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NUTRITION AND OBESITY (S MCCLAVE, SECTION EDITOR)

The Microbiome-Host Interaction as a Potential Driver of Anastomotic Leak

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Abstract

Purpose of Review The goal of this paper is to review current literature on the gut microbiome within the context of host response to surgery and subsequent risk of developing complications, particularly anastomotic leak. We provide background on the relationship between host and gut microbiota with description of the role of the intestinal mucus layer as an important regulator of host health.

Recent Findings Despite improvements in surgical technique and adherence to the tenets of creating a tension-free anastomosis with adequate blood flow, the surgical community has been unable to decrease rates of anastomotic leak using the current paradigm. Rather than adhere to empirical strategies of decontamination, it is imperative to focus on the interaction between the human host and the gut microbiota that live within us. The gut microbiome has been found to play a potential role in development of post-operative complications, including but not limited to anastomotic leak. Evidence suggests that peri-operative interventions may have a role in instigating or mitigating the impact of the gut microbiota via disruption of the protective mucus layer, use of multiple medications, and activation of virulence factors.

Summary The microbiome plays a potential role in the development of surgical complications and can be modulated by peri-operative interventions. As such, further research into this relationship is urgently needed.

Keywords Microbiome · Microbiota · Anastomotic leak · Anastomosis · Surgical site infection · Colorectal surgery · Gastrointestinal surgery · General surgery · Mechanical bowel prep

Introduction

Despite major advances in surgical technology and technique, anastomotic leak (AL) remains a feared complication after intestinal resection and is responsible for high morbidity and mortality. AL is caused by a disruption in the healing gut epithelium that allows for intestinal contents—succus or stool—to leak into the peritoneal cavity. The consequences range from subtle subclinical leaks to abscesses requiring percutaneous drainage to potentially fatal systemic sepsis. Leakage is associated with increased risk of severe consequences, including permanent stoma, pelvic sepsis, fistula, and increased all-cause mortality. The overall incidence of AL in colorectal surgery is approximately 11% with a range of 1 to 24% [1, 2], with increasing incidence for more distal colorectal and coloanal anastomoses [3]. AL contributes to greater length-of-stay, cost, poor quality of life, and need for further interventions; it is also associated with increased recurrence and decreased survival after oncologic resection [4, 5]. The overall mortality rate from AL is typically reported as high as 15% [6].

Despite extensive research into possible risk factors and optimal surgical technique, the pathogenesis of AL remains unknown [2]. The development of AL has been attributed to multiple patient variables including age, comorbidities, obesity, diet, smoking, radiation therapy, and immunosuppression among others. The success of an anastomosis is historically thought to be secondary to surgical technique and adherence to the basic tenets of ensuring good blood flow, a tension-free

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connection, and carefully executed apposition of mucosa either via a hand-sewn or stapled technique. While there is no doubt that these three factors are important for a healthy anastomosis, this paradigm does not account for the role of the endogenous microbiome and the protective mucus layer that lines the gut epithelium. Recent evidence suggests a potential relationship between microbial virulence and anastomotic breakdown.

Advancements in sequencing technology over the past three decades have exponentially expanded our ability to characterize the gut microbiota (i.e., microbial strains) and have identified correlations between gut microbiota composition and a host of conditions, including diet, environment, and disease state (e.g., inflammatory bowel disease, colorectal cancer, autism, obesity, and metabolic syndrome). As we move toward microbiota-based therapies, it is critical that we employ additional technologies (e.g., metabolomics, proteomics), high-dimensional analytics, culture-based techniques, and animal models to determine the functional role of the microbiota in effecting host physiology [7–10]. We have begun to understand that nuances and inter-patient variability of the microbiome may contribute to the development of post-operative complications. This review will highlight areas to focus future research for limiting iatrogenic perturbations to the host-microbiota relationship during the peri-operative period. To improve outcomes and make surgery safer, we need to increase our understanding of the molecular, genetic, and functional response of the host in reaction to alterations in microbiota [11]. Over the course of this review, we will begin to explore how surgical intervention initiates alterations in the host microenvironment that can cause a shift in microbiota composition and behavior that subsequently may confer increased risk of developing post-operative complications.

