Minireview

Host-microbe interaction in the gastrointestinal tract

Aimée Parker,*† Melissa A. E. Lawson,† Laura Vaux, and Carmen Pin

Quadram Institute Bioscience, Norwich Research Park, NR4 7UA, UK.

Summary

The gastrointestinal tract is a highly complex organ in which multiple dynamic physiological processes are tightly coordinated while interacting with a dense and extremely diverse microbial population. From establishment in early life, through to host-microbe symbiosis in adulthood, the gut microbiota plays a vital role in our development and health. The effect of the microbiota on gut development and physiology is highlighted by anatomical and functional changes in germ-free mice, affecting the gut epithelium, immune system and enteric nervous system. Microbial colonisation promotes competent innate and acquired mucosal immune systems, epithelial renewal, barrier integrity, and mucosal vascularisation and innervation. Interacting or shared signalling pathways across different physiological systems of the gut could explain how all these changes are coordinated during postnatal colonisation, or after the introduction of microbiota into germ-free models. The application of cell-based in-vitro experimental systems and mathematical modelling can shed light on the molecular and signalling pathways which regulate the development and maintenance of homeostasis in the gut and beyond.

Introduction

Our gut is home to a large and complex community of microorganisms termed the intestinal microbiota. The dynamic environment within the intestine presents a challenge to both the host and the intestinal microbiota to maintain a mutualistic relationship throughout life (Tannock, 2007). In this review, we focus on the factors which influence bacterial composition throughout the gastrointestinal tract, and on the cross-talk between the microbiota and the host at the gastrointestinal (GI) barrier, which results in the development of a precise GI organisation and functionality. We discuss this in the context of what is currently known about gut microbiota interaction with host defences, and research tools and models that can be used to study these interactions.

Following pioneering experiments in clinical and animal models over a century ago (Cushing and Livingood, 1900) researchers have generated a variety of tools, including animal models devoid of microorganisms (germ-free/axenic models) providing insight into the host processes regulated by the presence and/or composition of the gut microbiota in health and disease (Reyniers, 1959; Smith et al., 2007). Based on their interaction with the host, members of the microbiota are loosely classified as beneficial/commensal species [including ‘probiotic’ bacteria, such as Bifidobacterium (Fanning et al., 2012), and benign organisms such as members of the defined “altered Shaedler flora” (Biggs et al., 2017)], or pathogenic species, including pathobionts such as Helicobacter pylori (Marshall, 2002) and opportunistic pathogens.

Those bacteria which initially colonise neonatal guts establish a mutualistic relationship with the gastrointestinal tract that can last a lifetime (Human Microbiome Project C, 2012). The birthing process has been reported to influence the type of bacteria that first colonise the infant gut; as infants acquire bacteria either by vertical transmission from the mother through the vaginal canal, and/or their environment (including the mother’s skin) after caesarean section. Vaginal delivery results in the gut colonisation by pioneer bacteria including Streptococcus, Escherichia, and Klebsiella, which grow and establish a favourable environment (i.e., by reducing oxygen levels) for other anaerobic species including Bifidobacterium, Lactobacillus and Bacteroides, which dominate the infant gut (Houghteling and Walker, 2015). In infants born by caesarean section, early life gut microbiota tends to mimic the skin (dominated by Staphylococcus) and other environmental bacteria (Rutayisire et al., 2016). Recent research suggests...
maternal vaginal swabs used to inoculate caesarean delivered infants can partially restore the levels of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* to those observed in vaginally delivered babies (Dominguez-Bello *et al.*, 2016) although longer term effects on microbial composition and host health are not yet known.

The infant gut microbial community is characterised by low diversity with high instability and is susceptible to modification by exogenous factors such as antimicrobial drugs and/or diet. Disturbances during microbiota establishment and development, for instance by antibiotic use (both through breast feeding and postnatally), can have long lasting effects on microbial composition by selecting for resistant species (Mathew, 2004; Jemberg *et al.*, 2010). Infant diet also affects which type of bacteria establish the early life microbiota (Lee *et al.*, 2015). In infants that are solely breast-fed, the microbiota is simple in structure, and is dominated (upwards of 80% of the total microbiota composition) by *Bifidobacterium*, a beneficial bacterium that is often associated with probiotic traits (Serafini *et al.*, 2012). Formula fed infants have a much more complex microbiota composition with significantly increased levels of *Enterobacteriaceae* and reduced levels of *Bifidobacterium* (Harmsen *et al.*, 2000; Rinne *et al.*, 2005; Praveen *et al.*, 2015). Differences in microbial composition induced during the early development of the gut microbiota, and their longer-term effects on host health and immunity, are likely to be affected by when, where and which species colonise the gastrointestinal tract during development.

**The host luminal environment determines the spatial distribution of a dynamic microbial community along the gastrointestinal tract**

Microbial density and composition varies dramatically throughout the gastrointestinal tract and this spatial distribution seems to be independent of the mode of colonisation (e.g., co-housing versus oral gavage in germ-free mice), suggesting that host-microbe interactions are the strongest determining factor for the microbial colonisation of each region (Seedorf *et al.*, 2014). The gastrointestinal tract comprises a series of connected specialised segments with different environmental pressures, which affect bacterial colonisation. Below we describe the main conditions affecting the microbiota progressing along the gastrointestinal tract.

The highly acidic and enzymatic environment of the stomach, in combination with the detection of very low levels of culturable bacteria, previously led to the assumption that the stomach was a somewhat sterile environment. It is now known that the stomach harbours a highly diverse bacterial community, with Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria and Actinobacteria as the most abundant phyla, and colonisation densities ranging from $10^1$ to $10^3$ bacteria/g of content (Bik *et al.*, 2006; Sheh and Fox, 2013). This diversity in the gastric microbiome is however drastically reduced in individuals harbouring *H. pylori*, the strongest known risk factor for developing gastric adenocarcinoma (Cho and Blaser, 2012), which is present in 50% of the human population (Brown, 2000).

Microscopic finger like projections into the intestinal lumen, termed villi, increase the surface area for absorption in the small intestine, decreasing in length from the proximal duodenum towards the distal ileum (Fig. 1). Villi are covered by a monolayer of epithelial cells comprising enterocytes, for nutrient absorption, and a variety of secretory and enteroendocrine cells. The combination of rapid transit of luminal contents and the presence of detergent-like compounds, such as bile acids and digestion enzymes, makes the content of the proximal small intestine an unfavourable environment for bacterial growth.
colonisation (Donaldson et al., 2016). As a result, relatively low numbers of bacteria are found in the proximal small intestine, which tends to be dominated by a few fast-growing facultative anaerobes and acid-tolerant bacteria carried over from the stomach, such as Helicobacteriaceae, Streptophyta, Enterobacteriaceae and Lactobacillaceae (Seedorf et al., 2014). The luminal contents reaching the distal ileum form a bacterial growth-permmissive environment, comprising primarily indigestible dietary material with a pH value close to neutral, lower concentrations of compounds challenging microbial growth, and reduced oxygen availability, resulting in an increase in microbial load and complexity in the ileum as it advances towards the large intestine.

The large intestine is significantly shorter and wider than the small intestine resulting in a decreased transit rate, facilitating the epithelial absorption of large volumes of luminal fluid and permitting the establishment of a high density bacterial population of up to $10^{12}$–$10^{14}$ bacteria/g of content. The majority of species belong to four distinct phyla: Proteobacteria, Actinobacteria, Bacteroides and Firmicutes (Gu et al., 2013; Seedorf et al., 2014) contributing to an extremely diverse and complex microbial environment. Ongoing work attempts to identify the many species and strains which form part of a healthy versus dysregulated or pathogenic colonic microbiota.

In addition to the increase in microbial diversity and abundance in oral-caudal direction along the gut, there is evidence supporting differences in microbial distribution in the axial direction, from the lumen through the mucus layer toward the intestinal epithelium (Li et al., 2015). The mucus layer, produced by epithelial goblet cells, is present to varying degrees throughout the gastrointestinal tract but is thickest and most complex in the colon, where mucus-producing goblet cells are more abundant (McGuckin et al., 2011; Juge, 2012). The mucus layer provides a barrier to protect the epithelium from direct contact with the gut content. In the colon, the mucus consists of two defined layers; a compact inner layer which is reported to be sterile, and an outer loosen layer colonised with bacteria (Juge, 2012). In the small intestine, mucus is less abundant, the layers are less well defined, and may be more permeable to bacteria (Ermund et al., 2013). The colonic outer mucus layer is a niche that functions not only as an attachment platform, but also as a nutrient source for bacteria such as Akkermansia muciniphila, and members belonging to the genus such as Lactobacilli, Bacteroides and Bifidobacteria (Pretzer et al., 2005; Garrido et al., 2011; Pudlo et al., 2015). Bacterial selection within the mucus-associated niche is the suggested reason for the reported detection of Firmicutes, Lachnospiraceae and Ruminococcaceae enriched communities in discrete ‘inter-fold’ regions of the colonic mucosa and Bacteroidetes, Prevotellaceae, Bacteroidaceae and Rikenellaceae in the lumen (Nava et al., 2011; Donaldson et al., 2016).

The mucus layer is subject to a relatively high rate of turnover. For instance, the colonic outer mucus layer, together with the attached microbiota, is dislodged by peristaltic movements propelling luminal contents along the gastrointestinal tract, and is continually replenished by the inner layer produced by epithelial goblet cells (McGuckin et al., 2011). Recent research has highlighted the importance of metabolism and bacterial kinetics enabling E. coli and Bacteroides thetaiotaomicron to persist in the outer mucus layer (Li et al., 2015). The microbial composition of the mucus therefore results from the balance between microbial attachment and proliferation and shedding of mucus-attached bacteria into the lumen.

