Potential Use of Human Stem Cell–Derived Intestinal Organoids to Study Inflammatory Bowel Diseases

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Inflammatory bowel disease (IBD) is a chronic remitting disorder with increasing incidence worldwide. The intestinal epithelial barrier plays a major role in IBD, contributing to its pathogenesis, evolution, and perpetuation over time. Until recently, studies focused on exploring the role of the intestinal epithelium in IBD were hampered by the lack of techniques for the long-term culturing of human primary epithelial cells ex vivo. Recently, however, a methodology for generating stable human 3D epithelial cultures directly from adult intestinal stem cells was established. These long-term cultures, called organoids, mimic the tissue of origin and can be generated from small-size intestinal tissue samples, making it a promising tool for modeling the course of IBD.

In this review, we provide an overview of the versatility of human organoid cultures in IBD modeling. We discuss recent advances and current limitations in the application of this tool for modeling the contribution of the intestinal epithelium alone and in combination with other key cellular and molecular players in the context of IBD pathophysiology. Finally, we outline the pressing need for technically standardizing the laboratory manipulation of human epithelial organoids for their broader implementation in clinically oriented IBD studies.

Key Words: IBD, disease modeling, intestinal epithelium, human epithelial organoids

INTRODUCTION

Crohn’s disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBDs) of uncertain etiology, characterized by alternating periods of activity and remission.1, 2 The current consensus holds that IBD may result from an inappropriate immune response to commensal microorganisms, with genetic susceptibility and environmental factors contributing, to varying degrees, to the disease risk.3 Incidence of IBD is increasing worldwide, in both pediatric and adult populations,4, 5 strongly suggesting that environmental cues play some role.

Growing evidence suggests that the intestinal epithelium, a monolayer of epithelial cells that governs the interactions between the luminal microbiota and the underlying lamina propria, plays a key role in the pathogenesis of IBD (Fig. 1).6, 7 Several IBD-associated genetic risk loci affect pathways active in epithelial cells, which are involved in essential functions such as bacterial sensing, autophagy, and endoplasmic reticulum stress.8 In addition, dysfunctions in the epithelial barrier, secondary to acute or chronic inflammation, are also characteristic of IBD.9 During active disease, pro-inflammatory factors are released in the intestinal mucosa, progressively damaging the epithelial layer.10, 11 This exposes the underlying lamina propria to luminal antigens and consequently amplifies the inflammatory response, which may become chronic and difficult to manage. The epithelium is also actively involved in repair processes that are endogenously activated to re-establish mucosal homeostasis.12, 13 Re-epithelialization is an essential process in achieving mucosal healing and represents a gold standard in both UC and CD management. Indeed, mucosal healing is associated with more favorable prognoses, fewer hospitalizations, and lower surgery rates.14 Nonetheless, we have previously shown that the intestinal epithelium of patients with UC can harbor lasting alterations despite complete endoscopic and histologic remission.15 We hypothesized that these persistent alterations in the epithelium of remitting IBD patients could also contribute to disease perpetuation, including neoplastic degeneration, which has been observed in long-standing IBD.16

