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***The Microbiome: Modeling for Health and Disease***

**Bleich and Fox. The Mammalian Microbiome and Its Importance in Laboratory Animal Research, pp. 153-158**

Domain: 3 - T3

SUMMARY: This article is the summary of the 10 articles contained in the ILAR Journal 2015 vol 56 No 2. The general theme of this ILAR discusses bacteria in the gut (“microbiome/microflora/microbiota”) and how it can affect research.  They discuss defining of the microbial landscape, the indigenous microbiota, the composition of the microbiota, impact of microbiota on animal models, analyzing the effects of microbiota, the relevance of the microbiome for animal using scientists, and considerations of the IACUC.  This summary article does not discuss viruses, fungi, protozoa, and other parasites.

The ten articles discussed are:

1. Information on the definition of microorganisms according to the virulence potential (Honef et al 2015)

2. Description of the microbiota of mammals (Nelson et al 2015)

3. Review of the history, composition, and use of ASF (altered Schaedler flora) (Brand et al 2015)

4. Effects of intestinal flora microbiota on the host (Becker et al 2105)

5. Effects of intestinal flora microbiota on the host (Hormannsperger et al 2105)

6. Approaches to manipulate the gut microbiota of research animals (Ericsson and Franklin 2015)

7. Interactions of xenobiotics with gut microbiota (Lu et al 2015)

8. Technical aspects regarding standards for analyzing microbiomes (Hiergeist et al 2015)

9. Description of the maintenance and monitoring of gnotobiotic rodents (Nicklas et al 2015)

10. Applied veterinary aspects with regards to the impact of the gut microbiota on rodent models (Hansen et al 2015)

QUESTIONS (True or False)

1. The enlarged cecum is a common finding in germ free animals?

2. The reason the research in the field of microbiomes has become more interesting is because microbiota has an enormous impact on the phenotype of various animal models and that molecular methods have become more available allowing the analysis of complex microbial communities?

ANSWERS

1.  True. The enlarged cecum occurs from osmosis due to nondegraded mucopolysaccharides that bind sodium and enhance intestinal atonia.

2.  True. No more waiting for cell cultures thanks to advanced DNA sequencing methods and an example of the impact on animal’s modes: IL-10 KO mice of IBD can develop spontaneous or Helicobacter induced disease at differing rates and severity

**Hornef. Pathogens, Commensal Symbionts, and Pathobionts: Discovery and Functional Effects on the Host, pp. 159-162**

Domain 3: Research

SUMMARY: There is a large amount of information on the composition of the microbiota, its benefits and potentially harmful consequences of altering it. The present review aims at summarizing for the reader the general concept of pathogenic and commensal bacteria and their particular features. It also discusses the more recently defined pathobionts, members of the microbiota that exert specific effects on the host's mucosal immune system associated with the development of clinical disease.

The golden age of microbiology brought about the discovery of pathogenic microorganisms that were characterized and studied extensively including, pathogenesis, role of toxins and capsule expression etc. These included *B. anthracis*, *M. tuberculosis*; *Y. pestis*; *N. gonorrhoeae*; *V. cholerae*; *C. tetani*; *C. diptheriae*; and *S. dysenteriae* among others. These bacteria all fulfilled the third Koch's postulate, which stated that causative isolates are able to evoke a similar disease in healthy animals, defining the ability of pathogens to mediate disease in a healthy host.

Due to progress in medicine, a new group of bacteria emerged that causes significant morbidity and mortality, not in healthy individuals, but in hospitalized patients or individuals with preexisting medical conditions. These included pneumonia caused by *Pseudomonas aeruginosa* coagulase-negative staphylococci, among them *Staphylococcus epidermidis*, *Clostridium difficile*, an important causative agent of antibiotic-associated diarrhea. Following antibiotic treatment and suppression of the competitive enteric microbiota, *C. difficile* proliferates and secretes toxins. These bacteria do not infect healthy individuals but require a certain degree of immunological (or microbiota) impairment to induce disease. To indicate this somewhat restricted pathogenic ability, the term *opportunistic pathogens* has been coined.

The presence of apparently non-pathogenic bacteria (commensals) has been recognized since the 17th century from oral mucosal swabs, body surfaces and in the environment. Their role in host physiology has been studied such as, the protective function of the colonization of the vaginal mucosa with lactobacilli, which lower the local pH thus protecting it from colonization by pathogenic microorganisms.  Germfree chickens and rodents have previously been developed to help understand the functional role of commensal bacteria. This led to the determination that in the intestine, the microbiota synthesizes essential nutrient constituents such as vitamins, facilitates access to complex nutritional polysaccharides, and drives the development of the mucosal immune system. They have also been show to demonstrate colonization resistance; the ability of the naturally occurring commensal bacteria to outcompete pathogens during the early phase of infection.

Most commensal bacteria are probiotic (live microorganisms which, when administered in adequate amounts, confer a health beneﬁt on the host). Probiotics have been used to help in individuals with an overgrowth of pathogenic bacteria that result in conditions such as chronic diarrhea when intestinal bacterial overgrowth occurs. Probiotics are most helpful in their natural unaltered forms. Such is the case in fecal transplantation (i.e. the direct transfer of a complete “healthy” non-cultured enteric microbiota into a host suffering from *C. difficile* infection) and has been shown to be highly efficacious. There is a risk of also transmitting pathogenic microorganisms in this scenario.

Pathobionts are groups of organisms of the microbiota that have the ability to promote immune maturation or inflammation.  These organisms show that there is a constant, dynamic, and intimate interaction between the highly dynamic and competitive microbiota and the efficient mucosal immune system. Good examples are segmented filamentous bacteria (SFBs) that have long been noted to exert a particularly potent stimulation on the host's mucosal immune system. Although pathobionts coexist in the absence of overt disease in the healthy, immunocompetent host and significantly support maturation of the immune system, their influence might, under certain circumstances, drive autoimmunity and promote the development of clinical disease.

There is need for better characterization of these bacteria and their prevalence in humans, and their functional influence on the host might provide new insight into the pathogenesis of chronic inflammatory disease and provide new strategies in the clinical management of patients.

QUESTIONS

1. What are pathobionts?

a. Live microorganisms which, when administered in adequate amounts, confer a health beneﬁt on the host

b. Groups of organisms of the microbiota that have the ability to promote immune maturation or inflammation

c. Another name for commensals that are organisms that coexist without any harmful effects to their hosts

d. These are symbionts that coexist and impart beneficial effects to each other.

2. Opportunistic pathogens \_\_\_\_\_?

a. Cause significant morbidity and mortality, in hospitalized patients or individuals with preexisting medical conditions

b. Have the ability to mediate disease in a healthy host

c. Support the maturation of the immune system through the gut

d. Only populate mucous membranes

3. These encompass the aggregate of microorganisms that are found on the skin, mucosa, saliva, and gut?

a. Pathogens

b. Pathobionts

c. Microbiota

d. Opportunistic pathogens

ANSWERS

1. b

2. a

3. c

**Nelson. An Update on the Status of Current Research on the Mammalian Microbiome, pp. 163-168**

Domain 3: Research

SUMMARY: The microbiome is described as the thousands of microbial species that inhabit a host or environment. These microbial species benefit the host through the production of vitamins, metabolism of plant structural compounds and sugars and education of the immune system. An expansion of studies focused on microbiomes has resulted from the development and expansion of next-generation sequencing (NGS) technologies for generating genomic data in a shorter time frame and informatics tools for interpreting these data. This article outlines recent studies of the mammalian microbiome.