The microbiota influences gut biology

The luminal contents, and the mucus layer, condition the spatial distribution of the microbiota along the tract. In return, the rapid microbial colonisation of the infant gastrointestinal environment has profound effects on the development and functionality of host gut physiological processes and mucosal defence mechanisms (Tannock, 2007). Our current understanding of these effects owes much to a large body of in-vivo work employing a range of germ-free animal models, including mice, rats, pigs, drosophila, zebrash, chickens and others (a number of which are summarised in Table 1). Below, we discuss interactions between gut microbes, the intestinal epithelial layer and immune and nervous systems.

Microbial targeting of epithelial turnover

During development, the initially flat mucosa develops into the characteristic crypt-villus architecture via a series of tissue-patterning and cell fate determination processes coordinating the development of the epithelium and the underlying mesenchyme and smooth muscle (Walton et al., 2012). Ongoing maturation of crypts and villi continues through the postnatal and weaning periods in parallel with microbial colonisation of the gut, eventually culminating in mature crypts containing stem cells, Paneth cells and enterocytic/secretory precursors, and villi composed of differentiated enterocytes, goblet cells, tuft cells and a variety of enteroendocrine cells. Cell proliferation within crypts is the principal force driving cell migration on the villi (Parker et al., 2017) and the equilibrium of cell number and turnover is maintained by compensatory cell shedding from the villus tip into the gut lumen (Gerbe et al., 2011; Clevers, 2013). The continual production, migration and shedding of

© 2017 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd., Environmental Microbiology, 20, 2337–2353

Host-microbe interaction in the GI tract 2339
Epithelial cells presents a dynamic barrier to microbial attachment and persistence.

The small intestines of germ-free mice have reduced overall mass and surface area, thinner villi, shallower crypts with decreased cell proliferation and reduced migration along the crypt-villus axis (Fig. 2) (Sommer et al., 2015). Likewise, in the colon of germ-free or conventionally raised but antibiotic-treated mice, cell proliferation rate is reduced and crypts contain fewer cells than those of conventional mice. In Drosophila, Lactobacilli can modulate gut stem cell proliferation via release of reactive oxygen species (Jones et al., 2013) and microbe-induced JAK-STAT signalling is essential for stem cell division (Buchon et al., 2009). Together these data suggest the

Table 1. Summary of germ-free phenotypes in animal models.

| Feature                        | Altered phenotype in germ-free                                                                 | Model         | References                                                                                           |
|--------------------------------|-------------------------------------------------------------------------------------------------|---------------|------------------------------------------------------------------------------------------------------|
| Transit of luminal contents    | Delayed gastric emptying and prolonged transit                                                   | Mouse (Abrams and Bishop, 1967; Iwai et al., 1973) |                                                                                                    |
| Crypt-villus morphology        | Thinner villi with shallower crypts, reduced thickness of LP                                     | Rat (Meslin et al., 1973; Meslin and Sacquet, 1984) |                                                                                                    |
|                                | Thinner villi with shallower crypts, reduced thickness of LP                                     | Mouse (Abrams et al., 1963; Lesher et al., 1964; Glaister, 1973) |                                                                                                    |
|                                | Thicker villi with shallower crypts, reduced thickness of LP                                     | Guinea pig (Sprinz et al., 1961) |                                                                                                    |
|                                | Reduced cell proliferation and migration along the crypt-villus axis                              | Chicken (Furuse and Okumura, 1994) |                                                                                                    |
| Epithelial microvilli          | Impaired formation                                                                               | Mouse (Gordon, 1959) |                                                                                                    |
| Tight junctions                | Increased barrier permeability                                                                    | Mouse (Smith et al., 2007) |                                                                                                    |
| Epithelial turnover            | Reduced proliferation, migration and renewal in the gut                                           | Mouse (Khoury et al., 1969; Rakoff-Nahoum et al., 2015) |                                                                                                    |
|                                | Thinner villi with shallower crypts, reduced thickness of LP                                     | Rat (Guenet et al., 1970) |                                                                                                    |
|                                | Reduced cell proliferation and migration along the crypt-villus axis                              | Pig (Kenworthy and Allen, 1966) |                                                                                                    |
|                                | Reduced cell proliferation and migration along the crypt-villus axis                              | Dog (Heneghan, 1965) |                                                                                                    |
|                                | Reduced cell proliferation and migration along the crypt-villus axis                              | Chicken (Rolls et al., 1978) |                                                                                                    |
|                                | Reduced cell proliferation and migration along the crypt-villus axis                              | Zebrafish (Bates et al., 2006) |                                                                                                    |
|                                | Reduced in number, release of antimicrobial peptides and increased bacterial contact with epithelium | Drosophila (Buchon et al., 2009; Cronin et al., 2009) |                                                                                                    |
| Paneth cells                   | Reduced in number, release of antimicrobial peptides and increased bacterial contact with epithelium | Mouse (Cash et al., 2006; Yu et al., 2016) |                                                                                                    |
|                                | Reduced in number                                                                               | Rat (Satoh, 1984) |                                                                                                    |
| Goblet cells                   | Reduced in number                                                                               | Mouse (Yu et al., 2016) |                                                                                                    |
|                                | Reduced in number                                                                               | Rat (Gustafsson and Maunsbach, 1971) |                                                                                                    |
|                                | Reduced in number                                                                               | Chicken (Cheled-Shoval et al., 2014) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Johansson et al., 2015; Li et al., 2015) |                                                                                                    |
|                                | Reduced in number                                                                               | Rat (Szentkuti and Enss, 1998) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Abrams et al., 1963; Shroff et al., 1995; Yamanaka et al., 2003; Bousskra et al., 2008; Tsuji et al., 2008) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Kanther et al., 2014) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Macpherson and Harris, 2004; Geuking et al., 2011) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Benveniste et al., 1971b; Benveniste et al., 1971a) |                                                                                                    |
|                                | Reduced in number                                                                               | Guinea pig (Sprinz et al., 1961) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Cahenzli et al., 2013) |                                                                                                    |
|                                | Skewed isotype switching in the gut (IgA—IgE)                                                   | Zebrafish (Kanther et al., 2011) |                                                                                                    |
|                                | Reduced activation of NF-κB signalling                                                         | Mouse (Stappenbeck et al., 2002; Reinhardt et al., 2012) |                                                                                                    |
|                                | Reduced activation of NF-κB signalling                                                         | Zebrafish (Kanther et al., 2011) |                                                                                                    |
|                                | Diminished villus capillary density and complexity                                              | Mouse (Collins et al., 2014; McVey Neufeld et al., 2015) |                                                                                                    |
|                                | Morphological and functional alteration of neurons and glia                                     | Rat (Husebye et al., 2001) |                                                                                                    |
|                                | Reduced in number                                                                               | Mouse (Kabouridis and Pachnis, 2015) |                                                                                                    |

© 2017 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd., Environmental Microbiology, 20, 2337–2353
presence of microbes not only promotes, but is required for normal epithelial development and turnover.

On the other hand, pathogenic bacteria can specifically inhibit epithelial turnover processes to facilitate their spread. Some bacteria specifically target cell turnover processes in order to affect the barrier integrity and persist in the epithelium, for example, by altering crypt cell gene expression programs governing cell cycle control and proliferation (Rakoff-Nahoum et al., 2015; Sommer et al., 2015). Recent studies in mouse models and in-vitro organoid models, detailed below, demonstrate that microbial signalling can alter epithelial turnover via activation of pattern recognition receptors (PRR) expressed on crypt stem cells, to alter cell proliferation and survival decisions (Neal et al., 2012; Hormann et al., 2014; Nigro et al., 2014). Microbial effects on epithelial turnover can also occur indirectly, by the induction of neurotransmitter and cytokine release from lamina propria propria cells (e.g., neuroglial, immune and stromal cells) acting on the epithelium (Hyland and Cryan, 2016; Obata and Pachnis, 2016).

Controlled cell death processes (apoptosis, necroptosis, pyroptosis) can serve to restrict microbial persistence and translocation across the epithelium (Negroni et al., 2015). Some bacterial species, including enterohemorrhagic E. coli (EHEC), H. pylori and Campylobacter jejuni, inhibit epithelial cell death, thus preserving their replication niche for longer and maximising their chance of translocating to underlying tissues (Lim et al., 2017; Song et al., 2017) (Fig. 3). In addition, intracellular autophagic pathways, which can also regulate cell death and proliferation to restrict bacterial invasion (Benjamin et al., 2015).

Fig. 2. Major components of the ileal mucosa and differences between conventionally raised and germ-free mice. The presence of microbiota influence mucus composition, crypt-villus morphology, epithelial immune receptor expression and AMPs release, immune structure and cell composition, vascularisation, innervation, glial networks and mucosal thickness. SIgA: Secretory Immunoglobulin A, PP: Peyer's patch, AMPs: antimicrobial peptides.
et al., 2013) can be targeted by species including Orientia tsutsugamushi and Mycobacterium tuberculosis (Shin et al., 2010; Choi et al., 2013) to facilitate epithelial penetrance.