Until recently, the study of the functional role of the intestinal epithelium in IBD has been hampered by the inability to stably maintain cultures of these cells in vitro. A major technical breakthrough in the practice of laboratory manipulation of human intestinal epithelial cells is the recent development of 3D culture systems, also known as organoid cultures, derived from self-organizing pluripotent stem cells (PSCs) or intestinal adult stem cells (ASCs).17, 18 When supplied with
FIGURE 1. Human adult epithelial stem cell–derived organoids in inflammatory bowel disease modeling. The healthy gut epithelial barrier (Panel 1, left side of the picture) is a monolayer of columnar cells organized into invaginations called crypts. It represents the interface between the intestinal lumen, containing the gut microbiota on 1 side, and the lamina propria (LP), where immune and mesenchymal cells reside, on the other. Adult stem cells (ASCs) reside at the bottom of each crypt (ASC niche) and continuously give rise to daughter cells that differentiate into the various epithelial subtypes. The disruption of the epithelial layer contributes to both the development and the perpetuation of inflammatory bowel disease (IBD). Injury to the mucosa, which is the hallmark of active IBD, can result from a predisposing genetic and/or epigenetic background (Panel 1, right side of the picture) in some patients, combined with aberrant pro-inflammatory signals from the surrounding luminal and LP environment (Panel 2). Intrinsic mechanisms are then activated within the intestinal mucosa that promote wound healing and thus restore tissue homeostasis (Panel 3). However, permanently disturbed epithelial functions that are not restored during remission may contribute to the persistence of IBD over time (Panel 4). Patient-specific organoid cultures generated from the ASCs of non-IBD and IBD mucosa represent a versatile tool for exploring the role of the epithelium in the diverse IBD-relevant scenarios depicted in the figure (A–C). By using “omics” technologies, for instance, cohorts of pure epithelial cultures (A) can be used to obtain in-depth profiles of the epithelial genetic/epigenetic background or the effect of engineered genetic mutations (Panel 1). Similarly, “omics” approaches can also be applied to organoid cultures to interrogate the effects of this background or of epigenetic marks (acquired from the persistent exposure of the epithelial lining to pro-inflammatory signals) (A) in IBD perpetuation (Panel 4). Alternatively, 3D organoid cultures can be adapted, for example, to a 2D platform, to study the effects of the direct or indirect (eg, through secreted mediators) interactions of the epithelium with the luminal microbiota (B) and LP cells (C) during the different stages of IBD pathophysiology (Panels 2, 3, and 4).
growth factors and signals that mimic the intestinal stem niche environment, organoids can expand for several months. These cultures can reflect the architecture, regional specification, and cell composition of the epithelium in vivo, and in the case of PSC-derived organoids, they are able to generate adjacent stromal cells. Increasing evidence shows that both PSC- and ASC-derived organoid cultures represent promising approaches for studying the role of the intestinal epithelium in homeostasis and in several human disorders, including inflammatory diseases.

In this review, we provide an overview on the present understanding and future applications of ex vivo human organoid cultures with a specific focus on the ASC-derived culture system, their potential uses in clinically oriented research, and the current advantages and technical limitations in IBD modeling.

**POSSIBLE USES OF HUMAN ADULT STEM CELL–DERIVED INTESTINAL ORGANOIDS IN IBD MODELING**

The first organoid cultures from primary intestinal tissue were generated from sorted Lgr5+ ASCs derived from mouse small intestinal crypts. Mouse epithelial organoid cultures were then modified and optimized for the generation of 3D organoids from ASCs expanded directly from human intestinal crypts. ASC-derived organoid cultures can contain multiple cell types, or can be purely epithelial if generated from isolated intestinal crypts or single epithelial cells. The latter have also been referred to as enteroids or colonoids, depending upon whether they are generated from the small or large intestine. The versatility of the ASC-derived organoid culture system makes it a powerful tool to explore the role of the epithelium in IBD (Fig. 1). Indeed, these organoids can be expanded from intestinal tissue samples of healthy and IBD mucosa in both adult and pediatric patients, with comparable growth efficiency. Moreover, organoid cultures can be efficiently generated from both the human small intestine and colon and are able to retain the location-specific transcriptional and epigenetic profiles of the intestinal segment from which they have been drawn, even after prolonged culture. ASC-derived organoids are also genetically stable. Genetic and epigenetic stability represent a clear advantage of ASC-derived organoids over PSC-derived human intestinal organoids, which can acquire genetic and epigenetic variations during the reprogramming process or culturing. In contrast to transformed intestinal cell lines, which are usually composed of only 1 cell type (e.g., Caco2, enriched in enterocyte-like cells, or HT29, enriched in Goblet-like cells), ASC-enriched organoids can give rise to all of the differentiated lineages that populate the intestinal crypt, even those less represented, like enteroendocrine, tuft, or M cells. This aspect is very important for disease modeling due to the fact that defects in several epithelial cell type functions have been observed in IBD.

