Immune-mediated disease and the Micro/Macro biome

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Outline - Learning Objectives

• Immune-mediated disease and the Environment
• Brief into to Immune Regulation
• Micro/Macro-biome as a context setter
• Microbiome and disease
  – C. difficile colitis role of FMT
  – IBD
  – Celiac
  – MS
  – Helicobacter pylori
• Please interrupt and ask questions during talk
  – Conversation instructs better than lecture
Immune-Mediated Disease

• There are over 80 well-recognized immune-mediated diseases. Examples include; T1D, RA, MS, Celiac, IBD, Psoriasis, Lupus, Asthma, Grave’s, ITP, ...

• Most are increasing in frequency and are rare in areas with less industrialization.

(from Bach, N.E.J.M. 347:911)
Inflammatory Bowel Disease

Normal Colon

Crohn’s Colitis
Crohn’s Disease
Dysregulated Inflammation

Normal
Regulated Inflammation
>160 Genes/Loci are Implicated in IBD by GWAS

Etiology of IBD

Nature
Genes

Nurture
Environment

IBD
“Ecologic” Events in Immunity

• Genetic predisposition explains a minor component of the risk for IBD
  – ~50% concordance for Crohn’s disease in monozygotic twins
  – <20% concordance for UC in monozygotic twin pairs
  (Halfvarson, Gastroenterology 124:1767)

• The immune response is sensitive to context.
  – Strength of receptor engagement (stochastic selection)
  – Selection of co-signaling molecules (perception of danger)
  – Proteoglycan matrix signals (perception of tissue damage)
  – Pre-existing cytokine milieu (dictates response profiles)

• The Microbiome helps to set immunologic context
#Biomics

- Current focus is on bacteria
  - >10,000 publications on gut microbiome in last 5 years
  - ~1000 to 1200 bacterial species inhabit human gut
  - Each person has ~160 bacterial species in gut (functional core)
  - Healthy identical twins will have similar but different bacteria population
    - Population is relatively stable but can shift – “PreBiotics”
  - Dysbiosis = pathologically skewed population of gut bacteria
    - Cause vs Effect of illness
      (Lloyd-Price, et.al. The healthy human microbiome Genome Med 8:51 2016)

- More neglected are the ecological contribution of:
  - Archaea (e.g. *Methanobrevibacter smithii* )
  - Virus (+/- RNA, ss/ds DNA, bacteriophages)
  - Fungi (yeast - Candida, Malassezia, and Saccharomyces)
  - Protozoa (*Blastocystis, Entamoeba, etc...*)
  - Helminths (worldwide 50-90 % of people carry helminths)
  - Old friends hypothesis – disappearing biome
Altering the ‘Biome to Treat Immune-Mediated Inflammatory Disease (IMID)

• Assumptions:
  – Immune mediated diseases are due to immune dysregulation
  – Immune dysregulation is contextual
  – The microbiome helps set context
  – Changing the ‘biome may treat disease

• Examples
  – C. difficile colitis (not an IMID but best example for biome Rx)
  – IBD
  – Celiac
  – MS
  – Helicobacter pylori gastritis
**C. difficle colitis**

- Plasmid-directed production of toxins that mediate destruction of colonic epithelium
- Caused by overgrowth of Clostridium difficile often after antibiotic treatment
- Treated with specific antibiotics but Clostridia are spore formers and recurrent infections are common
- Recurrent/refractory *C. difficle* can be treated with Fecal Microbiota Transplant (FMT)
FMT – refractory C. diff

• AKA - Stool transplant
• FMT is an established treatment for RCDI
  – ~92% effective (better than repeated vancomycin)
  – Lower delivery ~95% better than upper delivery (~88%)
  – No difference between fresh or frozen
  – Repeated attempts add incremental efficacy
  – Donors need extensive screening
    (Quraishi, et.al. Systematic review with meta-analysis: ...Aliment Pharmacol Ther. 46:479 2017)
• OpenBiome (commercial source of frozen poo)
  – You too can become a stool donor (Boston only) !!!!
• SynPoop - groups are trying to create standardized cultured FM
IBD