Further physical challenges to microbial penetrance are provided by the epithelial cell brush border and tight junctions between neighbouring epithelial cells (Zihni et al., 2016), the formation of which is promoted by the presence of the normal microbiota [germ-free mice have impaired brush-border microvillus formation and increased barrier permeability (Gordon, 1959; Smith et al., 2007)]. Pathogenic bacteria, including Salmonella Typhimurium and invasive E. coli can target these defences by secreting proteases and neuro-immune stimulatory ligands to impair brush border formation (Lhocine et al., 2015) and disrupt epithelial tight-junctions (Fig. 3) (Awad et al., 2017; Shawki and McCole, 2017).

**Microbial targeting of epithelial immune defences**

Besides the physical impairments to colonisation provided by epithelial structure and turnover, epithelial cells of all types have immune defence mechanisms to limit and/or respond to microbial invasion. Accordingly, microbes also target these mechanisms to gain access to epithelial cells and underlying tissues.

Epithelial cells express a range of immune PRR, including various Toll-like receptors (TLR) and nucleotide-binding oligomerization domain-like receptors (NLRs), which survey both luminal and basolateral membranes of the barrier, and intracellular compartments. During development, alterations in epithelial expression and activity of these receptors occurs concomitantly with the morphological maturation of the epithelium and microbial colonisation and establishment. Furthermore, reduced epithelial TLR expression in germ-free and antibiotic-treated mice, and subsequent recovery in recolonised mice, suggests the normal microflora promote the expression of the receptor repertoire (Hormann et al., 2014). In neonatal mice, tuning of receptor repertoires [downregulation of the TLR4 signalling pathway (Lotz et al., 2006) and upregulation of TLR9 (Pott et al., 2012)] drives a more tolerant response to microbes. This, combined with upregulation of antiviral TLR3 expression at weaning (Pott et al., 2012), suggests the
TLR repertoire is customised to prepare the foetal epithelium for colonisation and facilitate development of a stable microbiota, preparing the host for the uptake of new foods and antigens. In adults, triggering of PRR signalling by pathogenic bacteria, or commensal species under inflammatory conditions, promotes a range of immune responses, which limit further invasion and clear infected cells. Some species therefore inhibit PRR signalling pathways to mask their presence or prevent death of the cells they have infected (McGuire and Arthur, 2015). Bacterial triggering of TLRs can also promote the local release of pro-inflammatory cytokines such as TNF-α, which can disrupt epithelial tight junctions leading to a ‘leaky’ barrier and allowing the ingress of opportunistic bacterial species.

Paneth cells, specialised epithelial cells which appear at the base of crypts during the suckling to weaning transition, not only express PRRs but can also release a variety of soluble antimicrobial peptides into the mucus, targeting of a vast range of organisms including gram-positive and gram-negative bacteria, parasites, fungi and some viruses (Kopp et al., 2015). Upon colonisation of the GI tract, Paneth cell release of RegIII proteins (α, β, γ) into the lumen contributes to regulating microbial composition by specifically targeting Gram-positive bacteria (Cash et al., 2006). In the absence of RegIII-γ there is increased bacterial contact with the epithelium and an increased adaptive immune response against commensals (Vaishnava et al., 2011). Not surprisingly, some bacteria have devised counter strategies to subvert these antimicrobial peptides. Helicobacter pylori for example exploits host cholesterol to obtain resistance to the antimicrobial peptide LL-37 in the gerbil intestine (McGee et al., 2011) and can also selectively inhibit human beta defensin 3 (hDB3) (Bauer et al., 2013).

In addition to producing mucus, goblet cells have been reported to deliver antigens across the epithelium via so-called goblet cell associated antigen passages (GAPs) (McDole et al., 2012; Knoop et al., 2015). Deletion of goblet cells in a mouse model of wild type Salmonella Typhimurium infection prevented the translocation bacteria to the draining mesenteric lymph nodes, indicating that S. Typhimurium uses goblet cells as an entry portal. Furthermore, luminal exposure to an invasive S. Typhimurium, shut off GAP-associated translocation suggesting that goblet cell sensing of an invasive pathogen to shut off GAPs is a host defence mechanism (Kulkarni et al., 2016; Knoop et al., 2017).

Additional specialised epithelial cells, ‘microfold’ or ‘M’-cells, located in the follicle-associated epithelium overlying Peyer’s patches and isolated lymphoid follicles, have shortened microvilli and altered extracellular matrix, allowing uptake and shuffling of antigens to innate and adaptive immune cells of the lamina propria and gut-associated lymphoid tissues (Mabbott et al., 2013) (Fig. 3). Pathogenic bacteria including S. Typhimurium and Shigella flexneri target M cells as sites of entry, via intracellular trafficking mechanisms, by specifically killing the M cells to create an entry portal, or more generally by inducing a local inflammatory immune response to create a leaky epithelium (Jones et al., 1994; Corr et al., 2008).

**Microbial interaction with the mucosal immune system**

Upon colonisation of the intestine, bacteria and bacterial products (including lipopolysaccharides, DNA, RNA, flagellin, etc.) are recognised by receptors including TLR and NLR not only on epithelial cells, but also on cells of the mucosal immune system including monocytes, macrophages, granulocytes, B cells, natural killer cells and dendritic cells, which direct appropriate pro-inflammatory or tolerogenic immune responses (Ausubel, 2005; Hooper and Macpherson, 2010). Intestinal dendritic cells (DCs) residing in the lamina propria and Peyer’s patches have been suggested to sample bacterial antigen in the lumen through a variety of methods. These include possible extension of transepithelial dendrites (Rescigno et al., 2001; Farache et al., 2013) and interception of antigens transcytosed antigens across epithelial goblet cells and M cells as described above. The presence of bacterial antigens in the lamina propria and Payer’s patches contribute to the development and maturation of the adaptive immune system and the epithelium, including the development of M cells positioned over PP in the follicle-associated epithelium. Integrated within the epithelium, a subset of innate lymphoid cells (ILCs) can also be activated by microbial stimuli to produce pro- or anti-inflammatory cytokines. Recent work suggests particular cell subsets (NKp46+ ILC3s and F4/80+ CD11c+ mononuclear cells) may be conditioned by maternal microbiota during gestation, such that offspring have a reduced inflammatory immune response to the adult gut microbiota compared to offspring of germ-free animals (Gomez de Aguero et al., 2016).

In the absence of a microbiota, the gastrointestinal tract is characterized by sparse infiltration of lymphocytes in the intestinal epithelial layer and lamina propria, that is particularly noticeable by the reduced number of Immunoglobulin A+ (IgA+) positive plasma cells (Benveniste et al., 1971a,b) and CD4+ T regulatory cells (Geuking et al., 2011) (Fig. 2). Upon colonisation, the lamina propria, Peyer’s patches and mesenteric lymph nodes are infiltrated with B- and T-lymphocytes, and characterised by high levels of the non-inflammatory IgA+ plasma cells (Benveniste et al., 1971b; Geuking et al., 2011; Hooper et al., 2012). This expansion
causes both an increase in size and number of hypoplastic B cell follicles and germinal centres in the mesenteric lymph nodes and Peyer’s patches (Yamanaka et al., 2003); indicating further immune maturation due to colonisation. In addition, bacterial colonisation of the large intestine also leads to the maturation of cryptopatches into isolated lymphoid follicles (Shroff et al., 1995; Tsuji et al., 2008). This process appears to be mediated by the presence of Gram negative bacteria, through direct bacterial antigens interacting with NLR (specifically NOD1) signalling on host cells (Hasegawa et al., 2006; Bouskra et al., 2008) suggesting a direct link between immune development and bacterial composition in the gut. Systemically, the immune system of germ free mice has low levels of isotype-switched immunoglobulins, as well as poor structural organisation within secondary lymphoid organs that are all reversible by colonisation (Hooper et al., 2012).

Effect of the microbiota on the enteric nervous system

The enteric nervous system (ENS) comprises complex networks of neurons and glial cells involved in complex cross-talk with immune, bacterial and epithelial cells. Under the epithelial layer, the intestinal mucosa is densely vascularised and innervated, with neuronal fibres in close proximity to, and in some cases direct contact with (Bohorquez et al., 2014), overlying epithelial cells and surrounded by other mesenchymal and immune cells. Together, the vascular capillary network and the neural networks of the ENS regulate many aspects of homeostatic gut function, including motility, permeability, secretion and absorption (Schemann and Neunlist, 2004; Van Landeghem et al., 2009), and are also involved in coordinating responses during damage and repair (Veiga-Fernandes and Pachnis, 2017). While the generation and early patterning of the vasculature and ENS takes place during embryogenesis in parallel with epithelial development (Hatch and Mukouyama, 2015), further morphological and functional maturation of these systems is profoundly influenced by the microbiota, both in early life (Stappenbeck et al., 2002; Kabouridis and Pachnis, 2015; Rakoff-Nahoum et al., 2015) and in adulthood (Laranjeira et al., 2011; Reinhardt et al., 2012; Kabouridis and Pachnis, 2015). Germ-free mice for example have diminished capillary density and complexity (Stappenbeck et al., 2002; Reinhardt et al., 2012), altered neuronal patterning and composition (Collins et al., 2014; McVey Neufeld et al., 2015) and impaired glial cell development and migration (Young et al., 2003; Kabouridis and Pachnis, 2015) (Table 1 and Fig. 2).

