Current Trends in Microbiology
Vol. 15, 2021

Review

Intestinal organoids as a model for unravelling bacteria-host interactions

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ABSTRACT

Gastrointestinal organoids represent a meaningful advance in structural and functional complexity over underlying *in vitro* cell culture models of the human gastrointestinal epithelium while maintaining much of the genetic and molecular acquiescence that makes *in vitro* experimentation so enchanting. Specific human models for studying the crosstalk between commensal bacteria and host interactions are crucial for better understanding gastrointestinal infections. Gastrointestinal organoids are *ex vivo* models that can be used to explore the various functions of healthy intestines in comparison with pathogen-infected tissues or to examine the effects of colonisation with one of several species of commensal bacteria. A robust human microbiome-gut-brain axis model with a potential to expand our understanding of this complex system and properly explore novel, microbiome-based pathogenesis needs to be developed to imitate the *in vitro* model to carry comprehensive studies of the mechanisms underlaying complex cross-talk within the microbiome-gut-brain axis and functional gastrointestinal disorders. A human epithelial organoid culture can be derived *ex vivo* from intestinal crypts isolated from biopsies and surgical specimens and has been shown to retain the specificity of the intestinal segment of origin. Therefore, the study of gastrointestinal organoids represents an effective strategy for dissecting the mechanisms underlying disease progression and the establishment of a healthy interplay. In this review, we provide an overview highlighting the importance of this model, its applications for the study of various gastrointestinal infections, and approaches for investigating how commensal bacteria contribute to eubiosis.

KEYWORDS: organoids, colonoids, crosstalk, bacterial pathogens, bacterial commensals.

INTRODUCTION

Organoids are three-dimensional cell assemblages derived from stem cells or organ-specific progenitor cells that resemble the organs from which they are derived [1]. Gastrointestinal (GI) organoids retain their molecular and genetic tractability while presenting a degree of functional and structural complexity that make organoids generally advantageous over traditional *in vitro* cell cultures. The first introduced *in vitro* GI model was the Caco-2 cell model [2], which was used to uncover significant findings that enhanced our understanding of intestinal physiology and cancer biology [3]. Similarly, organoids represent a breakthrough in the understanding of both intestinal development and the complex interplay underlying bacteria-host interactions.

Organoids are derived from primary tissues and have the capacity for long-term growth. They contain

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varying levels of cellular complexity and physiological similarity to native organ systems. Human pluripotent stem cell (hPSC) derived intestinal organoids (HIOs) lack some cellular populations found in the native organ, including vasculature. Intestinal stem cells are closely associated with a diverse but poorly characterized network of mesenchymal cell types. Analyses of patient-derived organoids established that PGE (2)-PTGER4 also regulates stem-cell function in human. The resulting three-dimensional intestinal organoids consisted of a polarized, columnar epithelium that was patterned into villus-like structures and crypt-like proliferative zones that expressed intestinal stem cell markers. With the goal of modeling human disease of the large intestine, we sought to develop an effective protocol for deriving colonic organoids (COs) from differentiated human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs). The organoids can be derived from two stem cell sources: induced pluripotent stem cells (iPSCs) and organ-restricted adult stem cells (ASCs) [4-11]. The primary advantage of organoids is their ability to form defined structures that mirror cytoarchitectures (e.g., the villi and crypts of the small intestine). Moreover, organoids can undergo self-renewal and self-organisation for prolonged periods [12-14].

GI organoids have been used in combination with emergent technologies, such as genome editing (clustered regularly interspaced small palindromic repeats [CRISPR]–CRISPR associated [Cas9]) to study how certain cancer-promoting mutations affect cellular signalling [15-18]. Additionally, one study used single-cell RNA sequencing (scRNA-Seq) to uncover detailed transcriptional patterns and monitor how each cell type within the organoid responds to the addition of intestinal bacteria to the organoid culture, which revealed specific pathways that were activated in certain cells [19]. scRNA-seq has also been used to delineate the response of colorectal cancer cells to a first-line cancer drug, which demonstrated heterogeneous cellular behaviour (i.e. chemosensitive and chemotolerant cellular subgroups) and successfully recapitulates clinical heterogeneity [20-22]. The use of organoids, single-cell transcriptomics, and genome editing are likely to have deep impacts on human health, highlighting the importance of using new technologies to improve well-being, particularly for personalised medicine. This review aims to provide a wide perspective on the interactions that have been uncovered in host-bacteria interplay (between pathogenic and commensal species) in studies using intestinal organoids as a platform.

Intestinal organoid development and classification

In 2009, two independent groups achieved the *in vitro* long-term growth of non-transformed, GI-derived tissue for the first time [13, 23]. These breakthroughs laid the basis for propagating 3-dimensional (3D) intestinal tissues in culture, which were termed organoids (Figure 1). Organoids can be grown from two cell sources: i) iPSCs and ii) embryonic stem cells [3]. iPSCs are first allowed to develop into a 3D aggregate, and the induction of specific developmental signals mimicking the *in vivo* process is used to obtain a specific organ. To obtain organoids from embryonic stem cells, specific, fully differentiated stem cells are acquired from the tissue of interest, and growth factors are added to generate organoids, which consist of stem cells and organ-specific cell types. The establishment of organoids from either cell type requires the maintenance of physical and chemical niches that support the long-term growth of intestinal tissue, particularly an extracellular matrix (ECM) or Matrigel (transcriptome-wide analysis revealed hallmarks of human intestine development and maturation *in vitro* and *in vivo*) and growth factors (including epidermal growth factor [EGF], Noggin, and R-spondin-1). When placed in Matrigel in the presence of an adequate concentration of growth factors [7, 12, 13, 24-26], $Lgr5^{+}$ stem cells differentiate and proliferate into 3D organ-like structures, even in the absence of mesenchymal and stromal cells, that can then be differentiated into human intestinal organoids (HIOs). Although these HIOs can perform some transport functions (dipeptide absorption), they are foetal in nature, failing to express specific proteins necessary to mimic the segment specificity of the jejunum, ileum, duodenum, or colon [27]. However, the HIOs' maturation can be achieved when they