Another advantage of ASC-derived organoid cultures is that they can be generated from a very limited amount of intestinal tissue (i.e., endoscopic biopsies). This offers a 2-fold benefit: 1) endoscopic biopsies provide a source of organoid cultures from endoscopically inactive or mildly active areas that are rarely obtained from surgical samples; 2) control samples can be accessed from a more appropriate age-matched non-IBD group (patients undergoing colorectal cancer (CRC) screening colonoscopies and who are free of cancerous or precancerous lesions).

Recent technical advances in the culturing of human organoids will further foster their use in IBD modeling. For example, 2D cultures derived from 3D organoids represent a valuable alternative to other 2D cultures of primary epithelial cells, such as the air–liquid interface cultures of intestinal stem cells, for studying host–microbiota interactions in IBD. The observation that 2D cultures can be differentiated toward secretory cell populations makes this organoid-derived culture system a useful tool for exploring how epithelial factors from specific cell subpopulations, like the recently discovered sentinel goblet cells, for example, can modulate the adhesion of IBD-associated bacterial strains. Similarly, an increasing number of studies show promising results using co-cultures of pure epithelial ASC-derived organoids that include mesenchymal or immune cells. The addition of this level of complexity to the organoid system could yield a more physiologically accurate model for dissecting IBD pathogenesis; however, no studies using this approach have been published thus far in the context of IBD.

Moreover, organoid cultures are amenable to genetic modifications. Indeed, genetic editing based on CRISPR/Cas9 technology has been successfully tested in organoid cultures from patients with CRC and cystic fibrosis. In addition, the gymnosis technique for knocking down gene expression has recently been validated in mouse intestinal organoids. Inflammatory bowel disease could also benefit from these approaches, especially in those cases, such as in very early-onset IBD (VEO-IBD; see the next section), where a specific genetic mutation is responsible for a defect in the intestinal epithelial barrier.

Finally, intestinal ASC-derived organoid cultures can be cryopreserved with high rates of recovery upon thawing. This practice facilitates the establishment of living biobanks of patient-derived organoids that can be very useful to translational studies in which accessibility to patient samples is often a limiting factor. Studies showing a high genetic concordance between cryopreserved organoid cultures and corresponding biopsy specimens from CRC patients have already been published. Indeed, IBD organoid culture biobanks are already available to researchers.

In the following sections, we will explore in more detail the use of human intestinal organoid cultures in IBD modeling. We will focus on the role of the epithelium in contributing to IBD...
predisposition via its genetic and epigenetic background and to mucosal injury, wound repair, and disease perpetuation and how organoids could be used to study all of these phenomena.

