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The opposing forces of the intestinal Microbiome and the emerging pathobiome

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Abstract

Objective—The purpose of this article is to summarize emerging concepts on the role of the intestinal microbiome in surgical patients.

Background—Revolutionary research over the past decade has shown that human beings live in close and constant contact with boundless communities of microbes. This new reality has wide-ranging implications for the care of surgical patients.

Methods and Results—Recent innovations in the study of the human microbiome are reviewed. To demonstrate the applicability of these studies to surgical disease, we discuss in detail what is known about the role of microbes in the pathogenesis of perioperative complications including anastomotic leak, sepsis, and surgical site infections.

Conclusions—Enhanced awareness of the human microbiome will empower clinicians to adopt novel practices in the prevention and treatment of a wide variety of surgical conditions.

Keywords

Microbiome; Bacteria; Anastomotic leak; Sepsis; Infection; Metabolomics; Proteomics

I. Introduction

For centuries, humans have dismissed the importance of microbial organisms whose mere existence is essential to our ability to survive and remain healthy. Microbes and the complex community structures in which they assemble and function have remained invisible for decades, only occasionally garnering recognition during times of plague and strife. Over time, however research has shown that human beings live in close and constant contact with boundless communities of microbial organisms. These organisms are not only our

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cohabitants, but they are the majority of live forms around and within us, outnumbering all other living beings on earth. The human body is a vessel to trillions of microbes, and it has been estimated that the number of microbes in or on the human body outnumber the total number of human cells by a factor of ten. The human body consists of around ten trillion cells while *a hundred* trillion bacteria¹ colonize its surfaces and intestinal tract directly participating in health maintenance. For every gene in the human genome, there are 100 bacterial genes. The human associated bacterial communities and their genomes are collectively referred to as the 'Microbiome' and are present in the gut, mouth, skin and all surfaces exposed to the external environment including the lungs. The role of the microbiome in health maintenance is emerging as having a profound influence on overall homeostasis. Human behavior, in turn, can have a significant impact on the structure and function of the microbiome. Thus humans and their microbiota are engaged in a mutualistic relationship that is crucial for the wellbeing of both living bodies. Everything from the food or medications ingested to the way an individual is born influences the species, community structure, and function of microbiota that take up residence in our bodies. The recent recognition that the pharmacodynamics and efficacy of cancer chemotherapy are dependent on the structure and function of our microbiota^{2,3} is forcing a reconsideration of the way we approach disease and its treatment. As such it is becoming increasingly untenable to dismiss the health of the microbiota when considering both the pathogenesis of disease and its treatment. For this reason, we are forced to adopt new life practices, as well as, re-evaluate the many established methods of medical and surgical management of disease. The purpose of this article is to summarize what is currently known about the role of the microbiome in health and disease and to provide instances in which consideration of the host- microbiota interaction might affect future research and care of surgical patients.

II: Rising incidence of surgical infections, antibiotic resistance, bowel preps, and beyond

Prior to the past 70 years, surgical infection radically limited surgeons' ability to perform advanced operations. Before the 1850s, postoperative "irritative fever" developed in most patients, leading to overwhelming sepsis and death.⁴ Surgeons seldom washed their hands and students were encouraged to place bare hands in the operative field for educational purposes.⁵ The famed surgeon William Halsted went so far as to operate in tents placed outdoors at Bellevue Hospital for better infection control.⁶ In the mid-19th century, Joseph Lister introduced antisepsis, providing the foundation for modern aseptic technique. By the end of the century, Louis Pasteur's germ theory was gaining acceptance. The combined work of these two pioneers led to significantly decreased perioperative morbidity and mortality.