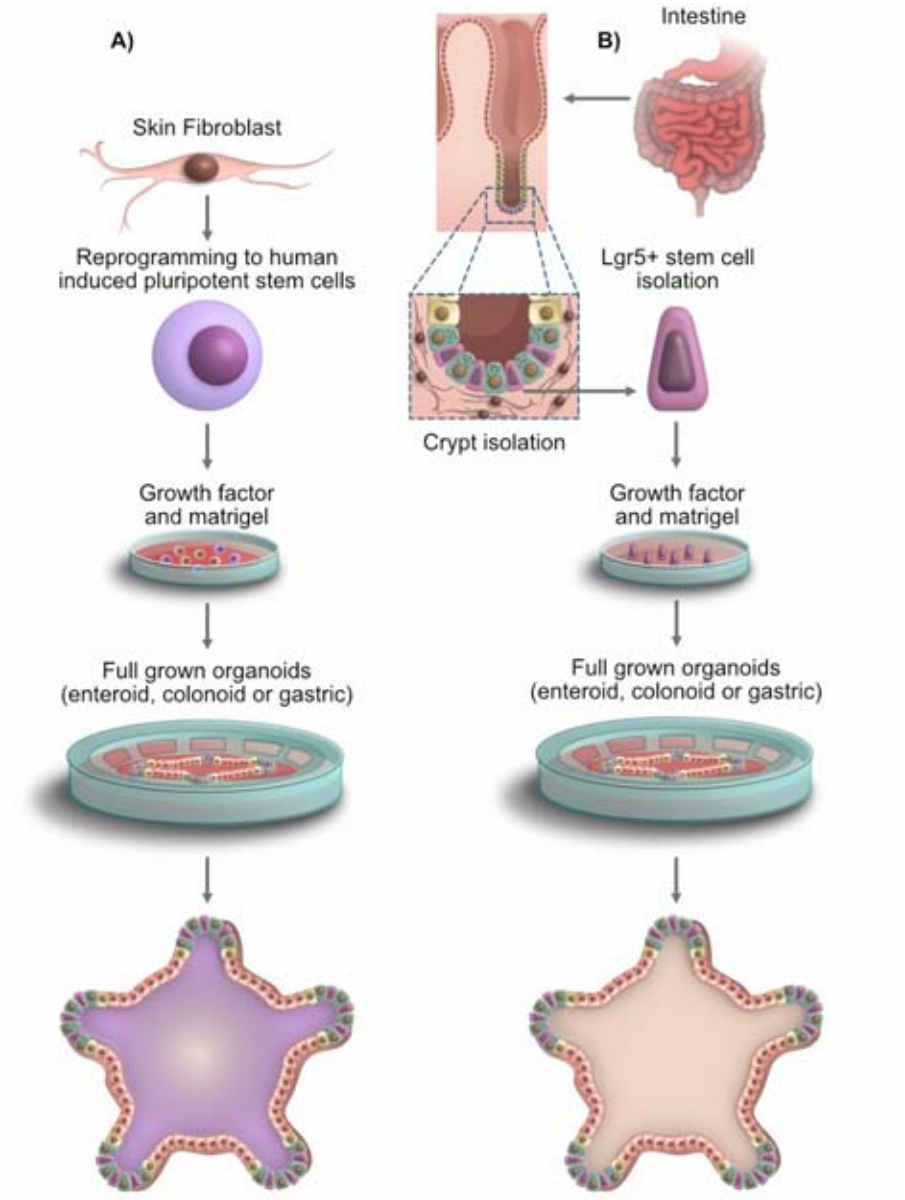

Figure 1. Two different strategies were applied for gastrointestinal organoid development. A) Human intestinal organoids derived from embryonic/pluripotent stem cells were foetal in nature. B) Colonoid/enteroid/gastric organoids were derived from adult stem cells.

are transplanted into mouse kidney capsules, resulting in the development of mature villus compartments and crypts, in addition to adult-like intestinal features [8]. The development of iPSCs into 3D HIOs was pioneered in 2011 [10] through the manipulation of signalling pathways to mimic *in vivo* intestinal development.

Currently, no universal consensus exists regarding the classification of intestinal organoids, despite an effort to systematise the nomenclature [28]. The term "organotypic" has been used to define tissues capable of recapitulating some *in vivo* functions and cellular diversity; however, this term has been applied to both *in vitro* organoid cultures and *ex vivo* whole tissue explants [10, 29, 30]. Several protocols have now been developed for the generation of intestinal organoid cultures, resulting in significant variations. In one approach, organoids are grown from isolated $Lgr5^{+}$ crypt stem cells

obtained from mature tissue using Matrigel to provide artificial niche-specific scaffolding and signalling molecules [13], whereas another approach uses mesenchyme for this purpose [23]. These organoids are not fully comparable due to differences in the provided developmental niches. As previously mentioned, iPSC-derived organoids resemble foetal tissues and should be expected to provide different information in comparison with mature tissue-derived organoids. The term enteroid describes 3D organoids derived from the ileum, duodenum, or jejunum, whereas colonoid describes 3D organoids derived from the distal or proximal colon [26, 31, 32]. Both enteroids and colonoids are composed of epithelial cells that have undergone differentiation and self-organisation into mature subtypes, including Paneth cells, goblet cells, enteroendocrine cells, enterocytes, and tuft cells [13].

Following the establishment of 3D intestinal organoids, 2D organoid monolayers can be generated containing entire differentiated cell populations (enterocytes, Paneth cells, tuft cells, goblet cells, and enteroendocrine cells). The 3D organoid is first dissociated into a single-cell suspension that is plated into porous membranes previously coated with ECM. The cells form a monolayer when in the presence of adequate growth factors. The polarised monolayer then expands, expressing all of the differentiated intestinal cell types [33, 34]. The advantage of 2D monolayer intestinal organoids is easy accessibility to basolateral and apical surfaces, offering an uncomplicated method for studying host-bacteria interactions or metabolite-host interactions using high-throughput approaches [35, 36]. However, an automated microinjection technique was recently developed for the performance of high-throughput assays on 3D organoids, allowing for the study of host-bacteria, host-metabolite, or host-drug interactions (Figure 2). In addition, 3D organoids have been characterised with a decreasing internal oxygen gradient that is suitable for the modelling of strict anaerobic bacteria-host interactions [37, 38].

Bacteria-host interactions uncovered using intestinal organoids as a study platform

One major advantage of GI organoids (either 2D or 3D) is cell differentiation. In addition, organoids can be grown from any cancer or normal intestinal sample and propagated over the long term. This intrinsic feature makes organoids a powerful tool for translational research. Recently, remarkable findings in the field of bacteria-host research have been reported by studies using intestinal organoids as a platform to dissect interactions. Most of these studies focused on the co-cultivation of organoids with specific bacterial species (pathogens or probiotics); however, the use of bacterial metabolites to analyse cellular responses also yielded significant discoveries (Table 1). Recently, a new approach was developed to study whole bacterial communities obtained from stool samples that is capable of delineating interactions. New insights into the mechanisms by which those agents may prevent or trigger diseases significantly widen our knowledge of diet-microbiome-host interactions. This review aims to provide a broad perspective on the interactions that have been uncovered in host-bacteria interplay (pathogenic and commensal) using intestinal organoids as a platform.

Clostridioides difficile

Clostridioides difficile is an obligate anaerobic pathogenic bacterial species that represents the leading cause of nosocomial diarrhoea, leading to 14,000 deaths each year [39]. Leslie *et al*. [40] used HIOs derived from human pluripotent stem cells (hPSCs) to determine the virulence of the *C. difficile* strain VPI 10463 using a nontoxigenic *C. difficile* strain as a control. Using a microinjection technique, the authors colonised the HIO lumen with viable vegetative *C. difficile* VPI 10463, which disrupted the organoid epithelium. By contrast, the nontoxigenic *C. difficile* strain did not affect the organoid. To further validate the model for this specific bacterium, purified *C. difficile* toxin A (TcdA) and TcdB were injected into the organoid to analyse their effects. Only TcdA recapitulated the damage observed following the injection of the VPI 10463 strain. This study was the first report in which HIOs were successfully colonised with anaerobic pathogens, paving the way for future detailed studies and highlighting how organoids can be used to untangle the specific contributions of toxins to bacterial pathogenesis. One breakthrough using enteroid/colonoid models to dissect pathogen-host interactions was the identification of frizzled proteins as receptors for TcdB. Using an elegant

