Evolution of the Gut and Mutualism

Prokaryotes lack a “gut organelle”: “food” degradation occurs in the cytosol

Unicellular eukaryotes have “intracellular guts” in the form of phagolysosomes

Multicellularity improved on the “quorum sensing” of prokaryotes by providing physical intercellular connections and displaying surface recognition markers and developing a division of labor

1. Dispersed sponge cells re-associate according to species

2. In simple jellyfish, cells in loose association develop a division of labor have distinct functions e.g. stinging cells etc.

Complex invertebrates, e.g. insects, developed segmented guts with different functions in each segment
All Vertebrates have Similar Segmented Gastro Intestinal Tracts (GIT)

The human GIT is representative of omnivores

Some herbivores diverged to develop segments hosting specialized microbiomes that extract nutrients from plant fiber
1. The rumen of artiodactyl ruminants
2. The enlarged caecum (~appendix of humans) of rabbits and other rodents

Conditions and Bacterial Concentrations Differ Along the GIT

**Oxygen, pH and Temp**

**Bacterial Concentration**

Feces contain \( \sim 10^{14} \) bacteria of \( \sim 1000 \) species
60 % of fecal mass in humans is their microbiome
90% of fecal bacteria are anaerobic (Firmicutes & Bacteriodes) [Session2; Lecture 2]
Muscle Tube

Parenchymal cells ("stromal") accumulate within the tube.

A basement membrane forms on which a mucosal epithelium develops.

Neutrophils (PMN) & dendritic cells (DC) infiltrate the parenchyma to comprise the innate immune system.

Guts have an “afterlife” in Western culture.
Innate Immunity is the First Line of Defense
- Enterocytes provide the membrane barrier
- Goblet cells provide the mucus to cover the surface (blue)
- Neutrophils (PMN) engage translocating pathogens
- Dendritic (DC) and M-cells sample the lumen

Enter the Lymphoid System and Adaptive Immunity
- T-lymphocytes kill infected cells
- Lymphocytes activate macrophages and neutrophils
- Lymphoid follicle develop beneath M-cells where T-lymphocytes help B cells to become plasma cells and secrete antibodies
- Dendritic and M-cells continue to sample the lumen but now communicate with follicular lymphocytes
The Renewal and Shedding of Enterocytes is a Defense Strategy

New epithelial cells that are produced in the crypts:
1. Move up the villi as they mature in form and function (See pink arrows)
2. The oldest and most damaged enterocytes are shed into the lumen and appear in the feces along with the microbiome

Some enterocytes are specialized as sensors such as flattened M-cells
- M-cells overlay lymphoid follicles
- M-cells and dendritic cells sample the lumen and pass their information and products to the lymphoid follicle

Lymphocytes in the follicle give rise to plasma cells that secrete antibodies against pathogens and other luminal contents

Certain pathogens like *Salmonella* take advantage of the sampling behavior of the M-cells to translocate across the mucosal membrane barrier (Trojan horse) and cause bacteremia.
Healthy Tight Junctions Maintain the Integrity of the Enterocytes Barrier

Tight junctions are maintained by: (a) SCFA produced by anaerobic microflora. (b) intestinal alkaline phosphatase (IAP) and (c) heat shock proteins (see Lalles model later).

An increase in e.g. Proteobacteria can weaken tight junction integrity leading to bacteremia.

In newborns (Session 2 Lecture 2) tight junctions are relaxed and IAP activity reduced to allow maternal antibodies to be absorbed intact.
Evolution of the Immune Systems

1. Immunity in Prokaryotes is only Innate
   - Release toxins
   - Produce antibiotics
   - Undergo rapid reproduction and genetic change

2. Innate immunity remains the sole immune defense for early eukaryotes
   - Unicellular
   - Simple
   - Multicellular
   - Invertebrates
   - Prochordates
   - Toxins
   - Stinging Cells
   - Venoms
   - Phagocytic cells
   - Rapid reproduction
   - genetic change

3. Long-lived Eukaryotes evolved an adaptive immune defense while retaining innate immunity

4. Lymphocytes, capable of somatic genetic change, appeared and “saved their bacon”
   - Jawless fish
   - Fishes
   - Amphibians
   - Mammals
   - Birds
   - Reptiles
   - Lymphocyte-like
   - True Lymphoid Systems
The changeover from Innate to Adaptive Immunity occurs during the “Critical Window of Immunological Development”

