Archaea, Fungi, Viruses and Parasites

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Source: WGO Handbook on Gut Microbes (World Gastroenterology Organisation)
Overview

- Extremely complex & difficult to study
- We understand the bacteria component much better than the others
- Extensive interactions in health & disease
- Some interactions becoming better understood
  - Candida-bacteria interactions
  - Parasite-bacteria interactions
  - Virus (bacteriophage)-bacteria interactions
“There is considerable clinical and experimental evidence that dysbiosis of the intestinal bacteria, with developing evidence for fungi and viruses, contributes to development of Crohn’s disease, ulcerative colitis, pouchitis, and chronic experimental intestinal inflammation”
Giardia alters commensal microbial diversity throughout the murine gut.

Barash NR¹, Maloney JG², Singer SM², Dawson SC¹.

Abstract

*Giardia* lamblia is the most frequently identified protozoan cause of intestinal infection. Over one billion people are estimated to have acute or chronic giardiasis, with infection rates approaching 90% in endemic areas. Despite its significance in global health, the mechanisms of pathogenesis associated with giardiasis remain unclear as the parasite neither produces a known toxin nor induces a robust inflammatory response. *Giardia* colonization and proliferation in the small intestine of the host may, however, disrupt the ecological homeostasis of gastrointestinal commensal microbes and contribute to diarrheal disease associated with giardiasis. To evaluate the impact of *Giardia* infection on the host microbiota, we use culture-independent methods to quantify shifts in the diversity of commensal microbes throughout the entire gastrointestinal tract in mice infected with *Giardia*. We discovered that *Giardia*’s colonization of the small intestine causes a systemic dysbiosis of aerobic and anaerobic commensal bacteria. Specifically, *Giardia* colonization is typified by both expansions in aerobic *Proteobacteria* and decreases in anaerobic *Firmicutes* and *Melainabacteria* in the murine foregut and hindgut. Based on these shifts, we created a quantitative index of murine *Giardia*-induced microbial dysbiosis. This index increased at all gut regions during the duration of infection, including both the proximal small intestine and the colon. Giardiasis could be an ecological disease, and the observed dysbiosis may be mediated directly via the parasite’s unique anaerobic fermentative metabolism or indirectly via parasite induction of gut inflammation. This systemic alteration of murine gut commensal diversity may be the cause or the consequence of inflammatory and metabolic changes throughout the gut. Shifts in the commensal microbiota may explain observed variation in giardiasis between hosts with respect to host pathology, degree of parasite colonization, infection initiation, and eventual clearance.
Ménage à trois in the human gut: interactions between host, bacteria and phages.

Mirzaei MK¹, Maurice CF¹.

Abstract

The human gut is host to one of the densest microbial communities known, the gut microbiota, which contains bacteria, archaea, viruses, fungi and other microbial eukaryotes. Bacteriophages in the gut are largely unexplored, despite their potential to regulate bacterial communities and thus human health. In addition to helping us understand gut homeostasis, applying an ecological perspective to the study of bacterial and phage communities in the gut will help us to understand how this microbial system functions. For example, temporal studies of bacteria, phages and host immune cells in the gut during health and disease could provide key information about disease development and inform therapeutic treatments, whereas understanding the regulation of the replication cycles of phages could help harness the gut microbiota to improve disease outcomes. As the most abundant biological entities in our gut, we must consider bacteriophages in our pursuit of personalized medicine.
Archaea
Archaea

- One of three domains of life (+ Bacteria, Eukaryota)
- Initially identified in extreme environments; were thought to be primitive (“archaic”) life forms
- More recently identified as common gut commensals (e.g., humans and ruminants) & in soil, plants, etc.
- Most common species in human gut microbiome:
  - Methanobrevibacter smithii (30% to 90% of population)
  - Methanosphaera stadtmanae (1% to 11% of population)
Archaea: Clinical Relevance

- Strict anaerobes, some are methanogens: produce methane from $H_2 + CO_2$ (or acetate)
- Methanogenesis promotes more efficient carbohydrate fermentation by removing excess $H_2$
- Excess methane / methanogens associated with:
  - Abdominal bloating, flatulence, pain
  - Slowed GI transit / constipation, IBS/SIBO-C, obesity
- Susceptible to some antibiotics, resistant to others
Archaea: Methanogen Commensals

