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)

**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.

**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. 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 or as epithelium plus supporting stromal/mesenchymal tissue. 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. Collectively, these organotypic cultures represent a complex system for studying the intestine and commonly are called organoids because they retain organoid-like features, such as the plethora of differentiated epithelial cell types (goblet, Paneth, enteroendocrine, enterocyte).

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. 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. 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).

Human organoids, whether derived from donor tissue or from hPSCs, represent important tools to probe human gastrointestinal biology, physiology, and pathophysiology.
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. Perturbations in this dynamic symbiosis underlie the pathogenesis of inflammatory disease, gastrointestinal cancer, metabolic syndrome, and other conditions. 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 “organo-typic,” a term encompassing both cultured whole-tissue explants and organoids. 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. For example, tissue-derived organoids can be grown with or without mesenchyme, and are ideal for modeling adult homeostasis or disease. On the other hand, hPSC-derived organoids are more similar to immature (fetal) tissue than to adult tissues, but become more adult-like after transplantation into a living mouse host. 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. 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
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. 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. 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. Organoid-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. 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. 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, and established tissue-derived or hPSC-derived organoids can be dissociated enzymatically and reseeded onto Transwell (Corning Inc) monolayer cultures. This approach allows for the study of epithelial barrier dynamics and facilitates efficient exposure of the apical epithelium to experimental treatments, virus, or bacteria. Other researchers have used microinjection techniques to deliver experimental molecules or bacteria to the apical epithelial surface. 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, whereas 3D organoids can be readily passaged and may be better suited for long-term culture.

### 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. | 1,16,36    |
| Tissue-derived, epithelium, mesenchyme | Enteroid | 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|

### 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. 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 successional shaped by both dietary nutrients and the host response. Recent work has suggested a critical period of intestinal development and gut ecologic succession in infancy establishes key features of metabolism, digestion, neurocognitive function, and immune function.
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 United States, with in-hospital mortality occurring in up to 30% of cases. NEC risk increases by 7-fold among premature and low-birth-weight infants, and the prevalence of NEC may be increasing. 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. However, it remains unclear how changes in the microbiota promote healthy gut function or the development of NEC. 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.

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

**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, and recent work has established gastrointestinal organoids as a powerful model of microbial pathogenesis at the mucosal interface. Thus, 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. Clinical reports indicate that a loss-of-function mutation in *NEUROG3* causes congenital malabsorptive diarrhea, a condition in which the intestine lacks enteroendocrine cells. 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. 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 has shown an association between pathogenic traits in individual *E. coli* strains and NEC risk. 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. 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. 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 microbiologically 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. 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.94 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.73

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.96 However, model systems have been developed recently, including patient-derived organoids, which allow for replication of human norovirus in vitro.37,98 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)β, 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 al41 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).105 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.\textsuperscript{104} Outcomes of \textit{C. difficile} acquisition are highly heterogeneous and range from asymptomatic carriage to life-threatening toxic megacolon,\textsuperscript{105} with recurrent infection occurring in a significant subset of patients.\textsuperscript{105,106} In the United States, more than 14,000 patients die of CDI annually,\textsuperscript{107} yet very little is known regarding the molecular pathogenesis of CDI in the human gastrointestinal tract.

Although rodent models of CDI are available,\textsuperscript{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 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.\textsuperscript{51,52} \textit{C. difficile} has been shown to persist within the lumen of hPSC-derived intestinal organoids for at least 12 hours after microinjection.\textsuperscript{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 \textit{C. difficile} toxin A, but not \textit{C. difficile} toxin B (TcdB), or colonization of organoids with a toxin-producing \textit{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.\textsuperscript{51} The role of TcdB in human \textit{C. difficile} infection also was investigated in tissue-derived epithelial colonic monolayer cultures derived from human fetal colonic tissue.\textsuperscript{31} In these cultures, TcdB treatment produced extensive histologic damage, loss of epithelial barrier integrity, and dose-dependent increases in inflammatory genes including \textit{IL1B}, \textit{CXCR4}, and \textit{DUOX2}.\textsuperscript{31}

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

Together, these works show that stem cell–derived human intestinal organoids show physiologically relevant responses to \textit{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 \textit{C. difficile} metabolites,\textsuperscript{111} viral co-infection,\textsuperscript{112} or chronic inflammation\textsuperscript{113} on CDI pathogenesis using organoid systems.

**Helicobacter pylori**

\textit{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.\textsuperscript{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 \textit{H. pylori} at any given time.\textsuperscript{115} Morbidity and mortality associated with \textit{H. pylori} infection occurs as a result of the chronic interplay between \textit{H. pylori} and the gastric mucosa rather than fulminant bacterial infection. \textit{H. pylori} now is appreciated as a major risk factor for peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma.\textsuperscript{114} Experimental models that facilitate a better understanding of \textit{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\textsuperscript{116} in 2010, with methods for culture of primary human gastric epithelium following later.\textsuperscript{5,10} Gastric organoids also can be derived efficiently through differentiation of hPSCs.\textsuperscript{15}

\textit{H. pylori} colonization may persist for many years before the emergence of clinical symptoms or sequelae such as ulcers or gastric carcinoma,\textsuperscript{117} 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 \textit{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.\textsuperscript{117} Tissue-derived gastric organoids can be directed to express markers of the gastric pit or gastric gland epithelium.\textsuperscript{10} \textit{H. pylori} efficiently colonizes the gastric glands, where direct interaction with Lgr5\textsuperscript{+} stem cells enhances proliferation of the epithelial progenitor population. This effect was dependent on bacterial \textit{CagA} expression as well as microbial chemotaxis necessary for colonization of the antral glands.\textsuperscript{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 \textit{H. pylori}.\textsuperscript{119} Remarkably, organoids derived from primary gastric gland epithelium mount a robust inflammatory response to \textit{H. pylori} in comparison with gastric pit organoids,\textsuperscript{10} potentially attributable to restricted expression of Toll-like receptors\textsuperscript{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 signaling molecule in the mammalian epithelium.126 The effects of SCFA-producing bacteria on the indigenous microbiota may reduce disease risk in human hosts,24,27,28 identifying bioactive microbial products and evaluating their potential effects on the host epithelium.

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 propionate 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 propionate 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 propionate 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 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 bacteria11,52,53,80,81,101,102,110,118,119,128,129 and viral challenges.47,49 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.
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. HPSC-derived organoids currently are limited to immature stages. Although maturation is possible by transplantation into the mouse kidney capsule, it is unclear what effect this has on inflammatory and innate defense capabilities. Microbial pathogenesis and commensalism frequently are specific to the level of tissue maturation and anatomic location. 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-throughput multiplexed arrays of organoids will allow for efficient screening and culture of complex microbial communities and metabolites. 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, patient-derived organoids may be useful for determining clinical therapy or prognosis.

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.

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