Commensal and pathogenic microbes can alter ENS functions by electrical signalling (Kunze et al., 2009), release of neurotoxins (Yang and Chiu, 2017) or by the release of neurotransmitters including serotonin, neurotrophic factor, acetylcholine and nitric oxide (Sobko et al., 2006; Carabotti et al., 2015). Bacterial proteases or TLR ligands can also modulate neural and glial cell functions by signalling through protease-activated receptors and TLR expressed on cells of the ENS (Brun et al., 2013; Burgueno et al., 2016). Bacterial metabolites, such as short-chain fatty acids (SCFAs) can also stimulate the local sympathetic nervous system directly via neuronal G-protein coupled receptors (GPCR) (Kimura et al., 2011) or indirectly, via intriguing epithelial-neuro-immune cellular networks. For example, SCFA binding to enteroendocrine cells may trigger a signalling pathway involving pseudopods, neurons and glia to alter adjacent immune cell activity and gut motility (Sommier et al., 2015; Obata and Pachnis, 2016).

In-vitro techniques to study microbial-gut interactions

The specific niches which exist throughout the gastrointestinal tract are largely dictated by diet/nutrients and host-derived factors. Disentangling how different species colonise and modify these niches can be difficult in previously colonised animal models, in which “colonisation resistance” prevents the establishment of new bacterial strains, unless the resident gut microbiota is first depleted with antibiotics (Stecher et al., 2013). Germ-free animal models have alterations in intestinal morphology, immunity, and physiology, and do not reproduce a natural colonisation model. To circumvent some of these issues, and as an alternative or complement to the use of animal experiments, a multitude of in-vitro techniques have been established including continuous culture systems, the generation of intestinal tissue cell lines and organoids from intestinal explants, and mock community analysis in-silico to study host-microbe interactions. Many of these systems, such as continuous culture systems, can replicate flow dynamics and the microbial and physicochemical characteristics of the luminal content in the proximal and distal colon of a variety of human and animal models (Macfarlane et al., 1998), and are extensively used to understand microbial effects on the host, or how specific aspects of the host immune system affect microbial dynamics.

The development of intestinal epithelium ex-vivo culture techniques (Sato et al., 2009) has provided an in-vitro system to study the effect of the microbiota on stem and other crypt cells. Colonoids and small intestinal enteroids can be generated from primary tissues, biopsies and adult and induced pluripotent stem cells (iPSCs) from humans, mice and other species to form self-organising 3D cultures containing multiple differentiated epithelial cell types which recapitulate many...
functions of the original organ (Spence et al., 2011; Clevers, 2016). Organoids can be subjected to lineage tracing, live imaging, genetic engineering, e.g. by CRISPR-Cas9 or viral methods, drug screening, coculture and infection studies (Schwank et al., 2013; Maru et al., 2016). Organoid technology has been used to study microbial interaction with stem cells, detailing stem cell responses to bacterial products (Neal et al., 2012; Nigro et al., 2014). This technology can be used to probe host-microbe interactions relating to different regions of the intestinal tract, as intriguingly, organoids generated from distinct regions of the intestine appear to retain transcriptional and functional similarities with their site of origin (Basak et al., 2017). For example, application of LPS reduced proliferation and increased apoptosis in ileal crypts (Neal et al., 2012) while there was no such response in jejunal crypts (Davies et al., 2015). Similar site-specific responses to LPS are seen in vivo models (Pritts et al., 2000) and may reflect the expression of different TLR repertoires in different regions of the intestine (Gourbeyre et al., 2015).

One disadvantage for the use of organoids in infection studies with bacteria is that they form closed structures where the luminal side of the epithelium is not readily accessible (Fig. 4). Recent work using luminal microinjection techniques has allowed interrogation of epithelial responses to S. Typhimurium species (Wilson et al., 2015) in small intestinal enteroids and H. pylori in gastric organoids (Wroblewski et al., 2015). Organoids can also be co-cultured with other host cell types including myofibroblasts (Hirokawa et al., 2014), enteric neurons (Pastuba et al., 2015), intraepithelial lymphocytes (Rogoz et al., 2015), and macrophages (Noel et al., 2017) allowing studies of cross talk between the microbiota and multiple, but specific, combinations of host cell types simultaneously. Going further, multi-tissue units have been generated from human iPSCs by the joint development of epithelial, mesenchymal and neural-muscular layers which resulted in gut-like units with multiple functionality (Fig. 4) (Workman et al., 2017) facilitating investigation of microbe-epithelial-neuronal interactions.

A further limitation of organoids is that they fail to replicate the crypt-villus architecture of the small intestine and do not exhibit the characteristic steady state of the epithelium under continuous cell renewal, instead undergoing successive cycles of cyst formation, crypt enlargement and cyst bursting (Sato et al., 2009; Clevers, 2016). In an attempt to circumvent this issue, laser UV lithography can be used to generate patterned templates resembling the intestinal microarchitecture (Fink et al., 2007). The derived silicone microstructures act as scaffolds to support the proliferation of seeded immortalized cell lines, such as colonic Caco-2 cells (Kim and Wu, 2012; Costello et al., 2014), although these fail to reflect many other aspects of the intestinal epithelial niche. More encouragingly, intestinal stem cells seeded on crypt patterned silicone microstructures can give rise to epithelium and mimic cell proliferation migration and shedding along the microstructure (Wang et al., 2014) (Fig. 4). In these models, the basal cell side in contact with the silicone is not accessible for studies on transport across the epithelial barrier or epithelial cell interactions with the vascular, nervous or immune systems, however this may be resolved by using micro-patterned collagen porous scaffolds with accessible luminal and basal cell sides (Wang et al., 2017).

Mathematical and computational modelling to study microbial-gut interactions

The application of mathematical and computational techniques to study biological systems has enabled the study of dynamics and complex organisations and, in doing so, has often provided a mechanistic understanding of these systems not achievable by other means. Experimental information on the microbiome emerges...
from state-of-the-art high-throughput methods, such as metagenomics, metatranscriptomics and metabolomics. After optimal bioinformatics analysis, the resulting data enable the quantification of species abundance within the DNA sequences and viability with RNA sequencing (Heinken and Thiele, 2015). The analysis of network topology has proven useful to study the complex organisation of microbial communities (Stelling, 2004) while the integration of flux balance analyses and metagenomic studies has succeeded in reporting the metabolic activity of whole microbial communities (Klitgord and Segre, 2011). The study of host-microbe metabolic interactions increases remarkably the complexity of network-based models and the computational demand on flux simulations. Global human-microbe metabolite networks have been reconstructed to describe dietary input and metabolic exchange between host and microbes (Heinken et al., 2013). While flux balance analysis assumes that the system is in a steady state, dynamic models comprising similar differential equations, combined with network models, can be used to predict the temporal (transient) dynamics of the microbial population across colonic compartments (Munoz-Tamayo et al., 2011) or continuous spatial frameworks (Moorthy et al., 2015) that emerge in response to changes of the gut environment. For instance, these models have been instrumental in demonstrating that microbiota-epithelial-metabolic interactions have a greater impact on microbial selection than luminal compounds, which preferentially affect microbial cells that are eventually shed into the lumen (Schluter and Foster, 2012).

The term ‘host-microbiota interactome’ was coined, under a broader vision, to describe molecular and physical interactions between the microbiota and the host, considering not only the metabolic but also the antimicrobial activity of the epithelium and mucosal immune system. The analysis of this type of system dynamics has been carried out using computational modelling techniques (Christley et al., 2015). Such computational models have been used to describe the spatiotemporal interactions between pathogens, T cells, macrophages, dendritic cells and epithelial cells during infection (Wendelsdorff et al., 2012; Alam et al., 2015). Simulations of experimentally inaccessible scenarios have for instance predicted that the removal of neutrophils and epithelial-derived anti-microbial compounds would enhance commensal bacteria growth and promote recovery against Clostridium difficile infection (Leber et al., 2015). Systems of ordinary (Arciero et al., 2013) and partial differential equations (Barber et al., 2013) have been proposed to model the epithelial and inflammatory responses to the microbiota driving the progression of necrotising enterocolitis in premature infants.

Most developed models for the microbiota do not account for the spatiotemporal cell dynamics of intestinal epithelial turnover. Analytical models have been used to describe temporal cell dynamics across separated cell compartments in the crypt-villus axis (Johnston et al., 2007; Parker et al., 2017) and in a continuous spatial framework (Maclaren et al., 2017) to gain qualitative insights into the population-level mechanisms underlying epithelial homeostasis and carcinogenesis. In the intestine, stochastic modelling of monoclonal expansion of stem cells, together with Cre/LoxP-based and other lineage tracing experimental strategies, have been instrumental in demonstrating that small intestinal epithelial stem cells are equipotent regarding their ability to occupy with their descendants the entire gland, divide symmetrically and be replaced at random according to a neutral drift pattern (Lopez-Garcia et al., 2010; Snippert et al., 2010; Leushacke et al., 2016). Among computational models, individual-based models have been widely used to describe the spatio-temporal dynamics of single cells, biomechanical properties, cell–cell and cell–matrix interactions, cell density effects and signalling pathways within the crypts (Pitt-Francis et al., 2009; Fletcher et al., 2012; Dunn et al., 2013; Pin et al., 2015). Both analytical and computational models of the epithelium are suitable for extensions, in alignment with data gathering, to account for interactions with the microbiome, immune system and enteric nervous system.

Currently, significant resources are directed to understand the impact of the gut microbiome on health and disease throughout all life stages and corresponding cohort studies are being planned and conducted. Modelling strategies are an invaluable tool to face the challenge of understanding long term dynamics of the microbiome–host interactions. This type of complex system can exhibit behaviours varying from stable to erratic or emergent, and is characterised by the uncertainty of the information gathered in discontinuous sampling times and individuals. Mathematical and computational tools are essential to provide an organisational framework for complex information, and to reveal the properties and dynamics of complex long-term microbiome-host interactions.

