cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Gastrointestinal Organoids: Understanding the Molecular Basis of the Host–Microbe Interface

David R. Hill[1] and Jason R. Spence[1,2,3]

[1]Department of Internal Medicine, [2]Department of Cell and Developmental Biology, [3]Center for Organogenesis, University of Michigan Medical School, Ann Arbor, Michigan

SUMMARY

New methods enable prolonged culture of human intestinal tissue in the laboratory. This review summarizes the use of these tools in the study of host–microbe interactions and suggests future avenues of research.

In recent years, increasing attention has been devoted to the concept that microorganisms play an integral role in human physiology and pathophysiology. Despite this, the molecular basis of host–pathogen and host–symbiont interactions in the human intestine remains poorly understood owing to the limited availability of human tissue, and the biological complexity of host–microbe interactions. Over the past decade, technological advances have enabled long-term culture of organotypic intestinal tissue derived from human subjects and from human pluripotent stem cells, and these in vitro culture systems already have shown the potential to inform our understanding significantly of host-microbe interactions. Gastrointestinal organoids represent a substantial advance in structural and functional complexity over traditional in vitro cell culture models of the human gastrointestinal epithelium while retaining much of the genetic and molecular tractability that makes in vitro experimentation so appealing. The opportunity to model epithelial barrier dynamics, cellular differentiation, and proliferation more accurately in specific intestinal segments and in tissue containing a proportional representation of the diverse epithelial subtypes found in the native gut greatly enhances the translational potential of organotypic gastrointestinal culture systems. By using these tools, researchers have uncovered novel aspects of host–pathogen and host–symbiont interactions with the intestinal epithelium. Application of these tools promises to reveal new insights into the pathogenesis of infectious disease, inflammation, cancer, and the role of microorganisms in intestinal development. This review summarizes research on the use of gastrointestinal organoids as a model of the host–microbe interface. ***(Cell Mol Gastroenterol Hepatol 2017;3:138–149; http://dx.doi.org/10.1016/j.jcmgh.2016.11.007)***

Keywords: Epithelium; Enteroids; Intestine; Symbiosis; Pathogenesis; Model Systems.

2009 was a landmark year in the field of gastroenterology, because it was the first time that primary non-transformed tissues derived from the gastrointestinal (GI) tract was shown to have the capability of long-term growth in vitro.[1,2] These studies successfully led to the maintenance and propagation of 3-dimensional (3D) tissue that maintained some properties of the complex intestine in vivo. For example, isolated intestine could be grown as epithelium-only structures[1] or as epithelium plus supporting stromal/mesenchymal tissue.[2] A defining feature of these seminal works was the creation of an artificial niche in vitro, which promoted the maintenance of the highly proliferative intestinal stem cell population. This artificial niche is complex and requires the proper physical environment (extracellular matrix [ECM]) and chemical environment (growth factor signaling) to mimic, in part, the environment found in the native gut.[1,2] Collectively, these organotypic cultures represent a complex system for studying the intestine and commonly are called *organoids* because they retain organ-like features, such as the plethora of differentiated epithelial cell types (goblet, Paneth, enteroendocrine, enterocyte).[3]

Since these early days, well shy of a decade ago, diverse tissues of the gastrointestinal tract have been grown from primary human and mouse tissue sources, including esophagus, liver, pancreas, stomach, and colon.[4–13] In addition to long-term culture of organ-derived tissues, the development of an artificial niche also prompted advances in research involving the differentiation of human embryonic and induced pluripotent stem cells (collectively called hPSCs) into 3D organoids. To date, hPSCs have been differentiated into GI tissues including small intestine, stomach (antrum), and liver.[14–19] A rapidly expanding body of literature has emerged surrounding the applications of organoids, and intestinal organoids in particular, as model systems to study human development and disease in vitro (Figure 1).[20,21]

Human organoids, whether derived from donor tissue or from hPSCs, represent important tools to probe human gastrointestinal biology, physiology, and pathophysiology.

Abbreviations used in this paper: CDI, *Clostridium difficile* infection; ECM, extracellular matrix; GI, gastrointestinal; HIO, human intestinal organoids; hPSC, human pluripotent stem cell; IFN, interferon; IL, interleukin; NEC, necrotizing enterocolitis; SCFA, short-chain fatty acid; TcdB, *C difficile* toxin B; 3D, 3-dimensional.

Most current article

© 2017 The Authors. Published by Elsevier Inc. on behalf of the AGA Institute. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2016.11.007

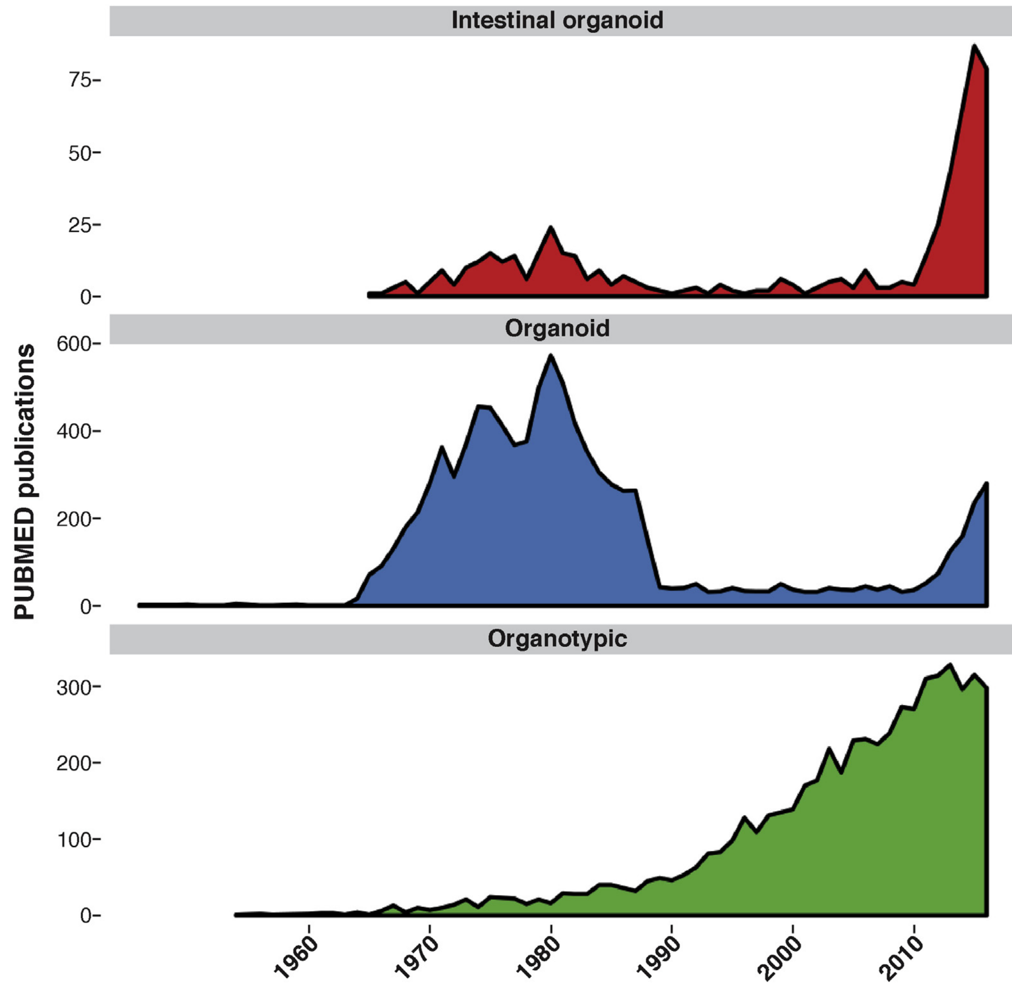

Figure 1. The number of citations referencing intestinal organoids has increased dramatically over the past decade. Notably, the term *organoid* was at one time commonly used in reference to organoid nevus, an uncommon type of benign hair follicle tumor that is now known as a sebaceous nevus.[29]

Among the essential roles of the intestinal epithelial barrier is the maintenance of a continuous surface that must perform necessary absorptive functions, and that must interface with microbes to create an environment that simultaneously permits colonization by beneficial organisms and excludes opportunistic pathogens.[22] Perturbations in this dynamic symbiosis underlie the pathogenesis of inflammatory disease,[23,24] gastrointestinal cancer,[25] metabolic syndrome,[26] and other conditions.[27,28] However, the mechanistic basis of host–pathogen and host–symbiont interactions in the human intestine is not well understood. This review focuses on the application of gastrointestinal organoids as a model of the host–microbe interface.