Background

The Microbiome and Its Metabolome

The human gut is home to a diverse microbial community (i.e., bacteria, viruses, fungi); the term microbiome refers to these microbes, their genes, and their metabolic products. More than 1500 bacterial species make up the gut microbiota; the majority are anaerobic and belong to the *Firmicutes* and *Bacteroidetes* phyla [12, 13]. However, there is substantial inter-individual variability largely based on environmental factors (diet, age, exercise, antibiotic use, smoking, inflammation, etc.) [14•, 15, 16, 17•] and genetic inheritance [18]. In addition to this vast taxonomic diversity, the gut microbiota contains an extraordinary genomic potential; the metagenome of the gut microbial community is approximately 150-fold larger than the human gene complement [19]. The bacterial community—both the structure of the community and the metabolic activities of individual taxa—is impacted by substrate delivered from the diet. The microbiota uses diet as the substrate to produce metabolites that in turn influence host physiology [20–24]. Consisting of tens of trillions of bacterial cells in the colon, the gut microbiota is the main source of thousands of small bioactive molecules that can trigger host metabolism and immunity [25]. Collectively, host-derived small-molecule compounds and these bacterial-derived metabolites within the host's blood stream are referred to as the systemic metabolome.

The vast majority of human microbiome studies have used fecal samples as a proxy for the gut microbiome. However, it is important to note that the composition, diversity, and function of the gut microbiota vary both longitudinally (i.e., along the GI tract from the mouth to anus) and radially (i.e., from tissue-mucosal interface to the lumen) within the GI tract [26]. Longitudinally, although only a short physical distance separates the small intestine and the colon, they contain vastly different microbiota. Contrary to the highly diverse and relatively stable colonic microbiota, the small bowel microbiota has a lower diversity (up to 88.3% of bacteria from one genus [27, 28]) and is subject to sub-daily fluctuations [29]. These fluctuations are likely driven by a response to dietary variation, as metatranscriptomic analysis has shown that the metabolic focus of the small bowel microbiota is transport and metabolism of simple carbohydrate substrates [23]. Radially, microbiota composition varies between the epithelial cell layer, the mucus layer, and the lumen [30].

This anatomic and spatial diversity impacts microbial interaction between different species of bacteria and between bacteria and host. In addition to being influenced by lifestyle factors and host biology, bacteria can impact each other through intra- and inter-species communication via quorum sensing [31]. For example, the virulence of *Pseudomonas aeruginosa* is modulated by the presence of specific fermentation products [32]. Additionally, host defense and immunity play an important role in creating specific niches for bacterial colonization via the production of secretory immunoglobulins and antimicrobial peptides, as well as the activation of bactericidal mechanisms in response to mucosal injury.

In healthy subjects, the commensal bacteria in the human GI tract exist symbiotically with their host and contribute to the development of metabolism, immunity, epithelial cell growth and development, energy harvesting, gut motility, barrier function, and absorption of nutrients [33]. While daily variations in lifestyle—including diet, exercise, pets, and environment—can cause marked alterations in the microbiome, composition and function of the gut microbiome in healthy humans remain relatively stable [34]. However, upon injury—including elective surgery—the gut microbiota can undergo a swift phenotypic shift and dramatic change in microbial density and metabolite production. Over the course of a few hours after an insult, the microbiome demonstrates a 90% reduction in Bacteroidetes and Firmicutes species with a

concomitant rise in potentially pathogenic gamma-Proteobacteria within the GI tract, which includes virulent phenotypes of *Escherichia coli*, *Enterococcus faecalis*, and *P. aeruginosa* that are often associated with post-operative infections [11]. These changes have been demonstrated to persist up to 90 days after surgery in a mouse model [35•]. Notwithstanding, the human response to surgical injury can be positively influenced through early administration of IV fluids, oral nutrition, and judicious medication use, which can support refaunation of the commensal microbiota [36].