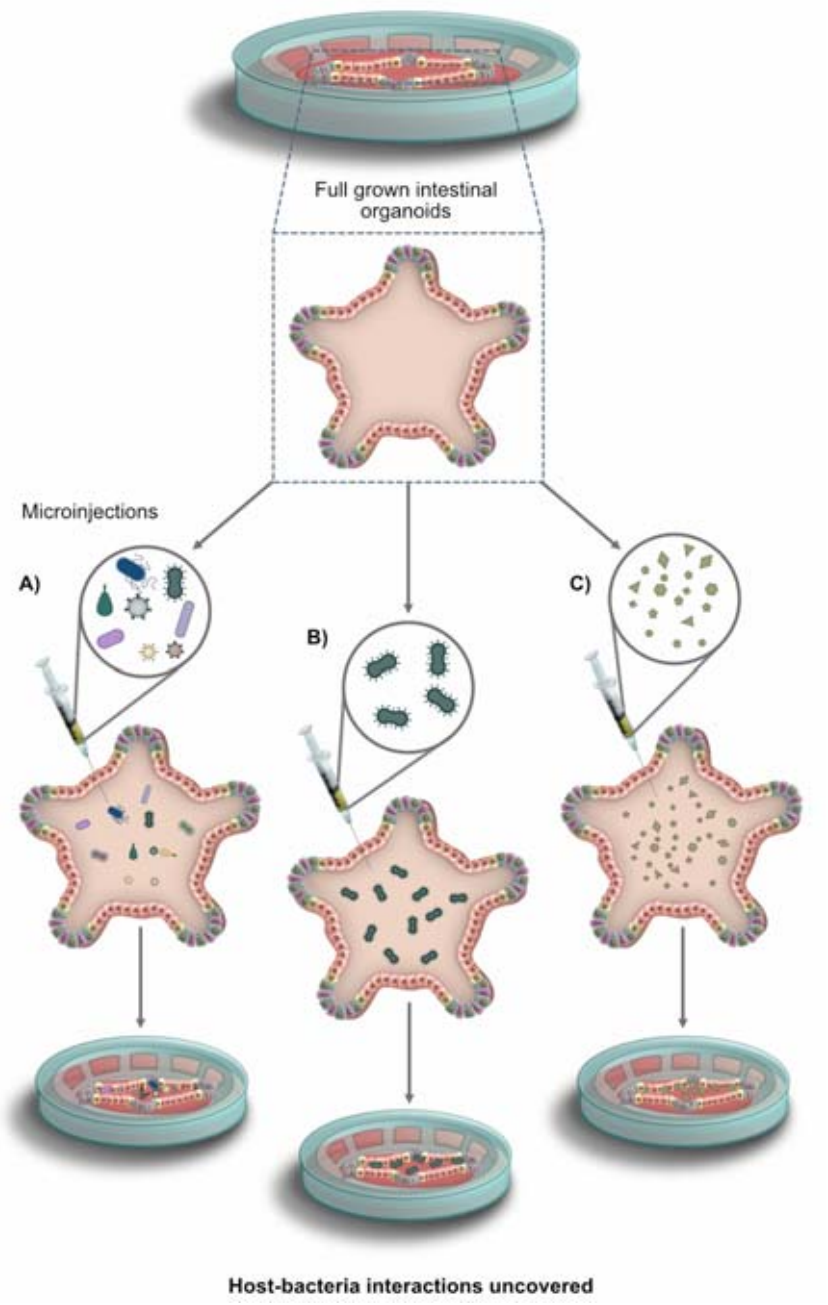

Figure 2. Three approaches were applied to study bacteria-host interactions. A) Complex bacterial communities filtered from faecal samples were injected into organoids. B) A single bacterial species obtained from culture was injected into organoids. C) Well-established bacteria-produced metabolites were injected either individually or collectively into organoids. In all three scenarios, after incubation, organoids were harvested to further characterise interactions.

methodology to dissect the mechanism through which TcdB binds to the epithelium, Tao *et al*. [41] applied CRISPR-Ca9 at the whole-genome level and identified the Wnt receptor frizzle family receptors (FZDs) 1, 2 and 7 as high-affinity TcdB receptors in the colonic epithelium. Using colonoids isolated from an *FZD7*-knockout mouse strain (in addition to the knockdown of *FZD1* and *FZD2*),

Table 1. Significant bacteria-host interactions using intestinal organoids.

Clostridioides difficile	• Frizzled proteins identified as enterocyte receptors for the establishment of Toxin B virulence factor [41]. • Mucin 1 (MUC1) detected as the major component of acidic mucus [42]. • NHE3 (Na^+/H^+ exchanger) is decreased during infection, providing a favourable environment for infection [43].
Salmonella	• Antimicrobial peptides produced by Paneth cells effectively restrict the bacterial growth of *S. enterica* ser. Typhimurium [48]. • The early infection mechanism for *S. typhi* differs substantially from that of *S. enterica* ser. Typhimurium [52]. • VexD emerged as a potential vaccine candidate for *S. typhi* [52].
Escherichia coli	• The earliest targets of enterohemorrhagic *E. coli* (EHEC) are MUC2 and protocadherin 24 (PCDH24), which are tackled sequentially [54]. • Macrophage-enteroid co-cultures showed a significant increase in macrophage projections when exposed to enteropathogenic *E. coli* (EPEC). When macrophage-enteroid co-cultures were exposed to enterotoxigenic *E. coli* (ETEC), the bacteria attached to the apical side of the enteroid monolayer and macrophages physically interacted with the bacteria on the apical surface [55].
Helicobacter pylori	• CagA virulence factor associates with the c-Met receptor shortly after exposure to the culture [58]. • Long-term cultures showed that sheathed flagella did not induce a host response, which confirmed that this characteristic was crucial to initiating cell colonisation [60].
Lactobacillus rhamnosus	• Gut permeability was maintained when faecal supernatants were incubated with *L. rhamnosus* in enteroids, demonstrating the mechanism through which the probiotic can alleviate some irritable bowel syndrome (IBS) symptoms [67].
Akkermansia muciniphila	• *Hdac3* and *Hdac5* were upregulated after the injection of *A. muciniphila* supernatants into enteroids, whereas *Fiaf*, *Gpr43*, and *Pparg* were downregulated [71].
Short-Chain Fatty Acids (SCFAs)	• Butyrate, propionate, and acetate were independently and collectively injected into ileal organoids. Acetate upregulated *Hdac3* and *Hdac5*, whereas butyrate upregulated *Fiaf*, *Hdac3*, and *Hdac5*, and propionate upregulated *Fiaf*, *Hdac3*, and *Hdac5*. This suggested that SCFAs are sufficient to promote changes in specific genes involved in the cell cycle [71].

