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 $\rightarrow$ 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
- Association of Obesity with Serum Leptin, Adiponectin, and Serotonin and Gut Microflora in Beagle Dogs *Park, et al, JVet Intern Med 2015;29:43–50*
- 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?
- Microbiotia 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
  JOURNAL OF VETERINARY INTERNAL MEDICINE Volume 28, Issue 1, January/February 2014, Pages: 59–65, Z. Ramadan, et al

- 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
- Allergy, Parasites, and the Hygiene Hypothesis Yazdanbakhsh, et al Science 19 April 2002: Vol. 296 no. 5567 pp. 490-494
- 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

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

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

- No adverse events
- Support argument that helminths induce regulatory circuits that could prevent and treat IBD?
- Many patients on immunosuppresive 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
- Glucooligosaccharides
- Xylooligosaccharides
- Palatinose
- Gentiooligosaccharides
- 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
        - Lactobacillis strains
    - Constipation
    - IBD
        - Bifidobacteria
        - Chron's exception
            - Lactobacillis
- 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
- Effect of the Probiotic Enterococcus faecium SF68 on Presence of Diarrhea in Cats and Dogs Housed in an Animal Shelters JOURNAL OF VETERINARY INTERNAL MEDICINE J Vet Intern Med 2011;25:856–860, Pages: 1368–1371, Bybee et al
- 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
- Effect of feeding a selected combination of galacto-oligosaccharides and a strain of Bifidobacterium pseudocatenulatum on the intestinal microbiota of cats Biagi, et al, Am J Vet Res 2013;74:90–95
- Evaluate effect of feeding selected probioticprebiotic 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
- Decreased Immunoglobulin A Concentrations in Feces, Duodenum,and Peripheral Blood Mononuclear Cells of Dogs with Inflammatory Bowel Disease Meada et al J Vet Intern Med 2013;27:47–55
- 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
- Microbiome restorative therapy: successful treatment of dogs and cats with fecal transplants J Am Holistic Vet Med Assoc. 2015 Winter;38(0):8-12.

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

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