The Intestinal Mucus Layer

The intestinal mucosa is lined by epithelial cells that are covered by a functional mucus system [37]. Colonic mucus is composed of two layers [38]: an inner layer of net-like sheets of MUC2—gel-forming mucin polymers—that is impenetrable to bacteria in healthy hosts [39], and a non-attached outer mucus layer that serves as a habitat for distinct colonic microbiota. The mucus layer is essential for protecting the colonic mucosa from injury and is regenerated by goblet cells through the production and secretion of mucin on an hourly basis [38]. Alterations to the host can affect this process; for example, in an ischemia-reperfusion model, colonic ischemia led to detachment of mucus that placed bacteria in direct contact with the intestinal epithelium. Following reperfusion, crypt goblet cells secreted stored mucus and effectively cleared the bacteria that had come in contact with the epithelium [40]. Additionally, mucus secretion is in part regulated by microbiota, including through bacterial metabolites generated from diet, prostaglandins, and certain bacterial strains [41]. The thickness of the mucus layer, which is rich in polysaccharides [38], has been positively correlated with microbial community diversity [42] and negatively correlated with dietary fiber intake [43]. A defective mucus layer that allows bacteria to contact the mucosa may be a pathophysiological mechanism for infectious disease, metabolic syndrome, and colitis [44, 45].

Host mucus production also impacts the composition of the microbiota, as specific microorganisms are capable of utilizing mucus glycoproteins as a nutrient for growth [46, 47]. While similar bacterial species exist in both the outer mucus layer and the lumen, differential resource utilization and genetic expression are observed between the separate compartments [30]. In healthy hosts, an increased presence of *Actinobacteria* and *Proteobacteria* is found in the mucosa-associated microbiome. This increase in aerotolerant organisms could, in part, be based on a radial oxygen gradient between aerobic tissue and the anaerobic lumen [48, 49]. Mucosally associated microbes differ significantly from luminal microbiota [50] and perform a distinct role in the host-microbiota relationship. Molecular exchange of bioactive molecules occurs bidirectionally between the host and the outer mucus layer [30].

The Impact of Peri-operative Interventions on the Microbiome-Host Relationship

Despite extensive studies linking the human gut microbiome with human health and disease (e.g., obesity, inflammatory diseases, malignancy), our understanding of its role in surgical diseases and outcomes remains limited. It will be essential to find a balance between our evolving understanding of the microbiome's role in immune function and wound repair with the traditional dogma of intestinal antisepsis before gastrointestinal surgery. Surgeons make multiple peri-operative perturbations (e.g., fasting—nil per os [NPO], mechanical bowel preparation [MBP], and antibiotic use—both oral and intravenous administration) that impact the microbiota with minimal understanding of the impact on the structure or function(s) of the bacterial community [51, 52]. Evidence suggests that the microbiome may play an integral role in the development of post-operative complications, particularly AL and surgical site infections (SSI). While our understanding of the microbiome's impact on the repair of anastomotic tissues is in its infancy, emerging data suggests that the insult of surgery itself may alter host-microbiota virulence and increase the risk of post-op complications, especially AL.

Peri-operative medications can also alter microbiome composition. Antacids neutralize gastric secretions, which disrupt the balance of acid-sensitive organisms in the foregut. Vasoactive medications, which are often used in critically ill patients, impact perfusion and oxygen delivery to the bowel lumen and induce luminal hypoxia and hypercarbia, which can induce a shift in bacterial virulence. Opioids disrupt peristalsis and impair GI motility, thereby decreasing the mechanical removal of luminal material (including bacteria). This can result in ileus, dysbiosis, and/or bacterial overgrowth. The host-pathogen balance is also altered in the setting of colonic enteral nutrient deprivation—either due to fasting, total parenteral nutrition, or highly processed foods whose absorption is complete in the small bowel. This imbalance is characterized by an absence of commensal bacteria, a plethora of highly virulent microorganisms, and a dampened immune response with alterations in intestinal barrier function that ultimately can lead to systemic inflammation (e.g., gut-derived sepsis) [53].

Peri-operative interventions have the potential to induce a bloom of virulent bacterial taxa (e.g., *Enterococcus*, *Pseudomonas*) that are able to shift toward a more aggressive and tissue-destroying phenotype in response to environmental cues [54••, 55]. For example, catecholamines secreted in response to stress can affect the growth of certain bacterial taxa, including *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *Yersinia enterocolitica*, and *Campylobacter jejuni*, as well as their

expression of virulence factors [56]. Likewise, intermittent intestinal hypoperfusion and subsequent reperfusion injury can alter the established oxygen gradient within the lumen of the GI tract and lead to increased nitrogen concentration, which favors the growth of opportunistic pathogens [48]. These alterations may contribute to the pathogenesis of AL (Fig. 1).

