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Modeling human gastrointestinal inflammatory diseases using microphysiological culture systems

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Abstract

Gastrointestinal (GI) illnesses are a significant health burden for the US population, with 40 million office visits each year for gastrointestinal complaints and nearly 250,000 deaths. Acute and chronic inflammation are a common element of many GI diseases. Inflammatory processes may be initiated by a chemical injury (acid reflux in the esophagus), an infectious agent (*Helicobacter pylori* infection in the stomach), autoimmune processes (graft versus host disease after bone marrow transplantation), or idiopathic (as in the case of inflammatory bowel diseases). Inflammation in these settings can contribute to acute complaints (pain, bleeding, obstruction, diarrhea) as well as chronic sequelae including strictures and cancer. Research into the pathophysiology of these conditions has been limited by the availability of primary human tissues or appropriate animal models that attempt to physiologically model the human disease. With the many recent advances in tissue engineering and primary human cell culture systems, it is conceivable that these approaches can be adapted to develop novel human *ex vivo* systems that incorporate many human cell types to recapitulate *in vivo* growth and differentiation in inflammatory microphysiological environments. Such an advance in technology would improve our understanding

of human disease progression and enhance our ability to test for disease prevention strategies and novel therapeutics. We will review current models for the inflammatory and immunological aspects of Barrett's esophagus, acute graft versus host disease, and inflammatory bowel disease and explore recent advances in culture methodologies that make these novel microphysiological research systems possible.

Keywords: inflammation, oxidative stress, autophagy, gastrointestinal (GI) disease, gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), esophageal adenocarcinoma (EAC), graft-versus-host disease (GvHD), inflammatory bowel disease (IBD), human 3D organotypic model systems (OTC)

Introduction

Gastrointestinal inflammatory conditions in humans, both acute and chronic, are a significant source for disease-related morbidity and mortality, both in the U.S. and worldwide. In addition to the human costs in pain, disability, and premature death, these disorders carry a considerable financial impact as well. Productivity loses and the enormous expense of medical care strain both familial and societal budgets. Indeed, the costs for care of U.S. patients with inflammatory bowel disease (IBD), a single chronic inflammatory condition, were estimated to be over $6 billion in 2008 ([1]). Altogether, this severe impact on patient well-being and the enormous resulting financial burden confirms the importance of research efforts to better understand and treat these disorders.

Microphysiological culture systems hold great promise to revolutionize research into these important disease conditions. Although tissue and cell culture methodologies as laboratory techniques have been established for well over a century, and have significantly contributed to the advancement of biomedical research and therapeutics, it has become increasingly clear that current standard culture techniques remain highly artificial ([2],[3]). Limitations include an over-reliance on transformed and neoplastic cell lines, utilization of stiff plastic and glass culture plates, and a dependence upon undefined serums and matrices to support and sustain cell growth. Microphysiological culture systems, in contrast, seek to better replicate *in vivo* microenvironments in an *in vitro* setting suitable for research laboratories.

Microphysiological culture systems, in contrast, attempt to better model human tissues through a combination of innovative bioengineered platforms, novel materials, and the use of primary or immortalized human cells rather than transformed cell lines. This edition of *Experimental Biology and Medicine* explores novel bioengineered devices and 3-dimensional culture conditions that are attempting to replicate every major organ system of the human body. Each utilizes novel approaches to study the biology and physiology of these engineered tissue representations. Moreover, many of these systems are being explored as platforms for toxicology testing and drug screening, two very powerful applications of this technology. However, no single platform currently recapitulates all the cell types and biology inherent in the endogenous tissues. In this review we propose another innovative use of these novel human culture systems: to model the effects of acute and chronic inflammatory microenvironments on human gastrointestinal epithelium. We will explore several inflammatory diseases of the esophagus and

intestine, briefly discuss current cell culture and animal models employed to study them, and propose how innovative modifications to microphysiological culture conditions can endow them with new functionalities such as improved modeling of human inflammatory diseases in the laboratory.

Gastroesophageal Reflux Disease and Barrett's Esophagus

Acute and chronic inflammatory conditions of the esophagus including gastroesophageal reflux disease (GERD), reflux esophagitis, eosinophilic esophagitis (EOE), Barrett's esophagus (BE), and esophageal adenocarcinoma (EAC), cumulatively afflict a significant portion of the US population each year (4). These diseases progress sequentially, with gastric acid and bile refluxed to the esophagus in GERD inflicting tissue injury and inflammation that leads first to esophagitis (Figure 1) (5). An estimated 10–15% of these patients then proceed to develop BE, a metaplasia characterized by replacement of normal esophageal stratified squamous epithelium with an intestinal columnar epithelium (Figure 2) (6). These metaplastic regions then progress to low-grade dysplasia (LGD), followed by high-grade dysplasia (HGD), culminating in esophageal adenocarcinoma (EAC). EAC is a deadly cancer with an increasing incidence rate in the US, and the risk of EAC development for patients with BE is approximately 0.5% per year (7). Despite its prevalence, its association with EAC, and the increasing incidence of EAC, the biology of BE and its progression to cancer are poorly understood and are therefore critically important clinical and basic research imperatives.
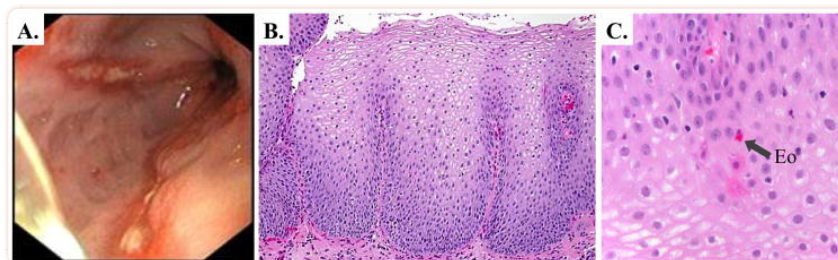
Figure 1

