Negative Energy Balance, the Microbiome and the Immune System: The Perfect Storm

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Topics

• Mucosa Epithelium and Immunity
• Innate Immunity
• Acquired Immunity/Mucosal Immunity
• Microflora, Diet and Cytokine Storm

Credits

• Patricia LeFleur HHMI
• Kuby Immunology
• Immunobiology, 8th edition
• David Topham, University of Rochester
• Berk et al Science 2012
• Locke et al, Science 2011
What is the immune system?
• The body’s defense against disease causing organisms, cancer cells, and foreign particles

Think of the body as a hollow plastic tube…
We are surrounded internally and externally by our microbiota.

What is the Immune System?
• Dynamic
• React
Escherichia coli is common and plentiful in all of our digestive tracts. Why are we all not sick?

- These bacteria are technically outside the body and aid in digesting material we cannot
- Only if E.Coli are introduced in an unnatural manner can they break through the first line of defense and harm us

Immune responses

1st Line of Defense
Barriers
- mucus, tears, gastric pH, saliva, skin

2nd Line of Defense
Cellular and humoral defenses
- interferon, cytokines (pro-inflammatory and T stimulatory), complement proteins, phagocytosis, NK cells

3rd Line of Defense
Cellular and humoral defenses
- Antibodies, cytokines, T helper cells, cytotoxic T cells

Epithelial Cells and First Line of Defense

Decrease water intake- dehydration barrier
Epithelial Cells

- **Location**
  - GI tract
  - Respiratory tract
  - Reproductive tract
  - Skin

Epithelial Cells

- **Physiological Function**
  - Absorption
  - Digestion
  - Secretion
  - Barrier
  - Immune

Epithelial Cells

- **Immune Function**
  - Danger recognition- HYPORESPONSIVE- anti-inflammatory
  - Anti-bacterial Peptides
  - Cytokine production
  - Antigen Presentation
Epithelium-Anatomy

Epithelial and Immune Function

Microbiota (Microflora)

• The collection of organisms found in and on our body- very location specific and individual specific (genetic component)
• Gastrointestinal microbiota is the most diverse and has the largest interaction both with mucosa and ingesta- “superorganism”
Superorganism-Nutritional Stress


Commensal Organisms

• $10^{14}$ organisms-eukarya, archae and bacteria
  – $10^{12}$ commensal bacteria/ml- 10X higher than the combined somatic and stem cells in man
• Commensals are essential for immune development
• Composition of commensal bacteria influenced by the host immune status

Commensal Bacteria

[Diagram showing commensal bacteria distribution across different parts of the digestive tract]
Hygiene hypothesis

- The presence of microorganisms and allergens is essential for immune development of the neonate.

Commensal Bacteria - Inhibit proinflammatory responses

- Fatty acids butyrate
Weaned Calf—poor background program

Epithelial Cells—Immunomodulation
- Pro-inflammatory cytokines-
- Epithelial Cell Enemy# 1
  - Tumor necrosis factor-alpha

Leaky Gut

• **Innate Immunity - 2nd Line of Defense**
  
  - injury & infection
  - macrophages are on patrol - Sensor - Smoke Alarm
  - cytokine chemicals attract other "troops" - Recruiter
  - Absolutely essential for vaccine responses

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**Inflammatory Response**

- IL-1 Proinflammatory cytokines

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**The Second Line of Defense ~White Blood Cells~**

- If invaders actually get *within* the body, then white blood cells (WBCs) begin their attack
- WBCs normally circulate throughout the blood, but will enter the body's tissues if invaders are detected
- These white blood cells are responsible for eating foreign particles by engulfing them
Uncontrolled Inflammatory Response

What does pro-inflammatory response do to Neutrophil Function?

Biofilms - What are they?

- Bacteria + Secreted Matrix
- Multiple organisms
- *M. hemolytica* forms biofilms only with tissue culture fluid

Biofilms

Biofilm


Biofilm 3-D

How can a phagocytic cell or antibody attack the biofilm?


2nd Line of Defense

Acute Inflammatory Response-Pathogens

Systemic response
Inflammation Summary

- Innate Pro-inflammatory response
  - Cell Recruitment
  - Neutrophil Migration
  - Acute Inflammation

Inflammation Summary

- Innate Anti-inflammatory response
  - Maintains gut homeostasis
  - Prevents Acute Inflammation

Microflora and Diet

- Nutrition isn’t just feeding the “animal” it is also feeding the “microbes”
- Production of vitamins
- More efficient bacteria- increase opportunity for obesity
Inflammation and Obesity- “Fat” Weaning Calf, Finishing Cattle and the Fresh Dairy Cow

Brain-Gut-Microbiota axis
Inflammation and Obesity- Overconditioned Weaned Calves, Fat Cattle, Transition Dairy Cow

Cytokine Storm
- High Temps- 104°-106°F
- Respiratory Disease-
  - Acute Lung Injury
  - Acute Respiratory Disease Syndrome
- Is Vaccination or Aspirin the Answer?
Acute Respiratory Disease Syndrome


Cytokine Storm

Overactive Inflammatory Response

Tissue Damage
Tissue Damage - Overactive Immune System

Immune responses

1st Line of Defense
- Dehydration

2nd Line of Defense
- Cellular and humoral defenses
  - Interferon, cytokines (pro-inflammatory and T stimulatory), complement proteins, phagocytosis, NK cells
  - Eosinophils

3rd Line of Defense
- Cellular and humoral defenses
  - Antibodies, cytokines, T helper cells, cytotoxic T cells

Summary

- Immune System is the Body’s Defense System - 3 lines of defense
- Acquired - Active and Passive
- Stress - physical, psychological and environmental - immune system
- Interaction Brain, Gut and Immune System
Take Aways

• Avoid turning on pro-inflammatory response at times of stress
  – Vaccination
  – Surgery
• Can we modulate pro-inflammatory responses?
  – Using NSAIDS-Timing- Need to give before inflammation-
  – Need modulate initial pro-inflammatory response

Take Aways

• Microbiome needs to be managed and not upset- diet, dehydration, intakes
• Pre- and Probiotics- where do they fit in?