**Use of Epithelial Organoids to Study the Impact of the Genetic and Epigenetic Background on the IBD Epithelium**

Increasing evidence shows that genetic alterations affecting intestinal epithelial functions can play a pivotal role in the pathogenesis of IBD (Fig. 1, Panel 1). Most forms of IBD are polygenic, with multiple susceptibility loci contributing to the overall risk of disease. Thus far, genome-wide association studies have identified approximately 250 risk loci. Some of the variants associated with intestinal epithelial cell functions have been shown to correlate with abnormal epithelial cell phenotypes, such as in the case of small intestinal Paneth cells. In addition to polygenic IBD, there are rare forms of IBD with very early onset (e.g., VEO-IBD, occurring in children younger than 5 years old), where the role of host genetics on disease development is prominent. In these Mendelian forms of IBD, genetic defects affecting epithelial function have also been identified in some patients (e.g., ADAM17, TRIM22, TTC7A). As organoid cultures retain the genetic makeup of the original tissue, they offer suitable material for exploring genetic–phenotypic associations in IBD, similar to what has been done in mono-factorial stable material can be obtained and applied to a wide array of laboratory techniques, from standard DNA-, RNA-, and protein-based approaches to advanced “omics” technologies (e.g., single-cell RNA sequencing, MethylC sequencing, mass spectrometry) and live imaging (Fig. 1A). However, due to the low frequency of these phenotype-associated genetic alterations, the introduction of mutations into normal organoids (e.g., by CRISPR/Cas9, TALEN, or ZFN technologies) may represent a better alternative for studying the impact of these mutations on epithelial cell function (Fig. 1A). The use of the organoid system has also contributed to the identification of age-dependent differences in the human intestinal epithelium that could affect its function. For example, it has been shown that epithelial ASCs accumulate spontaneous genetic mutations during their lifetime, which could promote intestinal tumorigenesis. In line with this, it has been observed that the epithelium from fetal, vs adult, subjects showed intrinsic differences in response to external stimuli when a monolayer derived from a human organoid culture was challenged with pro-inflammatory cytokines. Taken together, these observations suggest that organoid cultures represent reliable systems to reproduce intrinsic epithelial alterations ex vivo.

The presence of epigenetic marks in the epithelium of IBD patients (Fig. 1, Panel 1) has also been recently identified. In a recent work, Howell et al. showed that changes in the DNA methylation profile of purified IBD epithelium present at diagnosis were stably maintained during follow-up regardless of the mucosal inflammatory status. Such changes were partly retained in derived organoid cultures, reinforcing the idea of the primary nature of this epigenetic imprinting.

**Use of Epithelial Organoids to Model the Interactions of the Epithelium With the Environment in Mucosal Injury and Wound Repair**

In the context of acute or chronic intestinal inflammation, the epithelium contributes to the overall damage by amplifying the inflammatory response induced by microbial and lamina propria factors (Fig. 1, Panel 2). The ASC-derived organoid culture system provides a promising platform for examining ex vivo the result of the crosstalk of the epithelium with the surrounding pro-inflammatory environment (including both soluble and cellular components) in the context of IBD. In particular, organoid cultures from healthy subjects can serve to explore the specific biological role played by IBD-associated pro-inflammatory factors in epithelial barrier integrity. Conversely, healthy organoids that have been genetically edited with IBD-associated mutations (as discussed above) or those from IBD patients can be exploited to explore the cumulative effect of genetics and a pro-inflammatory environment on epithelial integrity. This feature can be particularly useful in cases where genetic and epigenetic variants do not translate to a direct effect on the epithelial behavior or phenotype, unless the contribution of immune, stromal, or luminal factors is also considered.

The ASC-derived organoid culture system has been already successfully used to investigate the effects of diffusible compounds on the intestinal epithelium, supporting their potential use in IBD modeling (Fig. 1B, C). For example, 2 of the best-characterized pro-inflammatory cytokines in IBD, IFN-γ and TNFα, have been shown to influence epithelial functions in 3D-cultured organoids. Thus, organoid cultures could also be helpful in elucidating the function of other cytokines, the role of which on the epithelial barrier remains controversial. Moreover, evidence that specific microbial communities are associated with CD and UC makes the study of the complex interactions of the epithelium with whole or isolated components of the gut microbiota another promising application of the organoid system. For example, it could help elucidate the contribution of alterations in the microbiota–host crosstalk to mucosal injury. Indeed, the organoid culture system has also been implemented to assess the effects of microbial-derived metabolites, such as short-chain fatty acids, on the epithelium. Interestingly, Kaiko et al. showed that, although protective to the upper epithelial crypt, butyrate had detrimental effects on the epithelial stem cell compartment, challenging the supposed beneficial role played by this
metabolite in active IBD. Based on their observation, they proposed that intestinal crypts play a role in protecting stem cells at the bottom of the crypt from microbial metabolites by limiting their access to this cell subset, which relies on continuous proliferation and is essential for crypt regeneration. One could argue that in the context of crypt destruction in the ulcerated mucosa of IBD patients, stem cells may be exposed to higher concentrations of metabolites and other luminal factors normally excluded from the lower crypt compartment. Organoids therefore represent a relatively simple system for studying interactions with diffusible molecules such as cytokines or metabolites.