Pre-operative Manipulation of the Microbiome and the Mucus Layer

Pre-operative manipulations have the potential to have long-lasting consequences for the gut microbiota and the host. Both enteral and parenteral antibiotics shift the composition of the bacterial community, alter host-microbe symbiosis, and decrease protection against pathogenic bacteria conferred by commensal microbes [57, 58]. There are multiple mechanisms by which commensal organisms confer protection against pathogenic infections, including competitive niche exclusion (i.e., competition for space and nutrients), production of inhibitory compounds (e.g., antimicrobial peptides), and intra- and inter-species communication (i.e., quorum sensing). Antibiotic use can disrupt this defense mechanism via depletion and/or alteration of the commensal gut microbiota [30]. Although the microbiota recovers quickly following termination of antibiotic use, alterations can persist for up to 2 years after treatment, including decreased diversity, alteration of taxonomic composition, and upregulation of antibiotic resistance genes [59].

MBP can be employed with or without oral antibiotics. There are two mechanisms of action by which MBP alters the gut microbiota. Increased fluid in the colonic lumen washes out fecal content and stimulates gut motility in response to distension. This increase in gut motility also leads to a decrease in bacterial load and alteration in community structure [51, 60, 61]. The specific impact(s) of lavage itself on the colonic microbiota is poorly understood; however, there are differing effects on the mucosa-associated and luminal colonic (i.e., stool) bacteria [62]. In addition to clearing bulky matter and improving visualization, purgative cleansing preparations also induce mucosal inflammation and create a transient shift in microbial composition [51, 63]. A randomized controlled trial evaluating the effect of pre-op MBP on fecal flora found significant reduction in total number of bacteria, including *Clostridium*, *Bifidobacteria*, *Lactobacillus*, and *Enterobacteriaceae*. In contrast, there was no demonstrable decrease in the potentially pathogenic taxa *Enterococcus* and *Staphylococcus* [64, 65]. MBP has also been shown to impact the mucus layer, decrease the concentration of SCFAs in the lumen, and stimulate a bloom in *Proteobacteria* by altering luminal pH [66••, 67]. Alterations to the mucosa can ultimately impact intracellular signaling pathways.

While nearly all colorectal surgeons in the USA reported using MBP in 2003, there has been a slight decline over the past 15 years due to increasing scrutiny over the efficacy of MBP [68, 69•]. Several recent trials were unable to show a statistically significant difference in SSI between patients operated on with or without MBP (however, this analysis did not include the use of oral antibiotics) [68, 70, 71]. Further, a 2011 Cochrane review [72] showed no decrease in AL, post-operative complications, or mortality in the colorectal population following MBP alone. Since then, there is conflicting information from multiple studies regarding use of MBP with or without oral antibiotics in the era of prophylactic parenteral antibiotics.

In large retrospective analyses, pre-operative MBP combined with oral antibiotics significantly reduced (~ 50%) the rate of complications following colorectal surgery, including surgical site infection, ileus, and AL; however, this study did not address if both interventions are necessary for this outcome [62]. An analysis of the Veterans Affairs Surgical Quality Improvement Program (VASQIP) revealed no difference between the rate of SSI for patients receiving oral antibiotics, irrespective of whether they also had MBP (9% vs.

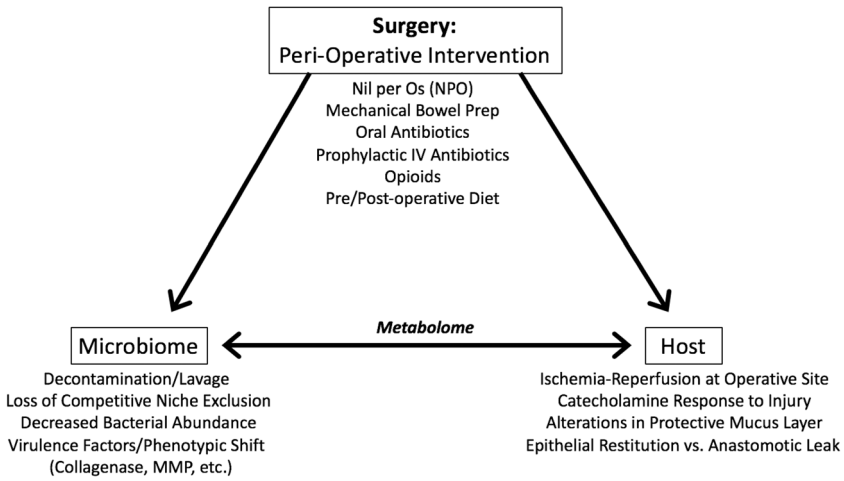

Fig. 1 Multi-dimensional interactions between peri-operative interventions (diet, medications, bowel prep, etc.), gut microbiome, and the human host