- Can be detected in microbiome tests, breath tests
- Associated with higher carbohydrate intake
- Associated with other microbiome groups
  - Candida, Prevotella, Ruminococcus
- Preliminary evidence that colonization is associated with (raw) organic dairy consumption
  - Grazing and reduced antibiotic usage may be factors related to organic dairy association
“A well-known carrier of M. smithii and M. stadtmanae is the rumen of beef and cows. Therefore, it is likely that products derived from cows, such as dairy products, may contain some of these taxa, which was reflected in our results. Moreover, these specific methanogenic archaea have also been found in soil which could be the route of origin to cows”
“The hydrogen in the gut is mainly the result of bacterial fermentation, and accumulation of hydrogen subsequently inhibits this process of breaking down food components for energy. Therefore, reduction of hydrogen levels by methanogens stimulates food fermentation by saccarolytic bacteria”
Irritable Bowel Syndrome, Particularly the Constipation-Predominant Form, Involves an Increase in Methanobrevibacter smithii, Which Is Associated with Higher Methane Production.

Ghoshal U¹, Shukla R¹, Srivastava D², Ghoshal UC².

Abstract

**BACKGROUND/AIMS:** Because *Methanobrevibacter smithii* produces methane, delaying gut transit, we evaluated *M. smithii* loads in irritable bowel syndrome (IBS) patients and healthy controls (HC).

**METHODS:** Quantitative real-time polymerase chain reaction for *M. smithii* was performed on the feces of 47 IBS patients (Rome III) and 30 HC. On the lactulose hydrogen breath test (LHBT, done for 25 IBS patients), a fasting methane result ≥10 ppm using 10 g of lactulose defined methane-producers.

**RESULTS:** Of 47, 20 had constipation (IBS-C), 20 had diarrhea (IBS-D) and seven were not sub-typed. The *M. smithii* copy number was higher among IBS patients than HC (Log₁₀ 5.4, interquartile range [IQR; 3.2 to 6.3] vs 1.9 [0.0 to 3.4], p<0.001), particularly among IBS-C compared to IBS-D patients (Log₁₀ 6.1 [5.5 to 6.6] vs 3.4 [0.6 to 5.7], p=0.001); the copy number negatively correlated with the stool frequency (R²=−0.420, p=0.003). The *M. smithii* copy number was higher among methane-producers than nonproducers (Log₁₀ 6.4, IQR [5.7 to 7.4] vs 4.1 [1.8 to 5.8], p=0.001). Using a receiver operating characteristic curve, the best cutoff for *M. smithii* among methane producers was Log₁₀ 6.0 (sensitivity, 64%; specificity, 86%; area under curve [AUC], 0.896). The AUC for breath methane correlated with the *M. smithii* copy number among methane producers (r=0.74, p=0.008). Abdominal bloating was more common among methane producers (n=9/11 [82%] vs 5/14 [36%], p=0.021).

**CONCLUSIONS:** Patients with IBS, particularly IBS-C, had higher copy numbers of *M. smithii* than HC. On LHBT, breath methane levels correlated with *M. smithii* loads.
Methanogens, methane and gastrointestinal motility.

Triantafyllou K¹, Chang C², Pimentel M².

Abstract

Anaerobic fermentation of the undigested polysaccharide fraction of carbohydrates produces hydrogen in the intestine which is the substrate for methane production by intestinal methanogens. Hydrogen and methane are excreted in the flatus and in breath giving the opportunity to indirectly measure their production using breath testing. Although methane is detected in 30%-50% of the healthy adult population worldwide, its production has been epidemiologically and clinically associated with constipation related diseases, like constipation predominant irritable bowel syndrome and chronic constipation. While a causative relation is not proven yet, there is strong evidence from animal studies that methane delays intestinal transit, possibly acting as a neuromuscular transmitter. This evidence is further supported by the universal finding that methane production (measured by breath test) is associated with delayed transit time in clinical studies. There is also preliminary evidence that antibiotic reduction of methanogens (as evidenced by reduced methane production) predicts the clinical response in terms of symptomatic improvement in patients with constipation predominant irritable bowel syndrome. However, we have not identified yet the mechanism of action of methane on intestinal motility, and since methane production does not account for all constipation associated cases, there is need for high quality clinical trials to examine methane as a biomarker for the diagnosis or as a biomarker that predicts antibiotic treatment response in patients with constipation related disorders.
Archaea: Key Take-Homes

- Methanogens present in many but not all (30-90%)
- May contribute to constipation, SIBO, obesity
- Associated with higher carb intake, organic dairy, Candida, Prevotella, Ruminococcus
- Influenced by antibiotics (some may increase, some decrease)
Fungi

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Fungi (a.k.a. “Mycobiome”)

