The gastrointestinal microbiome in health and disease

Overview

- Introduction to microbiota
- Microbiome in health
- Microbiome in disease
- The helminth hypothesis
- Therapies
  - Prebiotics
  - Probiotics
  - Poo

Introduction:

- Aggregate of all living microorganisms that inhabit GI tract
  - Bacteria
  - Fungi
  - Protozoa
  - Viruses

The Intestinal Microbiota

- Until recently culture was principal method to identify bacteria inhabiting canine GI tract
- Still useful when employed for detection of specific pathogens
  - *Salmonella*
  - *Campylobacter jejuni*
- Vast majority of intestinal microbes present in GI tract remain undetected using culture-based methods
- Culture independent approach
- Allows bacteria identified in much more reliable way
- 16S rRNA sequencing
- Bacterial DNA extracted from intestinal sample
- 16S rRNA gene amplified via PCR
- Comprehensive identification of bacteria present
- Canine GI tract home to highly complex microbial ecosystem
- Intestinal microbiome
  - Consists of
  - Several hundred different bacterial genera
  - Probably > thousand bacterial phylotypes
- Intestinal microbiome ~ 10x more microbial \((10^{12} - 10^{14})\) than host cells
- Microbial gene pool ~100x larger than host’s
- Highly complex microbial ecosystem plays crucial role in regulation
- Host health
- Host immunity
- Demonstrated in studies in
- Humans
- Rodent models
- Dogs & cats (recently)

The GI Microbiome of Healthy Dogs

- Gut microbes benefit host in several ways
  - Defensive barrier against transient pathogens
  - Nutrient breakdown & energy harvest from diet
  - Provide nutritional metabolites for enterocytes
  - Help regulate host’s immune system
Molecular phylogenetic analysis bacterial 16S rRNA gene → detailed inventory bacterial groups in GI tract

Revolutionized understanding of gut ecology

Small intestine mainly

Aerobic

Large intestine almost exclusively

Anaerobic

Facultative anaerobic

Each animal harbors unique microbial profile

May explain differing responses to therapies designed to modulate intestinal microbiota

Microbiome Metabolites:

- Produced by resident microbiome
  - Important driving force behind co-evolution of GI microbiota with host

- Major nutrient sources of bacteria
  - Complex carbohydrates
    - Intestinal mucus
    - Starch
    - Dietary fiber
      - Pectin
      - Inulin
  
- Fermenting these substrates mainly → short-chain fatty acids (SCFA)
  - Acetate
  - Propionate
  - Butyrate

- Plus other metabolites important for energy for host

- SCFA
- Important growth factors for intestinal epithelial cells
- Have immunomodulatory properties
- Inhibit overgrowth of pathogens
  - Modulate colonic pH
  - Influence intestinal motility
- Butyrate protects against colitis
  - ↓ oxidative damage to DNA
  - → apoptosis in cells with damaged DNA
- Acetate beneficially modulates intestinal permeability
  - ↓ systemic translocation microbiota-derived endotoxins
- Different members of intestinal microbiota produce various other immunomodulatory metabolites
  - Histamine
  - Indole
- In vitro studies shown microbial derived indole
  - ↓ IL-8 expression
  - → expression mucin genes
  - ↑ gene expression that strengthens tight junction resistance

**Microbiota in Immunity and Health**

- Balanced microbial ecosystem crucial for optimal health
- Resident microbiota important in development of physiological gut structure
  - Germ-free animals exhibit altered mucosal architecture
    - ↓ number lymphoid follicles
    - Smaller villi
- Microbiome in early life crucial for establishing oral tolerance
  - Prevents inappropriate responses to bacterial & food antigens
- Associated with chronic GI inflammation
- Constant “cross-talk” between intestinal bacteria & host immune system
- Mediated through combination
  - Microbial metabolites
  - Surface molecules
- Activates innate immune receptors in intestinal lining
  - Toll-like receptors (TLRs)
- Resident intestinal microbiota crucial part of intestinal barrier system that protects host from invading pathogens & deleterious microbial products (e.g. endotoxins)
  - Compete for nutrients
  - Occupy mucosal adhesion sites
  - Create physiologically restrictive environment for non-resident bacterial species
    - Secrete antimicrobials
    - Alter gut pH
    - Produce hydrogen sulfide