Technologies used to describe the microbiome traditionally included non-sequencing-based methods, such as culture. High-throughput sequencing (HTS) has the advantage of removing culture-based biases, such as growth conditions. In addition, only a small fraction of microbial species can be cultivated at all, while NGS allows for the characterization of all microbial species in a sample. Examples of next generation sequencing include Sanger sequencing, 454 technology (first commercially available platform), Illumina technology, MiSeq and HiSeq.

Analysis of the 16S rDNA gene sequence has become routine. Early studies were performed on production animals, using high-throughput sequencing to study the rumen microbiome, showing that all of the animals had the required microbial gene components to break down plant cell wall components.

Gut, oral and skin microbiota have been described for healthy companion animals and in dogs with active disease such as inflammatory bowel disease and allergies and in dogs receiving antibiotics to detect changes in these states. One study presented evidence that pets play a role in microbial transfer between humans in the same household.

The fecal microbiome of horses was shown to have a bacterial richness less than that of cattle, greater than that of pigs and comparable to that of humans. Microbial changes were observed in horses that underwent an abrupt dietary transition and those receiving different antibiotics. Higher bacterial diversity was observed in the feces of horses with chronic laminitis.

Microbial colonization of rumen epithelial cells in goats was shown to be age-dependent, achieved at 2 months. In white pigs, the composition of microbes was different in various segments of the GI tract until about 6 months of age, when the pig gut microbiota became relatively stable.

In microbiome studies of non-human primates, the dominant phylum in black and white colobus, red colobus and red-tailed guenon was Firmicutes. Microbiomes among individuals of the same NHP species were more similar than between those of difference species and distinct from the human gut microbiome.

Similarly studies comparing microbiomes across several animal species have demonstrated that same-species hosts had more similar microbiomes than those of different host species and that diet was significant in microbiome characterization.

The authors mention that many studies suffer from small numbers of animals and that the majority of data reflect correlative relationships rather than cause and effect. Work to understand the mechanisms of action of microbial species in the microbiota and host genetic contributions is needed. Future studies may focus on health/disease associations, animal performance, and development of antimicrobial resistance in animal feed systems and microbes at the animal/human interface.

QUESTIONS

1. Which of the following is NOT an example of new generation sequencing?
   1. 454
   2. Culture
   3. Illumina
   4. MiSeq
      1. TRUE or FALSE: When comparing culture to high-throughput sequencing approaches, culture introduces fewer biases and can identify and characterize more microbial species that exist in nature
      2. Which of the following have been shown to change the gut microbiome?
   5. Inflammatory bowel disease in dogs
   6. Various antibiotic administration in horses
   7. Age in pigs
   8. All of the above

ANSWERS

1. b

2. False

3. d

**Wymore et al. The Altered Schaedler Flora: Continued Applications of a Defined Murine Microbial Community, pp. 169-178**

Domain 3, T3

Primary Species: Mouse (*Mus musculus*)

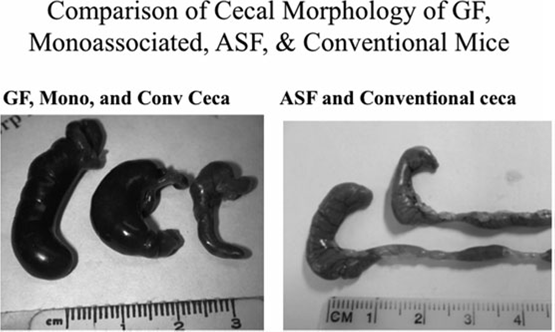
SUMMARY: Microorganisms have adapted to colonize niches with varying conditions within the gastrointestinal tract.  Gnotobiotics and germfree mice allow researchers to study host-microbe and microbe-microbe interactions.  Germfree mice have anatomical differences such as an enlarged cecum.  Altered Schaedler flora (ASF) is a gnotobiotic model that was developed by Russell W. Schaedler in 1965.  Originally Schaedler developed a defined microbiota mouse colony with *Streptococcus,* 2 *Lactobacillus* species, a bacteroides, an *Enterococcus*, and a coliform strain.  In 1978, Roger P. Orcutt refined the microbiota of the Schaedler flora.  The new microbiota replaced 4 of the original bacteria with microorganisms from CD-1 mice.  The new organisms represented autochthonous microbiota.  It consisted of 2 lactobacilli that could grow aerobically, which could allow for detection of contamination under aerobic conditions.  In 1999, 16S rRNA genes of the ASF were sequenced.  This found that ASF 361 was identical to *L. murinus* and *L. animalis*, ASF 360 was similar to *L. acidophilus and L. lactis*, ASF 519 was related to *Prophyromonas*, ASF 457 was a distinct phylum classified as *Mucispirillum schaedleri*, ASF 492 was identical to *Eubacterium plexicaudatum,* ASF 500 was not related to any other microorganism, *ASF 519* wasParabacteroides goldsteinii*,* ASF 360 was *Lactobacillus intestinalis,* and ASF 502 is a *Clostridium.* Fluorescence in situ hybridization (FISH) has been used to monitor changes in bacterial mucosal association and was utilized to evaluate spatial distribution of ASF.  The ASF have mostly been used in mice, but also in rats and pigs.  These models have been used to study the impact of enterobacteria colonization, and evaluate the dynamics of the mucosal IgA response, as well as evaluate changes in distribution of the microbes following GI perturbation.  ASF has also been used to study nutritional needs of the host, such as how the GI microbiota produce short chain fatty acids.  It has been found that IL-10-/- mice kept in traditional housing develop enterocolitis while SPF IL-10-/- mice develop localized colitis.  With ASF models there are limitations as well.  ASF mice lack functional redundancy and metabolic capacity and it has been argued that ASF mice can be considered dysbiotic.  It would also be difficult to construct “altered” ASF communities that lack one or more of the ASF microorganisms.  And there are also limitations to cultivation of ASF in vitro.

QUESTIONS

1. What microorganisms were originally included in the first ASF created by Russell W. Schaedler in 1965?
2. ASF 457 16S rRNA was sequenced and found to be a distinct phylum.  What was it classified as?
3. What has fluorescence in situ hybridization (FISH) been used for in regard to ASF?
4. What animal species has ASF been used in?

ANSWERS

1. Originally Schaedler developed a defined microbiota mouse colony with *Streptococcus,* 2 *Lactobacillus* species, a bacteroides, an *Enterococcus*, and a coliform strain.
2. *Mucispirillum schaedleri*
3. Fluorescence in situ hybridization (FISH) has been used to monitor changes in bacterial mucosal association and was utilized to evaluate spatial distribution of ASF.
4. The ASF have mostly been used in mice, but also in rats and pigs.