Parting Thoughts

• Stressors
  – Heat stress- temp swings
Parting Thoughts

• Stressors
  – Heat stress- temp swings
  – Non-feed events-
  – Transport
Microflora and Immune Development

- Germ free animals (gnotobiotic) do not develop normal immune systems
  - Studies with various Bacterial cocktails to fine “optimal microflora”
- Effect in the pregnant mom on the development of the babe

Serendipity

Erika von Mutius MD Pediatric Immunology
Head of the Asthma and Allergy Department of Munich University Children’s Hospital
Munich, Germany.

“Hygiene Hypothesis”

- Reduction/lack of in infections and microbial exposures prenatally or early in life may be associated with increased risk of allergy, asthma and autoimmune diseases
- Based on observations and speculation on:
  - Urban/rural differences
  - Farming/non-farming differences
  - Birth order / small families / day care
  - Early exposure to parasites, allergens, viruses, etc.
Imprinting and Immune Response during Gestation

- Epigenetic - occur outside of normal inheritance
- In domestic animals, usually have thought mineral deficiencies during pregnancy result in changes in development
- Humans
  - Allergy - The exposure of the mother during pregnancy influences the immune response in the baby

The Hygiene Hypothesis

- Infection history makes a difference

Hygiene Hypothesis - Infection history makes a difference
Fetal Immune Development

• Are Gestation Sows too “Clean”?
• Does lack of helminth infection in sows decrease immune development?
• Can we provide “exposures” to the pregnant animal that will enhance fetal immune development?

Primary Immune Response-
Underlying Assumption

• The animal must be immunocompetent
• Are “today’s piglets” as immunocompetent as their ancestors from the 80’s?
• Have management changes “changed” immunocompetence?
  – Gestation Management
  – Young Pig Management-age of postnatal vaccine and weaning age

Neonatal Immunology

• All food animals are immunocompetent at birth.
• Colostrum supplies immediate specific and non-specific immunity.
• The normal neonate is agammaglobinemic.
• Usually consider 55-60 days of gestation breakpoint for immunocompetence
• At this point, the pig has the capacity to recognize all the antigens they can
Maternal Immune Suppression

Maternal and Environmental Influences

- PGE2
- IL-4
- IL-10

Prevent immunological rejection of fetus

The development of immunity in the pig

Conception Puberty Weaning Birth

Innate Immunity

Passive Immunity

Adaptive Immunity

Window of Susceptibility

Total Immunity


Colostral Immune Suppression

- Th2 cytokines- TGF-beta, IL-4, IL-10
- Maternal Interference
Mononuclear Cells in Young Pig

Immunity in the Neonate

- Essentially all components of the immune system develop in utero in domestic animals
  - Less efficient than in the adult
  - Gradually improve over the first weeks of life

Maternal Antibody Interference

- Antibody produced by the dam
- Transferred via the colostrum to the piglet
- Maternal antibody half-life in pigs is 11.3–20 days in pigs compared to a half-life of 16–28 days in cattle
- The half-life for swine maternal antibodies is
  - 16.2 days for PRRSV
  - 14 days for SIV
  - 19 days for PCV2
  - 11.3 days for PRV
  - 11 days for classic swine fever virus
  - 20 days for parvovirus
  - 15.8 days for M. hyopneumoniae
How Do We Speed up Neonatal Immune Development and Overcome Maternal Interference

- Adjuvants - increase immune responsiveness and protect against pre-existing immunity

Weaning age and its effect on mast cells in the gut

- Mast cells have also been shown to have a major effect on intestinal barrier integrity particularly in pigs weaned <21 days
- These pigs maintain a higher # mast cells - poor absorption and mucosa health

Stages in the development of the mucosal immune system of the piglet

<table>
<thead>
<tr>
<th>Stage</th>
<th>The newborn pig</th>
<th>Stages 1-2, immature Peyer's patches, small numbers of mucosal APCs and T-cells</th>
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</thead>
<tbody>
<tr>
<td>Stage 2</td>
<td>1 Day-2 weeks</td>
<td>Non-specific expansion of Peyer's patches and B-cells. Appearance of some conventional, activated, helper T-cells, influx of MHCII+ cells</td>
</tr>
<tr>
<td>Stage 3</td>
<td>2 weeks-4 weeks</td>
<td>Appearance of mature helper T-cells</td>
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<tr>
<td>Stage 4</td>
<td>4 weeks-6 weeks</td>
<td>Expansion of B-cell repertoire, appearance of cytotoxic T-cells</td>
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Vaccinating the Young Pig - What do we do?

- Responsiveness at less than 3 weeks of age is less than ideal
- Window of opportunity to vaccinate in newborn 1-3 days of age - how good is the memory?
- Avoid vaccinating at weaning time

Rules of Immunological Common Sense

- The Pig’s Immune System Doesn’t Lie
- The Best Defense is a Good Defense
- The Immune Response doesn’t get a Free Ride - Nutrient dependent
- The older the better.
- The longer between vaccination the better.

The Primary Immune Response

- Humoral response
  - B cell
  - Plasma cell
  - Antibody production
  - T helper cell involvement

- Cell mediated response
  - APC, DC’s
  - T helper cell
  - Cytokine production
  - Th cytokines
  - T cell cytokines