8%, with and without MBP, respectively); however, for the group that did not receive oral antibiotics, MBP alone was not effective in preventing infections (SSI rate doubled to 20%). After adjusted analysis, they found that MBP with oral antibiotics was associated with a 57% decrease in SSI (OR = 0.43, 95% CI 0.34–0.55). Even more striking, oral antibiotics alone resulted in a 67% decrease in SSI (OR = 0.33, 95% CI 0.21–0.5 0) [73]. This finding was re-demonstrated in Morris et al.'s evaluation of NSQIP data from 2011 to 2012, which demonstrated a 50% reduction in SSI with oral antibiotics versus no oral antibiotics (6.5% vs. 13%, respectively) [74].

These analyses suggest that benefits of oral antibiotics may outweigh their effects on the microbiota; it is less clear if the benefits of MBP, beyond making surgery technically easier, outweigh the impact on the microbiome. However, further work examining the specific mechanisms by which these interventions alter the microbiota in the setting of surgical interventions, as well as the functional consequences, is required before clinical protocols are significantly altered.

Surgical Injury and Host Response: Disruption of Microbiome Has Functional Consequences

Anastomotic Tissues—Bacterial and Host Response

Despite efforts to minimize trauma to tissues and adherence to the tenets of robust blood flow, decreased tension, and proper construction of anastomoses, the rates of AL have not changed in the twenty-first century [2]. Further, while grounded in pragmatism, these tenets are not based on level 1 evidence, which suggests the need to expand our understanding of the dynamic response to the creation of an anastomosis. Commensal microbes are influenced by surgical injury and peri-operative management; microbes continuously sample their microenvironment and respond to host cues to optimize their own survival. Tissue ischemia has been demonstrated to impact local microbiota composition. In a mouse model, mesenteric ischemia and subsequent reperfusion induced a relative increase in *E. coli* and decrease in *Lactobacillus* of the ileum and colon. This change persisted for approximately 6 h after reperfusion until colonic microbiota began to recover [75]. This shift in colonic microbiota is accompanied by breakdown in intestinal barrier function with loss of mucus layer integrity, which allows for clinically relevant localized tissue inflammation and translocation of potentially pathogenic bacterial species [11]. The transient ischemia endured during the process of creating an anastomosis induces reversible damage to the mucus layer as long as reperfusion occurs in under an hour [40]. Upon reperfusion, Goblet cells rapidly replenish the mucus layer via mass exocytosis of mucin. Because mucin biosynthesis takes time, the anastomosis should be protected from an additional period of ischemia.

Over 60 years ago, Cohn and Rives developed a dog model of colonic anastomosis, which included ligation of mesenteric vessels to induce ischemia of the bowel. They then used an indwelling catheter to infuse either topical antibiotics or saline directly at the site of anastomosis. Animals that received antibiotics demonstrated complete healing of anastomosis, as opposed to animals receiving saline alone who developed major leakage, peritonitis, and death [76]. The topical administration of antibiotics clearly interacted with the bacterial species in a beneficial way. The protective effect of non-absorbable enteric antibiotics was re-demonstrated by Cohen et al. in 1984 who also showed avoidance of AL despite mesenteric ischemia [77]. A decade later, Schardey and colleagues used a rat model to identify a potentially causative species of AL, *P. aeruginosa* [78]. In this model of total gastrectomy with esophagoduodenostomy, oral gavage of *P. aeruginosa* was compared to oral antibiotic treatment. The introduction of pathogenic *Pseudomonas* resulted in significantly greater transmural histological defect at the anastomosis and functionally resulted in lower bursting pressure—two features that reflect increased risk of leak rate. These experiments demonstrate the detrimental role that virulent microbiota can play in development of AL.

