have made it past the mouth and stomach
Barrier-Associated Immune Cells - GIB

- Dendritic Cells - Bacterial internalization
  - CX3CR1+ → Stimulation of Th17 Cell Response → Increased Inflammation
  - CD103+ → Development of T regulatory cells → Tolerance
- Macrophages -- Ingestion of invaders
- Intraepithelial T-Cells -- Tissue Repair
  - Most abundant γδ
  - Stimulated by microbiota
- Natural Killer Cells - Immunoregulation
- Plasma Cells - Ig-A
- Paneth Cells -- Chemical Component
GIB Video

https://youtu.be/gnZEge78_78
Mini Pathway for NK Cells

1. Cytokines IL-2, and 23 induce phosphorylation of STAT3
2. Activates Rorc(γt) Gene
3. Formation of RORγt+ T-Cells
4. Induces expression of IL-17, 22 cytokines
Barrier-Associated Immune Cells - BBB

- **Perivascular Macrophages**
  - Constantly Regenerated
  - Mice Study 80% replaced w/in 3 months
  - Innate immune
  - What happens if we don’t have these?
    - Mannosylated clodronate Liposomes
  - Adaptive Response
    - Professional Antigen Presenting Cell
    - “Trojan Horse”

- **Microglial Cells**
  - Innate Immune
  - Microgliosis
  - Wound Healing - produce Growth factors
  - Adaptive Immune - Antigen Presenting
What’s the Same?

Activation triggers:
neuromediators,
inflammatory mediators and cytokines

Mast cells

Release via granules
- Proteases
  - Tryptase → PAR-2 cleavage
  - Tight junction rearrangement
- Histamine

De novo synthesis
- Cytokines
  - i.e. IL-1β, TNFα

Epithelial permeability
Barrier Associated Immune References

- [https://www.clodrosome.com/other-info/mannosylated-liposomes/](https://www.clodrosome.com/other-info/mannosylated-liposomes/)
Intestinal Disorders: Inflammatory Bowel Disease and Irritable Bowel Syndrome

- Chron’s Disease (CD) and Ulcerative Colitis (UC)
  - Multifactorial disorders
  - Immune reactions to autologous flora
  - Homeostasis altered by inflammatory potential of immune cells

- IBS
  - Heterogenous multifactorial disorder
  - Related to stress via CRH
Chron’s Disease

- Symptoms: abdominal pain, severe diarrhea, fatigue, weight loss/malnutrition
- Chronic, discontinuous inflammation
  - Terminal ileum
  - Colon
- Whole mucosa layer affected
  - 50% of patients show granuloma
- Parent or sibling with CD
  - 20% chance of developing disease
- Mediated/triggered by diet
- Autoimmune disorder?
Ulcerative Colitis

- Involves only colon
- Inflammation restricted to superficial mucosa
- Symptoms:
  - Diarrhea + blood/pus
  - Abdominal pain/cramping
  - Rectal bleeding
  - Urgency to defecate
  - Weight loss
  - Fatigue
- Diet exacerbates
Chron’s and UC

- Genetic and environmental components
- Bacterial involvement
  - Antibiotics help severity
  - Mutations in genes encoding CARD15 (NOD2), CARD4 (NOD1) or TLR4 (sensors of bacteria) and autophagy related genes
  - No clear association with any pathogenic bacteria
  - Immune-react to autologous flora
- Primary defect in intestinal permeability?
- Noninflammatory phenotype of macrophages and DCs conferred by local microenvironment
  - Defects → inability to control inflammatory potential of immune cells
Irritable Bowel Syndrome

- Symptoms: pain and cramping
  - Diarrhea
  - Constipation
  - Changes in bowel movements
  - Gas + bloating
  - Food intolerance
  - Fatigue, difficulty sleeping

- Stress induces changes via CRH
  - Activate immune cells
  - Induces mast cell degranulation and increased intestinal permeability

- Increased number of mast cells in rectal biopsies
Irritable Bowel Syndrome

- Pathologically altered gut-brain axis homeostasis
- Comorbidity with anxiety and depression
- Visceral hypersensitivity to pain
  - Early life stress / chronic stress
  - Antibiotics in early life induce changes in visceral pain responses and pain signaling pathways
  - Microbiota influence wiring of pain pathways
- Gut microbiota
  - Prolonged exposure to HFD → intestinal inflammation, microbiota composition changes, increased bacterial transmission across intestinal mucosal barrier
- Stress alters colonic motor activity → change microbiota profiles
- Conflicting evidence for probiotics as treatment
Neuroinflammation: Stroke and Multiple Sclerosis