Defining Gastrointestinal Organoids

The rapid growth in the number of investigators using diverse 3D intestinal tissue culture systems (Figure 1) has outpaced the adoption of standardized nomenclature. Cultured tissues that retain some amount of complex in vivo function and cellular diversity are considered "organotypic,"[3] a term encompassing both cultured whole-tissue explants[30] and organoids.[1,14,31] However, given the plethora of models now available as research tools, it is important to keep in mind that not all organoids are directly comparable.[3] For example, tissue-derived organoids can be grown with[2,32] or without[1,33] mesenchyme, and are ideal for modeling adult homeostasis or disease.[32,34–36] On the other hand, hPSC-derived organoids are more similar to immature (fetal) tissue than to adult tissues,[16,37–39] but become more adult-like after transplantation into a living mouse host.[38,40,41] For the purposes of the current review, we refer to organoids derived from human tissue/organs as tissue-derived organoids and we specify if tissue-derived organoids are epithelium-only or grown with the epithelium and mesenchyme, and we refer to organoids derived from hPSCs as *hPSC-derived organoids* (Table 1).

Improved Models of the Gastrointestinal Tract

Cell lines have been an important work horse of in vitro gastrointestinal experimentation for decades, and have led to major insights, but also have some limitations.[42–44] Explant tissue models, in which intestinal tissues are removed from model organisms, dissected from patient samples or collected from human organ donors offer the full spectrum of cellular diversity and intestinal

Table 1. Human Intestinal Organic Model Systems and Nomenclature

Organoid model	Alternate names	Description	References
Tissue-derived, epithelium only	Enteroid	A single layer of epithelium with apical–basal polarity and a hollow internal lumen Generated from differentiated human or animal intestinal tissue	1,12,36
Tissue-derived, epithelium, mesenchyme		A single epithelial layer with apical–basal polarity and underlying mesenchyme Generated from intestinal tissue and grown in air-liquid interface Currently no human model	2
hPSC-derived	Organoid, mini-gut	A single epithelial layer with apical–basal polarity and a hollow internal lumen and underlying mesenchymal cells Generated by directed differentiation of induced pluripotent stem cells or embryonic stem cells in vitro and retaining fetal characteristics	14,16,38,41

function, but are hampered by limited viability outside the body and often are difficult to obtain in sufficient quantities, particularly in the case of human tissues.[30] Likewise, although animal studies are a mainstay of gastrointestinal research, the applicability of mouse models of human inflammatory disease recently has come under renewed scrutiny.[45,46]

In contrast to traditional cell lines, which often represent a homogeneous population of cells, organoids occupy 3-dimensional space and form complex microenvironments that facilitate differentiation and persistence of epithelial subtypes and the formation of villus-like structures.[1,14,38,41] hPSC-derived intestinal organoids possess mesenchymal cells that form critical interactions with the epithelium that are key to the maintenance of tissue identity and the vitality of the epithelium.[14,16,38,39,41] In this respect, hPSC-derived organoids closely resemble native tissues, in which intercellular interactions define diverse microenvironments that determine the differentiation and function of individual cells and cumulatively shape the gross structure of organs and tissues. The implication is that organoids consisting of many differentiated cell types organized in a structured fashion that resembles native tissue can model the behavior of cells and tissues in vivo more accurately. However, the complex 3D structure of organoids also imposes practical limitations on in vitro experiments that are not found in traditional cell culture approaches. For example, lumenal oxygen concentration in hPSC-derived organoids is approximately 5% whereas oxygen concentration in the surrounding media and Matrigel (Corning Inc, Corning, NY) is approximately 20%, suggesting limited diffusion of oxygen across the organoid epithelium (Hill and Spence, unpublished data). Other challenges of the 3D culture approach include difficulty accessing the internal luminal compartment, and the potential for heterogeneous growth and differentiation within and between individual organoids.

As in other experimental systems, the best solutions to the limitations of different organoid models will be tailored to the requirements of individual experiments. For example, the 3D structure of these tissues does not seem to be required for cellular differentiation,[47–49] and established tissue-derived or hPSC-derived organoids can be dissociated enzymatically and reseeded onto Transwell (Corning Inc) monolayer cultures.[47–49] This approach allows for the study of epithelial barrier dynamics and facilitates efficient exposure of the apical epithelium to experimental treatments,[49] virus,[47] or bacteria.[48,50] Other researchers have used microinjection techniques to deliver experimental molecules or bacteria to the apical epithelial surface.[51–53] This approach is more resource-intensive, but may preserve the internal microenvironment required by microorganisms. Typically, 2-dimensional monolayer preparations are used for relatively short-term culture and experimentation,[47,48] whereas 3D organoids can be readily passaged and may be better suited for long-term culture.[14,15,38,39,41] Notably, there is no evidence that either platform (2-dimensional or 3D) is generally superior in modeling the behavior of intestinal epithelium in vivo and it is likely that the experimental question will determine the most appropriate platform.

Host–Microbe Symbiosis During Development

Microbes Play a Key Role in Development and Neonatal Disease

Crucial maturation of the intestinal mucosal barrier and immune system occurs in late gestation and during infancy and perturbations in this maturation may affect susceptibility to inflammatory disease in adulthood.[54] This period of rapid intestinal maturation coincides with the first exposure of the intestinal epithelium to exogenous nutrients as well as both commensal and pathogenic microorganisms. Microbial colonization of the digestive tract involves a progressive ecologic succession[55,56] shaped by both dietary nutrients[57,58] and the host response.[59–61] Recent work has suggested a critical period of intestinal development and gut ecologic succession in infancy establishes key features of metabolism,[62] digestion,[63] neurocognitive function,[64,65] and immune function.[66–69]

Understanding the role of microbes in neonatal intestinal development is clinically relevant to the treatment of necrotizing enterocolitis (NEC), which is the leading cause of gastrointestinal-related mortality in premature infants. NEC affects as many as 1% of all newborns in the Unites States,[70] with in-hospital mortality occurring in up to 30% of cases.[71] NEC risk increases by 7-fold among premature and low-birth-weight infants,[72] and the prevalence of NEC may be increasing.[73] NEC is characterized by severe inflammation and tissue necrosis resulting from intestinal immaturity and abnormal colonization of the intestine by microbes. NEC is preceded by major changes in the microbial population of the intestine in comparison with healthy infants, most notably reduced microbial diversity and colonization by different species.[74] However, it remains unclear how changes in the microbiota promote healthy gut function or the development of NEC.[75,76] Recent reports have suggested a multifactorial etiology by which immature intestinal barrier function predisposes the preterm infant to intestinal injury and inflammation after postpartum microbial colonization. Moreover, published data have suggested that both specific microbes and an immature gut are both required to initiate NEC pathogenesis.[74,75,77]