**Microbiota in Dogs with GI Disease**
- Various GI disorders associated with alterations in composition of intestinal microbiota
  - Dysbiosis
- Chronic enteropathies
- Granulomatous colitis in boxers
- Changes in composition of microbiota can have significant impact on host health
- Can manifest themselves in GI tract
- Microbiota’s important effect on GALT means dysbiosis impacts extra-intestinal organ systems
- Examine adipokine, serotonin, microbiota in lean & obese dogs
- 14 Beagles over 6 months
  - 7 Obese (free fed, experimentally induced)
  - 7 lean (restricted)
- Leptin
- Adiponectin
- 5HT
- CSF-5HT
- Fecal samples
- Collected in lean & obese groups 6 months after obesity induced
- Leptin ↑ in obese group than lean
- Adiponectin & CSF-5HT ↑ in lean than obese
- Microbiome diversity of microbial community ↓ in obese
- Population shifts
  - Firmicutes (85%) dominant group microbiota lean
  - Proteobacteria (76%) dominant group microbiota obese
- ↓ 5HT levels in obese might ↑ risk obesity because ↑ appetite
- Microflora enriched with gram-negative bacteria related to chronic inflammation status in obese dogs?

Enteropathies Associated with mucosally invasive bacteria
- Boxers with granulomatous colitis have invasive bacteria in colonic mucosa
- Comparing gene libraries before & after antibiotic-induced remission → significant enrichment in gram-negative sequences
  - Highest similarity to *E. coli* and *Shigella*
- Subsequent studies show granulomatous colitis French bulldogs also associated with mucosally invasive *E. coli*
- Eradicate invasive *E. coli* in boxers & frenchies with granulomatous colitis → disease remission
  - Infers causal relationship
- Types *E. coli* isolated from boxers resemble those associated with Crohn’s disease in people.

- Predisposition of boxers & frenchies to *E. coli*-associated granulomatous colitis suggests they may harbor genetic defect(s) that impairs ability to kill invasive *E. coli*.

Antibiotic Responsive Enteropathies, noninvasive bacteria

- Dogs with chronic GI disease resolved with anti-microbial therapy → “idiopathic small intestinal bacterial overgrowth” (SIBO)

- Total bacterial numbers in these dogs similar to
  - Healthy dogs
  - Dogs with food or steroid-responsive enteropathies
  - EPI

- “Antibiotic-responsive enteropathy” (ARE) coined

- Certain breeds appear predisposed to ARE
  - German shepherd dog

- Histopathological findings in GSD & others with ARE frequently
  - Normal
  - Mild lymphocytic plasmacytic IBD

- Absence of florid inflammation or invasive bacteria
  - Reason for response unclear

- Recent studies in dogs with chronic enteropathies implicate
  - Abnormalities in innate immune system
    - ↑ inflammatory responses to resident microbiota?

- TLRs = membrane-spanning receptors
  - Play key role in immune system & digestive tract