the authors demonstrated increased resistance to TcdB injection compared with wild-type (WT) mouse colonoids. The epithelium of the *FDZ7*-knockout mouse strain demonstrated decreased *in vivo* damage when challenged with a toxigenic *C. difficile* strain, further indicating the contributions of these receptors to the establishment of TcdB as a virulence factor [41]. In a different approach, the mucus secreted by patients with *C. difficile* infections was analysed [42], and mucin 1 (MUC1) protein was identified as the major component in the acidic mucus. Patients infected with *C. difficile* also demonstrated reduced MUC2 expression, which suggested a defect in the mucus barrier. To further analyse this issue, HIOs were injected with *C. difficile*, which was sufficient to decrease MUC production but not sufficient to alter the mucus oligosaccharide composition. The co-injection of *C. difficile* together with a stool supernatant obtained from an infected patient was able to mimic the physiological changes observed in biopsy samples obtained from infected patients, which suggested the participation of yet another factor in the observed changes [42]. To further

investigate this phenomenon, the working hypothesis focused on the effects of Na^+/H^+ exchanger 3 (NHE3), which can be functionally inhibited by TcdB. An *NHE3*-knockout mouse strain showed an altered intestinal environment and dysbiosis. HIOs injected with *C. difficile* and stool supernatants from infected patients demonstrated reduced NHE3 mRNA and protein levels compared with those injected with *C. difficile* and supernatants from healthy subjects. Combined with the observation that *C. difficile* grows optimally *in vitro* under conditions of high Na^+ concentrations and alkaline pH, these findings suggested that *C. difficile* inhibits NHE3 *in vivo*, generating a favourable environment for infection [43]. In further support of this hypothesis, NHE3 protein and mRNA concentrations were reduced in biopsies obtained from *C. difficile* infection patients compared with healthy tissues. Using mouse and human colonoids, a process involving TcdB was shown to be involved in the *C. difficile*-induced disruption of the epithelium, affecting the cellular organisation. Moreover, the ability to repair the injured epithelium is diminished during the intoxication process due to an altered colonic $LGR5^+$ stem cell population and WNT-dependent signalling [44]. Evidence indicated that α-defensin-1 neutralises bacterial toxins, including TcdB, as demonstrated by the combined treatment of human HIOs with TcdB and α-defensin-1; α-definsin1 protected not only against TcdB alone but also against the combination of TcdA, TcdB, and *C. difficile* binary toxin (CDT), which mimics the combination of hypervirulent *C. difficile* strains. [45]. In co-culture experiments using human HIOs, a remarkable bacterial synergy between *C. difficile* and *Fusobacterium nucleatum* was described. *F. nucleatum* is primarily localised in the oral microbiota, but sequencing evidence indicated that patients with *C. difficile* infections also have an increased *F. nucleatum* population in the gut. When the two genera were grown together *in vitro,* they formed intimate aggregates through specific interactions due to the binding of the *F. nucleatum* protein RadD with *C. difficile* flagella, and the interaction can be inhibited through RadD deletion or the use of a *C. difficile* strain lacking flagella. This synergy also results in the formation of biofilms. Several strains of both species were used, and the results were consistent, providing outstanding evidence in a vastly unexplored field to support that the interactions between bacterial species within the microbiota community contribute to infection [46].

Salmonella

One of the first studies to characterise the interaction between *Salmonella* and host cells using a 3D system was reported by Radtke *et al*. [47]. Although the 3D epithelium model used by Radtke *et al*. [47] differs substantially from enteroid/colonoid models, they replicated previously reported *in vivo* findings. Mutants from different *Salmonella* pathogenicity islands (SPIs) belonging to the type III secretion system (T3SS) were used to evaluate their roles in epithelial cell invasion. SPI-1, SPI-2, and *flhCD* mutants, both alone and in combinations (SPI-1/SPI-2; SP1-1/SPI-2/*flhCD*), were used to demonstrate that these proteins were not necessary for the invasion of 3D epithelial cells, as none of these strains was associated with a significant decrease in invasive ability. By contrast, the individual and combined mutations reduced the capacity for intracellular replication.

Wilson *et al*. [48] developed a microinjection methodology to study host-pathogen interactions. They injected several noninvasive *Salmonella enterica* serovar Typhimurium strains into mouse ileal enteroids and evaluated whether the production of antimicrobial α-defensin peptides by the organoid's Paneth cells could disrupt bacterial growth. They developed enteroids from a WT mouse and an *Mmp7*$^{-/-}$strain, which lacks functional α-defensins in the small intestine. When challenged with *S. enterica* serovar Typhimurium, WT enteroids showed restricted bacterial growth for at least 20 hrs in culture; however, this phenomenon was significantly attenuated in colonoids derived from *Mmp7*$^{-/-}$ mice. However, when human defensin 5 was expressed transgenically in the *Mmp7*$^{-/-}$-derived enteroids, bacterial suppression was restored, which demonstrated that enteroids are responsive to host factors that influence Paneth cell function.

Using mouse ileum and jejunum crypt-derived enteroids, Zhang *et al*. [49] demonstrated that

Salmonella disrupted epithelial tight junctions in infected organoids. The inflammatory response in the organoids was measured, revealing the activation of the nuclear factor (NF)-κB pathway. Interestingly, stem cell markers, such as Lgr5 and Bm1 fused with GFP, decreased significantly when the organoids were infected by *Salmonella*. In a different study, a *Salmonella* strain featuring a mutation in *invA,* which is a crucial gene involved in *Salmonella* invasion, was injected into HIOs for 90 min using a WT *Salmonella* strain as a control [50]. After determining the colony-forming units (CFU)/mL in HIO cells, the invasiveness of the WT strain was found to be as high as 30-fold that of the *invA*-mutant strain. No *invA*-mutant *Salmonella* cells were detected inside the enteroids, which confirmed the pivotal role played by this protein in the internalisation and invasion of host cells. In comparison, the WT strain was localised in the vacuoles of the cells [50], which was similar to descriptions from other models [51].

In a compelling study, *Salmonella typhi* colonisation was evaluated using HIOs obtained from biopsies to uncover the underlying mechanisms involved in the early stages of this infection, which revealed significant mechanistic strategies [52]. Surprisingly, prior to this research, no previous studies had focused on the early stages of *S. typhi* infection, which was generally assumed to utilise similar infection mechanisms as those used by *S. enterica* serovar Typhimurium, despite differences in the incubation periods, symptoms, and genetic divergence between these two strains. Enteric biopsies and HIOs were infected with *S. typhi,* and transcriptomic analyses were performed, identifying the downregulation of 57 genes involved in B cell receptor signalling, innate and adaptive immune responses, and cell signalling. Monolayer HIOs infected with *S. typhi* revealed a role for the cytoskeleton in the infection process, and the addition of a microtubule inhibitor prior to infection prevented invasion compared with untreated controls [52]. VexD, a polysaccharide that serves as an integral component of the outer wall, was proposed as a candidate for vaccine development because it was the only upregulated gene identified in ileal biopsies during early infection [52]. The importance of caspases during *Salmonella typhimurium* was evaluated in mouse and human colonoids using untransformed and transformed cells. Both models were used because whether the observed differences between mice and humans could be attributed to difficulties using *in vivo* approaches or transformed cells was unclear. The data showed that caspase-1 plays a prominent role in restricting intracellular replication in mouse but not in human cells. In addition, caspase-4 restricts *S. Typhimurium* replication and interleukin (IL)-18 production in both untransformed and transformed cells [53].