Despite the widely recognized and urgent need to develop effective strategies for preventing NEC,[73] the rate of mortality in NEC has remained largely unchanged for decades.[71,78] One of the major limitations in our understanding of NEC has been the lack of an appropriate human model system to study the disease.[42,79]

In Vitro Models of Fetal Intestine

hPSC-derived intestinal organoids closely resemble fetal intestinal tissue and recapitulate in vitro the digestive and host-defense functions associated with immature human intestinal tissue,[14,37,38,41] and recent work has established gastrointestinal organoids as a powerful model of microbial pathogenesis at the mucosal interface.[15,31,53,80–83] Thus, HIOs (human intestinal organoids) grown in vitro represent immature human small intestine tissue and are capable of undergoing further maturation in response to external cues when transplanted in vivo. This gap between the in vitro maturation status of HIOs and their in vivo potential is a significant opportunity for biomedical research. Delineation of the signals guiding maturation promises to reveal new mechanisms of human intestinal development. For example, transplanted HIOs are vascularized by blood vessels originating from the mouse kidney,[41] and exposure to circulating factors as well as direct interaction with endothelial cells likely plays an important role in promoting intestinal maturation[84] that has yet to be evaluated in this system.

It therefore is becoming clear that in some cases organoids can facilitate experiments in human GI tissue that otherwise would be very difficult owing to limited tissue supply or bioethical considerations surrounding the use of human fetal tissue. A number of clinically important diseases are thought to originate from deficiencies in gastrointestinal development. Here, hPSC-derived organoids may help fill the gap between clinical need and a lack of understanding of the biological foundations of disease. For example, neurogenin-3 is required for differentiation of endocrine cells of the pancreas and intestine in mice.[85,86] Clinical reports indicate that a loss-of-function mutation in *NEUROG3* causes congenital malabsorptive diarrhea, a condition in which the intestine lacks enteroendocrine cells.[87] Functional depletion of *NEUROG3* transcript using short hairpin RNA in hPSC-derived organoids resulted in the absence of Chromogranin ACHGA^{+} enteroendocrine cells. Likewise, overexpression of *NEUROG3* in developing HIOs increased the number of enteroendocrine cells more than 5-fold.[14] This work exemplifies both the practical utility and clinical relevance of the HIO system for evaluating mechanisms of intestinal development and epithelial differentiation in human tissue.

Several studies have hinted at the potential utility of organoid models in NEC research. Metagenomic characterization of uropathogenic *Escherichia coli* isolated from healthy preterm infants and NEC patients have shown an association between pathogenic traits in individual *E coli* strains and NEC risk.[88] However, multiple studies have shown that *E coli* is among the earliest colonizers of the infant gut and is associated frequently with positive clinical outcomes.[55,89,90] Tissue-derived intestinal organoids have been used to evaluate adherence phenotypes among pathogenic *E coli*, resulting in novel insights into the site-specific interaction of *E coli* with organoids derived from distinct intestinal segments.[48] A similar approach using NEC-associated and health-associated bacterial isolates could be applied to elucidate the role of specific *E coli* traits in NEC pathogenesis and identify new strategies for targeted pharmacologic intervention to accelerate barrier maturation or prevent hyperinflammatory reactivity in the neonatal intestine.

The 3D hPSC-derived intestinal organoid tissue culture systems therefore may prove to be a powerful new tool in the development of therapies to treat NEC and other microbe-associated diseases of infancy.

Organoids in Infectious Disease Research

Perhaps one of the most compelling applications of human gastrointestinal organoids is in the study of infectious disease pathogenesis. Gastrointestinal organoids represent a useful compromise between high-throughput, low-complexity epithelial monolayer culture systems and low-throughput, high-complexity animal models.[83] Epithelial interactions are of particular interest in the pathogenesis of infectious disease as the site of initial attachment to and invasion of host organisms and organoid models excel in their ability to recapitulate functional GI epithelium. Moreover, because organoids represent a reductionist system, often devoid of stromal cells and immune cells, they provide a unique view of the epithelial response in the absence of an immune reaction. Recent research has shown that organoid and enteroid culture systems can be used to evaluate the molecular mechanisms of both bacterial and viral disease pathogenesis in human GI tissue.

Rotavirus and Norovirus

Viral infection is the most common cause of gastroenteritis in both children and adults and contributes to more than 60% of all cases of diarrhea.[91] These infections generally carry a low risk of morbidity and mortality in healthy individuals. Nevertheless, the widespread prevalence of viral gastroenteritis results in 2–3 million deaths worldwide each year.[92] The most common viruses of the gastrointestinal tract, norovirus and rotavirus, spread through fecal–oral transmission of contaminated food, surfaces, or other materials. Viral replication occurs within the gastrointestinal tract, requiring direct interaction with the intestinal epithelium.[93]

Development of effective therapies for viral gastroenteritis has been limited in part by the absence of reliable methods for propagating human gastrointestinal viruses in the laboratory. For example, the propagation of a laboratory rotavirus strain (simian SA11) in hPSC-derived intestinal organoids recently was shown.[94] Exposure of the apical epithelial surface to dilute *Rhesus* rotavirus or clinical rotavirus isolates was sufficient to stimulate the formation of viroplasms and increased viral RNA titers within the HIO epithelium. Interestingly, fluorescent immunostaining showed viral protein particles in cells that also expressed the mesenchymal marker vimentin, a previously unknown target of rotavirus infection.[94]

Human tissue-derived epithelial organoids also can serve as useful models for rotavirus pathogenesis.[95] Replication of simian SA11 rotavirus occurs in primary epithelial organoids derived from murine intestine or from adult intestinal biopsy tissue within 24 hours and enhances expression of host interferon-stimulated genes.[82] Treatment with interferon α (IFNα) or ribavirin, used clinically to treat viral infection, suppressed the replication of rotavirus in primary intestinal organoids. This observation led the investigators to evaluate the responsiveness of patient-derived rotavirus to IFNα and ribavirin in primary epithelial organoid cultures. Among the 7 samples tested, responsiveness to IFNα and ribavirin in the primary organoid culture model varied between samples and, remarkably, correlated with the clinical treatment response for the individual patients.[82] These experiments represent an intriguing proof-of-concept that patient-specific organoid model systems can be useful for developing personalized treatment regimens.

Despite extensive efforts,[96,97] models to study human norovirus infection and replication remained elusive for decades, significantly limiting the ability to investigate pathogenesis.[98] However, model systems have been developed recently, including patient-derived organoids, which allow for replication of human norovirus in vitro.[47,99] Patient-derived organoids were grown in 3D cultures and subsequently seeded as a monolayer in Transwell cultures and were shown to support robust replication of multiple clinical human norovirus isolates in vitro in epithelium derived from different regions of the small intestine, and in a histo-blood group antigen–dependent manner. Replication of some human norovirus strains requires the presence of bile, which can be introduced along with the viral isolate. Interestingly, human norovirus is unable to replicate in hPSC-derived intestinal organoids, indicating that important biological differences between the 2 systems exist, and that may be leveraged to gain additional insights into disease pathogenesis (Mary Estes, personal communication; Christiane Wobus, personal communication). Finally, heating or γ irradiation of human norovirus samples was shown to completely abrogate viral replication in patient-derived cultures, suggesting that this platform could be used to test novel infection control measures.[47] Thus, patient-derived, epithelium-only organoids that are seeded as monolayer cultures represent a significant advance toward improved understanding of the pathogenesis of the most common cause of gastroenteritis and may lead to development of vaccines, improved diagnostic and prognostic tests, and better therapeutics.

