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A Whole New Ball Game: Stem Cell-Derived Epithelia in the Study of Host-Microbe Interactions

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Abstract

Recent advances in developmental and stem cell biology have resulted in techniques that enable the generation and maintenance of complex epithelium in vitro. While these models have been utilized to study host development and disease, a renewed appreciation of host-microbe interactions has sparked interest in employing these new techniques to study microbes at the epithelial interface. Here we review the current advances in host-microbe interactions that have resulted from experiments using these complex epithelia. Furthermore we highlight aspects of these techniques that warrant further development to facilitate these the study of host-microbe interactions.

Keywords

enteroid; organoid; *S. Typhimurium*; *H. pylori*; *C. difficile*

Introduction

The study of host/microbe interactions has undergone a revolution over the past decade. One driver has been the interest in studying not only pathogens but also the indigenous microbiota of mammalian hosts. The difficulty in examining the relationship between pathogens, host and microbiome has resulted in the use of many germ-free and gnotobiotic animal models. To simplify these complicated relationships, a variety of cultured epithelial cells have also been used to study host/microbe interactions. Recent advances in stem cell and developmental biology resulted in procedures by which a complex, model epithelium can be generated and maintained in vitro. These systems are now being utilized to

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investigate host/microbe interactions at a scale that lies between intact mammalian hosts and cultured cell lines. In this review we will introduce this model and highlight recent work that has utilized these model epithelia to study of the interactions between microbes, both pathogenic and non-pathogenic, and the host epithelium (Figure 1).

Three-dimensional model epithelia

In vitro model systems for the study of mammalian epithelia have in the past primarily employed immortalized, often transformed, cell lines. While a great deal has been discovered using these systems, they fail to replicate important features of an intact epithelium, such as cellular polarization or production of mucus. An important advancement was the development of polarized epithelial monolayers by growing certain cell lines in the Transwell system [1]. While this more closely resembled the structure of the epithelium found at mucosal surfaces such as the gastrointestinal tract they generally contained a single cell type and thus still failed to replicate the physiology of the epithelium. More recently systems have been developed to initiate and maintain self-organizing epithelia that contain multiple cell types [2, 3]. The nomenclature for these self-organizing epithelia is somewhat variable and for the purpose of this review we will define the two main systems that we will discuss in detail. One system involves harvesting stem cells from terminally differentiated tissues and then maintaining them in culture. When supplied with appropriate growth and differentiation factors these stem cells give rise to a self-organized epithelium that can be maintained almost indefinitely in vitro [4]. This gives rise to globular epithelial-only structures referred to as enteroids.

An alternative approach was developed utilizing pluripotent stem cells, which can either be embryonic stem cells (ESCs) derived or in the form of reprogrammed, induced pluripotent stem cells (iPSCs) [5]. In this case the addition of specific growth and differentiation factors leads to a spherical collection of cells that includes both an epithelium and surrounding mesenchyme. To distinguish these from the enteroids, which contain only epithelium and lack the supporting mesenchyme, model epithelium derived from pluripotent stem cells are referred to as organoids. Some authors use the term organoid when referring to both of these systems, however for clarity we will maintain the distinction for the remainder of this review [6]. While there are technical and biological differences between organoids and enteroids (technical aspects of these systems are reviewed in more detail in [7]) both of these systems are being explored as model systems for studying host microbiome interactions. As an example, shortly after the publication of the development of the organoid model system, it was demonstrated that the system could be used to support the propagation of rotavirus [8].

Interactions between *Salmonella* and stem cell-derived model epithelia

Much of our understanding about the interactions between bacterial gastrointestinal pathogens and the intestinal epithelium has come from the study of *Salmonella enterica* Serovar Typhimurium. Initial work on the genetics and molecular function of bacterial type III secretion systems involved studying *S. Typhimurium* interactions with cultured cell lines. Investigators have recently utilized both the enteroid and organoid systems as potential novel models to study *S. Typhimurium*/epithelial interactions [9]. Zhang and colleagues

generated enteroids from murine colonic tissue and examined their response when exposed to *S. Typhimurium* [10]. The bacterium entered the epithelial cells of the enteroids and this resulted in disruption of the tight junctions. Furthermore this was associated with activation of the NF- κ B pro-inflammatory response in the infected epithelium. Utilizing organoids derived from human induced pluripotent stem cells, Forbester and colleagues extended this type of investigation between the model epithelium and *S. Typhimurium* by measuring host response through RNAseq analysis and measurement of cytokine responses [11]. By utilizing a *S. Typhimurium* that lacked the *invA* component of the SPI-1 type III secretion system, they demonstrated that this system could be utilized to functionally assess the pathogenesis of defined mutant strains.

In a recent report, gallbladder epithelium, regenerated from harvested mouse gallbladders, was used to investigate the relationship between chronic infection with *S. enterica* Serovar Typhi and the development of gallbladder cancer, an association that has been observed in the Indian subcontinent [12]. The investigators observed cellular transformation of the model gallbladder epithelium following infection with *S. Typhi*, as cells from infected gallbladder enteroids lost dependency on growth factors. These researchers demonstrated that this transformation was dependent on activation of the Akt and Map kinase pathways.

***Helicobacter pylori* and crypt-derived gastric epithelia**

The microaerobic epsilon-Proteobacteria *H. pylori* has been studied for its role in the pathogenesis of ulcer disease and gastric cancer. Recent publications have utilized an enteroid-like system using both mouse and human gastric mucosa as the starting point to develop stem cell-derived gastric epithelium [13–15]. A team led by Zavros and colleagues has used both mouse and human gastric enteroids to examine the epithelial response to infection with *H. pylori*. While it was known that *H. pylori* infection induced Sonic Hedgehog (Shh) expression during infection, using gastric enteroids they demonstrated that *H. pylori* triggers Shh expression via CagA dependent activation of NF- κ B [16]. As noted by the authors, gastric enteroids were particularly useful in this study as the absence of immune cells in this model enabled the authors to determine that NF- κ B activation resulted directly from *H. pylori* infection rather than recruitment of other cell types, a finding that would be more difficult to make in an animal model. Subsequently this group has continued to use enteroids to demonstrate that the host receptor CD44 played a functional role in the epithelial cell proliferation triggered by the *H. pylori* Cag pathogenicity island [17].