- TLR5 recognizes flagellin
  - Flagellin forms filament in bacterial flagellae

- Polymorphisms in TLR5, ↑ TLR4, & ↓ TLR5 expression demonstrated in GSDs compared to healthy greyhounds
- Polymorphisms in TLR5 confer hyperresponsiveness to flagellin
  - Antibiotic response observed in GSDs from ↓ intraluminal flagellin?
- Microbiota of GSDs with chronic enteropathies ↑ abundance Lactobacillales compared to healthy greyhounds
  - Lactobacillales lack flagella
- NOD2 gene
  - Product detects bacterial lipopolysaccharides
  - Activates pro-inflammatory cytokines
  - Part of pathways that → transcription of hundreds of genes involved in immune response
  - Mutations associated with Crohn disease & IBD
- Four non-synonymous single nucleotide polymorphisms (SNPs) identified in canine NOD2 gene
  - More frequent in IBD dogs than controls
  - Results mirrored in non-GSD breeds
- Relationship between dysbiosis, clinical disease, & enhanced inflammatory responses still not clear
- Microbial alterations documented in dogs with chronic GI disease comparable to those observed across species
  - Shift from gram + Firmicutes to gram - Proteobacteria
- Correlates with intestinal inflammation
- Depletion commensal groups impairs host’s ability to down-regulate aberrant intestinal immune response
  - Several of these bacterial groups secrete metabolites that have direct anti-inflammatory properties
- Fecal Microbiota of Cats with Naturally Occurring Chronic Diarrhea Assessed Using 16S rRNA Gene 454-Pyrosequencing before and after Dietary Treatment
- Evaluate GI microbiota changes associated with diet change and related improvement in diarrhea in cats with chronic naturally occurring diarrhea
  - 15 cats
- Controlled crossover dietary trial for management of diarrhea
- Significant microbial differences within cats when fed i/d vs. EN, & with i/d & EN vs. Fancy Feast
- Significant microbial differences within cats when fed i/d vs. EN, & with i/d & EN vs. Fancy Feast
  - FS improved at least 1 unit
    - 40% cats fed i/d
    - 67% cats fed EN
- Normal stools
  - 13.3% cats fed i/d
  - 46.7% cats fed EN
- Significant correlations between microbiome and FSs
- Suggests ↑ numbers certain organisms important to GI health
- Altered intestinal microbiota associated with improved FS
- Cannot conclude if
  - Changes in microbiome caused improvement
  - Improvement caused changed in microbiome

The Hygiene Hypothesis:
- Early data mostly stems from work on human asthma
- First discussed in ’60s & ’70s
  - Noted prevalence parasitic infections negatively associated with prevalence asthma
- First really summarized in Science in 2002
- As autoimmune/allergic diseases ↑ in developed countries, considerably ↓ prevalence allergic diseases in developing countries
- Clear difference in prevalence allergies between rural & urban areas within one country
- ↑ allergic diseases in industrialized world explained by ↓ in infections during childhood
- Immunological explanation
  - Functional T cell subsets with polarized cytokine profiles
    - T helper 1 (TH1)
    - T helper 2 (TH2)
- Bacterial & viral infections during early life direct maturing immune system toward TH1
  - Counterbalance pro-allergic response TH2 cells
- ↓ in microbial burden →
  - Weak TH1 imprinting
  - Unrestrained TH2 responses
  - ↑ allergy
  - Exposure to food & orofecal pathogens ↓ risk atopy by 60%
  - Hepatitis A, *T. gondii, H. pylori*
  - In late 1990's
  - ↑ prevalence type 1 diabetes (TH1-mediated disease)
  - Associated occurrence type 1 diabetes & asthma in population
  - Kind of first link made between allergies & autoimmune diseases
  - But
  - Prevalence TH1-autoimmune diseases also ↑
  - TH2-skewed helminth infections not associated with allergy
  - ↑ anti-inflammatory cytokines from long-term helminth infections inversely correlate with allergy
  - IL-10
  - Induction of robust anti-inflammatory regulatory network by persistent immune challenge offers unifying explanation for observed inverse association of many infections with allergic disorders
Worldwide helminth infections & allergic diseases do not overlap
- Despite both conditions being accompanied by strong TH2 immune responses
- Both helminth infections & atopic diseases associated with similar immunological profiles
- Clinical outcome opposite
- Despite IgE sensitization to dust mites, helminth-infested subjects protected from mast cell degranulation
- Inflammatory responses
- Would skin test + but no clinical disease
- Burden & chronicity of parasitic infection matters
- Helminth-infested populations divided into none, light, or heavy worm burdens
- Light helminth infections associated with amplification of allergen-specific IgE responses and high skin reactivity
- Heavily parasitized subjects protected from atopic skin reactivity despite high degree of sensitization
- Went on to show if dewormed, clinical allergic symptoms in people
- Alleviated if light helminth infections
- Exacerbated if heavy worm burdens
- Tied this to Toxocara infections in industrialized countries
- Exposure to *Toxocara* associated with ↑ prevalence of airway symptoms
- NOT protective
- Theorized such infections presumably light/sporadic
- Exposure to helminth antigens potentiate TH2 responses without inhibitory component associated with heavy/chronic infections
- Asymptomatic helminth parasitic infections correlated with high levels IgG4
- TH2-dependent isotype
- Parasite-specific IgG4 antibodies can inhibit IgE-mediated degranulation of effector cells
• High exposure to cat allergens ↑↑ IgG4 titers & ↓↓ atopy
• Support IgG4 antibodies ↓ allergic responses
• IL-10
• ↑ IgG4 production
• ↓ mast cell degranulation
• ↑ levels IL-10 in people
• Chronically infected with helminths
• Receiving allergen immunotherapy
• Receiving probiotic therapy
• All 3 conditions associated with ↑ IgG4
• People w/ allergies express ↓ levels IL-10
• Immunosuppressive effects chronic helminth infections can be transferred to fetus in utero (seriously, how cool is that?)
• Wiring of immune responses in populations living in tropics/exposed to variety chronic helminth infections distinct from those with minimal exposure
• Not just worms
• Several chronic infectious diseases associated with ↓ inflammatory response
• Hep A
• Childhood viruses
• Malarial parasites associated with profound immunosuppression
• Also linked to ↓ allergy
• Now think related to
• Athrosclerosis
• Anxiety
• Alzheimer’s disease
• Cancer
### Therapeutic Uses of Helminths