Escherichia coli

The development of colonoids in a monolayer was developed by In *et al*. [54], which provided a new pathophysiological colonic model for the study of host-pathogen interactions. Using the isolation of adult proximal colonic crypts from human biopsies, an approach similar to obtaining small intestinal enteroids was developed, allowing for the establishment of polarised monolayer colonoids. Enterohemorrhagic *Escherichia coli* (EHEC) was used to validate the model, leading to the description of the earliest host targets used by this bacterium to promote infection. Extracellular mucin 2 (MUC2) and protocadherin 24 (PCDH24) are targeted sequentially, which promotes attachment to the epithelium, triggering the typical attachment and effacement lesions associated with EHEC. The development of organoid models to characterise host-pathogen interactions continued with the introduction of increasingly sophisticated approaches. One limitation to the use of intestinal organoid models is the absence of immune signalling in organoids, which plays a pivotal role in infection regulation. Noel *et al*. [55] resolved part of this problem by performing the co-cultivation of macrophages with enteroids to dissect the fine interactions between bacterial pathogens. Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) were studied for their effects on macrophage-enteroid by co-cultivation in the absence or presence of macrophages. Appropriate controls were used to demonstrate that both enteroids and macrophages developed in a normal fashion. Following the addition of EPEC, the macrophage projections increased significantly in response to the infection, and adherent macrophages increased compared with uninfected co-cultures.

These findings suggested that microbial exposure was responsible for this observation. ETEC attached to the apical side of the enteroid monolayer, and macrophages physically interacted with the bacteria on the apical surface. The presence of macrophages in the co-culture caused a reduction in live bacteria recovery after 16 h exposure compared with cultures containing enteroids alone, indicating a biological role for macrophages in the infection process.

In a thorough report, the effects of Shiga toxin (Stx) secreted by EHEC were evaluated in HIOs derived from iPSCs. Mesenchymal and epithelial cells underwent necrotic and apoptotic cell death, attributed to changes in transcription and cellular proliferation. Surprisingly, the epithelial barrier was resistant to Stx for up to 48 h after infection, which indicated a potential avenue for future therapeutic interventions [56]. In a different approach, strains isolated from cancer patients with EPEC-associated diarrhoea were able to colonise HIOs with novel adherence patterns. However, the authors clarified that not all patients with diarrhoea were EPEC-positive, as assessed by either quantitative polymerase chain reaction (qPCR, 70%), or cultivation (46%), suggesting that other important enteropathogens likely contribute to disease development, which can delay cancer care or decrease defined doses of the chemotherapeutic agent [57].

Helicobacter pylori

One of the most studied pathogens using intestinal organoids is *Helicobacter pylori,* which causes chronic infection and is estimated to cause 10% of all peptic ulcer disease and gastric cancers globally. During the pioneering studies that led to the development of human gastric organoids (hGOs), McCracken *et al*. [58] identified that the virulence factor CagA was rapidly associated with the c-Met receptor following the inoculation of *H. pylori* into hGOs. Two years after the development of hGOs, Schlaermann *et al*. [59] grew 3D spheroids derived from several locations in the human stomach; after shearing the spheroids, a polarised 2D epithelial gastric cell model was established. When the 2D model was infected with *H. pylori* for 22 h, the characteristic "hummingbird" phenotype (cells switch from a uniform polygonal shape into an elongated state characterised by the formation of needle-like structures) was observed, mediated by the translocation of CagA into host cells. Using microarrays to investigate the global response to *H. pylori*, a strong upregulation was observed for IL-8, which is a member of the NF-κB signalling pathway. Other upregulated genes included tumour necrosis factor (TNF) targets, which are involved in the inflammatory response to lipopolysaccharide (LPS). Since the initial conditions for growing *in vitro* gastric organoids were initially established, a long-term (>1 year) culture methodology has been developed [60]. This long-term model allowed for the i*n vitro* simulation of a fundamental *H. pylori* feature: chronic infection. Three stimuli previously shown to trigger a response in the host were injected into the organoid: LPS, flagellin, and bacterial DNA. Interestingly, gastric organoids did not exhibit any alterations in response to purified LPS or DNA. However, the organoids displayed a strong IL-8 upregulation in response to purified flagellin or the positive controls TNF-α and IL-1β. To further dissect this response, non-flagellated mutants were injected into the organoids, which continued to induce IL-8 expression [60]. *H. pylori* typically produce three to seven sheathed flagella (i.e., covered with a membrane composed of many different proteins) [61, 62], which several studies have proposed to serve as a mechanism that was developed to evade the host immune response [63]. Therefore, a slight immune response in the presence of non-flagellated mutants was expected because several proteins found in the flagellum are also present on the bacterial external wall. Earlier studies demonstrated that WT bacteria could colonise gastric glands, whereas mutants carrying deletions in the chemotactic system only colonise the surface mucus [64]. The injection of CagA in cell cultures triggers an increase in cell motility, the loss of polarity and adhesion. Unbiased interactome evidence showed that apoptosis-stimulating of p53 protein 2 (ASPP2) was a prominent target of CagA. The interaction was delineated using gastric organoids, using a peptide as an inhibitor, which disrupted the interaction, preventing the loss of cell polarity. A receptor tyrosine kinase (RTK)/phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signalling pathway was found to be involved in the

recruitment of ASPP2 by CagA, providing mechanistic insights into mechanisms underlying *H. pylori* infection [65].

Commensal bacteria-host interactions using organoids as a model

Most of the interactions uncovered between the host and commensal microbiota have focused on probiotic bacterial strains or their beneficial metabolites, such as short-chain fatty acids (SCFAs). An interesting example, provided by Kitamura *et al*. showed that carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 1 and CEACAM 20 (both members of the immunoglobulin superfamily) have an increased expression, likely activated by commensal bacteria. They hypothesised that a metabolite (SCFA) produced by commensal bacteria might be responsible for this effect. Enteroids were used to dissect the individual and combined effects of the injection of three SCFAs, butyrate, acetate, and propionate, into enteroids, which revealed that butyrate alone was responsible for an increase in the observed *Ceacam20* mRNA and CEACAM20 protein levels. None of the combined or individual SCFAs altered the expression level of CEACAM1, which suggested the direct participation of butyrate in the regulation of CEACAM20 expression in intestinal epithelial cells, likely mediated by the effects of butyrate on histone deacetylases (HDACs) [66]. *Lactobacillus rhamnosus* is one of the most widely used probiotics, associated with the alleviation of symptoms caused by irritable bowel syndrome (IBS), although the mechanism remained unclear until a recent study by Han *et al*. [67]. In that study, colonoids and enteroids were co-injected with faecal supernatants obtained from IBS patients and *L. rhamnosus* GG to unravel the interactions that promote a beneficial effect; faecal supernatants obtained from healthy subjects were used as a control. Gut permeability, as measured by fluorescein isothiocyanate (FITC)-Dextran (FD4) flux through the epithelium, revealed that those colonoids incubated with faecal supernatants derived from patients with IBS patients and *L. rhamnosus* GG were unaffected, whereas colonoids incubated with only the IBS-derived supernatant displayed increased paracellular permeability. The protective effect of *L. rhamnosus* GG was not observed when cell wall extracts, DNA, or denatured bacteria were used, which indicate that this process might involve the secretion of a beneficial metabolite from the bacteria into the extracellular milieu [67].