- Most are ubiquitous (found throughout the environment and in foods)
- Not well-studied yet; existing studies conflict as to which are the most common groups and species
- Comprise 0.1% - 0.5% (or less) of the metagenome
- Includes commensals & transients (from diet)
  - Yeasts: Candida, Saccharomyces, Geotrichum, Saprochaete, Galactomyces, Rhodotorula
  - Fungi: Cladosporium, Aspergillus, Penicillium, Debaromyces, Mucor, etc.
Candida (genus)

- Common commensals, pathobionts and transients
- Colonize mucosa (oral, intestinal, vaginal) and skin, and can cause a variety of infections
- Overgrowth of some species (e.g., C. albicans) linked to some chronic diseases (autoimmune diseases, Crohn’s)
- Several species found in the microbiome
  - C. albicans, C. parapsilosis, C. glabrata, C. tropicalis, C. krusei
Prevalence: 30%-60 or higher

Associated with higher-carbohydrate diets

Abundance correlates with certain carb-fermenting bacteria and methanogens

Overgrowth in GI tract is associated with bacterial dysbiosis and antibiotic use

SCFAs and tryptophan metabolites help keep Candida in check, along with immune system

Candida albicans
Candida albicans

- Can exist in different states: unicellular yeast, pseudohyphal, hyphae (multicellular filaments that penetrate epithelial layer)
- Hyphae can penetrate epithelial layer, increasing permeability and inflammation
- Forms biofilms
- Provokes Th17 (IL-17) response, which is associated with certain autoimmune diseases
Candida albicans morphogenesis and host defence: discriminating invasion from colonization.
IL-17-Mediated Immunity to the Opportunistic Fungal Pathogen Candida albicans.

Conti HR, Gaffen SL.

Abstract

IL-17 (IL-17A) has emerged as a key mediator of protection against extracellular microbes, but this cytokine also drives pathology in various autoimmune diseases. Overwhelming data in both humans and mice reveal a clear and surprisingly specific role for IL-17 in protection against the fungus Candida albicans, a commensal microbe of the human oral cavity, gastrointestinal tract, and reproductive mucosa. The IL-17 pathway regulates antifungal immunity through upregulation of proinflammatory cytokines, including IL-6, neutrophil-recruiting chemokines (e.g., CXCL1 and CXCL5), and antimicrobial peptides (e.g., defensins), which act in concert to limit fungal overgrowth. This review focuses on diseases caused by C. albicans, the role of IL-17-mediated immunity in candidiasis, and the implications for clinical therapies for both autoimmune conditions and fungal infections.
Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease.

Hoarau G¹, Mukherjee PK², Gower-Rousseau C³, Hager C², Chandra J², Retuerto MA², Neut C¹, Vermeire S⁴, Clemente J⁵, Colombel JF⁶, Fujikawa H⁷, Poulain D¹, Sendid B⁸, Ghannoum MA⁹.

Abstract

Crohn's disease (CD) results from a complex interplay between host genetic factors and endogenous microbial communities. In the current study, we used Ion Torrent sequencing to characterize the gut bacterial microbiota (bacteriome) and fungal community (mycobiome) in patients with CD and their nondiseased first-degree relatives (NCDR) in 9 familial clusters living in northern France-Belgium and in healthy individuals from 4 families living in the same area (non-CD unrelated [NCDU]). Principal component, diversity, and abundance analyses were conducted, and CD-associated inter- and intrakindom microbial correlations were determined. Significant microbial interactions were identified and validated using single- and mixed-species biofilms. CD and NCDR groups clustered together in the mycobiome but not in the bacteriome. Microbiotas of familial (CD and NCDR) samples were distinct from those of nonfamilial (NCDU) samples. The abundance of Serratia marcescens and Escherichia coli was elevated in CD patients, while that of beneficial bacteria was decreased. The abundance of the fungus Candida tropicalis was significantly higher in CD than in NCDR (P = 0.003) samples and positively correlated with levels of anti-Saccharomyces cerevisiae antibodies (ASCA). The abundance of C. tropicalis was positively correlated with S. marcescens and E. coli, suggesting that these organisms interact in the gut. The mass and thickness of triple-species (C. tropicalis plus S. marcescens plus E. coli) biofilm were significantly greater than those of single- and double-species biofilms. C. tropicalis biofilms comprised blastospores, while double- and triple-species biofilms were enriched in hyphae. S. marcescens used fimbriae to coaggregate or attach with C. tropicalis/E. coli, while E. coli was closely apposed with C. tropicalis. Specific interkindom microbial interactions may be key determinants in CD.
Immunological Consequences of Intestinal Fungal Dysbiosis.