- Ideal agent would colonize intestine without invading host
- Source of helminth = pathogen free to ↓ risk co-transmitting other diseases

Elliott et al. 2003
- Mice exposed to eggs of Schistosoma mansoni
- Challenged rectally with caustic agent
- Schistosome egg exposure
- Attenuated colitis
- Protected mice from lethal inflammation

Weinstock et al. 2005
- 29 patients with long-standing Chron’s Disease refractory to standard treatments
- Individuals given repeated doses of eggs of T. suis, prepared from pathogen-free animals
- At week 24,
  - 21/29 were in remission
  - 23/29 improved
- No placebo control
- No subjects had ill effects

Summers et al. 2005
- 29 patients with active IBD enrolled in an open label study
- All patients ingested 2,500 live T. suis eggs every 3 weeks for 24 weeks
- Disease activity monitored
- At week 24
  - 23 of 29 (79.3%) responded
  - 21 of 29 (72.4%) remitted

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- Review series on helminths, immune modulation and the hygiene hypothesis: The broader implications of the hygiene hypothesis Immunology, Rook et al, 2008

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- Elliott et al. 2003
- Weinstock et al. 2005
- Summers et al. 2005
- No adverse events
- Support argument that helminths induce regulatory circuits that could prevent and treat IBD?
- Many patients on immunosuppressive drugs
- Adverse events still rare even with this group
- *Trichuris suis* ova: Testing a helminth-based therapy as an extension of the hygiene hypothesis
  - Jouvin, et al. *Journal of Allergy and Clinical Immunology* July 2012 Volume 130, Issue 1, Pages 3–10
- Helminths and the IBD hygiene hypothesis
  - Joel V. Weinstock David E. Elliott *Inflammatory Bowel Diseases* Volume 15, Issue 1, pages 128–133, January 2009
- Hunter et al. 2007
- Ability *H. diminuta* to affect course of oxazolone-induced colitis in rats
  - Disease severity assessed by
  - Gross and microscopic anatomy
  - Myeloperoxidase and eosinophil peroxidase activity
  - Cytokine synthesis
  - Infection with *H. diminuta* caused significant exacerbation of oxazolone induced colitis
  - Not all parasitic helminths considered therapy for different inflammatory disorders
  - Understand mech dz and life cycle parasite
- Mansfield et al. 2003
  - *T. suis* and appearance of secondary infections with *C. jejuni*
  - 3-day-old germfree pigs given either
  - Dual infections with *T. suis* and *C. jejuni*
  - No pathogens
  - Only *T. suis*
- Only C. jejuni
- Dual infection pigs more frequent/severe diarrhea, histopath disease
- Hemorrhage & inflammatory cell infiltrates in proximal colon where adult worms found
- Abscessed lymphoglandular complexes in distal colon with intracellular C. jejuni present
- Pigs given only C. jejuni had mild clinical signs & pathology
- Combined effects of T. suis/C. jejuni significant site-specific disease
- Aoyama et al. 2007
- Autoimmune liver disease modulated by active helminth infections
- 4,117 patients admitted to hospitals in Japan, 1988-2006
- Case-control study
- Described prevalence helminth infections among patients with autoimmune liver diseases
- Primary biliary cirrhosis
- Autoimmune hepatitis
- Primary sclerosing cholangitis
- Hypothesized immunomodulation by S. stercoralis infection may ↓ incidence autoimmune liver disease
  
  Pre, probiotics, and poop
  
  Prebiotics
  