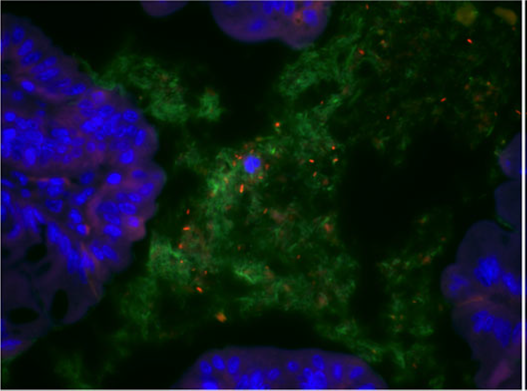


Figure 3. Photomicrograph depicting the use of fluorescent in situ hybridization (FISH) to evaluate the spatial distribution of the altered Schaedler flora (ASF) relative to the mucosal epithelium (DAPI stain - blue) of the proximal colon in a C3H/HeN mouse colonized with the ASF and Escherichia coli. The EUB338-FITC probe (green) was used as a nonspecific probe to detect the ASF with a species specific

Cy3-labeled probe for E. coli (orange).

**Hörmannsperger et al. Intestinal Microbiota in Animal Models of Inflammatory Diseases, pp. 179-191**

Domain 3: Research

SUMMARY: This review article gives an overview of major findings from experimental studies on the role of the intestinal microbiota in immunity and immune-relevant complex disease. The importance, but also the caveats of high standardization in this area of research are discussed as is the use of simplified microbial consortia (SMC) and microbial humanization.

The intestinal microbiota has long been known to play an important role in the maintenance of health. In addition, alterations of the intestinal microbiota have recently been associated with intestinal disorders like infections, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and colorectal cancer and also with a range of immune-mediated and metabolic disorders like type I diabetes, arthritis, obesity, type 2 diabetes and cardiovascular disease.

Several studies reported a significantly reduced microbial diversity in patients suffering from IBD compared with healthy controls. A recent large cohort study revealed significantly changed relative abundances of certain bacterial taxa in mucosal biopsies of children with new-onset Chrohn’s disease (CD) compared with healthy controls. However, prominent inter-individual differences in the composition of the intestinal microbiota are a major obstacle for the identification of disease-relevant changes, as well as for the use of observed (mean) changes as diagnostic markers. The high level of inter-individual variation in the intestinal microbiota can be explained by the fact that the development of the intestinal microbiota is dependent on various environmental factors like the kind of birth, diet, microbial environment (country, hygiene, infections, animal exposure, social interaction) and medication (especially antibiotics) as well as disease phenotypes (e.g. ileal versus colonic inflammation, fistulising, diarrheal in the context of IBD). Furthermore, arrays of host factors like genotype, age, psychological stress, health status, and gender have been shown to shape and influence the intestinal microbiota. Given the abundance of influential factors and their accidental interactions during the life span of a given individual, it is not surprising that there is no standard healthy intestinal microbiome.

The most straight forward way to investigate the role of the intestinal microbiota in the context of specific host functions or complex diseases is the comparison to germfree (GF) experimental disease models. If the host function or disease of interest is unchanged compared with that in colonized mice, it can be assumed that the intestinal microbiota is of minor relevance for disease development. If the respective disease is aggravated or reduced/diminished in the germfree state, the microbiota can be assumed to be disease relevant. Subsequent monoassociation studies or studies using selected consortia of microorganisms allow important mechanistic insights into specific microbe-microbe and microbe-host interactions under highly controlled and standardized conditions regarding genotype, diet and microbial colonization. Interestingly, many animal models for complex diseases (e.g. IBD-like chronic intestinal inflammation) show reduced or lack of disease under germfree conditions, indicating that the intestinal microbiota is involved in the development of most complex diseases. No obese diabetic (NOD) mice, a model for T1D show unchanged pathology under germfree conditions, but disease development can be modulated by association with specific bacteria.

The outcome of monoassociation studies in animal models for inflammatory diseases was found to be dependent on the respective disease model and the bacterial strains used, demonstrating that microbe-host interactions are highly specific and context dependent. Some bacteria that were found to induce colitis in one experimental colitis model do not induce colitis in other experimental colitis models. This finding demonstrates that the protective or detrimental nature of microbe-host interaction is strongly dependant on the respective bacteria and the susceptibility of the host. However it is still unknown which bacterial characteristics and functions determine whether a bacterial strain induces inflammation in a given experimental disease model. Interestingly, two bacterial strains that do not induce inflammation in monoassociated IL10-/- mice, Lactobacillus reuteri and H. hepaticus, trigger severe colitis when used in a dual-association setup. These results show that microbe-microbe interactions can affect microbe-host interactions or, alternatively that the interaction of the host with one or several microbes affect its interaction with additional microbes.

Gnotobiotic studies also offer the opportunity to analyse the impact of host functions or disorders on bacteria without confounding effects by additional microbes. Inflammation in E.coli monoassociated IL10-/- mice was found to induce stress response genes in E.coli. Surprisingly targeted deletion of these genes in E. coli resulted in more severe colitis. This result demonstrates that adaptation processes of the microbiota that positively correlate with the intestinal inflammation may not always be detrimental to the host. When interpreting results from gnotobiotic studies one must keep in mind that the observed effect of a specific microbe or microbial consortium on the host may be affected by the altered mucosal immune system, intestinal milieu and or metabolism in germfree animals. The results obtained from studies in gnotobiotic animals therefore require additional verification in order to support their physiological relevance in normally colonized hosts.

Environmental factors strongly impact the intestinal microbiota and in the last few years it became more and more evident that the results of experimental studies may vary from animal facility to animal facility, depending on the local microbiota, housing conditions and diet. One recent study showed that the intestinal microbiota as well as the severity of experimentally H. hepaticus induced colitis in IL10-/- mice varies between two different SPF facilities and fluctuations in the intestinal microbiota within a single animal facility were observed in this study. In addition to bacteria, commensal yeast, fungi, protozoa, virus and archaea may also be present but the currently extensively used high throughput sequencing analyses are blind toward these microbes. In conclusion, the composition and variations of the complex microbiota in a given animal facility are accidental and cannot be controlled for, and this high variability poses a challenge to the investigation of relevant microbe-host interaction in complex diseases and towards the comparability of results.

One important factor that can and should be controlled for is diet. Inflammation in TNFΔARE mice, a spontaneous model for ileitis was found to be significantly different between mice fed chow diet versus mice fed and experimental diet within the same animal facility. A dietary change in age- and sex-matched mice within the same animal facility was found to modulate the composition of the intestinal microbiota and the disease susceptibility for dextran sodium sulphate (DSS) colitis, infection, and EAE. In contrast the different diets used in this study did not affect DSS colitis in GF mice. Although dietary interventions in experimental animal models for inflammatory diseases are most often associated with alterations in the intestinal microbiota, suggesting an important role of indirect microbiota-mediated effects of diet on the respective disease, on must be aware that diet can also expert direct effects on host immunity.

Apart from the diet, the intestinal microbiota was found to differ between male and female animals. This difference is for example associated with the susceptibility for diabetes in NOD mice. Furthermore, healthy mice with different genetic background were found to harbour a different set of intestinal microorganisms, which were found to be associated with different susceptibility to DSS colitis. Cohousing of these different genotypes results in increased similarity of the intestinal microbiota (presumably mainly due to coprophagy) and also in increased DSS susceptibility of the formerly protected genotype. The transmission of disease phenotypes by cohousing or cross fostering was also observed in additional colitis models, therefore the hygienic conditions of the animal facility, as well as gender and cage effects are important confounders in experimental studies and should be controlled for.