Salmonella *typhimurium*

In addition to being an important human pathogen,[100] basic research on the pathogenesis of *Salmonella* infection has shown important aspects of microbial interactions with the gastrointestinal epithelium. Several studies have examined aspects of *Salmonella* pathogenesis in murine colonic enteroids,[101,102] although recent work in hPSC-derived intestinal organoids has shown novel features of the human epithelial response to *Salmonella* infection.[81] In this study, after microinjection of live *Salmonella enterica* serovar typhimurium into the organoid lumen and 3-hour coculture, RNA sequencing was conducted to examine the transcriptional signature of *Salmonella* infection in human small intestinal epithelium. This analysis showed significant up-regulation of cytokine-mediated signaling, nuclear factor κB activation, angiogenesis, and chemotaxis-related gene expression in *Salmonella*-infected hPSC-derived organoids. Measurement of cytokine secretion by *Salmonella*-infected hPSC-derived organoids confirmed enhanced release of proinflammatory mediators interleukin (IL)8, IL6, and tumor necrosis factor-α. Imaging of infected HIOs by electron microscopy showed adherent *Salmonella* positioned along the apical epithelium, with high-magnification images showing *Salmonella*-containing vacuoles present in the epithelial cytoplasm.[81] Such interactions are difficult to capture in murine infection models and have not been widely analyzed in patient tissue samples. The results of the study by Forbester et al[81] are consistent with findings from prior animal studies and serve as a useful proof-of-principle for the study of *Salmonella* in human organoids.

Clostridium difficile

Clostridium difficile is emerging rapidly as one of the most important infectious disease challenges to the health care system. Exposure to antibiotics, advanced age, gastrointestinal surgery, and chronic inflammatory disease are key risk factors for *C difficile* infection (CDI).[103] As a result, this spore-forming anaerobe is the leading cause of infectious nosocomial diarrhea in the United States. An aging population and continued use of broad-spectrum antibiotics is

expected to increase the prevalence of CDI in the coming years.[104] Outcomes of *C difficile* acquisition are highly heterogeneous and range from asymptomatic carriage to life-threatening toxic megacolon,[103] with recurrent infection occurring in a significant subset of patients.[105,106] In the United States, more than 14,000 patients die of CDI annually,[107] yet very little is known regarding the molecular pathogenesis of CDI in the human gastrointestinal tract.

Although rodent models of CDI are available,[108,109] in vitro tools to study CDI pathogenesis are remarkably limited for many of the same reasons discussed earlier, not least of which was the inability to co-culture human epithelial cells lines with obligate anaerobes. However, recent work has suggested that hPSC-derived intestinal organoids represent a novel platform for elucidating the cellular and molecular pathogenesis of CDI in vitro.[51,52] *C difficile* has been shown to persist within the lumen of hPSC-derived intestinal organoids for at least 12 hours after microinjection.[51] This suggests that the organoid lumen may possess anoxic microenvironments suitable for survival of obligate anaerobes over a period of time. Microinjection of purified *C difficile* toxin A, but not *C difficile* toxin B (TcdB), or colonization of organoids with a toxin-producing *C difficile* strain (VPI 10463), was associated with extensive epithelial damage and rapid loss of barrier function, whereas a non–toxin-producing strain (F200) had little impact on epithelial morphology. These effects were associated with changes in the distribution of epithelial tight junction protein zonula occludens-1, the cell–cell adhesion glycoprotein E-cadherin, and the actin cytoskeleton.[51] The role of TcdB in human *C difficile* infection also was investigated in tissue-derived epithelial colonic monolayer cultures derived from human fetal colonic tissue.[31] In these cultures, TcdB treatment produced extensive histologic damage, loss of epithelial barrier integrity, and dose-dependent increases in the expression of inflammatory genes including *IL1B*, *CXCR4*, and *DUOX2*.[31]

C difficile may have additional effects on epithelium that, although not directly damaging, can elicit changes in bowel function that promote *C difficile* growth. For example, the impact of *C difficile* on mucus layer composition in hPSC-derived organoids was evaluated recently.[52] Although decreased mucin 2 expression was associated with *C difficile* microinjection into organoids and with intestinal biopsies from CDI patients, there was no measurable change in mucus carbohydrate composition in *C difficile*–injected organoids. This was contrary to observations in the intestinal biopsy tissue, in which potential *C difficile* substrates *N*-acetylgalactosamine, *N*-acetylglucosamine, and galactose were altered relative to healthy tissue, calling into question the metabolic potential of obligate anaerobe *C difficile* in the oxygenated organoid culture system.[52] Microinjection of hPSC-derived organoids with *C difficile* or CDI stool samples resulted in decreased expression of the Na^+/H^+ exchanger NHE3, a condition that was shown to encourage growth of *C difficile* in vitro while healthy stool and *Clostridium butyricum* injection had no effect on NHE3 expression.[110]

Together, these works show that stem cell–derived human intestinal organoids show physiologically relevant responses to *C difficile* and that the cellular and molecular events underpinning CDI pathogenesis can be better understood through the use of 3D tissue culture models. Future studies may examine the effects of *C difficile* metabolites,[111] viral co-infection,[112] or chronic inflammation[113] on CDI pathogenesis using organoid systems.

Helicobacter pylori

Helicobacter pylori forms an intimate interaction with the gastric mucosa that enables colonization of the human host, which may persist for decades, often producing few clinical symptoms.[114] Infections often are acquired during childhood and adolescence through fecal–oral transmission, typically within families. Perhaps as much as 50% of the global adult population is infected with *H pylori* at any given time.[115] Morbidity and mortality associated with *H pylori* infection occurs as a result of the chronic interplay between *H pylori* and the gastric mucosa rather than fulminant bacterial infection. *H pylori* now is appreciated as a major risk factor for peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma.[114] Experimental models that facilitate a better understanding of *H pylori* pathogenesis therefore are useful not only as tools to develop new clinical therapies for treating an important public health problem, but more generally as systems for understanding the impact or microbial activity in the pathogenesis of epithelial cancers and inflammatory disease. Primary culture of murine gastric epithelium in a 3D tissue culture system was established by Barker et al[116] in 2010, with methods for culture of primary human gastric epithelium following later.[9,10] Gastric organoids also can be derived efficiently through differentiation of hPSCs.[15]

H pylori colonization may persist for many years before the emergence of clinical symptoms or sequelae such as ulcers or gastric carcinoma,[114] and the factors that induce this transition to the disease state remain poorly understood. Organoid model systems have proven to be valuable models of the pathogenesis of *H pylori* infection. The epithelial lining of the stomach is renewed by stem cells residing in structures called gastric glands. Multiple gastric glands containing both secretory cells and stem/progenitor cells empty into shared invaginations called gastric pits, which channel gastric gland secretions into the stomach lumen.[117] Tissue-derived gastric organoids can be directed to express markers of the gastric pit or gastric gland epithelium.[10] *H pylori* efficiently colonizes the gastric glands, where direct interaction with $Lgr5^+$ stem cells enhances proliferation of the epithelial progenitor population. This effect was dependent on bacterial *CagA* expression as well as microbial chemotaxis necessary for colonization of the antral glands.[118] By using tissue-derived gastric organoids, it was determined that urea emanating from the epithelium serves as a potent chemotactic agent guiding colonization of the gastric glands by *H pylori*.[119] Remarkably, organoids derived from primary gastric gland epithelium mount a robust inflammatory response to *H pylori* in comparison with gastric pit organoids,[10] potentially attributable to restricted expression of Toll-like receptors[120] in the gastric

gland or the physical barrier formed by mucus expressed in the gastric pit.[121]