Endoscopic and histologic appearance of gastro-esophageal reflux disease (GERD). **A.** LA Grade B esophagitis visualized endoscopically, characterized by non-confluent mucosal breaks in the distal esophagus. **B. and C.** Hematoxylin and Eosin (H&E) stain of GERD esophagitis at low power (**B**.) and higher power (**C.**) demonstrating non-specific immune cell infiltrate, including in some rare eosinophils (**Eo**).
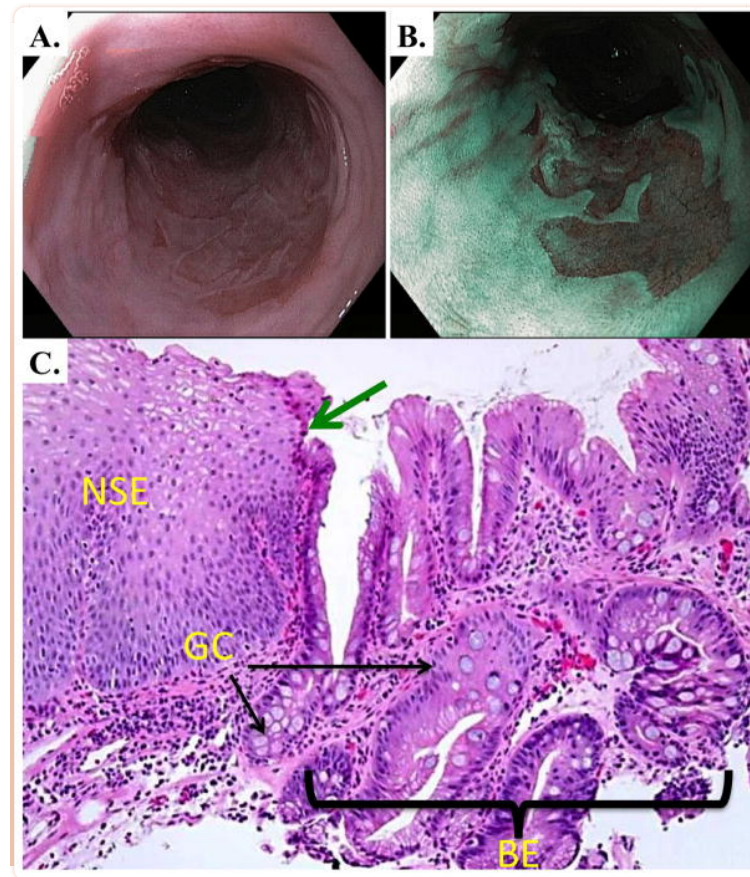
[Figure 2](#)

Endoscopic and histologic appearance of Barrett's esophagus. **A. and B.** Endoscopic appearance of Barrett's esophagus under white-light (**A.**) and narrow-band imaging (NBI) (**B.**). NBI uses filters to image using light in the blue and green wavelengths in order to enhance mucosal surface details. **C.** H&E stain of human Barrett's esophagus at the squamo-columnar junction (indicated by the green arrow). Normal multilayered squamous epithelium (NSE) is to the left and simple columnar Barrett's esophagus is to the right (**above bracket BE**). Within the BE field Goblet-cell (**GC**) meta-plasia and a chronic inflammatory infiltrate are observed.

Development of esophageal inflammatory diseases begins with acid reflux injury followed by the infiltration of immune cells to the site of the injury ([Figure 3](#)) ([5](#),[8](#)). Secretion of signaling molecules such as growth factors and cytokines orchestrate the activation of immune cells and direct their differentiation. Cytokines bind to naïve CD4+ T cells, upregulating master transcriptional regulators, and inducing differentiation into specialized T helper (T_H) subsets (T_H1, T_H2, T_H17, or regulatory T cells). Each of the four lineages then expresses its own distinct set of cytokines and transcription factors, conferring specific functionality ([9](#)). In the esophagus, activation of a T_H1 pro-inflammatory response, character-

ized by production of interferon (IFN)-γ, is typical for acid reflux esophagitis ([10]). Progression to BE is accompanied by a shift in cytokine expression patterns, including increased levels of interleukin (IL)-4, IL-5, IL-10, and IL-13, which are hallmarks of a T_H2 humoral immune response ([10]–[12]).
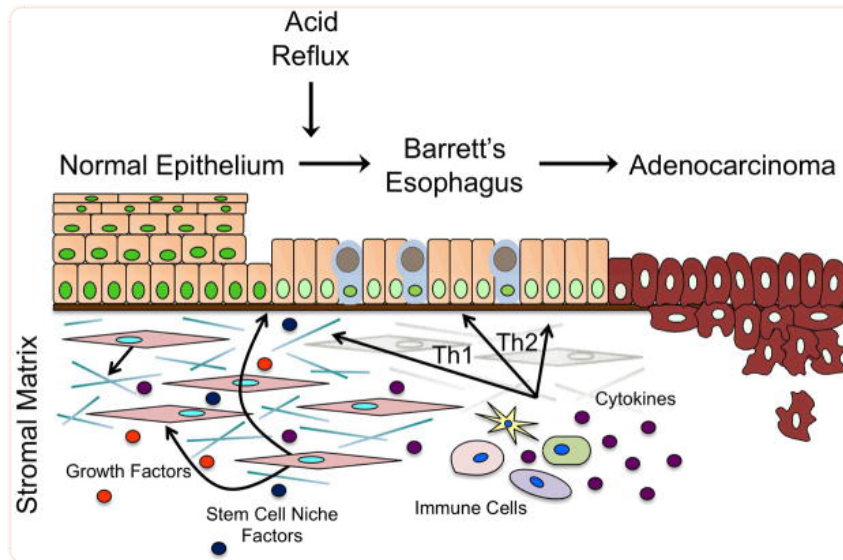

[Figure 3](#)

Model for progression from GERD to BE to EAC. Illustration summarizing the transition from normal esophageal epithelium to metaplastic Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC). Following reflux injury, epithelial cells experience several genetic events that, when combined with microenvironmental signals from stromal fibroblasts and infiltrating immune cells, trigger the development of intestinal columnar cells in place of stratified epithelium. BE is a precursor lesion that will continue to accumulate molecular alterations, ultimately leading to the development of dysplasia and invasive EAC.