Large observational clinical studies yield important insights into associations between the intestinal microbiota and the disease of interest. In IBD for example it was found that Faecalibacterium prusnitzii and Akkermansia muciniphila were negatively associated with the inflammatory disease, whereas Escherichia coli, Fusobacterium nucleatum, Haemophilus parainfluenzae, Veillonella parvula, Eikenella corrodens and Gemella moribillum were found to be positively associated. Intervention studies aimed at investigating the protective impact of the uptake of specific microbes or undefined microbial mixtures (fecal transplants) need to meet strict ethical and safety guidelines and are enormously cost and time intensive. In consequence, there is a clear lack of clinical data on the protective relevance of microbiota modulation for the onset and development of complex disease. The major gap in understanding the disease relevance of microbial dysbiosis or the presence/absence of specific bacterial taxa might only be overcome by experimental studies. Intervention studies in animal models for complex diseases can be used to preselect microbes with potential therapeutic relevance.

One way of trying to standardize and simplify the microbiota is to use gnotobiotic mice that are associated with a given simplified microbial consortia (SMC). This high level of standardization reduces the inter-individual variability and enables the targeted investigation of functional and mechanistically questions with regard to microbe-host interactions.

Germfree mice can also be associated with a complex microbiota derived from mice or human beings in an attempt to investigate the physiological impact of a given microbiota on a “standardized” host (analogous genotype, environment). The transfer of a complex microbiota from healthy or diseased organisms into germfree animals is a valuable tool to study the relative contribution of the respective microbiota to host dysfunctions or disease phenotypes. Furthermore the implementation of human-derived microbiota, the so called “microbial humanization”, enables interventional studies using for example different diets, microbes, or pharmacological agents in a controlled environment, which would be highly complicated or impossible in humans.

Interestingly it has been shown that the transfer of a given murine or even human microbiota into gnotobiotic recipients can result in the establishment of a rather stable and heritable intestinal microbiota that is compositionally and functionally similar to the donor microbiota. With regard to the transfer of human microbiota in to germfree mice this finding is surprising because the diets of humans and mice differ enormously and because diet is known to have a major impact on the composition of the intestinal microbiota. Also commensal microorganisms can be highly adapted to their respective host, which may result in altered microbial functionality of a given microbe in a different mammalian host.

Several studies have shown that disease susceptibility, disease phenotypes and clinical symptoms can be transmitted to germfree hosts by microbiota transfer. For example mice receiving microbiota from Roux-en-Y gastric bypass surgery operated animals showed increased weight loss compared with mice associated with the microbiota from sham-operated mice. In another study increased susceptibility to atherosclerosis was found to be transferable by microbiota transplantation in antibiotic pre-treated recipient mice. In another study increases body and fat mass were found to be directly transferable by humanization of mice with faecal microbiota from four obese twins compared with their lean twin counterparts, indicating that the intestinal microbiota in obese individuals significantly contributes to the progression of metabolic dysfunction. In contrast, humanization of mice using faecal microbiota of three colorectal cancer patients did not result in increased susceptibility to chemical induced tumorigenesis compared with mice humanized with fecal microbiota from three healthy individuals.

In summary studies using antibiotics, germfree mice or selective microbial associations of experimental modes for inflammatory disease have underscored the pivotal impact that microorganisms can have on immune functions. High standardization of intestinal microbiota, the host and the diet was found to be necessary in order to address specific functional questions. Knowledge about the advantages and caveats of each experimental model system is of pivotal importance for the generation of reproducible results and the interpretation of study outcomes.

QUESTIONS

1.  Define the term “microbial humanization”

2.  The current technique to sequence the microbiome focuses solely on bacteria. Which other intestinal organisms which are likely to impact host physiology are not taken into account?

3.  Which are the main types of studies which are used to understand the complex nature of host-microbe and interactions?

4.  Disease susceptibility and disease phenotypes can be transmitted to germfree hosts by microbiota transfer. Which of the following effects was not found to be true:

a.   Food dependent differences in the susceptibility to DSS colitis

b.   Increased weight loss in mice receiving microbiota from Roux-en-Y gastric bypass surgery operated animals compared to mice receiving microbiota from sham operated animals

c. Increased susceptibility to chemically induces tumorigenesis in mice humanized with microbiota from three colorectal cancer patients

d.  Increased body and fat mass in mice humanized with microbiota derived from obese twins compared with mice humanized with microbiota from lean twin counterpart

5.  True or False: the transfer of a given murine or even human microbiota into gnotobiotic recipients can result in the establishment of a rather stable and heritable intestinal microbiota

6.  Name one of the caveats when interpreting results from gnotobiotic studies

7. Which of the following factors are known to influence the intestinal microbiota?

a.  Diet

b.  Hygiene and housing conditions

c.  Gender

d. Genotype

e.  Antibiotics

ANSWERS

1. The association of germfree mice with a complex microbiota derived from humans

2.  Yeast, fungi, protozoa, virus and bacteriophages, archaea

3.  Germfree (gnotobiotic) mice which are subsequently associated with either one particular microorganism or associated with a simplified microbial consortia or associated with a complex microbiota derived from humans (microbial humanization) or mice.

4.  c

5. True

6. The observed effects of a specific microbe or microbial consortium in germfree mice may be affected by the altered mucosal immune system, intestinal milieu and metabolism and additional verification in normally colonized hosts is necessary in order to support the physiological relevance of the effects.

7.  All are correct answers

**Becker et al. The Intestinal Microbiota in Inflammatory Bowel Disease, pp. 192-204**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

T2: Control spontaneous or unintended disease or condition

Primary Species: Mouse (*Mus musculus*)

SUMMARY:The role of the intestinal microbiome in the pathogenesis of inflammatory bowel disease (IBD) is not fully understood, and further research may help define how manipulation of the intestinal microbiome can provide therapeutic options for treating IBD.

Introduction: The intestinal microbiome consists of large numbers of bacteria, viruses and fungi, and has important functions for the host’s metabolism, immune system development, and immune function.  Recent sequencing improvements have allowed researchers to gain details about the composition of the gut microbiome and relate that composition to the physiology and pathophysiology around the presence, absence and distribution of the microbiome.

The intestinal microbiome has important physiological functions, but bacteria are inherently foreign to the host and can induce an immune response if they come in contact with mucosal immune cells.  The bowel wall is lined with a single layer of epithelial cells that act as a barrier that if breached, can evoke inflammatory reactions such as those that occur in patients with IBD.

IBD refers to a group of idiopathic, chronic, relapsing inflammatory disorders of the GI tract (most common are Crohn’s disease (CD) and ulcerative colitis (UC)).  A critical component of the pathophysiology of IBD is a deregulated immune response against normal components of the gut microbiome.  In most animal models of IBD, bacteria appear to play a role in the triggering of intestinal inflammation in IBD.

The Intestinal Microbiome in IBD Patients

The Intestinal Microbiota

1. The intestinal microbiota is the largest reservoir of bacteria in the body (estimated in a healthy individual, ~1000 species, ~1012 cells)
2. There is significant inter-individual variability in the composition of the microbiota, and this is thought to be influenced by genetics.
3. The microbiota is relatively stable in adults, but is influenced by environment, nutrition, antibiotic use and environmental factors.