Conception

Birth

Weaning

Puberty

Passive Antibodies Protect

Pre-adaptive Antibodies Protect

Adaptive Immunity Develops

Adaptive Immunity

Passive Immunity

Immune Homeostasis Develops

Oral Tolerance

Gut Colonization

Innate Immunity

The Piglet as a Model for Development during the Critical Window

The model allows for greater control of experimental variable

1. Maternal regulatory factors are not transferred *in utero* as in humans or mice and do not “cloud” the issue

2. Newborn piglets are precosial and can be reared in so-called “autosows” or in germfree isolators without a mother.

3. The factors that act within the “critical window” are under the control of the investigator; something not possible in rodent models or in humans

4. This model allows the role of the microbiome to be studied from birth and even in utero.
Max-Planck Fellowship, Mariensee Germany 1973-1974
In utero fetal Catheterization

Surgical Team

One experimental design

Fetal IgM immune responses are dependent on encounter with bacterial products, i.e. MAMPs (see later) and nearly the same titer as in an adult sow.

Fetal response after immunization with FLU (○) or PC (→) conjugated to killed Brucella on day of gestation 94.
Studies using piglets show that a microbiome was required for: (1) proper weight gain, (2) development of the adaptive immune system and (3) secretion of specific antibodies.


GF= Germfree

TNP and FLU are chemically-defined antigens; TNP does not require T cell help.
Purified MAMPs, recognized by innate immune system receptors called TLRs, are more effective than living bacteria


MAMPs= Microbial Associated Molecular Patterns
GF= germfree, i.e. no contact with bacteria or viruses
CpG & MDP= MAMPs
TNP= Epitope of test antigen
What did we learn about the “Critical Window” and the role of the microbiome?

1. Fetal and GF piglets must encounter bacterial products (Microbial Associated Molecular Patterns or MAMPs) or a microbiome before their adaptive immune system can develop.

2. Food contaminated with bacterial endotoxin (LPS) can trigger development of adaptive immunity in germfree piglets.

3. In utero viral infection can also stimulate development of adaptive immunity like the GIT microbiome.

4. Fetal piglets are poor models for in utero catheterization (unlike sheep) since they are prone to abortion.

5. Fortunately, fetal inoculation followed by recovery at birth is an effective alternative and allows each litter to contain many replicates and controls.

6. Antibodies and certain labile factors in colostrum and milk regulate/suppress the rate of immune system development.
Cellular - Bacterial Cross-talk: A Summary

Enterocytes and dendritic cells have *innate immune receptors*, called Toll-like receptors (TLRs), that recognize structures and chemistries unique to bacteria, viruses and other pathogens

(1) These chemical moieties are referred to as MAMPs (Microbial Associated Molecular Patterns)
(2) These include:

- Flagellin
- Toxins
- LPS (endotoxin of Gram-negative bacteria)
- Peptidoglycans (Gram-positive bacteria cell wall component)
- Double-stranded RNA (viruses)

Recognition of bacterial (MAMPs) triggers:

(1) Development of adaptive immunity (*see previous experimental data*)
(2) The production of intestinal alkaline phosphatase (IAP) which:
   a. Stimulates tight junction protein synthesis
   b. De-toxifies pro-inflammatory bacterial products responsible for inflammation
   c. Can shift the make-up of the gut microbiome
   d. Inhibits neutrophil infiltration which is a feature of inflammation

(3) Intestinal heat shock protein (iHSP) released by enterocytes:
   a. Dampens release of anti-inflammatory factors in proportion to the bacterial load
   b. Regulate tight junction activity

The gut flora is also self-regulating in that *E. coli* probiotics cause release of β-defensins, i.e. “Kamikaze Probiotics”, that kill pathogens while bacitracin produced by other members of the microbiome is used to kill competitors
The Lalles model: One model of interactions between the GIT and the microbiome

The Lalles model assumes a simple mutualistic relationship between the human GIT and its microbiome in which:

1. Both nutrients and the microbiome stimulate production of iHSP and IAP by enterocytes and both play a protective role.

2. Events in the small intestine (duodenum and ileum) differ from those in the large intestine (colon) and SCFA are primarily produced in the colon.

3. Intervention by the host immune system in these interactions is not considered.

4. A simple mutualistic relationship between all member of the GIT microbiome is assumed. However, this is difficult to prove given that there are ~1000 species of bacteria in the GIT and less than 2% can be cultured and studied.

iHSP = intestinal heat shock protein
IAP = Intestinal alkaline phosphatase