Notably, the apical side of the organoid, which is targeted by microorganisms in vivo, is oriented toward the inner lumen of the spheroid. Several approaches have been introduced to facilitate access to microorganisms, making the system more physiologically relevant (Fig. 1B). For example, microinjection of bacteria into the organoids demonstrated that microorganisms can persist in a viable state in the spheroid inner lumen and affect several physiological functions of the epithelial barrier.

Similarly, by using human 2D-converted organoid cultures, the effect of microbial colonization on mucosal homeostasis has been explored. For example, recent studies show that pathogenic bacteria can not only cause significant changes in epithelial permeability and tight junction distribution, but can also compromise the mucus layer integrity, both important contributing factors to IBD pathophysiology. Although studies examining viruses in IBD are limited, growing evidence indicates a role of the gut virome in IBD pathogenesis. In that sense, the organoid system could also be used to model virus–epithelium interactions, for example, by infecting dissociated organoids with eukaryotic viral particles, similar to what has been done in manipulating organoid gene expression using the lentiviral system.

The organoid culture also provides a system to mimic ex vivo the cell contact–dependent or–independent interactions between the epithelium and lamina propria intestinal cell types in IBD (Fig. 1C). Promising results have been published on co-cultures of primary epithelial cell cultures with mesenchymal cells. Notably, the establishment of the epithelial monolayer–adipocyte co-culture showed that pro-inflammatory responses can be mutually activated in these 2 cell populations through paracrine signals without the influence of immune cells. This system could, therefore, offer a new tool for exploring the interactions of the epithelial barrier with mesenteric adipose tissue, for which a role in IBD has been recently postulated. Organoid cultures have also been used as a promising model to study the interactions of the epithelium with specific subpopulations of immune cells. Nozaki et al., for example, by generating co-cultures of murine organoids and intraepithelial lymphocytes (IELs), gained insights into the characterization of survival and motility mechanisms of IELs, which have recently been shown to be altered in IBD mucosa. Similarly, co-cultures of human organoid–derived monolayers with macrophages have helped elucidate how the epithelium and innate immune cells coordinate the response to enteric pathogens.

Similar to the induction of epithelial injury, restitution of the epithelial barrier is also an active process orchestrated by luminal factors, epithelial cells, and the stromal compartment (Fig. 1, Panel 3). During epithelial healing, the wound-associated epithelium (WAE) loses polarity and starts migrating toward the lesion to restore integrity. Multiple factors are released during this phase, both by mesenchymal cells and by the epithelium itself.

Organoid cultures can be a useful tool for elucidating the mechanisms of wound healing mediated by stromal factors (Fig. 1C). For example, studies co-culturing mouse organoids and fibroblasts have revealed that the latter improve epithelial growth and survival, suggesting that these cells play a role in providing prosurvival factors for epithelial stem cells. Similarly, recently established co-cultures of mouse intestinal organoids and primary neuronal cells have shed new light on the importance of signals from the enteric nervous system in promoting the survival of specific epithelial cell subpopulations. This co-culture system could provide a novel tool for revealing the role of enteric nervous system abnormalities in the pathophysiology of IBD. Miyoshi et al. recently proposed a novel mechanism of wound repair mediated by the epithelium. They showed that the addition of PGE2 to epithelial organoid cultures promotes their differentiation to WAE cells through the Ptger4-mediated signaling pathway, reporting similar results in vivo. The flexibility of 3D organoid cultures to grow in a monolayer also makes them useful as a tool for performing other assays geared toward exploring the role of the epithelium in wound healing, such as the Transwell migration or wound scratch assays. Organoids show high responsiveness to the stroma-derived soluble factors that are released during intestinal inflammation and promote epithelial regeneration, including IL-22 and IL-6, and to cytokines, such as IL-33, which stimulate the recovery of functionally differentiated lineages. Organoid cultures have also been useful tools for uncovering Wnt-dependent mechanisms associated with epithelial regeneration in IBD. For example, localized Wnt5a release on organoid cultures, which mimics wound channels, suppresses proliferation in a TGF-β-dependent manner, thus giving new insight into the role of noncanonical Wnts in new crypt formation after tissue injury. Based on these results, it is conceivable that human organoid cultures could also be useful for exploring the role of endogenous lipid mediators and lesser-known microbial metabolites (eg, derivatives of tryptophan and Gamma-amino butyric acid), which have been shown to contribute to the resolution of tissue inflammation but whose beneficial effects on the intestinal epithelium are only now becoming better understood (Fig. 1B).
Use of Epithelial Organoids to Model Changes in the IBD Epithelium Over Time