Faecalibacterium prausnitzii is a major butyrate-producing bacteria found in the human gut [68], and its absence has been associated with Crohn's Disease [69]. Furthermore, the presence of *Akkermansia muciniphila* has been inversely associated with obesity, diabetes, inflammation, and metabolic disorders, and one of its primary metabolites is propionate [70]. Using mouse ileal enteroids, Lukovac *et al*. [71] investigated the roles played by these bacteria and their corresponding SCFAs in host epithelial gene expression. Supernatants from single cultures of *F. prausnitzii* and *A. muciniphila* were injected into ileal enteroids, and the expression of five genes involved in cell cycle control was evaluated: *Fiaf*, *Gpr43*, *Hdac3*, *Hdac5*, and *Pparg*. *A. muciniphila* had the strongest impact on the host response, upregulating the expression of *Hdac3* and *Hdac5* and downregulating *Fiaf*, *Gpr43*, and *Pparg*. *F. prausnitzii* supernatants had no significant effects on the expression of the five genes analysed in this study. The expression levels of these host genes were also affected by the application of butyrate, acetate, or propionate, independently, as a single stimulus for the enteroids. Acetate upregulated *Hdac3* and *Hdac5*, whereas butyrate upregulated *Fiaf*, *Hdac3*, and *Hdac5* but downregulated *Gpr43* and *Pparg*. By contrast, propionate upregulated *Fiaf*, *Hdac3*, and *Hdac5* and downregulated *Gpr43* and *Pparg*. These results indicated that SCFA stimulation was sufficient to promote the expression of specific genes involved in the cell cycle. Interestingly, supernatants from *F. prausnitzii* were not sufficient to affect the expression of the analysed genes, which was likely due to high degrees of genetic variation among strains [72], as previous studies have reported that butyrate production by some *F. prausnitzii* strains can reach up to 40 mM [73].

E. coli Nissle is used primarily as a probiotic, but genetic evidence indicates a close relationship with uropathogenic *E. coli* (UPEC) strain CFT073. *E. coli* Nissle has been used as a therapeutic agent and has been shown to confer

protection against EHEC and UPEC. To discern whether *E. coli* Nissle protects against EHEC and UPEC, the co-cultivation with HIOs was performed. *E. coli* Nissle was not able to replicate inside HIO cells, unlike EHEC and UPEC, which also destroyed the epithelial barrier. After incubating HIOs with *E. coli* Nissle for 18 and 24 h, a challenge assay was performed using either EHEC or UPEC. Preincubation with *E. coli* Nissle inhibited apoptosis, the loss of epithelial barrier functions, and the loss of E-cadherin expression. Taken together, these results indicate that despite the close relationship with UPEC, *E. coli* Nissle is non-pathogenic and is able to confer protection against infections with EHEC and UPEC. *E. coli* Nissle appears to have higher fitness, outcompeting EHEC and UPEC due to highly effective intestinal colonisation, likely due to its intestinal adherence mechanisms and multiple iron acquisition systems. However, *E. coli* Nissle is prone to phage infections, and these lysogens can produce Shx. Thus, the wide use of *E. coli* Nissle as a probiotic and therapeutic agent could result in unwanted adverse effects [74].

A newly developed model is the "gut-on-a-chip" device, which was engineered to simulate the intestinal epithelial tissue, including peristalsis-like motion, providing a niche for the generation of a stable ecosystem that can be used to track interactions for longer than one week. Envisioned by Kim *et al*. [75], this microdevice was tested using a therapeutic probiotic mixture (VSL#3) co-cultured with enteroinvasive *E. coli* (EIEC). After one week of co-cultivation, a 50% increase in transepithelial electrical resistance (TEER; a parameter to measure intestinal barrier function) was observed compared with a control without VSL#3 colonisation. While the probiotic mixture was unable to completely prevent EIEC infection, the onset of intestinal injury was delayed by 18 h, which is similar to clinical findings reported for patients with ulcerative colitis during the early phase of the disease [75].

CONCLUSIONS

Intestinal organoids are culture systems that can self-organise and differentiate, typically derived from either mature intestinal stem cells or pluripotent stem cells. Intestinal organoids can be isolated from any patient, allowing for the development of model systems that reflect the unique genetic information of each individual, facilitating the development of personalised medicine and the testing of drugs or bacterial probiotic combinations that will work for each particular individual. Many avenues can be explore using organoids as a platform for the study of host-bacteria interaction, as existing studies have been limited to the explorations of interactions between the host and individual bacterial species or known purified metabolites. When combined with transcriptomics, approaches involving complex populations – either known and defined or complex (faecal samples) – will provide valuable information that can be used to delineate the interactions and model-specific bacterial diseases. Thus, these organoids may be invaluable for monitoring the progress of dysbiosis during certain diseases (e.g. Crohn's disease, IBS) and determining how *C. difficile* infections are established. The complex mechanisms underlying the transformation of these interactions into dysbiosis can be explored using various microbial combinations to challenge healthy organoids and observe interactions with healthy faecal microbiota. Prebiotics and probiotics are widely used to ameliorate minor diseases and promote a healthier gut. Organoids may be the key to dissecting how these beneficial bacteria interact with eubiotic or dysbiotic communities, which opens many possibilities for testing and improving combinations that offer a major host response. As most of the countless interactions that occur within faecal bacterial communities remain unknown, organoids represent a promising vehicle for observing and describing these interactions within an ecosystem that closely represents a physiological gut.