Dysbiosis and Reduced Diversity in IBD

1.   Studies in mice have shown that mucosal T lymphocytes in IBD respond to commensals, indicating that gut microbiota is a direct trigger of intestinal inflammation.

2.  Dysbiosis is a disorder characterized by having a markedly shifted intestinal microbial population.

3.  Dysbiosis has been shown to occur in individuals with IBD.  Their gut flora consists of fewer bacterial species and is more unstable over time compared with a healthy gut flora.

4.  A healthy gut is dominated by the phyla *Firmicutes* and *Bacteroidetes*, and to a lesser extent, *Actinobacteria* and *Proteobacteria*.

5.  In a gut with IBD, both *Firmicutes* and *Bacteroidetes* are decreased, while *Actinobacteria* and *Proteobacteria* are markedly increased.

Alterations of the Microbiota: Cause or Consequence of IBD?

1. In most mouse models of IBD, the colon or distal ileum are affected, similar to human IBD.
2. This correlates to where the concentration of bacteria is the highest.
3. In studies conducted with induced inflammation or where the inflammation was due to the genetic background (NEMO -/- mice, HLA-B27 transgenic rats, or IL-10 deficient animals) inflammation was absent when animals were maintained in a gnotobiotic state.  These animals rapidly developed disease when removed from the gnotobiotic state.
4. Studies in mice (T-bet deficient with a Rag2-deficient background) demonstrated that they develop spontaneous colitis in the absence of adaptive immunity. Transfer of bacteria from these colitogenic mice to healthy mice caused the healthy mice to develop colitis.
5. More than 160 genetic loci have been identified that confer protection from IBD or are associated with an increased risk of developing IBD.  This leads to the conclusion that IBD is a polygenic disease with many genetic factors involved in its development and pathogenesis.
6. The first gene locus identified as a risk locus for CD was NOD2 (nucleotide-binding oligomerization domain containing protein 2).  NOD2 heterozygotes have a 2X risk for developing CD, and homozygotes have a 20X risk for developing CD.  This discovery lends credence to the hypothesis that there are heritable factors involved in the development of CD.

A Still Ongoing Debate: Contribution of Specific Bacteria or Pathogens to IBD Development

1. Mouse studies have shown that infection of mice with specific pathogens can cause chronic colitis.
2. So far, no study has fulfilled Koch’s postulates (identified in all patient samples, can be transferred upon inoculation, and re-isolated after experimental infection).
3. However, it is likely that bacteria or pathogens influence the development of IBD by acting together with genetic and environmental factors.
4. Studies with immunocompromised mice have shown that bacteria such as *Helicobacter hepaticus* can have protective as well as pro-inflammatory functions and the host immune response is a critical determinant of that outcome.
5. Another mouse study using norovirus showed that some of the intestinal inflammation that developed after infection was due to commensal bacteria, suggesting that the primary infection predisposed the host for commensal-driven chronic inflammation (the mechanism suspected in IBD).
6. Another hypothesis is that infection with an enteric pathogen (such as salmonellosis or campylobacteriosis) causes an increased risk of developing IBD.  Damage to the epithelial cells would presumably allow commensal bacteria to translocate through the bowel wall, leading to inflammation.
7. Adherent/invasive E. coli (AIEC) has been associated with CD.  However, AIEC has only been associated with ileal disease, so would not explain the colitis seen with CD.
8. *Mycobacterium avium subsp. paratuberculosis* (MAP) is an obligate pathogenic bacterium that is considered to be potentially involved with the pathogenesis of IBD.  Some studies have demonstrated a higher level of MAP/MAP-DBA in mucosal tissues of CD patients vs. controls, but some have found just the opposite.  An argument against the involvement of MAP in IBD is that antibiotic treatment has proven ineffective against IBD.
9. *Clostridium difficile* has also been linked to the development of IBD.  Up to 10% of patients develop a *C. difficile* infection during the course of their disease.  However, IBD patients might be at higher risk of an infection because they frequently receive antibiotic treatments, immunomodulatory drugs, or they might have a genetic susceptibility.  It seems more likely that infection is secondary to IBD, instead of being a primary cause.
10. Segmented filamentous bacteria (SFB) are a bacterial species related to *Clostridia*.  Studies in mice showed that colonization of the gut by this bacteria could lead to both protection from pathogens, as well as inflammatory reactions.

Therapeutic Interventions Targeting the Intestinal Microbiota

1. Patients with IBD show imbalances in their intestinal microbiota, suggesting that manipulation of the microbiota would be valid treatment modality.
2. Antibiotics
   1. Widely used to treat complications related to IBD (fistulae, abscesses, infections)
   2. Can reduce overall bacterial load and/or reduce harmful bacterial species such as *Enterobacteriaceae*.
   3. Benefit is still questionable however, because antibiotic treatment results in marked changes in the gut flora that persist for longer periods of time.  It also reduces species diversity, which increases the risk of barrier loss and severe infections.
3. Probiotics and Prebiotics
   1. The mechanism of action is not fully understood, but oral treatment with Bifidobacteria or Lactobacilli has been shown to protect the host from intestinal inflammation by the downregulation of proinflammatory cytokines or stimulation of anti-inflammatory factors.
   2. Other mechanisms proposed for the protective effect of probiotics are: interbacterial competition with pathogens for nutrition, cell adhesion to the epithelial surface or toxin production that affects the growth of pathogens.
   3. Studies with the probiotic, VSL#3, showed improvements in gut bacterial richness and diversity vs. placebo treatments.  One study with mild to moderate UC treated with VSL#3 resulted in remission with no adverse effects.  However, other probiotic formulations tested did not show a benefit vs. placebo.
   4. Prebiotics (non-digestible oligosaccharides like inulin, lactulose, fructooligosaccharides and galactooligosaccharides) work by selectively supporting the growth of protective intestinal organisms.  Some studies have shown decreased mucosal inflammation after administration of prebiotics.
4. Fecal Microbiota Transplantation
   1. Fecal microbiota transplantation (FMT) has been shown through animal studies and in patients with severe relapsing *C. difficile* infections to be a potential treatment for a variety of intestinal and metabolic disorders.
   2. Stools from a healthy donor are introduced into the GI tract of the recipients (oral route using NG tubes or rectal route using enemas).
   3. Studies are ongoing and using a more defined donor microbial composition may prove beneficial.

Outlook

1.   It is clearly understood that the intestinal microbiota contributes to the development of IBD.

2.  The contribution of specific bacterial species or shifts in the overall composition is not well understood.

3.  Prospective studies using gnotobiotic mice colonized with individual bacteria or combinations of bacteria promise to help further clarify the role of the gut flora in IBD.

4. The genetic components of human IBD also require further study, and would likely require the use of genetically modified mice.