Several inflammatory mediators have been linked to BE and EAC pathogenesis and disease progression. Cyclooxygenase-2 (Cox-2) and its product prostaglandin E_2 (PGE_2) are highly elevated in BE, presumably as result of reflux injury, and aid in the recruitment of immune cells including neutrophils, macrophages, and mast cells ([13],[14]). PGE_2 also regulates T cell expansion by activating T_H2 effector cells while suppressing the T_H1 response, supporting a cytokine profile shift towards BE.

A number of other molecular events have also been implicated in the development of BE and progression to EAC ([Table 1](#)). Along the Barrett's metaplasia to dysplasia sequence (BE → LGD → HGD → EAC), allelic loss or silencing of the tumor suppressor and cyclin dependent kinase (CDK) inhibitor p16 is thought to be one of the earliest occurrences ([15]). Mutations of the p53 tumor suppressor gene, as well as enhanced expression of Bcl2 and cyclin D1 have also been detected in metaplastic/pre-dys-

plastic BE (16–18). Progression of BE to dysplasia and EAC is frequently accompanied by p21 upregulation and *K-Ras* activating mutations (19,20). Altogether, these genes regulate a broad range of cellular functions from cell adhesion to cell cycle checkpoints and apoptosis, illustrating the complexity of this esophageal disease and the challenges faced in modeling them in the research laboratory.

Table 1

Genes involved in BE onset and progression to EAC

	Gene	Protein	Alteration	Function	Associations	Ref
Adhesion	APC	Adenomatous polyposis coli	LOH	Tumor suppressor	BE - EAC	(21)
	CTNNB1	β-catenin	Nuclear Accumulation	Transcription factor	BE - EAC	(22)
	CDH1	E-cadherin	Decreased Expression	Cell-cell adhesion	BE - EAC	(22)
Apoptosis	BCL2	B-cell lymphoma 2	Increased Expression	Apoptosis inhibitor	BE - LGD	(17)
	FASLG	Fas Ligand	Increased Expression	Apoptosis driver	BE - EAC	(23)
	MDM2	E3 ubiquitin-protein ligase Mdm2	Increased Expression	Negative p53 regulator	EAC	(24)
	TP53	p53	Mutation	Tumor suppressor	BE - EAC	(16)
Cell Cycle	CCND1	Cyclin D1	Increased Expression	CDK regulation, proto-oncogene	BE - EAC	(18)
	CDKN2A	p16	LOH	CDK inhibitor, tumor suppressor	BE - EAC	(15)
	CDKN1A	p21	Increased Expression	CDK inhibitor	LGD - EAC	(19)
	CDKN1B	p27	Cytoplasmic Sequestration	CDK inhibitor, tumor suppressor	HGD - EAC	(25)
Cell Signaling	CDX2	Caudal type homeobox 2	Increased Expression	Transcription factor	BE	(26)
	ERBB2	Human epidermal growth factor receptor	Increased Expression	Growth factor receptor, proto-	EAC	(27)

Several different approaches have been pursued in the past to study BE and EAC, and while important advances have been made, each has its limitations. We will briefly review past and current models for BE and esophageal metaplasia, discussing the physiological limitations of each and then exploring how

novel human cell based organotypic and tissue engineering approaches can be applied to advance our understanding of BE and EAC pathogenesis.

Modeling Esophageal Diseases

Cell Culture Models: Esophagus

The earliest cell culture models for BE studies used human BE tissue explants and esophageal adenocarcinoma cell lines ([21]–[24]). More recently, primary normal human esophageal and Barrett's epithelial cell lines were established with retroviral transduction of human telomerase reverse transcriptase (hTERT) ([25],[26]). Five primary Barrett's cell lines were developed from various stages of BE progression, with CP-A and BAR-T cell lines originating from metaplastic tissue, while CP-B, CP-C, and CP-D were all derived from Barrett's with HGD. While the CP-A, B, C, and D cell lines have documented chromosomal abnormalities ([27]), the BAR-T cell line maintains a diploid status, displays normal morphology, and responds to contact inhibition ([28]), making them a particularly useful cell line for studies of disease progression to cancer. Many of the studies using these cell lines in 2-dimensional (2D) culture environments have explored the effects of various Barrett's etiologic agents upon esophageal keratinocytes, including chronic acid and bile acid treatment ([24],[29]), BMP-4 ([30]), retinoic acid ([31],[32]), and the intestine-specific transcription factors Cdx1 and Cdx2 ([33]–[35]). While these treatments did yield increased expression of intestine-specific genes and changes in cell morphology, none succeeded at inducing a complete BE phenotype in keratinocytes.

Animal Models: Esophagus

Animal models have become the cornerstone for biomedical research, as they allow for the evaluation of genetic alterations and/or environmental variables in a controlled manner. However, the development of a murine model that accurately recapitulates human BE continues to be a challenge. Major anatomical differences exist in mice and humans and, as a result, mice do not naturally experience GERD. Other anatomic differences include the absence of submucosal glands, which have been hypothesized as the cell of origin for BE ([36]), and the mouse squamous epithelium-lined forestomach. Due to this forestomach, the transition from squamous to columnar epithelial cells in rodents occurs in the stomach as opposed to the gastroesophageal junction where the squamo-columnar junction occurs in humans. Since rodents do not typically have GERD, surgical manipulation is necessary to introduce bile and gastric acid to the esophagus in order to mimic GERD and elicit an inflammatory response that progresses to metaplasia, dysplasia, and adenocarcinoma ([37]). Such procedures are technically challenging to perform and do not guarantee production of the desired phenotype. The utilization of transgenic mice has therefore been adopted as an alternative approach.