Induction of progenitor cell proliferation in the context of localized inflammation may create conditions that promote gastric cancer.[122] *H pylori* influences proliferation and differentiation processes in cultured gastric epithelium that may contribute to the emergence of adenocarcinoma in vivo. Microinjection of murine or human primary gastric epithelial organoids with *H pylori* results in epithelial proliferation. Notably, this response was dependent on expression of the *H pylori* virulence factor *CagA*, which was shown to interact with CD44 receptor on the host epithelium to induce downstream phosphorylation of c-Met and subsequent epithelial proliferation.[15,53,80] *H pylori* microinjection also modulates expression of cancer-associated tight junction components claudin-7 and snail in a β-catenin–dependent manner in primary murine gastroid cultures.[80] Taken together, these pioneering studies show that 3D gastric organoid culture techniques are tractable and reproducible systems for evaluating *H pylori* infection and the pathogenesis of infection-associated gastric cancers.

Microbial Metabolites

The products of microbial metabolism are a key component of microbial pathogenicity and symbiosis within the gastrointestinal tract.[27,28,106,111,123] Dietary intake supplies the major precursors for metabolite production by the indigenous microbiota and dietary changes alter the structure and function of the microbial community in the gut.[124,125] Modification of the metabolic activity of the gut microbiota may reduce disease risk in human hosts,[24,27,28] however, the overwhelming majority of microbial metabolites and byproducts have not been well characterized. Here, organoid culture methods may serve as a useful system for identifying bioactive microbial products and evaluating their potential effects on the host epithelium.

Short-chain fatty acids (SCFAs) such as butyrate, acetate, and proprionate are produced by microbial fermentation of dietary fiber in the gut and can serve as both an energy source and signaling molecule in the mammalian epithelium.[126] The effects of SCFA-producing bacteria on the intestinal epithelium have been evaluated recently using tissue-derived organoids. *Akkermansia muciniphila* is an abundant member of the human gut microbiota that colonizes the mucus layer, fermenting mucus glycoproteins into proprionate and acetate.[127] Mouse ileal organoids exposed to *A muciniphila* showed changes in the expression of a wide array of host transcription factors that suggest a broad influence of *A muciniphila* over host metabolic activity.[128] By contrast, another commensal SCFA-producing bacterium, *Faecalibacterium prausnitzii*, had relatively little effect on host gene expression. Notably, completely distinct responses were seen in ileal organoids treated with purified butyrate or proprionate alone, indicating that the transcriptional response to commensal organisms is multifaceted.[128]

A ground-breaking study by Kaiko et al[129] included a large-scale screen of microbial-derived metabolites in murine colonic organoids. A panel of 92 microbial metabolites was evaluated for effects on epithelial proliferation using colonic organoids derived from mice expressing a Cdc25A-luciferase construct. By using this assay, it was determined that butyrate dramatically suppresses proliferation of stem and progenitor cell–enriched colonic organoids in vitro at physiologic concentrations. The investigators cleverly manipulated organoid culture conditions to generate separate stem/progenitor-enriched organoids and colonocyte-enriched organoids to evaluate the cell-type–specific effects of butyrate. In contrast to the proliferation inhibition observed in stem/progenitor-enriched organoids, butyrate was metabolized rapidly by colonocyte-enriched organoids and had little effect on the rate of proliferation. This implied a novel link between the structure and function of the colonic crypt, specifically that distal colonocytes preserve stem and progenitor cell proliferative potential by rapid uptake and metabolism of luminal butyrate.[129]

Future studies may evaluate large panels of microbial metabolites for effects that are specific to intestinal segment, host tissue disease status or genotype, or developmental stage. Essential to any large-scale metabolite screen conducted in organoids will be the development of tools that enable rapid and reproducible measurement of responses to microbial metabolites.[129]

Conclusions and Future Directions

At present, intestinal organoid culture systems appear to have overcome several significant technical limitations that have been historical barriers to in vitro experimental work. By using these technologies, it now is possible to establish long-term cultures of primary gastrointestinal epithelium that retains much of the structural and functional characteristics of tissue in vivo.[1,10,12,36,116] Intestinal epithelium and associated mesenchyme can be differentiated de novo from human pluripotent stem cells, recapitulating many of the features of the fetal intestine and facilitating in-depth characterization of early events in human intestinal development.[14–16,38,41] These model systems are proving to be robust in the presence of bacterial[31,52,53,80,81,101,102,110,118,119,128,129] and viral challenges,[47,94] allowing for true co-culture experiments in which epithelium and microbe can be maintained for extended periods of time in the same culture dish. Organoids therefore represent a modular and highly adaptable model system for evaluating the molecular basis of the host–microbe interface (Figure 2). Single microbes or combinations of microbes representing reference strains, clinical isolates, or specifically engineered experimental organisms can be introduced into the organoid by microinjection or external application. The organoid itself may be tissue-derived or hPSC-derived, representing specific segments of the adult or fetal intestine, patient isolates, or genetically engineered cell lines. The ECM and media harboring the organoid is modifiable to simulate developmental or pathophysiologic conditions that may alter host–microbe interactions. Current research has just begun to explore the wide range of potential combinations.