Wheeler ML¹, Limon JJ¹, Bar AS¹, Leal CA¹, Gargus M¹, Tang J², Brown J², Funari VA², Wang HL³, Crother TR⁴, Arditi M⁴, Underhill DM⁵, Iliev ID⁶.

Abstract

Compared to bacteria, the role of fungi within the intestinal microbiota is poorly understood. In this study we investigated whether the presence of a "healthy" fungal community in the gut is important for modulating immune function. Prolonged oral treatment of mice with antifungal drugs resulted in increased disease severity in acute and chronic models of colitis, and also exacerbated the development of allergic airway disease. Microbiota profiling revealed restructuring of fungal and bacterial communities. Specifically, representation of Candida spp. was reduced, while Aspergillus, Wallemia, and Epicoccum spp. were increased. Oral supplementation with a mixture of three fungi found to expand during antifungal treatment (Aspergillus amstelodami, Epicoccum nigrum, and Wallemia sebi) was sufficient to recapitulate the exacerbating effects of antifungal drugs on allergic airway disease. Taken together, these results indicate that disruption of commensal fungal populations can influence local and peripheral immune responses and enhance relevant disease states.
Saccharomyces ("sugar fungus")

- **S. cerevisiae** (brewer’s yeast, baker’s yeast)
  - Wine, beer, bread, kombucha

- **S. cerevisiae var. Boulardii** (S. Boulardii)
  - Probiotic yeast (isolated in 1923 from lychee and mangosteen by Henri Boulard)

- **S. bayanus, S. eubayanus**
  - Wine, cider, lagers, ales
Other Yeasts

- **Geotrichum / Saprochaete**
  - S. clavata (G. clavatum) and S. capitata may cause infections in relatively rare cases
  - G. candidum: ubiquitous, essential for production of some soft cheeses, may cause infections in rare cases

- **Galactomyces**
  - Galactomyces geotrichum: Found in food products such as milk, cheeses and fermented beverages

- **Rhodotorula**
  - R. mucilaginosa, R. glutinis, R. munita may cause rare infections
Other Fungi

- **Aspergillus**
  - Many different mold species, some may cause infections
  - Especially common on starchy foods
  - A. oryzae: used to ferment soybeans and rice (sake)

- **Penicillium**
  - Common molds, some species used to make cheeses (blue cheese, roquefort, camembert, brie)

- **Cladosporium**
  - Common molds, rarely pathogenic
Fungi
Viruses

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Viruses ("Virome")

- Probably outnumber bacteria, but they are smaller and contribute less to metagenome
- Not well-studied yet, especially commensals
- Infect bacteria, archaea, protozoa, fungi and human cells – some integrate into genomes
- Pathogens: norovirus, rotavirus, adenovirus
- Bacteriophages - viruses that infect bacteria
  - Major effect on bacterial microbiome
Gut inflammation and immunity: what is the role of the human gut virome?

Focà A¹, Liberto MC¹, Quirino A¹, Marascio N¹, Zicca E¹, Pavia G¹.

Abstract

The human virome comprises viruses that infect host cells, virus-derived elements in our chromosomes, and viruses that infect other organisms, including bacteriophages and plant viruses. The development of high-throughput sequencing techniques has shown that the human gut microbiome is a complex community in which the virome plays a crucial role into regulation of intestinal immunity and homeostasis. Nevertheless, the size of the human virome is still poorly understood. Indeed the enteric virome is in a continuous and dynamic equilibrium with other components of the gut microbiome and the gut immune system, an interaction that may influence the health and disease of the host. We review recent evidence on the viruses found in the gastrointestinal tract, discussing their interactions with the resident bacterial microbiota and the host immune system, in order to explore the potential impact of the virome on human health.
The gut virome is a viral collective inhabiting the intestine, co-existing and closely integrated to the bacterial microbiome, fungi and other microbial communities that constitute the microbiome. In addition, due to the integrative capacity of many viruses, host genomes are frequently filled with virus-derived genetic elements (retroviral elements in eukaryotic genomes and prophages in bacterial genomes)."
"It is estimated that, in addition to integrated chromosomal viruses, each individual healthy human harbors more than ten permanent chronic eukaryotic viral infections that drive continuous activation of the immune system"
Increased fecal viral content associated with obesity in mice.

Yadav H¹, Jain S¹, Nagpal R¹, Marotta F¹.

Abstract

**AIM:** To investigate the presence of total gut viral content in obese mice, and establish correlation with obesity associated metabolic measures and gut microbiome.