**Concluding remarks**

Our intestinal microbiota and intestinal physiology is the result of millions of years of co-evolution and adaptation. Animal models, particularly germ-free animal models, have provided some understanding of the vast complexity of host-microbe interactions and their importance for our lifelong health. Most of these studies, although descriptive, report complex interactions that depend on the spatial location along the gut and on the life stage.

© 2017 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd., Environmental Microbiology, 20, 2337–2353
The underlying mechanisms of how cross talk between the luminal microbiota and host epithelial, immune and other systems are orchestrated along the gastrointestinal tract remain to be revealed. Microbiota dysbiosis is increasingly recognised as a key feature, cause, or effect, in disorders of metabolism and immunity. A deeper understanding of the host-microbiota interactions will therefore enable the generation of strategies to gastrointestinal/metabolic pathologies such as irritable bowel syndrome and necrotising colitis, obesity and type II diabetes.

Increasing evidence also suggests microbial influences can extend beyond the gastrointestinal and metabolic disorders to regulating development and disorders of the central nervous system (CNS). Postnatal brain development is paralleled by the maturation of the gut commensal microbiota, and CNS microglial morphology, maturation and function is (reversibly) altered in GF or antibiotic-treated mice (Erny et al., 2015). Recent studies have shown microbiota-dependent modifications in brain chemistry and behaviour, and there are reported associations between gut microbiota and CNS disorders ranging from Parkinson’s to autism (Rieder et al., 2017; Tognini, 2017), however the specific molecular mechanisms linking changes in microbes or their metabolites during postnatal development with effects on neural development and on neurological functioning later in life are at present unknown.

The development of stem-cell technologies such as in-vitro grown tissue or multi-tissue organic units has enabled the manipulation and visualisation of interacting components and it is already accelerating our understanding of microbial-host interactions in the gut. Moreover, the complexity and dynamic nature of these interactions demand the use of bioinformatics analysis techniques and mathematical models to fully capture the behaviour of the system. Future application and adaptations of these technologies may permit greater understanding not only host-environment cross talk in the gut and its importance for intestinal health, but provide insight into the wider impact of microbes in regulating systemic health and disease.

Acknowledgements

The authors gratefully acknowledge the support of the Biotechnology and Biological Sciences Research Council (BBSRC, UK). This research was funded by the BBSRC project number BB/K018256/1 and the BBSRC Institute Strategic Programme for The Gut Health and Food Safety (BB/J004529/1). ML is a Marie Skłodowska-Curie fellow and receives funding from Horizon 2020 (H2020-MSCAIF-2014; grant 661594).

References

Abrams, G.D., and Bishop, J.E. (1967) Effect of the normal microbial flora on gastrointestinal motility. Proc Soc Exp Biol Med 126: 301–304.

Abrams, G.D., Bauer, H., and Sprinz, H. (1963) Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. Lab Invest 12: 355–364.

Alam, M., Deng, X.W., Philipson, C., Bassaganya-Riera, J., Bisset, K., Carbo, A., et al. (2015) Sensitivity Analysis of an ENteric Immunity Simulator (ENISIS)-based model of immune responses to helicobacter pylori infection. PLoS One 10: e0136139.

Arciero, J., Bard Ermentrout, G., Siggers, R., Afrazi, A., Hackam, D., Vodovoz, Y., and Rubin, J. (2013) Modeling the interactions of bacteria and Toll-like receptor-mediated inflammation in necrotizing enterocolitis. J Theor Biol 321: 83–99.

Auszubel, F.M. (2005) Are innate immune signaling pathways in plants and animals conserved? Nat Immunol 6: 973–979.

Awad, W.A., Hess, C., and Hess, M. (2017) Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins (Basel) 9: E60.

Barber, J., Tronzo, M., Harold Horvat, C., Clermont, G., Upperman, J., Vodovoz, Y., and Yotov, I. (2013) A three-dimensional mathematical and computational model of necrotizing enterocolitis. J Theor Biol 322: 17–32.

Basak, O., Beumer, J., Wiebrands, K., Seno, H., van Oudenaarden, A., and Clevers, H. (2017) Induced Quiescence of Lgr5+ Stem Cells in Intestinal Organoids Enables Differentiation of Hormone-producing Enteroeodontocrine Cells. Cell Stem Cell 20: 177–190. e4.

Bates, J.M., Mittge, E., Kuhlman, J., Baden, K.N., Cheeseman, S.E., and Guillemin, K. (2006) Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev Biol 297: 374–386.

Bauer, B., Wex, T., Kuester, D., Meyer, T., and Malfertheiner, P. (2013) Differential expression of human beta defensin 2 and 3 in gastric mucosa of Helicobacter pylori-infected individuals. Helicobacter 18: 6–12.

Benjamin, J.L., Sumpter, R., Jr., Levine, B., and Hooper, L.V. (2013) Intestinal epithelial autophagy is essential for host defense against invasive bacteria. Cell Host Microbe 13: 723–734.

Benveniste, J., Lespinats, G., and Salomon, J. (1971a) Serum and secretory IgA in axenic and holoxenic mice. J Immunol 107: 1656–1662.

Benveniste, J., Lespinats, G., Adam, C., and Salomon, J.C. (1971b) Immunoglobulins in intact, immunized, and contaminated axenic mice: study of serum IgA. J Immunol 107: 1647–1655.

Biggs, M.B., Medlock, G.L., Moutinho, T.J., Lees, H.J., Swann, J.R., Kolling, G.L., and Papin, J.A. (2017) Systems-level metabolism of the altered Schaedler flora, a complete gut microbiota. ISME J 11: 426–438.

Bik, E.M., Eckburg, P.B., Gill, S.R., Nelson, K.E., Purdom, E.A., Francois, F., et al. (2006) Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci USA 103: 732–737.
Bohorquez, D.V., Samsa, L.A., Roholt, A., Medicetty, S., Chandra, R., and Liddle, R.A. (2014) An enteroendocrine cell-enteric glia connection revealed by 3D electron microscopy. *PloS One* 9: e89881.

Bouskra, D., Brezillon, C., Berard, M., Werts, C., Varona, R., Boneca, I.G., and Eberl, G. (2008) Lympohoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 456: 507–510.

Brown, L.M. (2000) Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol Rev* 22: 283–297.

Brun, P., Giron, M.C., Qesari, M., Porzionato, A., Caputi, V., ZoppeLlaro, C., et al. (2013) Toll-like receptor 2 regulates intestinal inflammation by controlling integrity of the enteric nervous system. *Gastroenterology* 145: 1323–1333.

Buchon, N., Broderick, N.A., Chakrabarti, S., and Lemaitre, B. (2009) Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. *Genes Dev* 23: 2333–2344.

Burgueno, J.F., Barba, A., Eyre, E., Romero, C., Neunlist, M., and Fernández, E. (2016) TLR2 and TLR9 modulate enteric nervous system inflammatory responses to lipopolysaccharide. *J Neuroinflammation* 13: 187.

Cahenzli, J., Koller, Y., Wyss, M., Geuking, M.B., and Cheled-Shoval, S.L., Gamage, N.S., Amit-Romach, E., Chang, J.H., Cheong, T.C., Ha, N.Y., Ko, Y., Cho, C.H., Jeon, I., and Blaser, M.J. (2012) The human microbiome: at the interface of health and disease. *Nat Neurosci* 15: 965–977.

Costello, C.M., Hongpeng, J., Shaffrey, S., Yu, J., Jain, N.K., Hackam, D., and March, J.C. (2014) Synthetic small intestinal scaffolds for improved studies of intestinal differentiation. *Biotechnol Bioeng* 111: 1222–1232.

Cronin, S.J., Nehme, N.T., Limmer, S., Liegeois, S., Pospišilík, J.A., Schramek, D., et al. (2009) Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. *Science* 325: 340–343.

Cushing, H., and Livingood, L.E. (1900) *Experimental and surgical notes upon the bacteriology of the upper portion of the alimentary canal*, with observations on the establishment there of an amicrobic state as a preliminary to operative procedures on the stomach and small intestine. John’s Hopkins Hospital Reports, 9: 543–549.

Davies, J.M., Santaloalla, R., von Furstenberg, R.J., Henning, S.J., and Abreu, M.T. (2015) The viral mimetic polysaccharide-polycytdial acid alters the growth characteristics of small intestinal and colonic crypt cultures. *PLoS One* 10: e0138531.

Domínguez-Bello, M.G., De Jesus-Laboy, K.M., Shen, N., Cox, L.M., Amir, A., Gonzalez, A., et al. (2016) Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med* 22: 250–253.

Donaldson, G.P., Lee, S.M., and Mazmanian, S.K. (2016) Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 14: 20–32.

Dunn, S.J., Nathke, I.S., and Osborne, J.M. (2013) Computational models reveal a passive mechanism for cell migration in the crypt. *PLoS One* 8: e80516.

Ermund, A., Schutte, A., Johansson, M.E., Gustafsson, J.K., and Hansson, G.C. (2013) Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. *Am J Physiol Gastrointest Liver Physiol* 305: G341–G347.

Erny, D., Hrabe de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., et al. (2015) Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* 18: 965–977.

Fanning, S., Hall, L.J., Cronin, M., Zomer, A., MacSharry, J., Goulding, D., et al. (2012) Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc Natl Acad Sci USA* 109: 2109–2113.