The process of resecting bowel and creating a new anastomosis induces a response in the host that causes release of soluble products. These chemical messengers are able to attract microbes and immune cells to the site of injury and send "cues" that induce a phenotypic shift among pathogenic bacteria, including *Pseudomonas* and *Enterococcus* [79•]. To understand how the normal dynamic response to surgery might lead to increased microbial virulence, Shogan et al. used 16S sequencing to characterize microbiota changes following partial colectomy and primary anastomosis [79•]. From postoperative day 0 to day 7, their group found a 200- and 500-fold increase in relative abundance of *Escherichia*/*Shigella* and *Enterococcus*, respectively. When examined on a functional level, there was a predominance in expression of bacterial virulence-associated pathways near the anastomotic tissue. Significantly, this bacterial gene expression was not present in the stool or luminal contents, which implies that there may induction of a phenotypic shift leading to increased local adherence of invasive bacteria to the anastomotic tissues.

Intestinal *P. aeruginosa* is capable of responding to host signals released during stress. By utilizing quorum sensing, opportunistic pathogens can sense host environmental changes (i.e., stress or injury) and respond by inducing a phenotypic shift in their own virulence [80]. In vitro, it has been demonstrated that products of hypoxic intestinal epithelial cells, like adenosine and dynorphin, can directly activate quorum sensing. For *P. aeruginosa*, this response is characterized by a shift to a more aggressive and barrier disrupting phenotype with high collagen-degradation activity [54••, 81] and increased intestinal tight junction permeability [82]. Theoretically, this

may also occur during times of ischemia and reperfusion in vivo. This is further supported by both hemorrhagic shock and ischemia-reperfusion models using germ-free mice that demonstrate improved survival compared to conventional control animals [83, 84]. In mice, morphine exposure led to a shift to a more virulent phenotype of *P. aeruginosa* that expressed greater biofilm formation, increased antibiotic resistance, and the ability to cause lethal gut-derived sepsis. Further, in the presence of morphine, these bacteria shifted to a mucus-suppressing phenotype, which disrupted the mucus layer and subsequently degraded gut epithelial integrity [85]. The increased virulence in *P. aeruginosa* has been attributed to a SNP mutation in the *mexT* gene that displays increased tissue destruction, collagenase expression, and swarming motility [86].

E. faecalis has been found to be highly prevalent in anastomotic tissues, likely due to its high adherence affinity to extracellular matrix proteins, including collagen. *E. faecalis* is capable of producing gelatinase (*GelE*), which contributes to the development of AL by breaking down collagen and activating intestinal matrix metalloproteinases (MMP), which are capable of degrading collagen. Interestingly, researchers were able to suppress MMP9 activation via direct application of topical antibiotics to intestinal tissues, but this protective effect was not replicated with IV antibiotics [54••]. This again suggests that the local interaction of mucus-associated microbes at the site of tissue injury with soluble factors may be causing a phenotypic shift to more virulent strains that contribute to collagen breakdown and thus increasing likelihood of developing AL. Shogan et al. concluded that incidence of AL is associated with microbiota (i.e., *E. faecalis*) that has both increased production of collagenase (aka *gelatinase*) and increased capacity to activate host intestinal MMP [54••]. They went on to examine the impact of a topical antibiotic regimen on an anastomotic model in rats and found decreased activation of MMP and collagen breakdown among rats treated with antibiotics, which correlated with decreased incidence of AL. However, this relationship was not conserved in rats that received intravenous (IV) Cefoxitin. Rather, administration of systemic IV Cefoxitin was associated with a bloom in high collagenase producing *E. faecalis*, which may explain why this group did not have a reduction in AL [54••]. Together, this again suggests that bacteria play a role in the development of AL via bacterial collagenase expression and excessive activation of host intestinal MMP leading to robust collagen degradation.

Alverdy and colleagues have proposed that AL is a product of the "right bacteria (*E. faecalis*, *P. aeruginosa*), with the right virulence genes (collagenase), expressed, in vivo, by the right activating cues (long operation, blood loss, difficult dissection), existing within a critically deficient microbiome (history of smoking, alcohol use, pre-operative chemoradiation, antibiotic use)," that upregulate the inflammatory process and allow for the development of necrosis and dehiscence [87••, 88].

Recovery After Surgical Injury

Modulation of the microbiome offers an opportunity to improve patient recovery following surgical interventions. A number of interventions, including dietary therapy [89], antibiotics [89], prebiotics [90], probiotics [91], and synbiotics (i.e., prebiotics and probiotics given together), could potentially be used to influence patient recovery through the microbiota.