**METHODS:** Fresh fecal samples were collected from normal and obese (Leptin deficient: Lep(ob/ob)) mice. Total viral DNA and RNA was isolated and quantified for establishing the correlation with metabolic measures and composition of gut bacterial communities.

**RESULTS:** In this report, we found that obese mice feces have higher viral contents in terms of total viral DNA and RNA (P < 0.001). Interestingly, these increased viral DNA and RNA content were tightly correlated with metabolic measures, i.e., body weight, fat mass and fasting blood glucose. Total viral content were positively correlated with firmicutes (R² > 0.6), whilst negatively correlated with bacteroidetes and bifidobacteria.

**CONCLUSION:** This study suggests the strong correlation of increased viral population into the gut of obese mice and opens new avenues to explore the role of gut virome in pathophysiology of obesity.
Enteric Viruses Ameliorate Gut Inflammation via Toll-like Receptor 3 and Toll-like Receptor 7-Mediated Interferon-β Production.

Yang JY¹, Kim MS², Kim E¹, Cheon JH³, Lee YS¹, Kim Y¹, Lee SH¹, Seo SU⁴, Shin SH⁵, Choi SS⁵, Kim B⁶, Chang SY⁷, Ko HJ⁸, Bae JW⁹, Kweon MN¹⁰.

Abstract

Metagenomic studies show that diverse resident viruses inhabit the healthy gut; however, little is known about the role of these viruses in the maintenance of gut homeostasis. We found that mice treated with antiviral cocktail displayed more severe dextran sulfate sodium (DSS)-induced colitis compared with untreated mice. DSS-induced colitis was associated with altered enteric viral abundance and composition. When wild-type mice were reconstituted with Toll-like receptor 3 (TLR3) or TLR7 agonists or inactivated rotavirus, colitis symptoms were significantly ameliorated. Mice deficient in both TLR3 and TLR7 were more susceptible to DSS-induced experimental colitis. In humans, combined TLR3 and TLR7 genetic variations significantly influenced the severity of ulcerative colitis. Plasmacytoid dendritic cells isolated from inflamed mouse colon produced interferon-β in a TLR3 and TLR7-dependent manner. These results imply that recognition of resident viruses by TLR3 and TLR7 is required for protective immunity during gut inflammation.
A Role for the Intestinal Microbiota and Virome in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)?

Navaneetharaja N¹,², Griffiths V³, Wileman T⁴,⁵, Carding SR⁶,⁷.

Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a heterogeneous disorder of significant societal impact that is proposed to involve both host and environmentally derived aetiologies that may be autoimmune in nature. Immune-related symptoms of at least moderate severity persisting for prolonged periods of time are common in ME/CFS patients and B cell depletion therapy is of significant therapeutic benefit. The origin of these symptoms and whether it is infectious or inflammatory in nature is not clear, with seeking evidence of acute or chronic virus infections contributing to the induction of autoimmune processes in ME/CFS being an area of recent interest. This article provides a comprehensive review of the current evidence supporting an infectious aetiology for ME/CFS leading us to propose the novel concept that the intestinal microbiota and in particular members of the virome are a source of the "infectious" trigger of the disease. Such an approach has the potential to identify disease biomarkers and influence therapeutics, providing much-needed approaches in preventing and managing a disease desperately in need of confronting.
Enteric bacteria promote human and mouse norovirus infection of B cells.

Jones MK¹, Watanabe M¹, Zhu S¹, Graves CL², Keyes LR¹, Grau KR¹, Gonzalez-Hernandez MB³, Iovine NM⁴, Wobus CE³, Vinjé J⁵, Tibbetts SA¹, Wallet SM², Karst SM⁶.

Abstract

The cell tropism of human noroviruses and the development of an in vitro infection model remain elusive. Although susceptibility to individual human norovirus strains correlates with an individual's histo-blood group antigen (HBGA) profile, the biological basis of this restriction is unknown. We demonstrate that human and mouse noroviruses infected B cells in vitro and likely in vivo. Human norovirus infection of B cells required the presence of HBGA-expressing enteric bacteria. Furthermore, mouse norovirus replication was reduced in vivo when the intestinal microbiota was depleted by means of oral antibiotic administration. Thus, we have identified B cells as a cellular target of noroviruses and enteric bacteria as a stimulatory factor for norovirus infection, leading to the development of an in vitro infection model for human noroviruses.
Ménage à trois in the human gut: interactions between host, bacteria and phages.

Mirzaei MK¹, Maurice CF¹.