Farrage, J., Koren, I., Milo, I., Gurevich, I., Kim, K.W., Zigmond, E., et al. (2013) Luminal bacteria recruit CD103+-dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity* 38: 581–595.

Fink, J., Thery, M., Azioune, A., Dupont, R., Chatelain, F., Bornens, M., and Piel, M. (2007) Comparative study and improvement of current cell micro-patterning techniques. *Lab Chip* 7: 672–680.

Fletcher, A.G., Breward, C.J., and Jonathan Chapman, S. (2012) Mathematical modeling of monoclonal conversion in the colonial crypt. *J Theor Biol* 300: 118–133.

Furuse, M., and Okumura, J. (1994) Nutritional and physiological characteristics in germ-free chickens. *Comp Biochem Physiol A Physiol* 109: 547–556.

Garrido, D., Kim, J.H., German, J.B., Raybould, H.E., and Mills, D.A. (2011) Oligosaccharide binding proteins from © 2017 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd., *Environmental Microbiology*, 20, 2337–2353
Bifidobacterium longum subsp. infantis reveal a preference for host glycans. *PLoS One* **6**: e17315.

Gerbe, F., van Es, J.H., Makrini, L., Brulin, B., Mellitzer, G., Robine, S., *et al.* (2011) Distinct ATOH1 and Neurog3 requirements define tuft cells as a new secretory cell type in the intestinal epithelium. *J Cell Biol* **192**: 767–780.

Geuking, M.B., Cahenzli, J., Lawson, M.A., Ng, D.C., Slack, E., Hofpelmeier, S., *et al.* (2011) Intestinal bacterial colonization induces mutatisubstantial regulatory T cell responses. *Immunity* **34**: 794–806.

Glaister, J.R. (1973) Factors affecting the lymphoid cells in the small intestinal epithelium of the mouse. *Int Arch Allergy Appl Immunol* **45**: 719–730.

Gomez de Aguero, M., Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., *et al.* (2016) The maternal microbiota drives early postnatal innate immune development. *Science* **351**: 1296–1302.

Gordon, H.A. (1959) Morphological and physiological characterization of germfree life. *Ann NY Acad Sci* **78**: 208–220.

Gourbeyre, P., Berri, M., Lippi, Y., Meurens, F., Vincent-Naulleau, S., Laffitte, J., *et al.* (2015) Pattern recognition receptors in the intestinal tract and the crypt/villus axis. *Physiol Rep* **3**: e12225.

Gu, S., Chen, D., Zhang, J.N., Lu, X., Wang, K., Duan, L.P., *et al.* (2013) Bacterial community mapping of the mouse gastrointestinal tract. *PLoS One* **8**: e74957.

Guenet, J.L., Sacquet, E., Gueneau, G., and Meslin, J.C. (1970) [Action of total microflora of the rat on mitotic activity of Lieberkuhn’s crypts]. *C R Acad Sci Hebd Seances Acad Sci D* **270**: 3087–3090.

Gustafsson, B.E., and Maunsbach, A.B. (1971) Ultrastructure of the enlarged cecum in germfree rats. *Z Zellforsch Mikrosk Anat* **120**: 555–578.

Harmsen, H.J., Wildeboer-Veloo, A.C., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G., and Welling, G.W. (2000) Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* **30**: 61–67.

Hasegawa, M., Yang, K., Hashimoto, M., Park, J.H., Kim, Y.G., Fujimoto, Y., *et al.* (2006) Differential release and distribution of Nod1 and Nod2 immunostimulatory molecules among bacterial species and environments. *J Biol Chem* **281**: 29054–29063.

Hatch, J., and Mukoyama, Y.S. (2015) Spatiotemporal mapping of vascularization and innervation in the fetal murine intestine. *Dev Dyn* **244**: 56–68.

Heikkinen, A., and Thiele, I. (2015) Systems biology of host-microbe metabolomics. *Wiley Interdiscip Rev Syst Biol Med* **7**: 195–219.

Heikkinen, A., Sahoo, S., Fleming, R.M., and Thiele, I. (2013) Systems-level characterization of a host-microbe metabolic symbiosis in the mammalian gut. *Gut Microbes* **4**: 28–40.

Henehan, J.B. (1965) Imbalance of the normal microbial flora. The germ-free alimentary tract. *Am J Dig Dis* **10**: 864–869.

Hirokawa, Y., Yip, K.H., Tan, C.W., and Burgess, A.W. (2014) Colonic myofibroblast cell line stimulates colonoid formation. *Am J Physiol Gastrointest Liver Physiol* **306**: G547–G556.

Hooper, L.V., and Macpherson, A.J. (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* **10**: 159–169.

Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012) Interactions between the microbiota and the immune system. *Science* **336**: 1268–1273.

Hormann, N., Brandao, I., Jackel, S., Ens, N., Lillic, M., Walter, U., and Reinhardt, C. (2014) Gut microbiol colonization orchestrates TLR2 expression, signaling and epithelial proliferation in the small intestinal mucosa. *PLoS One* **9**: e113080.

Houghteling, P.D., and Walker, W.A. (2015) Why is initial bacterial colonization of the intestine important to infants’ and children’s health? *J Pediatr Gastroenterol Nutr* **60**: 294–307.

Human Microbiome Project, C. (2012) Structure, function and diversity of the healthy human microbiome. *Nature* **486**: 207–214.

Husebye, E., Hellstrom, P.M., Sundler, F., Chen, J., and Midttvedt, T. (2001) Influence of microbial species on small intestinal myoelectric activity and transit in germfree rats. *Am J Physiol Gastrointest Liver Physiol* **280**: G368–G380.

Hyland, N.P., and Cryan, J.F. (2016) Microbe-host interactions: influence of the gut microbiota on the enteric nervous system. *Dev Biol* **417**: 182–187.

Iwai, H., Ishihara, Y., Yamanaka, J., and Ito, T. (1973) Effects of bacterial flora on cecal size and transit rate of intestinal contents in mice. *Jpn J Exp Med* **43**: 297–305.

Jernberg, C., Lofmark, S., Edlund, C., and Jansson, J.K. (2010) Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* **156**: 3216–3223.

Johansson, M.E., Jakobsson, H.E., Holmen-Larsson, J., Schutte, A., Ermund, A., Rodriguez-Pineiro, A.M., *et al.* (2015) Normalization of host intestinal mucus layers requires long-term microbial colonization. *Cell Host Microbe* **18**: 582–592.

Johnston, M.D., Edwards, C.M., Bodmer, W.F., Maini, P.K., and Chapman, S.J. (2007) Mathematical modeling of cell population dynamics in the colonic crypt and in colorectal cancer. *Proc Natl Acad Sci USA* **104**: 4008–4013.

Jones, B.D., Ghor, N., and Falkow, S. (1994) Salmonella typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer’s patches. *J Exp Med* **180**: 15–23.

Jones, R.M., Luo, L., Ardita, C.S., Richardson, A.N., Kwon, Y.M., Mercante, J.W., *et al.* (2013) Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J* **32**: 3017–3028.

Juge, N. (2012) Microbial adhesins to gastrointestinal mucus. *Trends Microbiol* **20**: 30–39.

Kabouridis, P.S., and Pachnis, V. (2015) Emerging roles of gut microbiota and the immune system in the development of the enteric nervous system. *J Clin Invest* **125**: 956–964.

Kanther, M., Sun, X., Muhlauer, M., Mackey, L.C., Flynn, E.J., 3rd, Bagnat, M., *et al.* (2011) Microbial colonization...
induces dynamic temporal and spatial patterns of NF-kappaB activation in the zebrafish digestive tract. Gastroenterology 141: 197–207.

Kanter, M., Tomkovich, S., Xiaolun, S., Grosser, M.R., Koo, J., Flynn, E.J., 3rd., et al. (2014) Commensal microbiota stimulate systemic neutrophil migration through induction of serum amyloid A. Cell Microbiol 16: 1053–1067.

Kenworthy, R., and Allen, W.D. (1966) Influence of diet and bacteria on small intestinal morphology, with special reference to early weaning and *Escherichia coli*. Studies with germfree and gnotobiotic pigs. J Comp Pathol 76: 291–296.

Khoury, K.A., Floch, M.H., and Hersh, T. (1969) Small intestinal mucosal cell proliferation and bacterial flora in the conventionalization of the germfree mouse. J Exp Med 130: 659–670.

Kim, B.J., and Wu, M. (2012) Microfluidics for mammalian cell chemotaxis. Ann Biomed Eng 40: 1316–1327.

Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., et al. (2011) Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). Proc Natl Acad Sci USA 108: 8030–8035.

Klitgord, N., and Segre, D. (2011) Ecosystems biology of microbial metabolites. Curr Opin Biotechnol 22: 541–546.

Knoop, K.A., McDonald, K.G., McCrate, S., Mc Doyle, J.R., and Newberry, R.D. (2015) Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. Mucosal Immunol 8: 198–210.

Knoop, K.A., Gustafsson, J.K., McDonald, K.G., Kulkarni, D.H., Kassel, R., and Newberry, R.D. (2017) Antibiotics promote the sampling of luminal antigens and bacteria via colonic goblet cell associated antigen passages. Gut Microbes 8: 400–411.

Kopp, Z.A., Jain, U., Van Limbergen, J., and Stadnyk, A.W. (2015) Do antimicrobial peptides and complement collaborate in the intestinal mucosa? Front Immunol 6: 17.