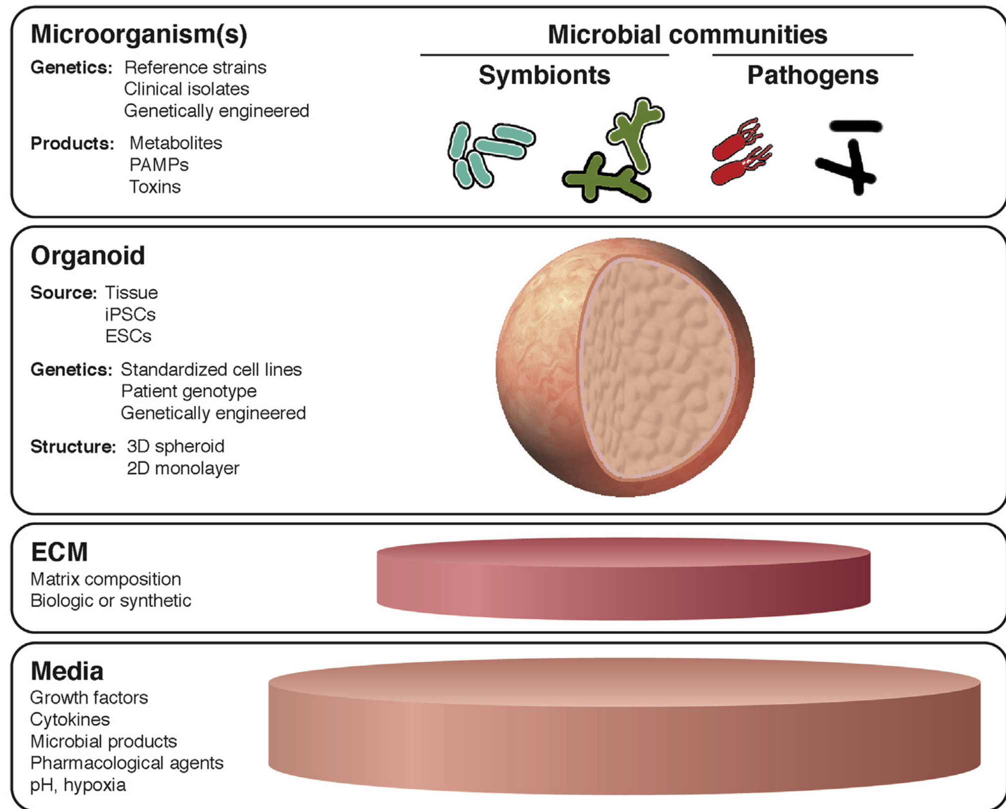

Figure 2. Organoid microbe co-culture systems are highly adaptable, with multiple interchangeable components that allow for a wide range of experimental uses. These features allow for the evaluation of microbial metabolites, toxins, pathogen-associated molecular patterns (PAMPs), single organisms or isolates, multi-organism cultures, or intact microbial communities in many distinct configurations. ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; 2D, 2-dimensional.

Advancement of organoid model systems will be contingent on further characterization of organoid model systems as well as technological developments that enhance the capabilities of these systems. At present, only tissue-derived organoids can replicate intestinal segment–specific tissue across the entire range of intestinal development.[21,34,36,82,129,130] hPSC-derived organoids currently are limited to immature stages. Although maturation is possible by transplantation into the mouse kidney capsule,[38,41] it is unclear what effect this has on inflammmatory and innate defense capabilities. Microbial pathogenesis and commensalism frequently are specific to the level of tissue maturation and anatomic location.[54,59–61] Further characterization of organoid model systems is necessary to facilitate the selection of appropriate culture systems for the study of host–microbe interactions.

The ability to construct high-thoughput multiplexed arrays of organoids[129] will allow for efficient screening and culture of complex microbial communities and metabolites (Figure 2). Moving forward, the ability to increase complexity in organoid model systems will enhance their utility further; this includes co-culture systems that incorporate immune cells, vascular tissue, and neurons, and assays that allow for real-time monitoring of organoid and microbial responses. In an era of medicine that is increasingly personalized,[131] patient-derived organoids may be useful for determining clinical therapy or prognosis.[36,82] Practical challenges also remain, such as the high cost of organoid maintenance and the need for well-defined ECM reagents that support robust organoid growth. Reduced cost and continued methodologic improvements will empower more researchers to incorporate organoid-based experimental designs.

References

1. Sato T, Vries RG, Snippert HJ, et al. Single lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–265.
2. Ootani A, Li X, Sangiorgi E, et al. Sustained in vitro intestinal epithelial culture within a wnt-dependent stem cell niche. Nat Med 2009;15:701–706.
3. Dedhia PH, Bertaux-Skeirik N, Zavros Y, et al. Organoid models of human gastrointestinal development and disease. Gastroenterology 2016;150:1098–1112.
4. Huch M, Dorrell C, Boj SF, et al. In vitro expansion of single lgr5+ liver stem cells induced by wnt-driven regeneration. Nature 2013;494:247–250.
5. Huch M, Bonfanti P, Boj SF, et al. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the lgr5/R-spondin axis. EMBO J 2013;32:2708–2721.
6. Huch M, Gehart H, van Boxtel R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299–312.
7. Reichert M, Takano S, Heeg S, et al. Isolation, culture and genetic manipulation of mouse pancreatic ductal cells. Nat Protoc 2013;8:1354–1365.

8. Reichert M, Rhim AD, Rustgi AK. Culturing primary mouse pancreatic ductal cells. Cold Spring Harb Protoc 2015;2015:558–561.
9. Gifford GB, Demitrack ES, Keeley TM, et al. Notch1 and notch2 receptors regulate mouse and human gastric antral epithelial cell homoeostasis. Gut 2016. Epub ahead of print December 9, 2016. doi: http://dx.doi.org/10.1136/gutjnl-2015-310811.
10. Bartfeld S, Bayram T, van de Wetering M, et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology 2015; 148:126–136.e6.
11. Boj SF, Hwang CI, Baker LA, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015; 160:324–338.
12. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 2011;141:1762–1772.
13. Mou H, Vinarsky V, Tata PR, et al. Dual sMAD signaling inhibition enables long-term expansion of diverse epithelial basal cells. Cell Stem Cell 2016;19:217–231.
14. Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011;470:105–109.
15. McCracken KW, Catá EM, Crawford CM, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature 2014;516:400–404.
16. McCracken KW, Howell JC, Wells JM, et al. Generating human intestinal tissue from pluripotent stem cells in vitro. Nat Protoc 2011;6:1920–1928.
17. Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481–484.
18. Hannan NR, Fordham RP, Syed YA, et al. Generation of multipotent foregut stem cells from human pluripotent stem cells. Stem Cell Reports 2013;1:293–306.
19. Hohwieler M, Illing A, Hermann PC, et al. Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling. Gut 2016. Epub ahead of print September 15, 2016. doi: http://dx.doi.org/10.1136/gutjnl-2016-312423.
20. Schweiger PJ, Jensen KB. Modeling human disease using organotypic cultures. Curr Opin Cell Biol 2016; 43:22–29.
21. Kretzschmar K, Clevers H. Organoids: modeling development and the stem cell niche in a dish. Dev Cell 2016; 38:590–600.
22. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014;14:141–153.
23. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008;453:620–625.
24. Chu H, Khosravi A, Kusumawardhani IP, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. Science 2016;352: 1116–1120.
25. Arthur JC, Perez-Chanona E, Mühlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012;338:120–123.
26. Cox LM, Blaser MJ. Pathways in microbe-induced obesity. Cell Metab 2013;17:883–894.
27. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575–1584.
28. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576–585.
29. Pinkus H. Organoid nevus. Mod Probl Paediatr 1976; 20:50–57.
30. Randall KJ, Turton J, Foster JR. Explant culture of gastrointestinal tissue: a review of methods and applications. Cell Biol Toxicol 2011;27:267–284.
31. Wang X, Yamamoto Y, Wilson LH, et al. Cloning and variation of ground state intestinal stem cells. Nature 2015;522:173–178.
32. Li X, Nadauld L, Ootani A, et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. Nat Med 2014;20:769–777.
33. Matano M, Date S, Shimokawa M, et al. Modeling colorectal cancer using cRISPR-cas9-mediated engineering of human intestinal organoids. Nat Med 2015;21:256–262.
34. Fujii M, Shimokawa M, Date S, et al. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. Cell Stem Cell 2016;18:827–838.
35. Drost J, van Jaarsveld RH, Ponsioen B, et al. Sequential cancer mutations in cultured human intestinal stem cells. Nature 2015;521:43–47.
36. van de Wetering M, Francies HE, Francis JM, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015;161:933–945.
37. Aurora M, Spence JR. HPSC-derived lung and intestinal organoids as models of human fetal tissue. Dev Biol 2016;420:230–238.
38. Finkbeiner SR, Hill DR, Altheim CH, et al. Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation in vitro and in vivo. Stem Cell Reports 2015. Epub ahead of print June 3, 2015. doi: http://dx.doi.org/10.1016/j.stemcr.2015.04.010.
39. Dye BR, Hill DR, Ferguson MA, et al. In vitro generation of human pluripotent stem cell derived lung organoids. Elife 2015;4.
40. Finkbeiner SR, Freeman JJ, Wieck MM, et al. Generation of tissue-engineered small intestine using embryonic stem cell-derived human intestinal organoids. Biol Open 2015;4:1462–1472.
41. Watson CL, Mahe MM, Múnera J, et al. An in vivo model of human small intestine using pluripotent stem cells. Nat Med 2014;20:1310–1314.
42. Balimane PV, Chong S. Cell culture-based models for intestinal permeability: a critique. Drug Discov Today 2005;10:335–343.
43. Ahmed D, Eide PW, Eilertsen IA, et al. Epigenetic and genetic features of 24 colon cancer cell lines. Oncogenesis 2013;2:e71.