To study BE pathogenesis, our laboratory previously generated a transgenic mouse in which an intestine-specific transcription factor, Cdx2, was ectopically expressed in the mouse esophagus using a Keratin-14 promoter (38). Cdx2 is required for intestine-specific gene expression, columnar cell shape and cell-cell adhesion, and is absolutely required for the normal differentiation of the intestinal epithelium (39–47). In human BE tissues, CDX2 mRNA and protein are uniformly expressed in biopsy samples (48, 49). Moreover, Cdx2 expression can be induced in cultures of esophageal keratinocytes treated with short pulses of acid and bile (24, 29). In addition, ectopic gastric expression of Cdx2 induced a gastric intestinal metaplasia (GIM) similar to human GIM . CDX2 is therefore expected to play a similar role in the pathogenesis of BE (33, 50). These K14-Cdx2 mice were found to have significantly reduced esophageal cell proliferation, as well as diminished barrier function. While a complete BE phenotype was not achieved, cells that exhibited ultrastructural features of both squamous and columnar cells were observed. These hybrid cells indicate that Cdx2 promotes the appearance of a transitional phenotype that, under a supportive microenvironment, may adopt a metaplastic columnar phenotype.

More recently, a transgenic mouse has been created that over-expresses the cytokine IL-1β in the esophagus, mimicking chronic esophagitis (51). Expression of IL-1β was specifically targeted to the oral cavity, esophagus, and squamous forestomach using an Epstein-Barr virus L2 promoter (52). By six months of age the IL-1β mice displayed moderate inflammation, followed by metaplasia at 12–15 months. Furthermore, the addition of bile acids in their drinking water resulted in significant acceleration of metaplasia, dysplasia, and cancer in the IL-1β mice but not their wild-type littermates. These data suggest a synergy between cytokine expression and bile acid exposure in the induction of metaplasia at the squamo-columnar junction. Future studies will explore the role of other genetic factors in disease progression using this model, however, inherent differences between murine and human biology persists. Perhaps a better way to accurately model the *in vivo* events occurring throughout the Barrett's metaplasia disease sequence will be to use novel human cell-based tissue-engineered culture systems.

Human Engineered Tissue Systems: Esophagus

To be clinically relevant, studies must strive to best mimic human *in vivo* physiology. However, animal models have significant limitations as we have seen, and traditional 2D cell culture using human cells can only represent the effects of a single cell type and fail to capture the significant contributions of the microenvironment. The development of the organotypic culture (OTC) system that allows for the co-culture of immortalized human epithelial cell lines together with primary fibroblasts in 3-dimensional (3D) tissue reconstructions represents a novel means by which to perform *in vitro* experiments that are still physiologically relevant (53). Under 3D OTC conditions, esophageal fibroblasts are embedded in a collagen matrix, overtop of which, after a period of growth and matrix modification, keratinocytes are seeded (Figure 4). Exposure of the keratinocytes to the air-liquid interface then triggers differentiation and stratification producing a fully mature epithelium (54). This physiologically relevant model thus provides a novel platform for the study of many human esophageal diseases.

[Figure 4](#)

OTC systems to model normal esophageal growth and inflammation. **A.** Illustration identifying the main components of the standard OTC method, as well as how to adapt this culture system for the inclusion of immune cells to invoke inflammation. **B.** H&E stain of normal human esophageal keratinocytes grown under OTC conditions.

When evaluated under OTC conditions, the Barrett's cell lines CP-A, CP-B, CP-C, and CP-D show surprising variability. The HGD-derived CP-B, CP-C, and CP-D lines displayed little to no stromal invasion, however, the metaplastic CP-A cells not only yielded mucin-producing goblet cells, but also were unexpectedly highly invasive ([55](#)). These major phenotypic differences were not apparent previously and were only observed as a result of the more physiological 3D OTC system. This study also underscores the fact that a high level of diversity exists not only between BE patients, but likely within their own BE segments, as well. As a result, it will be imperative in future studies to include a wide panel of cell lines and tissues in every stage of disease.

Our laboratory ventured to examine the early genetic and molecular events that precede the development of BE to gain further insight into its pathogenesis. We demonstrated that epithelial expression of the inflammation-associated enzyme Cox-2 in the hTERT-immortalized human esophageal keratinocytes produced elevated levels of PGE_2 and was sufficient to induce a metaplasia with features of BE ([56](#)). In addition, using this same OTC system, we determined through a genetic approach that the onset of a true BE is a multistep process that requires increased proliferation, senescence inhibition, and epigenetic alterations ([34](#)).

Future innovations will be directed toward increasing the complexity of the OTC cultures by including other relevant cell types including immune, endothelial, smooth muscle, and neural cells ([Figure 4](#)). In addition, investigators have recently identified conditions by which primary human Barrett's organoids can be maintained and expanded in a 3-dimensional culture matrix ([57](#)). Together, the novel OTC culture system and new primary human Barrett's cell culture methods will lead to engineered human esophageal and Barrett's tissues that better model physiologic responses and therefore, will be far superior research platforms than any that are presently available.

Graft versus Host Disease and Inflammatory Bowel Disease

Graft versus Host Disease (GvHD)

GvHD is an unfortunately common complication from allogeneic bone marrow transplantation (ABMT), the treatment of choice for a number of human malignancies, both hematologic and solid organ. It is a fatal complication to 15% of ABMT recipients, and can be a rare complication of solid organ transplantation as well ([58](), [59]()). It is caused by the donor cells responding to and attacking the recipient host alloantigens ([60]()). There are two types of GvHD, acute and chronic, originally defined based on whether they occurred within 100 days of the transplantation (acute) or after (chronic), but are now recognized to have distinctly different clinical presentations and underlying molecular pathogenesis.