• IBD does not develop in germ-free animals
• New onset pediatric Crohn’s disease (668 patients)
  – Increased abundance (*Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae*, and *Fusobacteriaceae*)
  – Decreased abundance (*Erysipelotrichales*, *Bacteroidales*, and *Clostridiale*)
  – Dysbiosis is exacerbated by antibiotic treatment
• Others find similar changes (dysbiosis)
• Cause vs Effect unknown
• FMT in IBD is being studied
  – Repeated (weekly x6) FMT appears to be effective in UC (remission: 24% FMT 5 % Placebo, p=0.03)
  – Stool diversity improved in recipients
  (Moayyedi, et. al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis... Gastroenterology 149:102, 2015)
  – No evidence yet for efficacy in Crohn’s
Rise in IBD – Eastern Europe

IBD Incidence in Western Hungary
Veszprem Province, 1977–2006

(from Lakatos, IBDJ 17:2558, 2011)
Eradication of Hookworm

- John D. Rockefeller initiated ~1909 – Sanitary Commission
- Global effort assisted by advances in hygiene
- Decrease in hookworm in USA over time

Hookworm is no longer endemic in USA.

Hookworm could help explain the prior north-south gradient.
Helminths and the Human Genome

- All vertebrate animals carry helminths
- Carriage of helminths appears to have influenced our gene variation
  
  ITGAV, COL4A1, ITGA8, FLNB, ITGA9, ITGB8, ITGAM, ITGBL1, COL4A2, COL1A2, DOCK2, PTK2B, ITGAX, FYN, MAPK13, LAMA2, ITGAL, LIMS1, COL24A1, ELMO1, COL15A1, DOCK1, MAPK14, COL9A3, VAMP3, SOCS6, PLCH2, PLCB1, GRB2, GNG10, ITPR1, CXCR6, MYH14, ITGA9, MYO3B, PLA2G4B, GNAQ, CAMK2A, PLCL1, CAMK2D, ITGAM, CCR9, COL1A2, ITGB7, PAK7, VAV2, PLCD3, ADCY2, PTK2B, PLCE1, RELA, ITPR2, CCL20, PLA2G4A, COL23A1, IL4, KCNS3, CTLA4, DPP10, TLR4, PTGER2, GATA3, PHF11, IL10, NPSR1, ADRB2, IKZF2, ADRA1A,

- Variation includes pathways conferring risk for inflammatory and autoimmune disease

(Fumagalli, BMC Evolutionary Biology 10:264, 2010)
Helminths and IBD Epidemiology

• Prior hookworm infection appears to protect from Crohn’s disease  (Kabeerdoss, Aliment. Pharmacol. Ther.; 34: 923, 2011)
  – Case control study in Vellore, India: 75 Control / 78 Crohn’s
  – Assayed in vitro PBMC reactivity to hookworm antigens
    • IFNγ ELISPOT (+): 48% control 26% Crohn’s disease (p<0.005)
    • % CD3+CD69+ shift: 3.16 ± 0.74 control, 0.90 ± 0.48 Crohn’s (p<0.001)

• Prior helminth exposure appears to protect from IBD  
  (Chu, Inflamm. Bowel Dis. 19:614, 2013)
  – Case control study in Cape Town, South Africa
    • 88 Crohn’s disease, 63 ulcerative colitis, 219 controls
    • 56% Crohn’s, 66% UC patients and 91% controls reported childhood helminth exposure (p<0.001)
    • Adjusted Odds Ratio CD = 0.2 [0.1-0.4] , UC = 0.2 [0.1-0.6]
Heligmosomoides (polygyrus) bakeri