44. Ilyas M, Tomlinson IP, Rowan A, et al. Beta-catenin mutations in cell lines established from human colorectal cancers. Proc Natl Acad Sci U S A 1997; 94:10330–10334.
45. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2013; 110:3507–3512.
46. Takao K, Miyakawa T. Genomic responses in mouse models greatly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2015;112:1167–1172.
47. Ettayebi K, Crawford SE, Murakami K, et al. Replication of human noroviruses in stem cell-derived human enteroids. Science 2016;353:1387–1393.
48. VanDussen KL, Marinshaw JM, Shaikh N, et al. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. Gut 2015;64:911–920.
49. Moon C, VanDussen KL, Miyoshi H, et al. Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. Mucosal Immunol 2014;7:818–828.
50. In J, Foulke-Abel J, Zachos NC, et al. Enterohemorrhagic Escherichia coli reduce mucus and intermicrovillar bridges in human stem cell-derived colonoids. Cell Mol Gastroenterol Hepatol 2016;2:48–62.e3.
51. Leslie JL, Huang S, Opp JS, et al. Persistence and toxin production by clostridium difficile within human intestinal organoids result in disruption of epithelial paracellular barrier function. Infect Immun 2015;83:138–145.
52. Engevik MA, Yacyshyn MB, Engevik KA, et al. Human clostridium difficile infection: altered mucus production and composition. Am J Physiol Gastrointest Liver Physiol 2015;308:G510–G524.
53. Bertaux-Skeirik N, Feng R, Schumacher MA, et al. CD44 plays a functional role in helicobacter pylori-induced epithelial cell proliferation. PLoS Pathog 2015; 11:e1004663.
54. Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nat Rev Immunol 2012;12:9–23.
55. Palmer C, Bik EM, DiGiulio DB, et al. Development of the human infant intestinal microbiota. PLoS Biol 2007; 5:e177.
56. Koenig JE, Spor A, Scalfone N, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A 2011;108(Suppl 1): 4578–4585.
57. Newburg DS, Morelli L. Human milk and infant intestinal mucosal glycans guide succession of the neonatal intestinal microbiota. Pediatr Res 2015;77:115–120.
58. Pacheco AR, Barile D, Underwood MA, et al. The impact of the milk glycobiome on the neonate gut microbiota. Annu Rev Anim Biosci 2015;3:419–445.
59. Salzman NH, Hung K, Haribhai D, et al. Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol 2010;11:76–83.
60. Schluter J, Foster KR. The evolution of mutualism in gut microbiota via host epithelial selection. PLoS Biol 2012; 10:e1001424.
61. Marcobal A, Southwick AM, Earle KA, et al. A refined palate: bacterial consumption of host glycans in the gut. Glycobiology 2013;23:1038–1046.
62. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012;488:621–626.
63. Erkosar B, Storelli G, Mitchell M, et al. Pathogen virulence impedes mutualist-mediated enhancement of host juvenile growth via inhibition of protein digestion. Cell Host Microbe 2015;18:445–455.
64. Diaz Heijtz R, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 2011;108:3047–3052.
65. Desbonnet L, Clarke G, Shanahan F, et al. Microbiota is essential for social development in the mouse. Mol Psychiatry 2014;19:146–148.
66. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. Am J Gastroenterol 2010;105:2687–2692.
67. Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut 2011;60:49–54.
68. Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low gut microbiota diversity in early infancy precedes asthma at school age. Clin Exp Allergy 2014;44:842–850.
69. Arrieta MC, Stiemsma LT, Dimitriu PA, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med 2015;7:307ra152.
70. Holman RC, Stoll BJ, Curns AT, et al. Necrotising enterocolitis hospitalisations among neonates in the united states. Paediatr Perinat Epidemiol 2006;20:498–506.
71. Lin PW, Stoll BJ. Necrotising enterocolitis. Lancet 2006; 368:1271–1283.
72. Sankaran K, Puckett B, Lee DS, et al, Canadian Neonatal Network. Variations in incidence of necrotizing enterocolitis in Canadian neonatal intensive care units. J Pediatr Gastroenterol Nutr 2004;39:366–372.
73. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364:255–264.
74. Morrow AL, Lagomarcino AJ, Schibler KR, et al. Early microbial and metabolomic signatures predict later onset of necrotizing enterocolitis in preterm infants. Microbiome 2013;1:13.
75. Torrazza RM, Ukhanova M, Wang X, et al. Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. PLoS One 2013;8:e83304.
76. Neu J. Probiotics and necrotizing enterocolitis. Clin Perinatol 2014;41:967–978.
77. Boccia D, Stolfi I, Lana S, et al. Nosocomial necrotising enterocolitis outbreaks: epidemiology and control measures. Eur J Pediatr 2001;160:385–391.
78. Schullinger JN, Mollitt DL, Vinocur CD, et al. Neonatal necrotizing enterocolitis. survival, management, and complications: a 25-year study. Am J Dis Child 1981; 135:612–614.
79. Tanner SM, Berryhill TF, Ellenburg JL, et al. Pathogenesis of necrotizing enterocolitis: modeling the innate immune response. Am J Pathol 2015;185:4–16.
80. Wroblewski LE, Piazuelo MB, Chaturvedi R, et al. Helicobacter pylori targets cancer-associated apical-junctional

constituents in gastroids and gastric epithelial cells. Gut 2015;64:720–730.