QUESTIONS

1. Which of the following is not considered a normal part of the intestinal microbiome?
   1. Bacteria
   2. Viruses
   3. Fungi
   4. Protozoa
2. T/F:  Translocation of commensal gut bacteria across the bowel wall epithelium is considered normal and not likely to induce inflammation in the host.
3. Which of the following is considered a hypothesis to explain how bacteria trigger the development of IBD?
   1. Direct contribution of known pathogens
   2. Bacteria with pathogenic potential
   3. Dysbiosis
   4. All of the above
4. Dysbiosis is defined as:
   1. A germfree environment
   2. A marked shift in intestinal microbial communities, with fewer bacterial species and an unstable population over time
   3. Having pathogens present
   4. The inability to digest food
5. T/F:  Inflammatory bowel disease and/or Crohn’s disease are always caused by specific bacteria.
6. Which of the following bacteria has not been associated with the development of inflammatory bowel disease and/or Crohn’s disease?
   1. *Helicobacter hepaticus*
   2. Adherent/invasive *E. coli*
   3. *Mycobacterium avium susp. paratuberculosis*
   4. *Bifidobacteria*
7. T/F: Antibiotics are not considered useful in the management of inflammatory bowel disease.
8. Which of the following is considered a mechanism by which prebiotics function in the gut?
   1. Interbacterial competition with pathogens for nutrition
   2. Cell adhesion to the epithelial surface
   3. Toxin production that limits the growth of pathogens
   4. All of the above
9. T/F: Fecal microbiota transplantation has been shown to be curative for inflammatory bowel disease and Crohn’s disease.
10. T/F: Prebiotics are dietary supplements that selectively inhibit the growth of potentially probiotic/protective bacteria such as *Lactobacilli* and *Bifidobacteria*.

ANSWERS

1. d
2. False
3. d
4. b
5. False
6. d
7. False
8. d
9. False
10. False

**Ericsson and Franklin. Manipulating the Gut Microbiota: Methods and Challenges, pp. 205-217**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Researchers have developed several techniques to experimentally manipulate the gut microbiota (GM) of research animals, allowing them to investigate causal roles of the GM in animal model phenotypes. The vast majority of bacterial microbes reside in the lower gastrointestinal (GI) tract, though due to their extreme oxygen sensitivity, only a small minority of gut microbes can be cultured. Animal models provide a way for relationships between GM and diseases can be evaluated using genetically identical animals in highly controlled environments.

The birth dam is probably the greatest factor in determining the GM composition by acting as the source of microbes colonizing the pups beginning immediately at parturition. A different GM may be introduced into a colony through rederivation, typically performed via surgical transfer of embryos into a surrogate dam. Mode of delivery influences the composition of the murine GM; vaginal delivery typically seeds offspring with microbes normally found in the maternal vaginal or intestinal microbiota while Cesarian delivery tends to result in greater proportions of microbes normally associated with the external body surfaces. Changing the composition of the diet can cause the GM to shift, due to changes in the amount of undigested polysaccharides reaching the colon and serving as an energy source for the GM. Autoclaving and irradiation are also variables that influence the microbial exposure to research animals. Since the GM of research animals can affect the phenotype of models of human disease, factors known to modulate the GM (such as animal source, sex, dietary formulation, housing density, time of sample collection) should be reported whenever possible.

Gnotobiotics is the study of animals that are free of all microorganisms or colonized only by known species. If it is desired to render mice with specific mutations axenic, the purchased germ free (GF) mice can be used as surrogate dams. Donor and surrogate mice are time-mated, with the surrogate timed to have pups 1 to 2 days before the donor. When parturition of the donor nears, pups are removed via hysterotomy or hysterectomy and sterilely transferred into the isolator and fostered onto the surrogate dam. GF mice are ideal for asking whether or not microbiota plays any role in a model phenotype. If GF mice fail to develop the phenotype seen in their conventionally raised counterparts, microbiota can be implicated in the development of this phenotype.

GF mice are also important to microbiota research because they can be reconstituted with agents ranging from a single bacterium (mono-associated) to defined microbiota to complex microbiota to xenografted microbiota. Mono- associated mice allow for the study of responses to a single agent or identification of bacterial species responsible for specific bacterial products. Mice reconstituted with defined microbiota were established, resulting in “altered Schaedler’s flora” (ASF) that is now most commonly used in gnotobiotic research. Mice reconstituted with ASF or similar defined microbiota offer several advantages: they are very well defined, they develop a mucosal immune system, have normal cecal volumes, and have normal reproductive performance. Disadvantages of using ASF-reconstituted mice center on the simplicity of this microbiota, which does not recapitulate the interactions that may occur in complex microbiota. Like GF mice, these mice must be generated in isolators and monitored routinely for the presence of appropriate microbiota and the lack of contaminants. GF mice may also serve as recipients of xenografted microbiota, most commonly from human fecal samples. The use of humanized microbiota may offer a more directly translatable model of human microbiota and dysbiosis. The ability to inoculate GF mice with complex microbiota may yield results more translatable to the microbiota donor species.

The two most common methods of generating mice that possess a desired complex GM are cross-fostering and rederivation. Cross-fostering requires foster dams harboring that GM. Pups must be transferred from the dam with the desired genotype to the foster as soon as possible after parturition. Pups acquire the GM of the foster dam gradually and in a physiologically natural manner. Rederivation via surgical embryo transfer (ET) can be used to render mice axenic or to eliminate pathogens incapable of transmission in utero.

Initial evidence supporting a role for the GM in a phenotype of interest is frequently provided by comparison of antibiotic-treated and un-treated animals. Multiple drugs with complementary spectra are administered in combination to enhance overall efficacy. Following reduction of the autochthonous bacteria via antibiotics or beginning from an axenic state, it is possible to repopulate the GIT with a desired complex microbial population via fecal microbiota transfer (FMT).

One of the simplest methods of assessing the influence of a complex GM on a recognized phenotype is cohousing of affected and unaffected animals already harboring complex microbial populations. If a phenotype is reliant on a single bacterial species, cohousing provides a simple means of demonstrating transmissibility via the GM. Cohousing offers several logistical advantages to other methods of altering an established complex GM, including minimal cost and necessary expertise.

As in most areas of biomedical research, mice are the most commonly used laboratory animal species in studies of the GM. Rats provide a biological system similar to mice but large enough to accommodate certain experimental techniques and possessing certain physiological parameters more closely related to humans. One other species gaining favor in the GM research community is zebrafish (Danio rerio). Zebrafish are much more cost effective with regard to housing and breeding efficiency. Additionally, zebrafish can be rendered axenic for studies requiring gnotobiotic hosts. Limitations of zebrafish in GM-related studies include the dissimilarity of zebrafish GM, anatomy, and physiology to that of mammalian hosts.

QUESTIONS

1. Which of the following species are NOT generally associated with external body surfaces?
2. Lactobacillus spp.
3. Staphylococcus spp.
4. Corynebacterium spp.
5. Propionibacterium spp.
6. In general, increases in the Firmicutes: Bacteroidetes ratio are associated with obesity and increased food intake.
7. Bacteroidetes: Firmicutes
8. Bacteroidetes: Proteobacteria
9. Firmicutes: Bacteroidetes
10. Proteobacteria: Bacteriodetes
11. Fecal microbiota transfer (FMT) is used in veterinary medicine as a treatment for dysbiosis, primarily in all of the following species EXCEPT:
12. Horses
13. Gerbils
14. Guinea pigs
15. Rabbits

ANSWERS

1. a. Lactobacillus spp. are normally found in the maternal vaginal or intestinal microbiota while Staphylococcus spp., Corynebacterium spp., and Propionibacterium spp. are normally associated with the external body surfaces.
2. c. Firmicutes: Bacteroidetes ratio increases are associated with obesity and increased food intake.
3. b. Gerbils

**Lu et al. Xenobiotics: Interactions with the Intestinal Microbiota, pp. 218-227**

Domain 3: Research, Task 2: Advise and consult with investigators on matters related to their research, K15: genomics, metabolomics, and proteomics

SUMMARY: The gut microbiome (GM) has important functions within the GI tract, but it also interacts with various body systems in such a way that dysregulated GM significantly contributes to a variety of diseases. This paper summarizes the role of GM in diseases, effects of xenobiotics on GM, and how GM affects xenobiotics.