Acute GvHD occurs in a third to half of all ABMT recipients, and is marked by strong inflammatory features including dermatitis, cutaneous blisters, crampy abdominal pain with or without diarrhea, nausea and vomiting, and hepatitis ([61]()). The endoscopic and histologic appearance of small intestine GvHD can be quite varied, depending upon the disease severity. In very mild disease, the small intestine mucosa can appear normal, with only focal crypt epithelial apoptosis and mild lymphocytic infiltrate characteristic of GVHD visible histologically. Moderately severe disease can present with small intestinal mucosal edema, increased friability, villus blunting, and small ulcerations ([Figure5]()) ([62]()). The histology in these cases often demonstrates apoptosis with focal dropout of crypt epithelial cells, small ulcerations, and lymphocytic infiltrates in the lamina propria are more numerous. In its most severe form, small intestine GVHD presents with a hemorrhagic epithelium from sloughing of substantial regions of mucosa and the presence of large ulcers. Histologically there is a dense lymphocytic infiltration, epithelial and crypt loss and ulcerations.

[Figure 5]()

Endoscopic and histologic views of acute GvHD. **A.** Clinically normal duodenum. **B.** Moderate acute GvHD diagnosed by mild mucosal edema, hyperemia, villous blunting, and small erosions. **C.** H&E stain of normal human duodenum demonstrating normal crypt/villous architecture. **D.** H&E stain of acute GvHD showing architectural distortion, loss of crypts, lymphocytic infiltration with small hemorrhage (H), and crypt cell apoptosis.

Molecularly, it is thought to be largely, though not exclusively, a T_H1 and T_H17 immune response, whereas chronic GvHD follows more typically a T_H2 pathway ([60](), [63](), [64]()). Treatment of acute GvHD is directed toward optimizing immunosuppressive therapy, and is typically effective, however about half of these patients will go on to develop chronic GvHD associated with greater long-term morbidity and mortality.

Chronic GvHD is the more serious and long-term complication of ABMT, occurring in a one to two-thirds of ABMT transplant recipients surviving more than 100 days (65). Unlike acute GvHD which is mediated by alloreactive cytotoxic effects, chronic GvHD is more indolent, resembling autoimmune vascular diseases (60). Patients can sometimes present with distinctive findings that establish the diagnosis, including sclerosis, esophageal webs, and bronchiolitis obliterans, however more typically they present with less diagnostic features (65). In these cases the diagnosis is confirmed by a biopsy of involved tissues which most commonly are the skin, oral cavity, liver, and intestine/gastrointestinal tract (including esophagus). As in acute GvHD, treatment typically requires immunosuppressive medications for a median of 2 to 3 years, and in some cases for 7 or more years. The mortality associated with chronic GvHD, which is as high as 30% to 50% at 5 years, is caused by two mechanisms, organ dysfunction and failure due to damage by the chronic GvHD, and infectious complications from chronic immune suppressive medications (60, 65). In summary, the significant morbidity and mortality from acute and chronic GvHD after ABMT significantly limits the effectiveness of ABMT for hematologic and solid malignancies and remains an important focus of research.

Inflammatory Bowel Disease (IBD)

Inflammatory bowel diseases such as Crohn's disease (CD) and ulcerative colitis (UC) are chronic disorders of the intestine and/or colon, in which patients suffer from rectal bleeding, severe diarrhea, abdominal pain, fever, and weight loss (66–68). In North America, 1.4 million people are affected by IBD (69). Our current understanding of the pathogenesis of IBD is that they are a result of abnormal host immune responses brought on by diet and lifestyle in genetically susceptible individuals (70). Host genetic susceptibility plays an important role in the risk of development of IBD. Several genes have been identified including those involved in innate immune signaling pathways (ITLN1) (71), autophagy (IRGM) (72), ER stress responses (ORMDL3) (73), and negative regulators of interferon-gamma (INF-γ) signaling (PTPN2) (74). The cornerstone therapies for IBD are immunosuppressive drugs and steroids, both of which provide variable benefit to the IBD population (75).

Clinical and histopathological observation of tissue from patients with IBD reveals gastric erosions and ulcerations, the presence of a large inflammatory cell infiltrate concurrent with extensive mucosal and transmural injury including edema, loss of goblet cells, decreased mucous production and crypt cell hyperplasia (Figure 6) (66). CD is characterized by an excessive recruitment of leukocytes from the blood circulation into the inflamed gut wall and massive infiltration of neutrophil granulocytes and macrophages into the affected mucosa (76–78). During acute flares of CD, neutrophil granulocytes are found in the stool and have served as a clinical and research tool to quantify levels of disease activity (76). Although it was previously thought that CD was a T_H1-mediated disease while UC was a T_H2-mediated disease, it has recently been suggested that a T_H17 immune response is also involved in the pathogenesis of IBDs; T_H17 cytokines are highly expressed in the intestinal mucosa of both CD and UC patients (79).

[Figure 6](#)

IBD visualized endoscopically and histologically. **A.** Clinically normal small intestine. **B.** Crohn's disease diagnosis presented as edematous, erythemous small intestine mucosa with nearly circumferential small bowel ulceration and over-lying inflammatory exudate. **C.** H&E stain of normal human small intestine, **D.** H&E stain of Crohn's disease marked by loss of villi, chronic inflammatory cell infiltrate extending into the submucosa, and an active inflammation in the epithelium.

Modeling Intestinal Diseases

Cell Culture Models: Intestine

The intestinal mucosa contains a large variety of cell types in close proximity to the epithelium and the complexity of this microenvironment is further increased during an inflammatory response with additional immune cells infiltrating the lamina propria. Immune and epithelial cells release a variety of signaling factors and peptides to regulate the inflammatory response (cytokines), recruit phagocytes (chemokines) and facilitate epithelial repair (growth factors) ([75](#)) ([Figure 7](#)). To better understand these key components in IBD, *in vitro* models have focused on a variety of culture systems utilizing one or more cell types.