Inflammatory bowel disease is chronically relapsing in nature. This behavior suggests that subclinical mechanisms acquired during inflammation remain altered in IBD patients during remission, thereby contributing to disease perpetuation and progression over time (Fig. 1, Panel 4). Multiple studies reported that a number of transcriptional changes persist in the intestinal mucosa of IBD patients well after complete resolution of the inflammatory flare; many of these alterations are associated with the epithelium.15, 116–118 Moreover, in a recent study, we demonstrated that organoid cultures derived from IBD patients exhibit altered transcriptional signatures, suggesting that the stem compartment is engraved with changes potentially acquired during previous local exposure to inflammation.119 In line with these results, the contribution of prolonged inflammation in imprinting the epithelium with lasting expression changes appears evident in the recently established “inflamed organoid model.”120 In this system, prolonged treatment of organoid cultures with a cocktail of pro-inflammatory cytokines induced the persistent activation of NFκB signaling and reactive oxygen species production that continued long after removal of the inflammatory milieu. As proposed by the authors, this system could be useful for modeling how chronic inflammation leads to transformation of the epithelial compartment, a phenomenon often associated with CRC development in IBD patients.121 Chronic or recurrent inflammation also plays a major role in the initiation of intestinal fibrosis, another common complication in IBD.122 The recent establishment of the organoid-based epithelial-to-mesenchymal transition (OEMT) model123 showed how stimulation with pro-inflammatory cytokines, such as TGF-β and TNF-α, synergistically produced marked mesenchymal changes in the intestinal epithelium, making the OEMT a promising model with which to dissect the role of epithelial–mesenchymal transition in intestinal fibrosis.

In addition, studies involving organoid samples generated from well-defined subpopulations of IBD patients (Fig. 1A) could contribute to the molecular stratification of the disease, similar to what has been done using intestinal tissue samples.124 Ideally, identification of the links between epithelial expression phenotypes and IBD clinical behavior (eg, disease course, response to treatment, risk of progression to colitis-associated CRC) could complement the information derived from conventional clinical classification and genetic association studies.126

Growing evidence supports the idea that epigenetic marks are not only primarily present in the intestinal epithelium (as discussed above) but are also acquired by IBD mucosa during the course of the disease.127 Among them, alterations in the DNA methylation profile and in noncoding RNA (ie, miRNA and lncRNA) expression have been identified in the mucosa of IBD patients.128, 129 Multiple epigenetic mechanisms seem to specifically regulate epithelial function, both in homeostasis and IBD130–132; lamina propria and microbial signals are largely responsible for regulating these mechanisms.50, 133 Organoid cultures can be helpful to test the role of lasting epithelial changes induced by disease-dependent stromal and bacterial factors in promoting IBD persistence (Fig. 1B, C). This is possible because organoids, in contrast to short-term cultured crypts or primary epithelial cultures,134, 135 can be maintained for several weeks, and therefore allow for detection of the epigenetic changes that occur during extended stimulation.