Abstract
The human gut is host to one of the densest microbial communities known, the gut microbiota, which contains bacteria, archaea, viruses, fungi and other microbial eukaryotes. Bacteriophages in the gut are largely unexplored, despite their potential to regulate bacterial communities and thus human health. In addition to helping us understand gut homeostasis, applying an ecological perspective to the study of bacterial and phage communities in the gut will help us to understand how this microbial system functions. For example, temporal studies of bacteria, phages and host immune cells in the gut during health and disease could provide key information about disease development and inform therapeutic treatments, whereas understanding the regulation of the replication cycles of phages could help harness the gut microbiota to improve disease outcomes. As the most abundant biological entities in our gut, we must consider bacteriophages in our pursuit of personalized medicine.
b  Adult, healthy gut

'Arms-race' dynamics:
- Bacteria with generalized phage resistance and/or phages that have broad host range
- Decreases bacterial diversity

Fluctuating selection dynamics:
- Co-evolution of phages and hosts towards specialists
- Increases diversity
c Dysbiosis

- Increased phage abundance, potentially due to phage induction
- Underlying ecosystem dynamics unclear
Bacteriophages, the most ubiquitous organisms on Earth, are viruses that infect bacteria and, for that reason, have been employed since their discovery in the development of therapeutics against infections. They are highly specific, very safe, and effective against their target pathogenic bacteria and rapidly modifiable in order to address new threats.”
Bacteriophage biocontrol of foodborne pathogens.

Kazi M¹, Annapure US¹.

+ Author information

Abstract

Bacteriophages are viruses that only infect bacterial cells. Phages are categorized based on the type of their life cycle, the lytic cycle cause lysis of the bacterium with the release of multiple phage particles where as in lysogenic phase the phage DNA is incorporated into the bacterial genome. Lysogeny does not result in lysis of the host. Lytic phages have several potential applications in the food industry as biocontrol agents, biopreservatives and as tools for detecting pathogens. They have also been proposed as alternatives to antibiotics in animal health. Two unique features of phage relevant for food safety are that they are harmless to mammalian cells and high host specificity, keeping the natural microbiota undisturbed. However, the recent approval of bacteriophages as food additives has opened the discussion about 'edible viruses'. This article reviews in detail the application of phages for the control of foodborne pathogens in a process known as "biocontrol".
Bacteriophages, natural drugs to combat superbugs

Date: April 18, 2017
Source: Baylor College of Medicine
Summary: Viruses that specifically kill bacteria, called bacteriophages, might one day help solve the growing problem of bacterial infections that are resistant to antibiotic treatment.

FULL STORY

Bacteriophages can potentially be used to combat antibiotic-resistant bacterial infections.

Credit: Sabrina Green/Baylor College of Medicine
Viruses: Summary Points

- Shape the composition and function of the microbiome ecosystem (microbiota and mucosa)
  - Bacteriophages shape the bacterial microbiome
- Commensal viruses may have an anti-inflammatory function
- Virome dysbiosis can contribute to bacterial dysbiosis, possibly contributing to some diseases
- Some integrate into microbial or human genomes
Viruses

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Protozoa & Helminths (Parasites)
Protozoa & Helminths ("Parasites")

- **Protozoa**
  - Single-celled eukaryotes (e.g., amoeba, Giardia)
  - Very little known about overall role in microbiome

- **Helminths (worms)**
  - No longer common in industrialized regions
  - Three main groups:
    - Nematodes (Roundworms)
    - Trematodes (Flatworms – Flukes)
    - Cestodes (Flatworms – Tapeworms)
“Parasite” has a negative connotation – some are pathogenic, but many probably harmless or beneficial

Some are less common today than historically, contributing to overall loss of diversity

Loss of diversity thought to contribute to increase in immune imbalances (e.g., allergies & asthma)

Reintroduction therapies being explored for immune disorders (primarily helminths)
Protozoa: Pathogens/Pathobionts

- Entamoeba histolytica
- Giardia intestinalis
- Cryptosporidium parvum
- Cyclospora cayetanensis
- Toxoplasma gondii
- Blastocystis hominis
- Dientamoeba fragilis
Protozoa: Commensals/Nonpathogenic

- *Entamoeba* species: *E. coli*, *E. dispar*, *E. hartmanni*, *E. polecki*
- *Endolimax nana* (pathobiont? May cause diarrhea)
- *Iodamoeba buetschlii*
- *Pentatrichomonas hominis*
- Others
Entamoeba histolytica