ACKNOWLEDGEMENTS

S. Fabela is a postdoctoral fellow supported by Dirección General de Apoyo al Personal Académico (DGAPA), UNAM. Funded by DGAPA-PAPIIT-UNAM IT202020 and Facultad de Medicina, UNAM.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Attayek, P. J., Ahmad, A. A., Wang, Y., Williamson, I., Sims, C. E., Magness, S. T. and Allbritton, N. L. 2016, PloS one, 11(4), e0153795.
2. Artursson, P. E. R. 1990, Journal of pharmaceutical sciences, 79(6), 476-482.
3. In, J. G., Foulke-Abel, J., Estes, M. K., Zachos, N. C., Kovbasnjuk, O. and Donowitz, M. 2016, Nature reviews Gastroenterology & hepatology, 13(11), 633-642.
4. McCracken, K. W., Howell, J. C., Wells, J. M. and Spence, J. R. 2011, Nature protocols, 6(12), 1920-1928.
5. Middendorp, S., Schneeberger, K., Wiegerinck, C. L., Mokry, M., Akkerman, R. D., van Wijngaarden, S., Clevers, H. and Nieuwenhuis, E. E. 2014, Stem cells, 32(5), 1083-1091.
6. Date, S. and Sato, T. 2015, Annual Review of Cell and Developmental Biology, 31, 269-289.
7. Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., van Es, J. H., van Den Brink, S., van Houdt, W. J., Pronk, A., van Gorp, J., Siersema, P. D. and Clevers, H. 2011, Gastroenterology, 141(5), 1762-1772.
8. Watson, C. L., Mahe, M. M., Múnera, J., Howell, J. C., Sundaram, N., Poling, H. M., Schweitzer, J. I., Vallance, J. E., Mayhew, C. N., Sun, Y. and Grabowski, G. 2014, Nature medicine, 20(11), 1310-1314.
9. Tsai, Y. H., Czerwinski, M., Wu, A., Dame, M. K., Attili, D., Hill, E., Colacino, J. A., Nowacki, L. M., Shroyer, N. F., Higgins, P. D. and Kao, J. Y. 2018, Cellular and molecular gastroenterology and hepatology, 6(2), 218-222.
10. Spence, J. R., Mayhew, C. N., Rankin, S. A., Kuhar, M. F., Vallance, J. E., Tolle, K., Hoskins, E. E., Kalinichenko, V. V., Wells, S. I., Zorn, A. M. and Shroyer, N. F. 2011, Nature, 470(7332), 105-109.
11. Aurora, M. and Spence, J. R. 2016, Developmental biology, 420(2), 230-238.
12. Sato, T., Van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., Van Den Born, M., Barker, N., Shroyer, N. F., Van De Wetering, M. and Clevers, H. 2011, Nature, 469(7330), 415-418.
13. Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., Van Es, J. H., Abo, A., Kujala, P., Peters, P. J. and Clevers, H. 2009, Nature, 459(7244), 262-265.
14. Sugimoto, S. and Sato, T. 2017, In 3D Cell Culture, Humana Press, New York, NY, 97-105
15. Drost, J., Van Boxtel, R., Blokzijl, F., Mizutani, T., Sasaki, N., Sasselli, V., de Ligt, J., Behjati, S., Grolleman, J. E., van Wezel, T. and Nik-Zainal, S. 2017, Science, 358(6360), 234-238.
16. Drost, J., Van Jaarsveld, R. H., Ponsioen, B., Zimberlin, C., Van Boxtel, R., Buijs, A., Sachs, N., Overmeer, R. M., Offerhaus, G. J., Begthel, H. and Korving, J. 2015, Nature, 521(7550), 43-47.
17. Schwank, G., Koo, B. K., Sasselli, V., Dekkers, J. F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C. K. and Nieuwenhuis, E. E. 2013, Cell stem cell, 13(6), 653-658.
18. Matano, M., Date, S., Shimokawa, M., Takano, A., Fujii, M., Ohta, Y., Watanabe, T., Kanai, T. and Sato, T. 2015, Nature medicine, 21(3), 256-262.
19. Haber, A. L., Biton, M., Rogel, N., Herbst, R. H., Shekhar, K., Smillie, C., Burgin, G., Delorey, T. M., Howitt, M. R., Katz, Y. and Tirosh, I. 2017, Nature, 551(7680), 333-339.
20. Chen, J., Lau, B. T., Andor, N., Grimes, S. M., Handy, C., Wood-Bouwens, C. and Ji, H. P. 2019, Scientific reports, 9(1), 1-12.
21. Chen, K. Y., Srinivasan, T., Lin, C., Tung, K.L., Gao, Z., Hsu, D. S., Lipkin, S. M. and Shen, X. 2018, July. In 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), IEEE, 2378-2381.
22. Brazovskaja, A., Treutlein, B. and Camp, J. G. 2019, Current opinion in biotechnology, 55, 167-171.
23. Ootani, A., Li, X., Sangiorgi, E., Ho, Q. T., Ueno, H., Toda, S., Sugihara, H., Fujimoto, K., Weissman, I. L., Capecchi, M. R. and Kuo, C. J. 2009, Nature medicine, 15(6), 701-706.
24. Sato, T. and Clevers, H. 2012, In Epithelial cell culture protocols, Humana Press, Totowa, NJ, 319-328.

25. Foulke-Abel, J., In, J., Kovbasnjuk, O., Zachos, N. C., Ettayebi, K., Blutt, S. E., Hyser, J. M., Zeng, X. L., Crawford, S. E., Broughman, J. R. and Estes, M. K. 2014, Experimental biology and medicine, 239(9), 1124-1134.
26. Zachos, N. C., Kovbasnjuk, O., Foulke-Abel, J., In, J., Blutt, S.E., De Jonge, H. R., Estes, M. K. and Donowitz, M. 2016, Journal of Biological Chemistry, 291(8), 3759-3766.
27. Finkbeiner, S. R., Hill, D. R., Altheim, C. H., Dedhia, P. H., Taylor, M. J., Tsai, Y. H., Chin, A. M., Mahe, M. M., Watson, C. L., Freeman, J. J. and Nattiv, R. 2015, Stem cell reports, 4(6), 1140-1155.
28. Stelzner, M., Helmrath, M., Dunn, J. C., Henning, S. J., Houchen, C. W., Kuo, C., Lynch, J., Li, L., Magness, S. T., Martin, M. G. and Wong, M. H. 2012, American Journal of Physiology-Gastrointestinal and Liver Physiology, 302(12), G1359-G1363.
29. Dedhia, P. H., Bertaux-Skeirik, N., Zavros, Y. and Spence, J. R. 2016, Gastroenterology, 150(5), 1098-1112.
30. Wang, X., Yamamoto, Y., Wilson, L.H., Zhang, T., Howitt, B.E., Farrow, M.A., Kern, F., Ning, G., Hong, Y., Khor, C. C. and Chevalier, B. 2015, Nature, 522(7555), 173-178.
31. Ranganathan, S., Doucet, M., Grassel, C. L., Delaine-Elias, B., Zachos, N. C. and Barry, E. M. 2019, Infection and immunity, 87(4), e00740-18.
32. In, J. G., Foulke-Abel, J., Clarke, E. and Kovbasnjuk, O. 2019, JoVE (Journal of Visualized Experiments), 146, e59357.
33. Moon, C., VanDussen, K. L., Miyoshi, H. and Stappenbeck, T. S. 2014, Mucosal immunology, 7(4), 818-828.
34. Scott, A., Rouch, J. D., Jabaji, Z., Khalil, H. A., Solorzano, S., Lewis, M., Martín, M. G., Stelzner, M. G. and Dunn, J. C. 2016, Journal of pediatric surgery, 51(6), 995-1000.
35. Bartfeld, S. 2016, Developmental biology, 420(2), 262-270.
36. Moorefield, E.C., Blue, R.E., Quinney, N.L., Gentzsch, M. and Ding, S. 2018, BMC cell biology, 19(1), 1-11.
37. Gracz, A. D., Williamson, I. A., Roche, K. C., Johnston, M. J., Wang, F., Wang, Y., Attayek, P. J., Balowski, J., Liu, X. F., Laurenza, R. J. and Gaynor, L. T. 2015, Nature cell biology, 17(3), 340-349.
38. Williamson, I. A., Arnold, J. W., Samsa, L. A., Gaynor, L., DiSalvo, M., Cocchiaro, J. L., Carroll, I., Azcarate-Peril, M. A., Rawls, J. F., Allbritton, N. L. and Magness, S. T. 2018, Cellular and molecular gastroenterology and hepatology, 6(3), 301-319.
39. Hall, A. J., Curns, A. T., McDonald, L. C., Parashar, U. D. and Lopman, B. A. 2012, Clinical Infectious Diseases, 55(2), 216-223.
40. Leslie, J. L., Huang, S., Opp, J. S., Nagy, M. S., Kobayashi, M., Young, V. B. and Spence, J. R. 2015, Infection and immunity, 83(1), 138-145.
41. Tao, L., Zhang, J., Meraner, P., Tovaglieri, A., Wu, X., Gerhard, R., Zhang, X., Stallcup, W. B., Miao, J., He, X. and Hurdle, J. G. 2016, Nature, 538(7625), 350-355.
42. Engevik, M. A., Yacyshyn, M. B., Engevik, K. A., Wang, J., Darien, B., Hassett, D. J., Yacyshyn, B. R. and Worrell, R. T. 2015, American Journal of Physiology-Gastrointestinal and Liver Physiology, 308(6), G510-G524.
43. Engevik, M. A., Engevik, K. A., Yacyshyn, M. B., Wang, J., Hassett, D. J., Darien, B., Yacyshyn, B. R. and Worrell, R. T. 2015, American Journal of Physiology-Gastrointestinal and Liver Physiology, 308(6), G497-G509.
44. Mileto, S. J., Jardé, T., Childress, K. O., Jensen, J. L., Rogers, A. P., Kerr, G., Hutton, M. L., Sheedlo, M. J., Bloch, S. C., Shupe, J. A. and Horvay, K. 2020, Proceedings of the National Academy of Sciences, 117(14), 8064-8073.
45. Fischer, S., Ückert, A. K., Landenberger, M., Papatheodorou, P., Hoffmann-Richter, C., Mittler, A. K., Ziener, U., Hägele, M., Schwan, C., Müller, M. and Kleger, A. 2020, The FASEB Journal, 34(5), 6244-6261.
46. Engevik, M. A., Danhof, H. A., Auchtung, J., Endres, B. T., Ruan, W., Bassères, E., Engevik, A. C., Wu, Q., Nicholson, M., Luna, R. A. and Garey, K. W. 2021, Gastroenterology, 160(4), 1301-1314.