  - Selectively fermented dietary ingredients that
    
    - → specific changes in composition and/or activity of microbiota
  
  Other prebiotic approaches may target further ecosystems
  
  - Skin
  
  - Oral cavity
  
  - Urinary tract
  
  Targets microbiota already present within ecosystem
  
  - Acts as selective ‘food’ for target microbes beneficial to host
- Resistance of prebiotic to degradation by mammalian enzymes or hydrolysis
- Microbial fermentation of prebiotic elicits
  - Selective stimulation of growth & activity of beneficial indigenous microorganisms
- Most tested prebiotics directed towards
  - Bifidobacteria
  - Lactobacilli (less so)
- Most widely accepted prebiotics
  - Fructooligosaccharides (FOS)
  - Galactooligosaccharides (GOS)
- Polydextrose
- Soybean oligosaccharides
- Isomaltooligosaccharides
- Glucoooligosaccharides
- Xyloooligosaccharides
- Palatinose
- Gentioooligosaccharides
- Some starch derivatives and sugar alcohols
  - Lactitol
  - Sorbitol
  - Maltitol

Probiotics
- Mainly work in SI
  - Interact with all components of gut barrier
  - Bugs don’t have to live in colon because will live in SI and effect LI
- Probiotics in health vs. disease are two different things
- Best in health
- Genus, species, strain
  - Strain matters
    - Each strain different and has different effects
- Combinations better than single strains
- Some strains help with
  - Infectious disease
    - Lactobacillus strains
  - Constipation
  - IBD
    - Bifidobacteria
    - Chron’s exception
      - Lactobacillus
  - It may take several tries with different ones to see an effect
- Response dependent upon diet
- Visbiome
  - Most CFUs (450 billion)
  - $$$
  - Must be refrigerated
- As effective as prednisone and metronidazole in dogs with IBD
  - One study
- Cannot colonize
  - Once you stop they are gone
- Mucus promotes tolerance
- Taken daily may get treated like commensals?
- Intermittent?
- Antibiotics?
  - Can give Fortiflora with Flagyl
    - May not need living bugs for them to work?
- Change gut mobility and pain perception
- Change bacterial population
- Change T-cell differentiation
- Promote tolerance
- Increase IgA secretion
- Animals: 217 cats
- Double blinded and placebo controlled
- For 4 weeks, animals fed Enterococcus faecium SF68 placebo
  - After 1-week washout period switched & continued an additional 4 weeks
- The percentage of cats with diarrhea >2 days was significantly lower in probiotic group vs. placebo group
- Evaluate effect of feeding selected probiotic/prebiotic combination on intestinal microbiota in cats
- 10 healthy adult cats
- Cats received supplemental once-daily feeding of probiotic for 15 days
  - Fecal samples collected for analysis days 0, 16, and 25
- Feeding probiotic combination had some positive effects on intestinal microbiota in cats
- May
- ↓ IgE secretion
- ↑ IgA secretion
- Local and systemic effects on immune system
  - Immunity gap in puppies?
- Takes about 2 months to get level of “protection” against diarrhea
  - Not a cure but prevention
- 1 gm per day dry, whole spirulina
- Prospective study
  - 37 dogs with IBD
  - 10 dogs with intestinal lymphoma
  - 20 healthy dogs
- IgA and IgG concentrations in serum, feces, and duodenal samples
- Compared to healthy dogs, dogs with IBD had significantly ↓ concentrations of IgA in fecal and duodenal samples
  - Might contribute to development chronic enteritis in dogs with IBD