In addition to understanding how the host responds to infection with *H. pylori*, gastric enteroids have also been instrumental in demonstrating how *H. pylori* senses and responds to the host. Work by Huang et al., suggests an interesting mechanism by which *H. pylori* concurrently senses and modulates its environment while colonizing its gastric niche. Using spent media from both gastric enteroids and polarized cell lines they demonstrated that *H. pylori* uses the chemoreceptor TlpB to sense urea emanating from the epithelium [18]. Somewhat surprisingly the authors noted that TlpB is sensitive to low levels of urea and that physiological levels of urea in the stomach would inactivate the receptor. Ultimately, they found that *H. pylori*'s ability to break down urea via its urease facilitates its ability to sense host urea.

Anaerobes and intestinal epithelia

Most bacterial members of the mammalian gut microbiota are considered strict anaerobes. Understanding the relationships between these members of our microbial community and the epithelium has so far been difficult in vitro. That host tissues require oxygen to survive presents an obvious technical hurdle for the prospect of co-culture with anaerobes. However, using mouse ileal enteroids Lukovac et al. sought to overcome this technical limitation by injecting enteroids with spent culture supernatant from two members of the microbiota, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* [19]. Following injection they found that culture supernatant from *A. muciniphila* significantly altered epithelial gene transcription, specifically genes involved in lipid metabolism, while culture supernatant *F. prausnitzii* had minimal effect.

In addition to indigenous members of the microbiota, virulence factors like the toxins from the anaerobic nosocomial pathogen *Clostridium difficile* have been used to demonstrate the feasibility of using model epithelia in the study of host-anaerobe interactions [20]. Wang et al. showed that colonic epithelia from cloned ground state colonic stem cells, treated with Toxin B resulted in increased paracellular permeability and altered gene expression, similar to what is seen in human infections. Other groups have used induced human intestinal organoids, which are more similar to the tissue found in the ileum, to study how *C. difficile* might alter host physiology to create a favorable environment for colonization [5, 21, 22]. Recently, our group showed that these human intestinal organoids are sensitive to *C. difficile* toxin A, while being less sensitive to Toxin B. In addition to using purified toxin and spent culture media, we also showed that that two different isolates of *C. difficile* can persist in the lumen of human intestinal organoids and in the case of the toxigenic strain, produce toxin [23]. However, to fully study anaerobic bacteria in the context of these model epithelia there is a need to develop better techniques for co-culture of enteroids and/or organoids with anaerobes.

Concluding Remarks

While we have highlighted work that has used enteroids to study direct interactions between bacteria and host epithelium, others have used these enteroids to explore how host signaling alters the physiology of the epithelium and ultimately the outcome of an infection [24]. Using mouse derived colonic enteroids Pham et al. showed that in the absence of signaling through the IL-22 receptor, cells had decreased expression of genes responsible for antimicrobial peptides and epithelial glycosylation. The authors specifically saw a decrease in *Fut2* expression, a gene that encodes an α 1,2-fucosyltransferase. Moving into an animal model, they found that exogenous administration of an α 1,2-fucosylated oligosaccharide protected mice deficient in the IL-22 receptor from death following infection with *Citrobacter rodentium*. The authors went on to postulate that increased epithelial fucosylation as result of IL-22 signaling altered the microbiota and prevented outgrowth of opportunistic pathogens. Studies of this nature show how enteroids can be incorporated into our current repertoire of techniques, as a middle ground between simple monolayers of cells and animal models. Using model systems like enteroids and organoids can lead to better

understanding of the complex interplay between the host, its resident microbiota, and invading pathogens.

Model epithelia such as enteroids and organoids are a relatively recent advance for the study of tissue development and function, however they have already proven to be useful new tools to study host/microbe interactions. Currently there are several different methods for developing these model epithelia, including different sources of starting cell lines (mouse vs. human, ESCs vs iPSC etc.). However, much is still not known about the effect of these methodological differences on the physiology of enteroids or organoids. Recent reports demonstrate that human intestinal organoids are more similar to fetal intestine, however grafting these organoids to mouse kidney capsule results in more adult morphology [25, 26]. While this apparent flexibility in maturation phenotype enables specific questions to be addressed, this inherent variability must be kept in mind to permit proper interpretation of experimental results. As with any new technology, the apparent paradigm shift needs to be followed by a period where the change is put into context of what came before [27]. We are entering the era where the true utility of these novel systems can be appreciated.

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Highlights

- Stem cell-derived model epithelia recapitulate aspects of the GI tract.
- These models are an intermediate between polarized cell lines and animals.
- *S. Typhimurium*, *H. pylori*, and *C. difficile* have been studied using these systems.

Figure 1. Using enteroids and organoids, epithelial surrogates of gastrointestinal organs, to study host-microbe interactions

Clockwise from the right, enteroids derived from the stomach were utilized to examine how *H. pylori* responds to urea (18). Colonic organoids have been shown to be sensitive to treatment with *C. difficile* toxin (20). Organoids that resemble the ileum have been used to study *S. enterica* Serovar Typhi and *C. difficile* (11, 23). Gallbladder derived enteroids were used to investigate the association between infection with *S. enterica* Serovar Typhi and development of gallbladder cancer (12).