Kulkarni, D.H., McDonald, K.G., Knoop, K., Miller, M.J., and Newberry, R.D. (2016) Small intestinal Goblet cells and Goblet cell associated antigen passages assist translocation of pathogenic bacteria. J Immunol 196: 136.135.

Kunze, W.A., Mao, Y.K., Wang, B., Huizinga, J.D., Ma, X., Forsythe, P., and Bienenstock, J. (2009) Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. J Cell Mol Med 13: 2261–2270.

Laranjeira, C., Sandgren, K., Kessaris, N., Richardson, W., Potocnik, A., Vanden Berghe, P., and Pachnis, V. (2011) Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. J Clin Invest 121: 3412–3424.

Leber, A., Viladomiu, M., Hontecillas, R., Abedi, V., Philipson, C., Hoops, S., et al. (2015) Systems modeling of interactions between mucosal immunity and the gut microbiome during clostridium difficile infection. PLoS One 10: e0134849.

Lee, S.A., Lim, J.Y., Kim, B.S., Cho, S.J., Kim, N.Y., Kim, O.B., and Kim, Y. (2015) Comparison of the gut microbiota profile in breast-fed and formula-fed Korean infants using pyrosequencing. Nutr Res Pract 9: 242–248.

Lesher, S., Walburg, H.E., Jr., and Sacher, G.A. Jr. (1964) Generation cycle in the duodenal crypt cells of germ-free and conventional mice. Nature 202: 884–886.

Leushacke, M., Barker, N., and Pin, C. (2016) Quantifying Lgr5-positive stem cell behaviour in the pyloric epithelium. Sci Rep 6: 21923.

Lhocine, N., Arena, E.T., Bomme, P., Ubelmann, F., Prevost, M.C., Robine, S., and Sansonetti, P.J. (2015) Apical invasion of intestinal epithelial cells by *Salmonella Typhimurium* requires villin to remodel the brush border actin cytoskeleton. Cell Host Microbe 17: 164–177.

Li, H., Limenitakis, J.P., Fuhrer, T., Geuking, M.B., Lawson, M.A., Wyss, M., et al. (2015) The outer mucus layer hosts a distinct intestinal microbial niche. Nat Commun 6: 8292.

Lim, M.C.C., Maubauch, G., Sokolova, O., Feige, M.H., Diezko, R., Buchbinder, J., et al. (2017) Pathogen-induced ubiquitin-editing enzyme A20 bifunctionally shuts off NF-kappaB and caspase-8-dependent apoptotic cell death. Cell Death Differ 24: 1621–1631.

Lopez-Garcia, C., Klein, A.M., Simons, B.D., and Winton, D.J. (2010) Intestinal stem cell replacement follows a pattern of neutral drift. Science 330: 822–825.

Lotz, M., Gutle, D., Walther, S., Menard, S., Bogdan, C., and Hornel, M.W. (2006) Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. J Exp Med 203: 973–984.

Mabbutt, N.A., Donaldson, D.S., Ohno, H., Williams, I.R., and Mahajan, A. (2013) Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. Mucosal Immunol 6: 666–677.

Macfarlane, G.T., Macfarlane, S., and Gibson, G.R. (1998) Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. Microb Ecol 35: 180–187.

Maclaren, O.J., Parker, A., Pin, C., Carding, S.R., Watson, A.J.M., Fletcher, A.G., et al. (2017) A hierarchical Bayesian framework for understanding the spatiotemporal dynamics of the intestinal epithelium. PLoS Comput Biol (accepted).

Macpherson, A.J., and Harris, N.L. (2004) Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 4: 478–485.

Marshall, B. (2002) Helicobacter pylori: 20 years on. Clin Med (Lond) 2: 147–152.

Maru, Y., Orihashi, K., and Hippo, Y. (2016) Lentivirus-based stable gene delivery into intestinal organoids. Methods Mol Biol 1422: 13–21.

Mathew, J.L. (2004) Effect of maternal antibiotics on breast feeding infants. Postgrad Med J 80: 196–200.

McDole, J.R., Wheeler, L.W., McDonald, K.G., Wang, B., Konjufca, V., Knoop, K.A., et al. (2012) Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 483: 345–349.

McGee, D.J., George, A.E., Trainor, E.A., Horton, K.E., Lotz, M., Gutle, D., Walther, S., Menard, S., Bogdan, C., and Hornel, M.W. (2006) Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. J Exp Med 203: 973–984.
McVey Neufeld, K.A., Perez-Burgos, A., Mao, Y.K., Bienenstock, J., and Kunze, W.A. (2015) The gut microbiome restores intrinsic and extrinsic nerve function in germ-free mice accompanied by changes in calbindin. *Neurogastroenterol Motil* **27**: 627–636.

Meslin, J.C., and Sacquet, E. (1984) Effects of microflora on the dimensions of enterocyte microvilli in the rat. *Reprod Nutr Dev* **24**: 307–314.

Meslin, J.C., Sacquet, E., and Guenet, J.L. (1973) Action of bacterial flora on the morphology and surface mucus of the small intestine of the rat. *Ann Biol Anim Biochim Biophys* **13**: 203–214.

Moorthy, A.S., Brooks, S.P., Kalmokoff, M., and Eberl, H.J. (2015) A spatially continuous model of carbohydrate digestion and transport processes in the colon. *PLoS One* **10**: e0145309.

Munoz-Tamayo, R., Laroche, B., Walter, E., Dore, J., Duncan, S.H., Flint, H.J., and Leclerc, M. (2011) Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. *FEMS Microbiol Ecol* **76**: 615–624.

Nava, G.M., Friedrichsen, H.J., and Stappenbeck, T.S. (2011) Spatial organization of intestinal microbiota in the mouse ascending colon. *ISME J* **5**: 627–638.

Neal, M.D., Sodhi, C.P., Jia, H., Dyer, M., Egan, C.E., Yazji, I., et al. (2012) Toll-like receptor 4 is expressed on intestinal stem cells and regulates their proliferation and apoptosis via the p53 up-regulated modulator of apoptosis. *J Biol Chem* **287**: 37296–37308.

Negroni, A., Cucchiara, S., and Stronati, L. (2015) Apoptosis, necrosis, and necroptosis in the gut and intestinal homeostasis. *Mediators Inflamm* **2015**: 250762.

Nigro, G., Rossi, R., Commere, P.H., Jay, P., and Sansonetti, P.J. (2014) The cytosolic bacterial peptidoglycan sensor Nod2 affords stem cell protection and links microbes to gut epithelial regeneration. *Cell Host Microbe* **15**: 792–798.

Noel, G., Baetz, N.W., Staab, J.F., Donowitz, M., Kovbasnjuk, O., Pasetti, M.F., and Zachos, N.C. (2017) A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. *Sci Rep* **7**: 45270.

Obata, Y., and Pachnis, V. (2016) The effect of microbiota and the immune system on the development and organization of the enteric nervous system. *Gastroenterology* **151**: 836–844.

Palmer, M.F., and Rolls, B.A. (1981) The absorption and secretion of calcium in the gastrointestinal tract of germ-free and conventional chicks. *Br J Nutr* **46**: 549–558.

Parker, A., Macalren, O.J., Fletcher, A.G., Muraro, D., Kreuzaler, P.A., Byrne, H.M., et al. (2017) Cell proliferation within small intestinal crypts is the principal driving force for cell migration on villi. *FASEB J* **31**: 636–649.

PastuBa, A., Middelhoff, M., Brandtner, A., Tobiasch, M., Höhl, B., Nuber, A., et al. (2015) Three-dimensional gastrointestinal organoid culture in combination with nerves or fibroblasts: a method to characterize the gastrointestinal stem cell niche. *Stem Cells Int* Article ID 149636.

Pin, C., Parker, A., Gunning, A.P., Ohta, Y., Johnson, I.T., Carding, S.R., and Sato, T. (2015) An individual based computational model of intestinal crypt fission and its application to predicting unrestricted growth of the intestinal epithelium. *Integr Biol* **7**: 213–228.

Pitt-Francis, J., Pathmanathan, P., Bernabeu, M.O., Bordes, R., Cooper, J., Fletcher, A.G., et al. (2009) Chaste: a test-driven approach to software development for biological modelling. *Comput Phys Commun* **180**: 2452–2471.

Pott, J., Stockinger, S., Torow, N., Smoczek, A., Lindner, C., Mincerney, G., et al. (2012) Age-dependent TLR3 expression of the intestinal epithelium contributes to rotavirus susceptibility. *PLoS Pathog* **8**: e1002670.

Praveen, P., Jordan, F., Priami, C., and Morine, M.J. (2015) The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome. *Microbiome* **3**: 41.

Pretzer, G., Snel, J., Molenaar, D., Wiersma, A., Bron, P.A., Lambert, J., et al. (2005) Biodiversity-based identification and functional characterization of the mannos-specific adhesin of Lactobacillus plantarum. *J Bacteriol* **187**: 6128–6136.

Pritts, T.A., Moon, M.R., Wang, Q., Hungness, E.S., Salzman, A.L., Fischer, J.E., and Hasselgren, P.O. (2000) Activation of NF-kappaB varies in different regions of the gastrointestinal tract during endotoxemia. *Shock* **14**: 118–122.

Pudlo, N.A., Urs, K., Kumar, S.S., German, J.B., Mills, D.A., and Martens, E.C. (2015) Symbiotic human gut bacteria with variable metabolic priorities for host mucosal glycans. *mBio* **6**: e01282–e01215.