- Breakdown of BBB and infiltration of leukocytes
- Ischemic stroke
  - Symptoms:
    - Sudden numbness/weakness
    - Sudden confusion/trouble speaking
    - Loss of vision
    - Trouble with balance/dizziness
- Multiple sclerosis (MS)
  - Symptoms:
    - Vision problems
    - Tingling/numbness
    - Balance problems
    - Cognitive problems
Ischemic Stroke

● Most common is MCA stroke
● Loss of blood flow to specific regions of CNS
  ○ Breakdown of BBB
  ○ Infiltration of leukocytes, neutrophils, monocytes (but not lymphocytes)
● Animal models of middle cerebral artery occlusion
  ○ Secretion of cytokines IL-1, IL-6, TNF-a, TGF-b → upregulation of ICAM-1 and Selectins on vascular endothelium
    ■ Promotes leukocyte migration across BBB
  ○ Less damage in P-selectin-deficient mice and ICAM1-deficient mice
    ■ Delivery of antibodies
Multiple Sclerosis

- Autoimmune disease targeting myelin in CNS white matter
  - Mediated by T-cells
- Symptoms: fatigue, gait difficulties, numbness/tingling, spasticity, vision problems, dizziness/vertigo, pain, bowel problems, depression
- More common in women
- Lymphocytes enter CNS
  - Focal disruptions of BBB
  - Increased leukocyte adhesion molecules on inflamed BBB
MS cont’d

- **Mouse model: experimental autoimmune encephalitis (EAE)**
  - Lymphocyte integrin a4b1 binds to endothelial VCAM1 - recruitment of inflammatory cells
  - ICAM1 upregulated on CNS vessels
  - T cell interactions with VCAM1 necessary for adhesion to endothelium
  - ALCAM upregulated on CNS endothelial cells in MS lesions

- **Monocyte infiltration to CNS**
  - Cytokines from inflamed BBB induce migrating monocytes to form antigen-presenting DCs
  - Monocyte-endothelial cell coculture experiments
    - Monocytes induce tPA release by CNS endothelial cells → activates ERK1 and ERK2 kinases
    - Degradation of tight junction protein occludin
Gut inflammation vs. Neuroinflammation

- Gut inflammation → alterations in GIB + increased passage of bacteria
- GIB has crucial role in immune function
- Disruption →
  - IBD and IBS
- Therapeutics to inhibit inflammatory response

- Neuroinflammatory processes → breakdown of BBB + leukocyte infiltration to CNS
- Leukocyte infiltration → extensive damage
- Therapeutics: methods to inhibit inflammation
- BBB performs crucial role in regulating CNS immune surveillance
  - Disruption →
    - Stroke and MS
Disorders references


Evasion of Barriers by Pathogens - GIB
Evasion of Barriers by Pathogens – BBB

- How do we know?
  - Cultured Human Brain Microvascular endothelial cells (HBMEC)
    - Electron Microscopy
  - Paracellular
    - Trypanosoma
    - Borrelia
  - Transcellular
    - E. coli
  - Transmigration
    - HIV
  - BBB Breakdown
    - HIV
Transcellular Movement

- E. coli mechanism
- OmpA binds to GlcNAc (gp96) → expression of Fimbriae 1 → bind to CD48
- CNF1 protein helps the bacteria across the membrane
  - Acts on Laminin receptor on endothelial cells

*This laminin receptor is a common target*
Transmigration and BBB Breakdown

**Chemokines:**
- CCL2 and CXCL12

**Cytokines:**
- TNF-α, IL-1β, IL-6

**Neurotoxic Host Factors:**
- Nitric oxide, Excitatory amino acids,
  - Free radicals, Quinolinic acid

**Neurotoxic Viral Factors:**
- Tat, gp120, gp41, Nef, Vpr, Rev

**Breakdown of BBB**

- Infection of transmigrating monocytes
- Uninfected and HIV infected monocytes
- Astrocyte infection and activation
- Microglia infection and activation
- Macrophage infection and activation

**Neurons**
- Demyelination, pruning, neuronal injury and loss
Evasion of Barriers References

Conclusion

● Consist of cellular layer forming physical barrier + immune cells
  ○ Barriers regulated by interactions with local cells and environmental stimuli
● Studies of one barrier can inform knowledge of the other
  ○ IBD patients have higher incidence of MS
    ■ Autoimmune diseases affect dif tissues but have similar mechanisms
● Breakdown of barrier → onset of disease
● Function of one barrier affects the other
  ○ Food molecules passing through GIB can act on BBB
● Stress can influence GIB permeability and worsen IBS and IBD
  ○ Action of enteric nervous system on mast cells, glial cells, enterochromaffin cells
  ○ Mast cell degranulation + activation → systemic inflammatory response affecting permeability of BBB