[Figure 7](#)

Illustration of key mediators involved in the development of acute intestinal GvHD (aGvHD) and IBD. aGvHD is caused by donor T cells responding to and attacking recipient host alloantigens with cytotoxic products. Irradiation and BMT conditioning prior to the BMT injure the intestinal epithelium, leading to intestinal barrier disruption and exposure of the mucosal immune compartment to bacterial products. These bacterial components are potent activators of an innate immune response, which trigger a "cytokine storm", amplifying the immune response and favoring the development of acute GvHD. IBD, such as Crohn's disease, develops due to the presence of a large inflammatory cell infiltrate with extensive mucosal and transmural injury including edema, loss of goblet cells, decreased mucous production and crypt cell hyperplasia. Factors secreted by the intestinal microbiota and immune cells can activate epithelial cells to produce cytokines that regulate inflammation, chemokines that signal recruitment of phagocytes, and growth factors with autocrine actions that facilitate epithelial repair and/or damage.

Historically, there have been four different cell culture approaches to model and study human small intestine epithelium, 1) primary cell cultures, 2) cell lines established from colon cancers, 3) spontaneously immortalized cell lines from normal intestine, and 4) normal intestinal cell lines immortalized by oncogenes and other genetic approaches. Each approach has important strengths and weaknesses. Cell lines established from human colon cancers have been most widely used experimentally. This is because they are human cells, easy to grow and manipulate in the lab, relatively inexpensive to maintain, and widely available. Several colon cancer cell lines exhibit interesting phenotypes. Three cell lines Caco-2, HT 29 and T84 express morphological and functional characteristics of differentiated intestinal epithelial cells including polarized, columnar morphology, vectoral transport, and tight junction-dependent barrier ([80](#)–[83](#)). They can be grown on transwell membranes and other structural supports readily, facilitating studies of these properties and functions by cell biologists and physiologists. Bioengineers fabricating devices to model intestinal epithelium likewise favor them for their ease of use and functional epithelial barrier.

However, while individual cell lines may be suitable for certain limited purposes (study of tight-junction formation and barrier disruption, or transporters and channels, for instance), they fall far short of physiological models of normal human small intestine. All are colonic in origin, though one cell line, CaCo-2, express small intestine hydrolases (sucrase-isomaltase, lactase, aminopeptidase N and dipeptidylpeptidase IV) when they spontaneously differentiate after reaching confluency. Moreover, all of these cells have considerable gene mutations and epigenetic changes in gene expression, resulting in many abnormal cellular signaling pathways, stress responses, and cellular metabolism. Perhaps most importantly, they lack normal intestinal stem cells, and therefore are unsuitable for toxicology studies exploring the effects of toxins on the vulnerable intestinal stem cell compartment.

Given these disadvantages, investigators have employed other strategies, including isolating from primary intestinal epithelial cultures spontaneously immortalized cell lines, or immortalizing primary intestinal epithelial cells using oncogenic proteins like SV40 Large-T antigen, or more recently telomerase. Fetal rat intestinal epithelium yielded a half-dozen spontaneously immortalized cell lines (IEC and FRIC cell lines), whereas attempts using fetal human intestine yielded a single cell line (HIEC cells) ([84], [85]). Another approach was to immortalize cell lines from a primary intestinal epithelial cell culture using a temperature-sensitive SV40-virus Large-T antigen, an established oncogene, or, more recently, a combination of non-oncogenic Cdk4 with telomerase ([86]). In most cases, these cells were homogeneous, passaged readily in culture, and were typically near-normal genetically. However they typically exhibited characteristics of poorly differentiated intestinal epithelium, more like crypt cells than differentiated cells in the villi. Moreover, except for human colonic HCEC cells ([86]), they do not form a mature polarized epithelial barrier with tight junctions.

In vitro research efforts in IBD have been directed towards understanding the contribution of the epithelium in the inflammatory response using single cells treated with specific molecules. Several studies using colon cancer cell lines observed that common IBD epithelial responses, including injury and repair, apoptosis, and necrosis, can be induced by microbes or microbial products and immune activation ([75]). Another common approach to studying these responses is by co-culturing human colon crypt-like T84 epithelial cells with bacterial species or bacterial products and/or immune cells (or conditioned media from such cells). This approach has been particularly effective for studies exploring perturbations in epithelial permeability (i.e. transepithelial electrical resistance) brought out by inflammatory responses ([75], [87], [88]).

Primary human intestinal epithelial cell cultures in theory should be the most physiologic model, however, the isolation of the epithelial cells from the underlying supportive mesenchyme leads to rapid loss of their differentiated characteristics, loss of stem cell capacity, and limited viability in culture. In addition, the simple culture of cell monolayers in 2D plastic systems does not permit exploration of how cell-to-cell interactions and a physiologic extracellular matrix can influence mucosal immune cell responses. Recent advances in techniques to maintain human small intestinal epithelial cells in long-term culture employ a 3-dimensional matrix and physiologically relevant growth factors ([57], [89], [90]). This innovative culture system is a significant advance, permitting the long-term growth of intestinal stem cells while permitting normal differentiation along four intestinal lineages, and the formation of a polarized epithelium with normal vectoral transport and cell-cell adhesion including tight junctions. This novel culture system has the potential to significantly advance current GvHD and IBD research practices

Animal Models: Acute GvHD

Mice are far and away the main preclinical animal model for GvHD and studies in mice have made important contributions to our understanding of the pathogenesis of this disorder, though other large animal models have contributed to our improved understanding as well (60). Mouse models for GvHD involve transplantation of donor bone marrow along with different types of donor lymphocytes into irradiated allogeneic recipients. These recipients typically differ from the donor with regard to their MHC class I and/or II molecules. The use of these animal models has led to novel insights regarding disease pathogenesis, including the central role of gastrointestinal injury as an initiating event (91).