Fecal Transplantation
- aka Fecal Bacteriotherapy or Fecal Transplant
  - Infusion of fecal suspension from healthy, prescreened donor into GI tract of patient with goal of curing specific disease
- First reported in 4th century China for treatment of food poisoning & diarrhea
- In 16th century given orally to treat variety GI symptoms and disorders
  - “Yellow soup”
- First “documented” use in humans was 1958
  - Pseudomembranous colitis
First enema treatment for *C. diff* infection in 1983

Alternative routes of administration

- 1991 (NG/NE Tube)
- 1998 (EGD)
- 2000 (Colonoscopy)
- 2010 (Self administered enemas)

Current human literature comprised of

- Single-center case series/reports
- 1 meta-analysis
- 2 systematic reviews
- 1 recently published randomized controlled trial

Success rate of 92% for RCDI

Multicenter long-term follow up study showed a cure rate of 98%

Systematic review comprising 317 patients from 8 countries shows cure rate of 92%

Presentation at American College of Gastroenterology forum in 2013 stated that FMT is successful

Humans: only 1 long-term follow-up study

- 5 center
- 77 patients
- 3 month follow-up
- 91% primary cure rate (cured with single transplant)
- 98% secondary cure rate (cured with second transplant or follow-up antibiotics)

No published RCT’s

One recent case report of patient becoming obese after transplant

Use of Fecal Transplant in Eight Dogs with Refractory *Clostridium perfringens*-Associated Diarrhea
ACVIM 2014 Murphy, et al

- Eight dogs with *Clostridium perfringens* not cured with antimicrobial therapy alone underwent fecal transplants from an infection-free donor dog
  - Donor stool was mixed with saline and given as enema
- 8/8 had immediate resolution of diarrhea
- 6/8 negative on follow-up PCR panels for *Clostridium perfringens* alpha toxin gene expression
- Dogs had between one & three fecal transplants

Screening
- Donor
  - Ova & Parasites
  - Giardia
  - Culture & Sensitivity

Prep
- Dilute feces 1:4 with non-bacteriostatic saline
- Blenderize until slurry formed with no large particulate matter visible
- Feces should be collected fresh if possible, although studies in humans of frozen feces have demonstrated efficacy
- Suspension passed through sieve to remove large particles
- Administered via large-bore red rubber catheter introduced into transverse colon
- Don’t use too much so it will be retained
- 10ml/kg slowly, with dose scaled down for larger dogs
  - Optimal volumes not known
  - Ideally patient moved to different positions (left lateral, sternal, right lateral) during retention period
- Retained for 45 minutes
  - 4 hours in people
Our team has done several transplants

Success ranges from curative to minimally successful

Usually do single transplant via enema

- Have done via GED
- Multiple transplants via enema in certain cases

Conclusions:

- Relationship between microbial alterations and inflammation is not well understood
  - Is dysbiosis a cause or a consequence of inflammation?
- Beginning to unravel the complex interrelationships between the enteric microbiota, health, disease
- Elucidating factors that shape intestinal microbiome provide novel opportunities for prophylaxis and therapeutic intervention for IBD, and maybe other diseases

References:


cdc.gov website

fda.gov website