CHALLENGES IN USING ORGANOIDS TO STUDY IBD

The clinical applications and future perspectives of human organoids are very encouraging; however, they largely depend on the optimization of a few technical aspects. Growth conditions need to be further improved and standardized. Attempts in this direction include the replacement of Wnt3a-conditioned medium with commercial alternatives136 or the use of synthetic extracellular matrices, including hydrogel and collagen, instead of nonstandardized mouse sarcoma-derived Matrigel.137

Another source of variability and potential low yields when culturing organoids is the use of tissue derived from actively inflamed intestinal areas. Due to the loss or erosion of the epithelial layer (especially in UC), the number and quality of intestinal crypts may be too low to support the growth of sufficient organoids for further experiments. Using tissue from involved segments with mild inflammation or in remission can, in our experience, help solve this problem.

The establishment of co-cultures using lamina propria cells and either spheroids or epithelial monolayers is also a technically challenging endeavor that many investigators are trying to tackle. Indeed, ASC-derived cultures are purely epithelial and do not provide the mesenchymal scaffold required to sustain the proper functional development of lamina propria cell types. In this sense, PSC-derived cultures offer a more physiologically accurate model for studying the high complexity of mucosal interactions, as it has been recently demonstrated in PCS-derived epithelial organoids containing both mesenchyme and functional enteric nervous cells.138

Finally, the development of a reliable model of microbiota–epithelium interactions also presents some challenges. Despite the fact that, ideally, one could isolate and culture the whole gut microbial communities from human stool,139 the primarily anaerobic nature of gut microbiota makes its co-culture with aerobic cells a significant hurdle yet to be overcome. In addition to technical drawbacks, another key aspect to consider when using organoid cultures in translational medicine, as with any other primary culture, is the complex and biologically heterogeneous nature of IBD.124, 140, 141 The screening of large
cohorts of organoid samples based on the clinical subphenotypes of patients combined with the use of new high-throughput microfluidics technologies will be required to effectively reduce these technical and biological biases.

CONCLUDING REMARKS

Available therapeutic strategies in IBD aim to induce remission by suppressing the inflammatory response. However, current treatments fail in a significant percentage of patients. This strongly underscores the need to identify new therapeutic targets beyond pure immune suppression.

The intestinal epithelium has a crucial role in preserving tissue homeostasis and driving regeneration by interacting, responding, and controlling both microbial and lamina propria components. Moreover, epithelial cells act as amplifiers of the inflammatory cascade by responding to (via receptor expression) and secreting key inflammatory molecules. None of the currently available therapies specifically target the epithelial lining, although some of them can directly or indirectly affect it (eg, corticosteroids, 5-ASA, cyclosporine).

Whether organoids are utilized to obtain a better understanding of the individual disease pathogenesis and pathophysiology to evaluate the mechanistic actions underlying currently available treatments or to assess new therapeutic options, this system presents unique advantages and opportunities in drug development and personalization, and in toxicity and efficacy evaluations.

In addition, as discussed throughout this review, the introduction of a human ASC-derived intestinal organoid model could help accelerate research in IBD by providing an ideal compromise between human transformed cell lines and animal models.

By mimicking the tissue of origin, organoid cultures provide a reductionist approach for studying patient-specific epithelial phenotypes and functions while also potentially supporting interactions with other cellular and microbial components. Moreover, organoid cultures have been shown to remain stable after freezing and thawing, a feature that has aided the development of living biobanks of patient-derived organoids.

In conclusion, despite the still limited number of published studies involving the use of human organoid cultures in investigating IBD physiopathology, epithelial ASC-derived organoid cultures represent a very promising tool for modeling IBD and personalizing medicine. Their potential arises from the fact that they maintain site- and patient-specific characteristics and therefore mimic cell behavior in an extremely versatile ex vivo model.

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