- Anaerobic parasitic protozoan, causes amoebiasis
- Over 50 million infected worldwide, 100,000 deaths
- Widespread in poorer regions in developing countries
- Exists as trophozoites (cause disease) and cysts (environmentally resistant & infectious)
- Cysts transmitted via food and water
Giardia lamblia is the most frequently identified protozoan cause of intestinal infection. Over one billion people are estimated to have acute or chronic giardiasis, with infection rates approaching 90% in endemic areas. Despite its significance in global health, the mechanisms of pathogenesis associated with giardiasis remains unclear as the parasite neither produces a known toxin nor induces a robust inflammatory response. Giardia colonization and proliferation in the small intestine of the host, however, may disrupt the ecological homeostasis of gastrointestinal commensal microbes and contribute to diarrheal disease associated with giardiasis. To evaluate the impact of Giardia infection on the host microbiota, we use culture-independent methods to quantify shifts in the diversity of commensal microbes throughout the entire gastrointestinal tract in mice infected with Giardia. We discovered that Giardia colonization of the small intestine causes a systemic dysbiosis of aerobic and anaerobic bacterial taxa. Specifically, giardiasis is typified by both expansions in aerobic Proteobacteria and decreases in anaerobic Firmicute and Melainabacteria in the murine foregut and hindgut. Based on these shifts, we created a quantitative index of murine Giardia-induced microbial dysbiosis. This index increased at all gut regions during the duration of infection, including both the proximal small intestine and the colon. Thus giardiasis could be an ecological disease, and the observed dysbiosis may be mediated directly via the unique anaerobic fermentative metabolism of Giardia or indirectly via parasite induction of gut inflammation. This systemic alteration of murine gut commensal diversity may be the cause or the consequence of inflammatory and metabolic changes throughout the gut. Shifts in the commensal microbiota may explain observed variation in giardiasis between hosts with respect to host pathology, degree of parasite colonization, infection initiation, and eventual clearance.
Advances in understanding Giardia: determinants and mechanisms of chronic sequelae.

Bartelt LA¹, Sartor RB².

Abstract

Giardia lamblia is a flagellated protozoan that is the most common cause of intestinal parasitic infection in children living in resource-limited settings. The pathogenicity of Giardia has been debated since the parasite was first identified, and clinical outcomes vary across studies. Among recent perplexing findings are diametrically opposed associations between Giardia and acute versus persistent diarrhea and a poorly understood potential for long-term sequelae, including impaired child growth and cognitive development. The mechanisms driving these protean clinical outcomes remain elusive, but recent advances suggest that variability in Giardia strains, host nutritional status, the composition of microbiota, co-infecting enteropathogens, host genetically determined mucosal immune responses, and immune modulation by Giardia are all relevant factors influencing disease manifestations after Giardia infection.
An up-date on Giardia and giardiasis.

Einarsson E¹, Ma'ayeh S¹, Svärd SG².

Abstract

Giardia intestinalis is a non-invasive protozoan parasite infecting the upper small intestine causing acute, watery diarrhea or giardiasis in 280 million people annually. Asymptomatic infections are equally common and recent data have suggested that infections even can be protective against other diarrheal diseases. Most symptomatic infections resolve spontaneously but infections can lead to chronic disease and treatment failures are becoming more common world-wide. Giardia infections can also result in irritable bowel syndrome (IBS) and food allergies after resolution. Until recently not much was known about the mechanism of giardiasis or the cause of post-giardiasis syndromes and treatment failures, but here we will describe the recent progress in these areas.
Blastocystis hominis

- Common anaerobic protozoan (pathobiont)
  - 1.5% - 20% developed regions, 30% - 100% in developing regions
- Inhabits lower GI tract, transmitted primarily by fecal-oral route
- Several subtypes (vary in pathogenicity)
- Many or most carriers are asymptomatic carriers
- Those with symptoms often have other pathogens / pathobionts
Eradiation of Blastocystis in humans: Really necessary for all?
Kurt Ö1, Doğruman Al F2, Tanyüksel M3.

Abstract
Blastocystis (initially named as Blastocystis hominis) has long been known as a protist without any clinical significance. However, there is now a huge pile of case reports where Blastocystis is blamed for the symptoms and the infection described in the patients. Introduction of the presence of as many as 17 Blastocystis subtypes while many infected individuals are non-symptomatic initially brought about the correlation between the subtypes and pathogenicity; however, the outcomes of these trials were not consistent and did not explain its pathogenicity. Today, it is mostly acknowledged that Blastocystis may colonize many individuals but the infection's onset depends on the interaction between the virulence of parasites and host's immune competence. Eradiation of Blastocystis is essential in some cases where it is the only infectious agent and patient is suffering from some symptoms. In such cases, metronidazole is the drug of choice but its efficacy is relatively low in some cases. Other agents used include trimethoprim-sulfamethoxazole, paromomycin, and furazolidone. Recent studies on the interactions between human health and the role of gut microbiota introduces new data which may significantly change our point of view against some protists, which we tend to see as "parasites requiring urgent eradication for cure". May the presence or absence of some Blastocystis subtypes necessary for human health, or is the absence or presence of certain Blastocystis subtypes in human gut is associated with certain diseases/infections? The answers of these questions will surely guide us to select patients requiring treatment against Blastocystis infection in future.
Current status of Blastocystis: A personal view.