47. Radtke, A. L., Wilson, J. W., Sarker, S. and Nickerson, C. A. 2010, PLoS One, 5(12), e15750.
48. Wilson, S. S., Tocchi, A., Holly, M. K., Parks, W. C. and Smith, J. G. 2015, Mucosal immunology, 8(2), 352-361.
49. Zhang, Y. G., Wu, S., Xia, Y. and Sun, J. 2014, Physiological reports, 2(9), e12147.
50. Forbester, J. L., Goulding, D., Vallier, L., Hannan, N., Hale, C., Pickard, D., Mukhopadhyay, S. and Dougan, G. 2015, Infection and immunity, 83(7), 2926-2934.
51. Birmingham, C. L., Smith, A. C., Bakowski, M. A., Yoshimori, T. and Brumell, J. H. 2006, Journal of Biological Chemistry, 281(16), 11374-11383.
52. Nickerson, K. P., Senger, S., Zhang, Y., Lima, R., Patel, S., Ingano, L., Flavahan, W. A., Kumar, D. K. V., Fraser, C. M., Faherty, C. S. and Sztein, M. B. 2018, EBioMedicine, 31, 92-109.
53. Holly, M. K., Han, X., Zhao, E. J., Crowley, S. M., Allaire, J. M., Knodler, L. A., Vallance, B. A. and Smith, J. G. 2020, Infection and immunity, 88(7), e00017-20.
54. In, J., Foulke-Abel, J., Zachos, N. C., Hansen, A. M., Kaper, J. B., Bernstein, H. D., Halushka, M., Blutt, S., Estes, M. K., Donowitz, M. and Kovbasnjuk, O. 2016, Cellular and molecular gastroenterology and hepatology, 2(1), 48-62.
55. Noel, G., Baetz, N. W., Staab, J. F., Donowitz, M., Kovbasnjuk, O., Pasetti, M. F. and Zachos, N. C. 2017, Scientific reports, 7(1), 1-14.
56. Pradhan, S., Karve, S. S., Weiss, A. A., Hawkins, J., Poling, H. M., Helmrath, M. A., Wells, J. M. and McCauley, H. A. 2020, Cellular and molecular gastroenterology and hepatology, 10(1), 171-190.
57. Olvera, A., Carter, H., Rajan, A., Carlin, L. G., Yu, X., Zeng, X. L., Shelburne, S., Bhatti, M., Blutt, S. E., Shroyer, N. F. and Jenq, R. 2021, Clinical Infectious Diseases, 72(10), e620-e629.
58. McCracken, K. W., Catá, E. M., Crawford, C. M., Sinagoga, K. L., Schumacher, M., Rockich, B. E., Tsai, Y. H., Mayhew, C. N., Spence, J. R., Zavros, Y. and Wells, J. M. 2014, Nature, 516(7531), 400-404.
59. Schlaermann, P., Toelle, B., Berger, H., Schmidt, S. C., Glanemann, M., Ordemann, J., Bartfeld, S., Mollenkopf, H. J. and Meyer, T. F. 2016, Gut, 65(2), 202-213.
60. Bartfeld, S., Bayram, T., van de Wetering, M., Huch, M., Begthel, H., Kujala, P., Vries, R., Peters, P. J. and Clevers, H. 2015, Gastroenterology, 148(1), 126-136.
61. Geis, G., Leying, H., Suerbaum, S., Mai, U. W. E. and Opferkuch, W. 1989, Journal of clinical microbiology, 27(3), 436-441.
62. Geis, G., Suerbaum, S., Forsthoff, B., Leying, H. and Opferkuch, W. 1993, Journal of medical microbiology, 38(5), 371-377.
63. Skene, C., Young, A., Every, A. and Sutton, P. 2007, FEMS Immunology & Medical Microbiology, 50(2), 249-256.
64. Howitt, M. R., Lee, J. Y., Lertsethtakarn, P., Vogelmann, R., Joubert, L. M., Ottemann, K. M. and Amieva, M. R. 2011, MBio, 2(4), e00098-11.
65. Buti, L., Ruiz-Puig, C., Sangberg, D., Leissing, T. M., Brewer, R. C., Owen, R. P., Sgromo, B., Royer, C., Ebner, D. and Lu, X. 2020, Proceedings of the National Academy of Sciences, 117(5), 2645-2655.
66. Kitamura, Y., Murata, Y., Park, J. H., Kotani, T., Imada, S., Saito, Y., Okazawa, H., Azuma, T. and Matozaki, T. 2015,Genes to Cells, 20(7), 578-589.
67. Han, X., Lee, A., Huang, S., Gao, J., Spence, J. R. and Owyang, C. 2019, Gut Microbes, 10(1), 59-76.
68. Lopez-Siles, M., Duncan, S. H., Garcia-Gil, L. J. and Martinez-Medina, M. 2017, The ISME journal, 11(4), 841-852.
69. Quévrain, E., Maubert, M. A., Michon, C., Chain, F., Marquant, R., Tailhades, J., Miquel, S., Carlier, L., Bermúdez-Humarán, L. G., Pigneur, B. and Lequin, O. 2016, Gut, 65(3), 415-425.
70. Zhou, K. 2017, Journal of functional foods, 33, 194-201.
71. Lukovac, S., Belzer, C., Pellis, L., Keijser, B.J., de Vos, W. M., Montijn, R. C. and Roeselers, G. 2014, MBio, 5(4), e01438-14.
72. Fitzgerald, C. B., Shkoporov, A. N., Sutton, T. D., Chaplin, A. V., Velayudhan, V., Ross, R. P. and Hill, C. 2018, BMC genomics, 19(1), 1-20.

73. Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J. J., Blugeon, S., Bridonneau, C., Furet, J. P., Corthier, G. and Grangette, C. 2008, Proceedings of the National Academy of Sciences, 105(43), 16731-16736.
74. Pradhan, S. and Weiss, A. A. 2020, Mbio, 11(4), e01470-20.
75. Kim, H. J., Li, H., Collins, J. J. and Ingber, D. E. 2016, Proceedings of the National Academy of Sciences, 113(1), E7-E15.