Rakoff-Nahoum, S., Kong, Y., Kleinstein, S.H., Subramanian, S., Ahern, P.P., Gordon, J.I., and Medzhitov, R. (2015) Analysis of gene-environment interactions in postnatal development of the mammalian intestine. *Proc Natl Acad Sci USA* **112**: 1929–1936.

Reinhardt, C., Bergentall, M., Greiner, T.U., Schaffner, F., Ostergren-Lunden, G., Petersen, L.C., et al. (2012) Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. *Nature* **483**: 627–631.

Rescigno, M., Urbano, M., Valzasina, B., Francholini, M., Rotta, G., Bonasio, R., et al. (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* **2**: 361–367.

Reyniers, J.A. (1959) The pure-culture concept and gnotobiotics. *Ann NY Acad Sci* **78**: 3–16.

Rieder, R., Wisniewski, P.J., Alderman, B.L., and Campbell, S.C. (2017) Microbes and mental health: a review. *Brain Behav Immun* **50**: S0889-1591(17)30016-8.

Rinne, M.M., Gueimonde, M., Kalliomaki, M., Hoppu, U., Salminen, S.J., and Isolauri, E. (2005) Similar bifidogenic effects of prebiotic-supplemented partially hydrolyzed infant formula and breastfeeding on infant gut microbiota. *FEMS Immunol Med Microbiol* **43**: 59–65.

Rogoz, A., Reis, B.S., Karssenemeier, R.A., and Muicida, D. (2015) A 3-D enteroid-based model to study T-cell and epithelial cell interaction. *J Immunol Methods* **421**: 89–95.

Rolls, B.A., Turvey, A., and Coates, M.E. (1978) The effect of prebiotic-supplemented partially hydrolyzed infant formula and breastfeeding on infant gut microbiota. *Br J Nutr* **39**: 91–98.

Rutayisire, E., Huang, K., Liu, Y., and Tao, F. (2016) The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year.
of infants’ life: a systematic review. BMC Gastroenterol 16: 86.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., et al. (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459: 262–265.

Satoh, Y. (1984) Ultrastructure of Paneth cells in germ-free rats, with special reference to the secretory granules and lysosomes. Arch Histol Jpn 47: 293–301.

Schemann, M., and Neunlist, M. (2004) The human enteric nervous system. Neurogastroenterol Motil 16 (Suppl 1): 55–59.

Schluter, J., and Foster, K.R. (2012) The evolution of mutualism in gut microbiota via host epithelial selection. PLoS Biol 10: e1001424.

Schwank, G., Koo, B.K., Sasselli, V., Dekkers, J.F., Heo, I., Demircan, T., et al. (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13: 653–658.

Seedorf, H., Griffin, N.W., Ridaura, V.K., Reyes, A., Cheng, J., Rey, F.E., et al. (2014) Bacteria from diverse habitats colonize and compete in the mouse gut. Cell 159: 253–266.

Serafini, F., Turroni, F., Guglielmetti, S., Gioiosa, L., Foroni, E., Sanghez, V., et al. (2012) An efficient and reproducible method for transformation of genetically recalcitrant bifidobacteria. FEMS Microbiol Lett 333: 146–152.

Shawk, A., and McCole, D.F. (2017) Mechanisms of intestinal epithelial barrier dysfunction by adherent-invasive Escherichia coli. Cell Mol Gastroenterol Hepatol 3: 41–50.

Sheh, A., and Fox, J.G. (2013) The role of the gastrointestinal microbiome in Helicobacter pylori pathogenesis. Gut Microbes 4: 505–531.

Shin, D.M., Jeon, B.Y., Lee, H.M., Jin, H.S., Yuk, J.M., Song, C.H., et al. (2010) Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. PLoS Pathog 6: e1001230.

Shroff, K.E., Meslin, K., and Cebra, J.J. (1995) Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. Infect Immun 63: 3904–3913.

Smith, K., McCoy, K.D., and Macpherson, A.J. (2007) Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 19: 59–69.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., et al. (2010) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell 143: 134–144.

Sobko, T., Huang, L., Midtvedt, T., Norin, E., Gustafsson, L.E., Norman, M., et al. (2006) Generation of NO by probiotic bacteria in the gastrointestinal tract. Free Radic Biol Med 41: 985–991.

Sommer, F., Nookaew, I., Sommer, N., Fogelstrand, P., and Backhed, F. (2015) Site-specific programming of the host epithelial transcriptome by the gut microbiota. Genome Biol 16: 62.

Song, T., Li, K., Zhou, W., Zhou, J., Jin, Y., Dai, H., et al. (2017) A type III effector NleF from EHEC inhibits epithelial inflammatory cell death by targeting caspase-4. Biomed Res Int 2017: 4101745.

Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., et al. (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 470: 105–109.

Sprinz, H., Kundel, D.W., Damgin, G.J., Horowitz, R.E., Schneider, H., and Formal, S.B. (1961) The response of the germfree guinea pig to oral bacterial challenge with Escherichia coli and Shigella flexneri. Am J Pathol 39: 681–695.

Stappenbeck, T.S., Hooper, L.V., and Gordon, J.I. (2002) Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci USA 99: 15451–15455.

Stecher, B., Berry, D., and Loy, A. (2013) Colonization resistance and microbial ecophysiology: using gnotobiotic mouse models and single-cell technology to explore the intestinal jungle. FEMS Microbiol Rev 37: 793–829.

Stelling, J. (2004) Mathematical models in microbial systems biology. Curr Opin Microbiol 7: 513–518.

Szentkuti, L., and Enns, M.L. (1998) Comparative lectin-histochemistry on the pre-epithelial mucus layer in the distal colon of conventional and germ-free rats. Comp Biochem Physiol A Mol Integr Physiol 119: 379–386.

Tannock, G.W. (2007) What immunologists should know about bacterial communities of the human bowel. Semin Immunol 19: 94–105.

Tognini, P. (2017) Gut microbiota: a potential regulator of neurodevelopment. Front Cell Neurosci 11: 25.

Tsujii, M., Suzuki, K., Kitamura, H., Maruya, M., Kinoshita, K., Ivanov, I.I., et al. (2008) Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. Immunity 29: 261–271.

Vaishnava, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, X., Koren, O., et al. (2011) The antibacterial lectin RegIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science 334: 255–258.

Van Landeghem, L., Mahe, M.M., Teusan, R., Leger, J., Guisle, I., Houlgatte, R., and Neunlist, M. (2009) Regulation of intestinal epithelial cells transcriptome by enteric glial cells: impact on intestinal epithelial barrier functions. BMC Genomics 10: 507.

Veiga-Fernandes, H., and Pachnis, V. (2017) Neuroimmune regulation during intestinal development and homeostasis. Nat Immunol 18: 116–122.

Walton, K.D., Kolterud, A., Czerwinski, M.J., Bell, M.J., Prakash, A., Kushwaha, J., et al. (2012) Hedgehog-responsive mesenchymal clusters direct patterning and emergence of intestinal villi. Proc Natl Acad Sci USA 109: 15817–15822.

Wang, Y., Ahmad, A.A., Sims, C.E., Magness, S.T., and Allbritton, N.L. (2014) In vitro generation of colonic epithelium from primary cells guided by microstructures. Lab Chip 14: 1622–1631.

Wang, Y., Gunasekara, D.B., Reed, M.I., DiSalvo, M., Bultman, S.J., Sims, C.E., et al. (2017) A microengineered collagen scaffold for generating a polarized crypt-villus architecture of human small intestinal epithelium. Biomaterials 128: 44–55.
Wendelsdorf, K.V., Alam, M., Bassaganya-Riera, J., Bisset, K., Eubank, S., Hontecillas, R., et al. (2012) ENteric immunity simulator: a tool for in silico study of gastrointestinal infections. IEEE Trans Nanobiosci 11: 273–288.

Wilson, S.S., Tocchi, A., Holly, M.K., Parks, W.C., and Smith, J.G. (2015) A small intestinal organoid model of non-invasive enteric pathogen-epithelial cell interactions. Mucosal Immunol 8: 352–361.

Workman, M.J., Mahe, M.M., Trisno, S., Poling, H.M., Watson, C.L., Sundaram, N., et al. (2017) Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nat Med 23: 49–59.

Wroblewski, L.E., Piazuelo, M.B., Chaturvedi, R., Schumacher, M., Aihara, E., Feng, R., et al. (2015) Helicobacter pylori targets cancer-associated apical-junctional constituents in gas-troids and gastric epithelial cells. Gut 64: 720–730.

Yamanaka, T., Helgeland, L., Farstad, I.N., Fukushima, H., Midvedt, T., and Brandtzaeg, P. (2003) Microbial colonization drives lymphocyte accumulation and differentiation in the follicle-associated epithelium of Peyer’s patches. J Immunol 170: 816–822.

Yang, N.J., and Chiu, I.M. (2017) Bacterial signaling to the nervous system through toxins and metabolites. J Mol Biol 429: 587–605.

Young, H.M., Bergner, A.J., and Muller, T. (2003) Acquisition of neuronal and glial markers by neural crest-derived cells in the mouse intestine. J Comp Neurol 456: 1–11.

Yzu, Y., Lu, L., Sun, J., Petrof, E.O., and Claud, E.C. (2016) Preterm infant gut microbiota affects intestinal epithelial development in a humanized microbiome gnotobiotic mouse model. Am J Physiol Gastrointest Liver Physiol 311: G521–G532.

Zihni, C., Mills, C., Matter, K., and Balda, M.S. (2016) Tight junctions: from simple barriers to multifunctional molecular gates. Nat Rev Mol Cell Biol 17: 564–580.