- Intestinal nematode of mice.
  - completely enteric lifecycle

- Acquired by ingesting small L3 larvae.
  - larvae are about 0.3 mm long

- Larvae and adult worms reside in the duodenum.
Basic *H. bakeri* protocol

C57BL/6

Sham Treatment

Hpb L3 by Gastric Lavage

2 wks

2 wks

Analyze

Analyze
**H. bakeri** exposure alters LPMC cytokine expression

ELISA of 48 hr cultures from αCD3-stimulated cells.
H. bakeri in CD25-depletion transfer colitis protocol

- **B6 reconstituted Rag1^-/-**
- **B6 WT Spleen**
- **B6 Rag1^-/-**
- **Colitic**
- **Piroxicam for 2 wks**
- **Sham Rx**
- **Hpb L3**
- **2 wks**
- **Analyze**

Remove CD25+ and B220+ cells
*H. bakeri* exposure reverses established transfer colitis

\[
\begin{align*}
3.78 \pm 0.06 & \quad p < 0.01 \\
1.54 \pm 0.13 & 
\end{align*}
\]
Ulcerative Colitis Trial Results

**Phase I**

*Intention-to-treat*:
- Placebo: 16.7%
- T. suis: 43.3%
  - $p = 0.04$

*Per-protocol*:
- Placebo: 17.4%
- T. suis: 44.8%
  - $p = 0.04$

Response (UCDAI $\geq 4$)

**Change in UCDAI Component Scores**
Celiac Disease
What is it?

• Inappropriate immune response to gluten
  – Gluten is a term for storage proteins in cereal grains
    • Wheat, Rye, Barley
    • ?Oats
  – Delayed-type (Type IV) hypersensitivity response
  – Wheat allergy initially described by Willem Dicke

• Results in chronic intestinal inflammation

• Allergy vs. Immune Mediated Inflammation vs. Autoimmune Disease
Endoscopic Character

Normal

Celiac
Celiac disease - Genetics

MHC Class II $\alpha$ & $\beta$ molecules form dimers that present antigens to T cells.

- More than 95% of patients have either
  HLA - DQ2 ($\alpha_1*501$ $\beta_1*0201$) Class II
  HLA - DQ8 ($\alpha_1*301$ $\beta_1*0302$) Class II

- About 30% of the population also express these dimers.

- DQ2 or DQ8 are required and contribute but do not cause celiac disease.
HLA-DQ2 binds gluten peptides

PQPQLPY

\( \text{tTG} \)

\( \text{NH}_2 \)

PQPHELPY

(P = Proline, Q = Glutamine, L = Leucine, Y = Tyrosine, E = Glutamate)

(Kim, PNAS 101:4175, 2004)
5 Epitopes in a Poorly Digestible 33aa Gliadin Peptide

..LQLPFPQPQLPYQPQPQLPYPQPQPF..

P = Proline
Q = Glutamine
L = Leucine
Y = Tyrosine
F = Phenylalanine

(Shan, Science 297:2275, 2002)
Celiac and the ‘biome

• Case control study on 2,933 celiac patients matched with 28,262 controls found a positive correlation between antibiotic use, and subsequent celiac (odds ratio (OR) = 1.40; CI=1.27–1.53).  

• Duodenal bacteria from celiac patients given to germ-free mice alter digestion of gluten to increase immunopathogenic peptide availability.
  – *Pseudomonas aeruginosa* seen in celiac increased antigenicity
  – *Lactobacillus* strains from non-celiac controls reduced antigenicity
  (Caminero et. al. (2016) Duodenal bacteria from patients with celiac disease ... affect gluten breakdown and immunogenicity. Gastro. 151:670, 2016)

• Celiac patients have more G- and few G+ bacteria and populations change with gluten-free diet
Investigators are studying the effect of helminth exposure on celiac disease.

- Utilized hookworm (*Necator americanus*)
- Patients were given 20 larvae (transdermal) and then micro-challenged with low-dose gluten
- Gluten exposure did not cause expected duodenal atrophy or increase anti-tTG, quality of life scores increased, intestinal T cell IFNγ declined and Foxp3+ T regs increased.
- “Microbial diversity” was enriched in patients harboring *N. americanus*.