Gut Microbiome and Host Homeostasis:GM regulates metabolic-modulating host genes and through fermentation of nondigestible carbohydrates into metabolites such as shot-chain fatty acids.

*Obesity*: Cecal microbiota of obese mice and humans contains more *Firmicutes* and fewer *Bacteroidetes* than nonobese controls, suggesting obesity has a microbial aspect.

*Diabetes:* Humans with type 2 diabetes have decreased *Faecalibacterium* sp. and GM may regulate gut permeability, suggesting that GM composition may play a role in diabetes development.

*Liver Disease (Nonalcoholic Fatty Liver Disease [NAFLD]):* Gut bacteria can transform choline to a metabolite trimethylamine (TMA). Mice susceptible to NAFLD excreted increased urinary TMA.

*Cardiovascular Disease:* TMA is converted to trimethylamine N-oxide (TMAO) and TMAO is associated with increased risk for an adverse cardiovascular event.

*Cancer:* Obesity-induced GM alteration increases deoxycholic acid, which modifies hepatic cells into secreting pro-inflammatory, tumorigenic molecules that facilitate hepatic carcinoma formation.

*Mental Disease*: Autistic patients with GI problems have more *Clostridium* in their gut microbiome compared to controls, autism-associated dysbiosis of GM is linked to decreased enzyme expression, and autistic children have increased p-cresol, a GM metabolite.

Effects of Xenobiotics on Gut Microbiome: Culture-free methods, such as 16S rRNA sequencing, have allowed profiling specific changes in the GM community structure as a result of xenobiotics exposure.

*Antibiotics:* Humans exposed to amoxicillin-clavulinic acid and mice exposed to vancomycin have increased *Enterobacteriaceae*. Ampicillin increases Bacteriodetes in humans. Also, antibiotic alteration of the GM shows promise in treating metabolic and GI disorders.

*Pesticides:* Exposure to organophosphate is associated with decreased *Lacobacillus* spp. and *Bifidobacterium* spp. in rats. Both are considered to be probiotic so the decrease signifies microbial dysbiosis. Poultry exposed to glyphosate also showed a decrease in beneficial bacteria.

*Air Pollutants:* Exposure to particulate matter in a mouse model of colonic inflammation appears to encourage a pro-inflammatory colonic environment, increasing susceptibility to diseases such as IBD.

*Polychlorinated Biphenyls (PCBs):* In mice, PCBs result in decreased abundance of gut bacteria.

*Heavy Metals:* Mercury increases mercury-resistant bacteria as well as antibiotic-resistant plasmids in the GM. Probiotics have a protective effect against increased mercury. Cadmium may promote gut inflammatory diseases by diminishing Bacteriodetes and decreasing short-chain fatty acids.

Xenobiotics Change the Functions of Gut Microbiome

*Antibiotics:* Various antibiotics in mice affect the expression of thousands of gene clusters in the GM, resulting in an upregulation in genes related to stress response and antibiotic resistant.

*Arsenic:* Arsenic significantly decreased several species of the *Firmicutes* phylum in mice and it disturbed the GM metabolic profile at a functional level.

Impact of Gut Microbiome on Xenobiotic Biotransformation: Xenobiotics induce the GM to express genes having to do with the metabolism of xenobiotics. GM can indirectly regulate xenobiotic metabolism in the liver so the microbiome does not have to “see” a particular metabolite to affect its metabolism.

*Polycyclic Aromatic Hydrocarbons:* PAHs can be biotransformed by the GM into potentially toxic metabolites. Several are modified to produce estrogenic metabolites.

*Gut Microbiome and Mycotoxin:* Gut microbial biotransformation may reduce the toxicity of some environmental chemicals.

*Gut Microbiome and Heavy Metals:* Mercury-resistant bacteria in the fecal flora of primates can biotransform mercury in a detoxification pathway.

*Gut Microbiome and Arsenic Metabolism:* Biotransformation of arsenic is complicated, but an altered GM may interfere with the detoxification of arsenic.

Conclusion:Xenobiotics can affect the gut microbiome profile, create functional changes to the gut microbiome, and become biotransformed by the gut microbiome into metabolites that could be more or less toxic. Therefore, the gut microbiome and associated functional changes have the potential to serve as biomarkers for the development of various kinds of diseases and disorders.

QUESTIONS

1. T/F. Xenobiotics may affect gut microbiome, but cannot change the function of gut microbiome.

2. T/F. Exposure to pesticides alters most components of the gut microbiome.

3. Which of the following is known to produce metabolites that may activate estrogen receptors?

a. Heavy metals

b. Polycyclic aromatic hydrocarbons

c. Organophosphates

d. Air pollutants

ANSWERS

1. False - 2 examples in this article for altering function were antibiotics and arsenic

2. False - Appears to decrease only the probiotic, beneficial bacteria

3. b

**Hiergeist et al. Analyses of Intestinal Microbiota: Culture versus Sequencing, pp. 228-240**

Domain 3: Research

SUMMARY: Analyzing human as well as animal microbiota composition has gained growing interest because structural components and metabolites of microorganisms fundamentally influence all aspects of host physiology. The analyses of microbiomes have led to new interest in the communities of nonpathogenic microbes residing in distinct niches of the human and animal body to determine the role of the microbiome composition for developmental processes, host metabolism, and physiology as well as different diseases.

There is a wide range of disease phenotypes linked to the composition of the microbiota: chronic inflammatory diseases, obesity, diabetes, allergies, autism, depression, cardiovascular diseases, some cancer types, and even lung diseases have recently been reported to persist concomitantly with a distinct microbiome constellation.

Originally dominated by culture dependent methods for exploring these ecosystems, the development of molecular techniques such as high throughput sequencing (also known as ‘next-generation’ sequencing) has dramatically increased our knowledge.

Although molecular approaches, such as 16S rDNA amplicon or whole metagenome shotgun (WMS) sequencing, provide some clear benefits compared with culture-dependent methods by reason of their ability to provide direct and in-depth insights into the composition of the microbiota in a culture independent manner, they seem to lack in the detection of low-abundant organisms. Another encouraging and interesting approach synergistically combines molecular and culture approaches to enable access to bacterial strains that have been previously identified from extensive metagenomic surveys, for further physiological studies.

Massively parallel 16S rRNA gene sequencing is less costly and less time consuming than WMS approaches, and pooling of barcoded amplicon libraries allows the analysis and comparison of hundreds of samples at one time. To capture the whole genetic information, WMS-based methods require much more effort, although marker gene-based approaches are also benefiting from higher sampling depths. Irrespective of the sequencing approach for the analysis of microbial communities, both methods are subject to biases and systematic errors that can significantly affect downstream analyses. Strict observance of uniform sample handling and DNA extraction procedures is a prerequisite for the prevention of “home-grown” intrasample variations. The introduction of contaminating microbial DNA in nucleic acid-based microbiome analyses is a considerable burden for both 16S rRNA gene sequencing and WMS surveys.