Irradiation and other conditioning regimens to prepare the recipient host for the donor BMT also injures the intestinal epithelium, leading to intestinal barrier disruption and exposure of the mucosal immune compartment to bacterial products including lipopolysaccharides (LPS) and DNA (91). These bacterial components are potent activators of an innate immune response, which triggers a "cytokine storm", amplifying the immune response and favoring the development of acute GvHD (Figure 7). This current model for acute GvHD is supported by a broad array of observations. Early animal and clinical studies found that gut decontamination significantly reduced death rates and acute GvHD incidence (91). Moreover, depletion of T-cells from the graft can abrogate acute GvHD in experimental models (48,69) (60, 91). Additionally, mutation of Toll-like receptor 4 (TLR4), an innate immune receptor for bacterial LPS, can reduce GvHD risk whereas activation of TLR9 by bacterial DNA can enhance the risk for GvHD (92, 93). Based on these observations, strategies that manipulate the gut flora, deplete T-cells, or suppress innate immune responses are an important focus of ongoing research efforts.

The strength of the mouse as a research model for human GvHD lies in our ability to manipulate its genome and generate transgenic mice with strategic genes "knocked-out" by mutation. The generation of transgenic mice with inactivating mutations in a host of cytokines and TLRs has enabled our dissection of their role in the pathogenesis of this disorder (94, 95). For this reason, mouse models of GvHD will likely remain an important tool for the foreseeable future. However, these important mouse models do have significant limitations as well. Fundamentally, while these models can explore the general pathogenesis of GvHD in the setting of MHC incompatibilities, they cannot test specifically human MHC alloreactive responses. An equally important consideration is the effect of mouse strain and genetic drift on GvHD research. It was recently reported that differences in mouse strains which naturally occur over time appear to be altering alloreactive immune responses and GvHD severity (96). Finally, genomic studies of GvHD have identified an increasing number of human polymorphisms that may alter the risk for the development of GvHD (97), however demonstrating this association beyond observational studies in human BMT recipients would require the generation of transgenic mice bearing the polymorphism, a considerable expense. Together these limitations argue for the development of human engineered tissues and systems in which acute and, possibly, chronic GvHD can be credibly modeled using human cells, possibly from BMT patient's themselves.

Animal Models: IBD

A major contribution to our current understanding of the pathogenic mechanisms responsible for the induction and progression of IBD has been the availability of animal models that recapitulate some aspects of the human disease (66, 98). While not ideal, these rat and mouse models (25–30 models total) of intestinal inflammation have yielded considerable insights and served as platforms for hypothesis testing and IBD disease modeling for more than 20 years.

Based on the nature of the inflammation and the method of disease induction, rodent models of intestinal inflammation are grouped into five major categories: 1) chemically or biologically induced, self-limiting models of colitis (TNBS, DSS, *C. rodentium*, for example), 2) genetically engineered traditional transgenic/knock-out/knock-in mice (i.e. IL-7 transgenic, IL-10 knock-out, mutant gp130 knock-in), 3) disruption of T cell homeostasis by adoptive transfer (CD45RB, CD8 transfer models), 4) congenital, spontaneous mutations (C3H/HeJBir), and 5) spontaneous (cotton-top tamarin) (66, 98, 99). Two of the more common IBD mouse models currently used in many laboratories around the world are the IL-10-deficient (IL-10$^{-/-}$) mouse model and the T cell transfer model.

Disruption of the IL-10 gene in mice leads to spontaneous pancolitis and cecal inflammation by 2–4 months of age and histopathologically the mouse colon shows many of the same characteristics as those observed in human IBD (66, 100). The advantage of the IL-10$^{-/-}$ mice is that it is a well-established T_H1-mediated model of transmural colitis. Alternatively, the T cell transfer model utilizes the adaptive transfer of CD4$^+$CD45RB^{high} T cells (naive T cells) from healthy wild-type (WT) mice into syngeneic recipients that lack T and B cells. Following T cell transfer, pancolitis and small bowel inflammation develop at 5–8 weeks (66, 101, 102). The major advantage of the T cell transfer model is that it shows the earliest immunological events associated with induction of gut inflammation and perpetuation of the IBD. Furthermore, data suggests that transfer of naive T cells into recombinase activating gene-1-deficient (RAG$^{-/-}$) mice will provide a model similar to CD development with induction of colitis and small bowel inflammation (66, 103, 104). Recent evidence also supports that epithelial cell stress and abnormalities can lead to intestinal inflammation in mice following deletion in XBP1 (105), inositol requiring enzyme 1β (IRE1β) (106), caspase-8 (107, 108), anterior gradient 2 (AGR2) (109), the RNA editing enzyme ADAR1(110), or missense mutations in MUC2 (111).

Although these various rodent models lack the complete recapitulation of the clinical and histopathological characteristics of human IBD, three underlying principles and recurring themes have evolved. First, the reason that suggests the T cell-dependent models of IBD are significantly more relevant than the other models, is that chronic gut inflammation is largely mediated by T lymphocytes. Second, this inflammation is initiated and perpetuated by commensal enteric bacteria. And third, the genetic background of the mouse is an important but poorly understood modulator/modifier of disease onset and severity (66, 67, 99).

Human Engineered Tissue Systems: Intestine

The intestinal and colonic mucosa are both complex and dynamic tissues with epithelial cells arranged in crypts and villi in the small intestine and crypts in the colon (112–115). In 2011, several groups independently published descriptions of methods by which human intestinal and colonic stem cells can be maintained and expanded in culture to mimic *in vivo* microenvironments (57, 89, 116, 117), building upon an older method of culturing mouse small intestine stem cells (90). In this culture method, human intestinal and colonic crypts or purified stem cells are embedded in Matrigel (Figure 8). Over several days they form spherical or elongated oval structures with a crypt-like lumen, referred to as enterospheres (118). Small intestine and colon spheres can expand into multilobulated enteroids and colonoids that mimic the ordered structure of the epithelium complete with crypts containing multipotent columnar base stem cells and Paneth cells. Other intestinal epithelial cell types, including enterocytes, goblet cells, and enteroendocrine cells (57, 118) can be observed in differentiated cell regions away from the stem cell compartment. While the majority of studies conducted with human enteroids and colonoids thus far have focused on stem cell characterization and regenerative medicine, there is immense potential for this culture system in GI research, particularly to model GvHD and IBD. However, they have not been widely adopted as yet to model these and other human disease conditions.