Multiple Sclerosis (MS)

- MS is caused by immune-mediated destruction of insulating myelin in the central nervous system causing axon damage
- Two major forms
  - Relapsing/remitting → secondary progressive
  - Primary progressive
- Animal model – Experimental Autoimmune Encephalitis (EAE)
- Microbiome
  - No major findings but in a case control study; MS cases exhibited higher abundances of *Methanobrevibacter* (Archaea) and *Akkermansia* and lower *Butyricimonas*
    
MS and helminths

• Case control studies suggest that:
  – Helminth carriage reduces disease severity
  – Eradication of helminths exacerbates disease

• Therapeutic helminth exposure can alter immune circuits and may influence disease course (open-label study).
**Helicobacter pylori**
Gastritis/Duodenitis

- Motile Gram (-) spiral-shaped rod bacteria
- Most common infection worldwide (>50%)
- Infection associated with decreased socioeconomic status
- Prevalence varies by age and group (next slide)
- Most infections are asymptomatic
Helicobacter pylori

*Helicobacter pylori* Seroprevalence

U.S. Population (Total)

- 1990
- 2000

Non-Hispanic Whites

Non-Hispanic Blacks

Mexican Americans

(from Am. J. Epidemiol. 175: 54-59, 2012)
H. pylori and PUD

- Discovered as agent of PUD
- H. pylori causes both acute and chronic gastritis
- Most (~90%) of patients with PUD and not using NSAIDs have H. pylori.
  - Lifetime risk varies 3% (US) to 25% (Japan)
  - Hp + NSAIDs increases PUD risk 60 fold
- Eradication of H. pylori reduces ulcer recurrence
  - from ~90% to 10%.
**H. pylori** and peptic ulcer disease

- **H. pylori** does not invade (remains lumenal)
- **H. pylori** secretes enzymes and toxins
  - All make Urease
    - Splits urea into bicarbonate + NH3
    - Buffers acid
  - Most strains make Vac A and Cag A
    - Vac A causes vacuolation of epithelial cells
    - Cag A increases cytokine production by epithelial cells
    - Uncertain contribution to pathogenicity
- Immune response is what causes disease
  - Chronic inflammation drives PUD and other pathology
**H. pylori** – Other Illness

- **Atrophic Gastritis**
  - Common cause of $B_{12}$ deficiency in the elderly

- **Gastric Cancer (Adenocarcinoma)**
  - *H. pylori* is classified as Type 1 (definite) carcinogen
  - The major cause of gastric cancer worldwide

- **MALT Lymphoma (98% have *H. pylori*)**
  - MALT = Mucosa-associated Lymphoid Tissue
  - B-cell tumor infiltrating the gastric mucosa
  - About 80% regress with *H. pylori* eradication
**H. pylori** diagnosis/treatment

- **Diagnosis**
  - Serology (IgG Anti-Hp antibody)
    - Best screening test if on PPI
    - Remains + for ~2yrs after Rx
  - Urease breath test
  - Stool Ag test
  - Gastric biopsy (evaluation of PUD)
    - Histology
    - urease “CLO” testing

- **Treatment**
  - If found -> treat.
  - Eradication rate is about 75%
  - Triple therapy (PPI + 2 antibiotics for **2 weeks**)
    - Clarithromycin plus Ampicillin or Metronidazole
Helicobacter pylori Microbiome

- Eradication has no effect on general diversity but
  - reduces relative abundance of Bacterioidetes
  - with corresponding increase in Firmicutes

- Other bacteria inhabit the stomach
  - H. pylori pathology may be influenced by other bugs
  (Sheh The role of the gastrointestinal microbiome in Helicobacter pylori pathogenesis. Gut Microbes. 4:505, 2013)

- Helminth infection alters H. pylori pathology and may decrease cancer risk
Questions?