During the 16S rRNA amplicon sequencing, chimeric molecules formed during PCR or errors in the sequencing process appear. These artifacts lead to incalculable numbers of false positives and complicate tracking of individual bacterial taxa across time. To circumvent these problems, a novel method for 16S rRNA amplicon sequencing to assay the bacterial composition of the gut microbiota at higher depth and precision was developed (low-error amplicon sequencing LEA-Seq).

Technical advances should be channeled and emphasis should be shifted toward the biological function in order to get insight into regulatory relationships within a microbiome population as well as mutualistically between host and microbiota.

QUESTIONS

* 1. T or F: 16S rRNA gene amplicon sequencing can be contaminated by water- and soil-dwelling bacteria of the genera Burkholderia, Mesorhizobium, Hydrotalea, and Bradyrhizobium

2. For more than 30 years, culture-independent microbial profiling has been based on the sequencing of a very important and convenient gene, the 16S ribosomal RNA (rRNA) gene

3. ‘Third-generation sequencing’ is based on single-molecule real-time analyses. This technique is better than the next-generation sequencing (NGS) because

a.  Much longer reads beyond 10 kb are produced

b.  The reads are produced in a markedly reduced time

c.  Cost is lower

d.  There is no need for amplification of samples

e.   All of the above

ANSWERS

1. True

2.  True

3. e

**Nicklas et al. Maintaining and Monitoring the Defined Microbiota Status of Gnotobiotic Rodents, pp. 241-249**

Domain 3

Introduction: Gnotobiotic animals include both germ free and defined flora animals. They serve as unique tools with defined immunological, morphological and physiological parameters to study host microbiome relationship. They are derived from C-section or embryo transfer. The risk of vertical transmission of infectious agents is greater with C-section but embryo transfer may be harder to do due to large size of the cecum. The gnotobiotic animals are inoculated by putting fecal or cecal contents in drinking water or by gavaging. They have enlarged cecum but smaller heart, liver and lungs compared to conventional mice. They need vitamin supplementation in food. Cecal torsion and volvulus are relatively common cause of death.

Housing and Maintenance: Gnotobiotic animals can be maintained in sterile conditions in positive pressure isolators for many years. Most of them are flexible film isolators made of PVC. Each isolator has a germ tight air inlet filter, germ free outlet filter, long arm gloves, sterile supply cylinder. Short term housing can be done in gnotocage. All surfaces of the microsiolator should be disinfected with disinfectants like peracetic acid, chlorine dioxide, and hydrogen peroxide. All supplies should be sterilized with autoclaving for 30 min at 134oC. Gamma irradiated food is preferred because autoclaved feed becomes harder to gnaw. Change in behavior of animals, smell of ammonia, change in appearance or consistency of fecal pellets are some of the indicators of contamination.

Monitoring Germfree Rodents: Health surveillance of gnotobiotic animals is performed in accordance with FELASA recommendations. Besides animals, all equipment and isolator components should be regularly tested for sterility. Animals should be at least 12 weeks old to test for antibodies against infectious agents. The most important samples to test are fecal pellets because high concentration of bacterial contamination can be detected in these pellets. Bacterial culture can be performed using brain heart infusion broth for aerobic bacteria, thioglycolate broth for anaerobic bacteria and Sabouraud broth for fungi. Cultures should also be incubated at lower temp (30oC) or higher temp (56oC). The key is to not only demonstrate no growth of contaminating bacteria but also to verify the presence of defined flora bacteria. Other testing methods include PCR and serology to detect viral antibodies. Testing should be done every three months or more frequently for supplies. To ensure only desired agents are present in the animals, once a year monitoring using 16S rRNA sequencing is recommended.

QUESTIONS

1. Which of these bacteria are part of Schaedler flora?

* 1. Escherichia coli var. mutabilis, Streptococcus faecalis, Lactobacillus acidophilus, Lactobacillus salivarius, group N Streptococcus, Bacteroides distasonis, a Clostridium sp., and an EOS fusiform bacterium
  2. *Staphylococcus xylosus, Corynebacterium renale, Streptococcus aureus, Proteus*
  3. MHV, EDIM, MNV, MVM, TGE
  4. *Trichophyton*, Griseofulvin

2. T/F. Axenic mice are free of all micro-organisms including those that are normally found in the GI tract.

3. What gas smell around the isolator indicates contamination?

1. Ammonia
2. Methane
3. Argon
4. Nitrous oxide

4. Research projects with privately owned animals may require oversight of following

1. IACUC
2. Clinical Veterinary Medical Research Advisory Committee
3. Ethical Review Committee
4. All of the above

ANSWERS

1. a

2. a

3. a

4. d



Figure 2: Cecal torsion in a germfree mouse.



Figure 3: Loading of an isolator (A–C). The sterilizing cylinder into which the supplies are transferred is autoclaved, connected to a port on the isolator using a flexible plastic sleeve, and the inside of the sleeve is sterilized using peracetic acid (personnel are protected by wearing powered air purifying respirators). After disinfection, the internal door on the isolator and the external seal on the transport cylinder are opened, and the sterile materials are transferred into the isolator. A static microisolator (gnotocage) can be used for the temporary maintenance of germfree mice (D).

**Kornerup et al. A Review of Applied Aspects of Dealing with Gut Microbiota Impact on Rodent Models, pp. 250-264**

SUMMARY: The gut microbiota (GM) affects numerous human diseases, as well as rodent models for these. The article review the impact and summarize ways of handling the challenge in animal research. The GM is complex, with the largest fractions being the gram-positive phylum Firmicutes and the gram-negative phylum Bacteroidetes. Other important phyla are the gram-negative phyla Proteobacteria and Verrucomicrobia, and the gram-positive phylum Actinobacteria. GM members influence models for diseases, and immune system - such as inflammatory bowel diseases, allergies, autoimmunity, cancer, and neuropsychiatric diseases. GM characterization of all individual animals and incorporation of their GM composition in data evaluation may therefore be considered in future protocols.  The article mention some of the issues shortly -  such as the diet, and its micro nutrient, drinking water and its acidity, cage affect, IVC vs open cage, genetic affect etc. Germ free isolator-housed rodents or rodents made virtually germ free by antibiotic cocktails can be used to study diverse microbial influences on disease expression. Through subsequent inoculation with selected strains or cocktails of microbes, new “defined flora” models can yield valuable knowledge on the impact of the GM, and of specific GM members and their interactions, on important disease phenotypes and mechanisms. All of this within take into considerations the limitation of inoculation timing and method, strain and human GM correlation with the animal. Rodent husbandry and microbial quality assurance practices will be important to ensure and confirm appropriate and research relevant GM.

QUESTIONS (True or False)

1.  The rodent and the human GMs have a high qualitative similarity?

2.  Cage environment may account for approximately 60% of the GM variation?

3.  The most advanced technique to analyze GF mice is by 16S PCR?

ANSWERS

1.  T. Although there are some important differences

2.  F. Only 30% - Mice that are housed together will after some time cluster together according to their microbiota.

3.   T. Although qPCR is advance technique – it doesn't show there type... only if GM is exist.