Stensvold CR¹, Clark CG².

Abstract

Despite Blastocystis being one of the most widespread and prevalent intestinal eukaryotes, its role in health and disease remains elusive. DNA-based detection methods have led to a recognition that the organism is much more common than previously thought, at least in some geographic regions and some groups of individuals. Molecular methods have also enabled us to start categorizing the vast genetic heterogeneity that exists among Blastocystis isolates, wherein the key to potential differences in the clinical outcome of Blastocystis carriage may lie. In this review we summarize some of the recent developments and advances in Blastocystis research, including updates on diagnostic methods, molecular epidemiology, genetic diversity, host specificity, clinical significance, taxonomy, and genomics. As we are now in the microbiome era, we also review some of the steps taken towards understanding the place of Blastocystis in the intestinal microbiota.
“When is testing for Blastocystis appropriate? Data currently emerging indicate that Blastocystis can be more common in individuals with a healthy GI system than in patients with organic and functional bowel diseases. Therefore, the inclusion of Blastocystis as a specific target in screening panels, alongside known pathogens such as Giardia, Cryptosporidium, and Entamoeba histolytica, currently appears to make little sense in the clinical microbiology laboratory.”
“The presence of Blastocystis in stool samples most likely implies that the carrier has been exposed to fecal-oral contamination, which should prompt the laboratory to look more closely for the presence of pathogens transmitted in the same way.”
The Hygiene Hypothesis & Helminth Therapy
“The hygiene hypothesis, formulated in 1989, proposed that lower intensities of infections during early childhood could explain the emergence of asthma and hay fever later in life. The study suggested that declining family size, improvements in household amenities, and increases in personal cleanliness reduced opportunities for cross infections in young families, resulting in a more widespread clinical expression of atopic diseases.”
"Over time, this theory has broadened to include a catalog of chronic inflammatory diseases. Indeed, urban migration, increased access to clean water, and improved sanitation have reduced exposure to many infectious agents including helminths. Multiple epidemiological studies have shown an inverse correlation between microorganism exposure and the development of autoimmunity."
Helminth Immunomodulation in Autoimmune Disease.

Smallwood TB\textsuperscript{1}, Giacomin PR\textsuperscript{2}, Loukas A\textsuperscript{2}, Mulvenna JP\textsuperscript{1,2,3}, Clark RJ\textsuperscript{1}, Miles JJ\textsuperscript{2,3,4,5}.

**Abstract**

Helminths have evolved to become experts at subverting immune surveillance. Through potent and persistent immune tempering, helminths can remain undetected in human tissues for decades. Redirecting the immunomodulating "talents" of helminths to treat inflammatory human diseases is receiving intensive interest. Here, we review therapies using live parasitic worms, worm secretions, and worm-derived synthetic molecules to treat autoimmune disease. We review helminth therapy in both mouse models and clinical trials and discuss what is known on mechanisms of action. We also highlight current progress in characterizing promising new immunomodulatory molecules found in excretory/secretory products of helminths and their potential use as immunotherapies for acute and chronic inflammatory diseases.
Helminth Immunomodulation in Autoimmune Disease.
How host cells sense intestinal parasitic infection and initiate the appropriate immune response has long been a focus of many immunologists. Three new papers now identify a critical role for tuft cells, an epithelial cell type involved in perception of taste, as key players that kick-start type 2 immunity.”
Currently stage 2 and stage 3 clinical trials are being conducted on helminth therapy

Safety of helminth therapy is still controversial

Knowledge of mechanisms is still in very early stages

Long-term effects / consequences of helminth therapy not yet known
Some are parasites, many may be beneficial commensals.

Pathogenic potential depends upon other microbes, diet, immune function, genetics, etc.

Helminths are associated with altered Type 2 /allergic and autoimmune responses.

Helminth therapies are being explored as potential treatments for allergic and autoimmune disorders.

Eradication of all “parasites” probably unnecessary and could be potentially harmful long-term.

Summary: Protozoa & Helminths
Protozoa & Helminth (Parasites)