81. Forbester JL, Goulding D, Vallier L, et al. Interaction of salmonella enterica serovar typhimurium with intestinal organoids derived from human induced pluripotent stem cells. Infect Immun 2015;83:2926–2934.
82. Yin Y, Bijvelds M, Dang W, et al. Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. Antiviral Res 2015;123:120–131.
83. Leslie JL, Young VB. A whole new ball game: Stem cell-derived epithelia in the study of host-microbe interactions. Anaerobe 2016;37:25–28.
84. Chaaban H, Stonestreet BS. Intestinal hemodynamics and oxygenation in the perinatal period. Semin Perinatol 2012;36:260–268.
85. Jenny M, Uhl C, Roche C, et al. Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. EMBO J 2002; 21:6338–6347.
86. López-Díaz L, Jain RN, Keeley TM, et al. Intestinal neurogenin 3 directs differentiation of a bipotential secretory progenitor to endocrine cell rather than goblet cell fate. Dev Biol 2007;309:298–305.
87. Wang J, Cortina G, Wu SV, et al. Mutant neurogenin-3 in congenital malabsorptive diarrhea. N Engl J Med 2006; 355:270–280.
88. Ward DV, Scholz M, Zolfo M, et al. Metagenomic sequencing with strain-level resolution implicates uropathogenic E. coli in necrotizing enterocolitis and mortality in preterm infants. Cell Rep 2016;14:2912–2924.
89. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 2015;17:852.
90. Yassour M, Vatanen T, Siljander H, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Sci Transl Med 2016;8:343ra81.
91. Oude Munnink BB, van der Hoek L. Viruses causing gastroenteritis: the known, the new and those beyond. Viruses 2016;8:2.
92. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003; 81:197–204.
93. Estes MK, Kang G, Zeng CQ, et al. Pathogenesis of rotavirus gastroenteritis. Novartis Found Symp 2001; 238:82–100.
94. Finkbeiner SR, Zeng XL, Utama B, et al. Stem cell-derived human intestinal organoids as an infection model for rotaviruses. MBio 2012;3: e00159–12.
95. Saxena K, Blutt SE, Ettayebi K, et al. Human intestinal enteroids: a new model to study human rotavirus infection, host restriction, and pathophysiology. J Virol 2016; 90:43–56.
96. Duizer E, Schwab KJ, Neill FH, et al. Laboratory efforts to cultivate noroviruses. J Gen Virol 2004;85:79–87.
97. Herbst-Kralovetz MM, Radtke AL, Lay MK, et al. Lack of norovirus replication and histo-blood group antigen expression in 3-dimensional intestinal epithelial cells. Emerg Infect Dis 2013;19:431–438.
98. Ha S, Choi IS, Choi C, et al. Infection models of human norovirus: challenges and recent progress. Arch Virol 2016;161:779–788.
99. Jones MK, Watanabe M, Zhu S, et al. Enteric bacteria promote human and mouse norovirus infection of b cells. Science 2014;346:755–759.
100. Deng X, Desai PT, den Bakker HC, et al. Genomic epidemiology of salmonella enterica serotype enteritidis based on population structure of prevalent lineages. Emerg Infect Dis 2014;20:1481–1489.
101. Zhang YG, Wu S, Xia Y, et al. Salmonella-infected crypt-derived intestinal organoid culture system for host-bacterial interactions. Physiol Rep 2014;2:9.
102. Wilson SS, Tocchi A, Holly MK, et al. A small intestinal organoid model of non-invasive enteric pathogen-epithelial cell interactions. Mucosal Immunol 2015; 8:352–361.
103. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol 2009;7:526–536.
104. Lessa FC, Gould CV, McDonald LC. Current status of clostridium difficile infection epidemiology. Clin Infect Dis 2012;55(Suppl 2):S65–S70.
105. McFarland LV, Surawicz CM, Rubin M, et al. Recurrent clostridium difficile disease: epidemiology and clinical characteristics. Infect Control Hosp Epidemiol 1999; 20:43–50.
106. Seekatz AM, Rao AM, Santhosh K, et al. Dynamics of the fecal microbiome in patients with recurrent and nonrecurrent clostridium difficile infection. Genome Med 2016; 8:47.
107. Hall AJ, Curns AT, McDonald LC, et al. The roles of Clostridium difficile and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. Clin Infect Dis 2012;55:216–223.
108. Theriot CM, Koumpouras CC, Carlson PE, et al. Cefoperazone-treated mice as an experimental platform to assess differential virulence of clostridium difficile strains. Gut Microbes 2011;2:326–334.
109. Reeves AE, Theriot CM, Bergin IL, et al. The interplay between microbiome dynamics and pathogen dynamics in a murine model of Clostridium difficile infection. Gut Microbes 2011;2:145–158.
110. Engevik MA, Engevik KA, Yacyshyn MB, et al. Human clostridium difficile infection: inhibition of nHE3 and microbiota profile. Am J Physiol Gastrointest Liver Physiol 2015;308:G497–G509.
111. Theriot CM, Koenigsknecht MJ, Carlson PE Jr, et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. Nat Commun 2014;5:3114.
112. Stokely JN, Niendorf S, Taube S, et al. Prevalence of human norovirus and Clostridium difficile coinfections in adult hospitalized patients. Clin Epidemiol 2016; 8:253–260.
113. Nguyen GC, Kaplan GG, Harris ML, et al. A national survey of the prevalence and impact of clostridium difficile infection among hospitalized inflammatory bowel disease patients. Am J Gastroenterol 2008; 103:1443–1450.

114. Backert S, Neddermann M, Maubach G, et al. Pathogenesis of Helicobacter pylori infection. Helicobacter 2016;21(Suppl 1):19–25.
115. Leja M, Axon A, Brenner H. Epidemiology of helicobacter pylori infection. Helicobacter 2016;21(Suppl 1):3–7.
116. Barker N, Huch M, Kujala P, et al. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 2010; 6:25–36.
117. Karam SM, Leblond CP. Dynamics of epithelial cells in the corpus of the mouse stomach. I. identification of proliferative cell types and pinpointing of the stem cell. Anat Rec 1993;236:259–279.
118. Sigal M, Rothenberg ME, Logan CY, et al. Helicobacter pylori activates and expands lgr5(+) stem cells through direct colonization of the gastric glands. Gastroenterology 2015;148:1392–1404.e21.
119. Huang JY, Sweeney EG, Sigal M, et al. Chemodetection and destruction of host urea allows helicobacter pylori to locate the epithelium. Cell Host Microbe 2015; 18:147–156.
120. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. J Immunol 2005;174:4453–4460.
121. Magalhães A, Rossez Y, Robbe-Masselot C, et al. Muc5ac gastric mucin glycosylation is shaped by fUT2 activity and functionally impacts Helicobacter pylori binding. Sci Rep 2016;6:25575.
122. Bessède E, Dubus P, Mégraud F, et al. Helicobacter pylori infection and stem cells at the origin of gastric cancer. Oncogene 2015;34:2547–2555.
123. Sharon G, Garg N, Debelius J, et al. Specialized metabolites from the microbiome in health and disease. Cell Metab 2014;20:719–730.
124. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–108.
125. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:1241214.
126. Sanderson IR. Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium. J Nutr 2004;134:2450S–2454S.
127. Derrien M, Collado MC, Ben-Amor K, et al. The mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. Appl Environ Microbiol 2008;74:1646–1648.
128. Lukovac S, Belzer C, Pellis L, et al. Differential modulation by Akkermansia muciniphila and faecalibacterium prausnitzii of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. MBio 2014;5.
129. Kaiko GE, Ryu SH, Koues OI, et al. The colonic crypt protects stem cells from microbiota-derived metabolites. Cell 2016;165:1708–1720.
130. Zachos NC, Kovbasnjuk O, Foulke-Abel J, et al. Human enteroids/colonoids and intestinal organoids functionally recapitulate normal intestinal physiology and pathophysiology. J Biol Chem 2016;291:3759–3766.
131. Hamburg MA, Collins FS. The path to personalized medicine. N Engl J Med 2010;363:301–304.

Received October 3, 2016. Accepted November 9, 2016.

Correspondence
Address correspondence to: David R. Hill, PhD, or Jason R. Spence, PhD, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109. e-mail: hilldr@med.umich.edu or spencejr@med.umich.edu; fax: (734) 763-4686.

Conflicts of interest
The authors disclose no conflicts.

Funding
Supported by the Intestinal Stem Cell Consortium (U01DK103141), a collaborative research project funded by the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Allergy and Infectious Diseases, and by the National Institute of Allergy and Infectious Diseases Novel, Alternative Model Systems for Enteric Diseases consortium (U19AI116482) (J.R.S.); also supported by the Mechanisms of Microbial Pathogenesis training grant from the National Institute of Allergy and Infectious Disease (T32AI007528) and the Clinical and Translational Science award to the Michigan Institute for Clinical and Health Research (UL1TR000433) (D.R.H.).