Figure 8

Engineered human tissue culture system to model intestinal inflammatory-related diseases. **A.** Human intestinal enteroids grown from stem cell-rich crypts cultured with immune cells in Matrigel and media. **B.** Phase contrast microscopic imaging of a multilobular human intestinal enteroid in culture. **C.** Alcian Blue staining of a human intestinal enteroid identifying mucin-producing Goblet cells.

Ideally, it would seem to be then that the next step in the advancement of these micophysiological intestinal culture systems would be the inclusion of additional intestinal cell types such as fibroblasts, smooth muscle cells, and immune cells to better mimic normal and disease environments. The inclusion of immune cells is of particular interest to our laboratory as a way to induce inflammatory responses that model GvHD and IBD diseases (Figure 8). Human peripheral blood mononuclear cells can be easily and safely isolated from human volunteers and patients; these cells directly, or fractionated subpopulations, can be added to the intestinal cultures and treated with cytokines to induce T_H1 or T_H2 type responses. The effects of these inflammatory responses on intestinal epithelial stem cell viability and proliferation would be of interest, as would the effects of T_{Reg} and other suppressor populations on the inflammatory response. Furthermore, tissues and immune cells from individuals with specific genetic polymorphisms could be tested to determine the effect of these polymorphisms on the *in vitro* in-

duction of a GvHD or IBD inflammatory response. Interestingly, genetic polymorphisms in E-cadherin and hepatocyte nuclear factor 4 α are thought to contribute to UC risk ([119](#)) and would be ideal candidate genes to test *in vitro* for effects on tissue structure and function in the intestine.

In terms of personalized medical applications, growing intestinal epithelial cells from GvHD or IBD patients, and combining them with their own isolated immune cells could serve as a novel microphysiological platform to screen the effectiveness of various therapies for that particular patient. In addition, the inclusion of other microphysiological systems representing the liver, kidneys, and other organ systems onto the platform would also serve to identify potential unexpected toxic and idiosyncratic responses thereby maximizing therapeutic efficacy and minimizing unwanted side-effects associated with treatment and therapy. One could, for instance, examine the role for the cytotoxic cytokine IL-13 in UC pathogenesis, in particular its effect on epithelial apoptosis. Moreover, one could potentially screen for agents to suppress this IL-13 cytotoxicity in patient-derived organoid systems as a novel approach to drug discovery ([120](#)).

Future directions: integrating complex biological systems into innovative platforms

The OTC and enteroid culture systems discussed here for modeling gastrointestinal inflammatory conditions have several distinct advantages. First and foremost, they utilize normal, non-transformed human cells grown under physiological conditions. Second, these innovative culture environments allow for the maintenance of a stem cell population, which until recently was not possible for intestinal epithelial cell cultures ([57](#), [89](#), [90](#)). Third, and of equal importance, the culture conditions are permissive for full cellular and morphologic differentiation, including the formation of the multilayered squamous epithelium of the esophagus, or the development of crypt structures and multilineage daughter cells (enterocytes, enteroendocrine, Paneth, and goblet). Lastly, these culture systems can be easily modified to include other cell types of interest, such as fibroblasts, immune cells, and smooth muscle cells, for example. Therefore the complexities of the actual human tissue can be more completely modeled.

However, these novel culture systems do have two important limitations which will restrict their application to research labs. The complex conditions that yield these biologically relevant models are not easily adapted to a clinical lab for diagnostic purposes. Nor are they suitable for high-throughput approaches favored by industry and the FDA for screening of novel compounds for therapeutic or toxic effects. Moreover, while it is possible to study vectoral nutrient transport, channel and transporter activity, and epithelial barrier function in the enteroids ([121](#), [122](#)), it is more difficult than when cells are cultured on Transwell or similar platforms.

Innovative bioengineered platforms are under development that, if modified, may resolve these limitations. The effort to microfabricate human organs on small culture devices has yielded a number of successful designs([123](#)–[127](#)). These devices can mimic peristalsis, incorporate a microbiome, and absorb and metabolize drugs in a physiologically relevant manner. However, all these devices were tested using

human colon cancer CaCo-2 cells because of their ease of culture and their ability to differentiate into polarized, columnar epithelial cells with functional tight junctions. Modifications to these platforms, such that they can support intestinal enteroid growth, including the maintenance of a crypt compartment with viable stem cells, would yield a device with relevant biology and excellent functionality.

In conclusion, novel engineered human gastrointestinal tissue systems that recapitulate normal physiology provide an innovative and attractive approach to model inflammatory diseases of the gastrointestinal track including GvHD and IBD. Adapting these research platforms to the new proposed uses has the potential to yield new insights into human disease pathogenesis. It will also likely advance the development of novel therapeutics and provide tools to better personalize medical therapies.

Acknowledgments

List of Abbreviations

GI   gastrointestinal

IBD   inflammatory bowel disease

GERD   gastroesophageal reflux disease

EOE   esosinophilic esophagitis

BE   Barrett's esophagus

EAC   esophageal adenocarcinoma

LGD   low-grad dysplasia

HGD   high-grade dysplasia

T_H T-helper

IFN   interferon

IL   interleukin

Cox-2   cyclooxygenase-2

PGE_2 prostaglandin E_2

hTERT   human telomerase reverse transcriptase

2D   2-dimensional

OTC   organotypic culture

3D   3-dimensional

ABMT   allogeneic bone marrow transplantation

GvHD   graft-vs-host disease

IBD   inflammatory bowel disease

CD   Crohn's disease

UC   ulcerative colitis

TLR   Toll-like receptor

IRE1β   inositol requiring enzyme 1β

AGR2   anterior gradient 2

Footnotes

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

KGH and JDB were responsible for drafting the manuscript, diagram design, and image acquisition. GWF, GGG, and NJ were responsible for image acquisition and for the concept and critical manuscript revision. JY, MGM, and AKR were responsible for the concept and critical manuscript revision. JPL was responsible for the concept and experimental design, critical manuscript revision and final approval. All authors read and approved the final manuscript.

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