One example of the link between intervention, microbiome composition, and host health is inflammatory bowel disease (IBD). In the case of IBD, patients exhibit a dysbiotic (i.e., altered) microbiota composition during active disease [92]. Exclusive enteral nutrition is one treatment modality used to treat IBD, and it is possible that this therapy exhibits a therapeutic benefit in part due to modulation of the microbiota [93, 94]. A recent study showed that host inflammation, antibiotics, and dietary alterations each independently influence the composition of the dysbiotic microbiome in pediatric Crohn's disease [89]. It is possible that dietary therapy and/or other diet-related strategies may provide a way to promote restitution of the microbiome and prevent a bloom in more pathogenic species. Results from early evaluation of enhanced recovery after surgery (ERAS) protocols suggest that early enteral feeding may be beneficial for recovery following surgical injury [95]. The microbiota is able to produce SCFA through the fermentation of ingested complex carbohydrates and proteins that reach the colon, which lends credence to the proposal that early enteral feeding may be a strategy to enhance the protective effect of re-establishment of a commensal microbiota following surgical injury.

In response to epithelial injury and mucosal barrier degradation, the host initiates rapid wound healing to reestablish homeostasis. This process—called gastrointestinal epithelial restitution [96]—involves the closure of gaps in injured epithelial surfaces via migration from edges of injured epithelial cells. Restitution is influenced via a variety of factors including GI microenvironment and microbiota [97]. GI restitution is modulated by multiple growth factors, including epidermal growth factor (EGF), transforming growth factor-alpha (TGF-a), and platelet-derived growth factor (PDGF), among others. Likewise, cytokines, SCFAs, bile acids, and the microbiota are all involved in the restitutive response.

Bacterial species interact with Toll-like receptors (TLR) on epithelial cell surfaces; activation of TLR-dependent pathways leads to production of inflammatory cytokines (i.e., IL-6 and heat shock proteins), whereas lack of activation causes a reduction in epithelial cell proliferation [98]. Further, bacterial fermentation of indigestible fiber leads to production of SCFAs, which are known to be the primary energy source

for colonocytes and stimulate mucus secretion. Through the production of SCFAs and transformation of bile acids, endogenous bacteria play both a positive and negative role in modulating the GI restitution response to epithelial damage. In a rat model, Bloemen et al. showed that butyrate enemas improve intestinal anastomotic strength [99] by protecting epithelial integrity. In this study, they found that rats treated with butyrate had stronger anastomoses (measured as burst strength) at 7 days compared to placebo and control, and their anastomoses had increased mature-to-immature collagen ratio [99]. It has been hypothesized that SCFAs increase re-epithelialization after injury to the intestinal epithelial layer. A different experimental model using Muc2-deficient mice that lack the ability to produce an outer mucus layer, 20 out of 22 Muc2-deficient mice developed AL, compared with only seven of 22 control mice ($p < 0.001$) [37], which may be secondary to induced re-epithelialization and support of the functioning mucus layer. Muc2-deficient mice also had significantly higher I-FABP levels than matched controls (Muc2 +/− and +/+), which is released from enterocytes and is a surrogate for cellular damage. This epithelial damage implies that the mucus layer is important for protecting the vulnerable anastomotic site from bacterial encroachment, and thus may be important to the healing process [37].

The multidirectional interactions between the host, the mucus layer, and the microbiota require further elucidation; however, it represents an opportunity for therapeutic dietary interventions, including before or after surgery. One example of this link between diet, the gut microbiota, and intestinal barrier function is the increase in dietary mucus degradation, alteration in microbial community structure, and promotion of colitis following chronic or intermittent dietary fiber deficiency in animal models [100].

Conclusion

While the long-term consequences of surgery on the gut microbiome are not entirely known, the existing literature provides compelling evidence to suggest that the host-microbiota relationship may play a role in causing and/or exacerbating AL after intestinal resection. As such, it is imperative that we further elucidate this complex and dynamic relationship. Likewise, surgeons should strive to minimize excessive perturbations to this equilibrium by decreasing ischemia, eliminating the use of unnecessary medication (e.g., limit opioids as able), and appropriately utilizing oral antibiotics with MBP when indicated.

Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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