In the 1950s, prophylactic antibiotics came into use for surgery. Despite inconsistent results from early clinical trials, Burke et al⁷ demonstrated that timing of antibiotic administration relative to the beginning of the procedure was crucial with respect to decontaminating the skin. In the 1960s, Bernard and Cole⁸ furthered the earlier work by performing a randomized controlled trial demonstrating the benefit of preoperative intravenous antibiotic prophylaxis in gastrointestinal tract operations. Work in the 1970s-1990s helped to define the role of the intestinal microflora on postoperative sepsis in general and on wound

infection specifically. However these studies were limited by results generated by culture alone and have not been repeated using newer genome based identification methods^{9,10}. There is concern that we are using antibiotics with broader coverage than is necessary which has the unwanted consequence of selecting out for more resistant organisms while at the same time destroying the health promoting microbiota. During critical illness such as occurs following trauma and burn injury, a countermeasure to the unavoidable use and consequences of broad antibiotic coverage has been to administer selective digestive decontamination (SDD) with oral non-absorbable antibiotics to eliminate potential pathogens that remain in the gut. This approach has not been widely accepted due to the fear of resistance. Another approach has been to repopulate the gut microbiota with probiotic organisms administered orally during critically illness. The use of such probiotic organisms has been controversial and the results disappointing. Both SDD and probiotic feeding to critically ill patients suffer because they are based on empirical evidence and there still exists major gaps in knowledge to explain how the microbiome maintains health during physiologic stress. Clinical trials of selective digestive decontamination suffer from a lack of detailed understanding of the precise effect it has on the community structure, taxa, and function of the intestinal microbiota. Numerous human and animal studies using 16s rRNA based genomic analysis of the microbiota demonstrate that loss of the core microbiome during physiologic stress and critical illness directly affects immune function and is associated with poor outcome. To date there are no studies that have examined the effect of SDD on the function, structure, and detailed taxa of the gut microbiome at multiple time points during critical illness. Whether microbial genes, such as virulence genes and antibiotic resistance genes, are activated within the transcriptome of the microbiota during SDD develops remains unaddressed. Similarly, the effect of a single strain of probiotic bacteria, or even several strains, can recapitulate the core functionality of the microbiota on the immune system during critical illness remains unstudied. Use of genomic, metagenomic, transcriptomic, and metatranscriptomic approaches to define how to best eliminate threatening microbes and preserve those that are health promoting is now available. These approaches represent an immense opportunity to shift from the empiricism of correlating a “broadly kill” or “selectively repopulate” approach to clinical outcomes to one in which the robustness and resilience of the core microbiome can be defined and maintained.

Similar line and approach of investigation could be applied to bowel preparation before elective gastrointestinal surgery. It has taken decades for surgeons to accept that mechanical cleansing of the bowel with purgative agents may be unnecessary, even harmful in elective gastrointestinal tract surgery^{11,12,13,14}. Historically, cleansing mechanical bowel preparations (MBP) were used to “de-contaminate” the GI tract as well as make the bowel easier to work with intraoperatively. However, MBP does little to decrease the concentration of bacteria in the bowel mucosa and lumen; it does, however, serve to upset the local microbiome allowing opportunistic pathogens the opportunity to thrive.¹⁵⁻¹⁷

The use of oral antibiotics in elective colon surgery has varied tremendously as a result of the work performed on mechanical bowel prep. Use of oral antibiotics to modify the intestinal microflora before surgery started in the 1930s and continued over the ensuing forty years as more antibiotics came into use. In the 1970s Clarke et al perform a randomized control trial at the Veterans Administration Hospitals in which patients received either oral

erythromycin-neomycin or placebo after mechanical bowel preparation. No intravenous antibiotics were used on the control group. Results showed a decrease from a 35% surgical site infection rate in the placebo group to a 9% rate in the oral antibiotic group ($p < 0.05$).¹⁸ Further studies confirmed this early finding and oral antibiotics became standard of care. While fecal cultures were examined initially, they proved to be of little use in directing subsequent practices and antibiotic choices. As mortality rates and surgical site infections decreased from 1970 to 2000, oral antibiotics were eliminated, intravenous antibiotics were empirically chosen, guidelines were developed, and the science to verify all these changes and choices was left abandoned. As clinical outcomes slowly improved, the need to understand the causality between antibiotic choice and outcome faded in interest. The downside of this disinterest is the emergence of antibiotic resistance, persistent surgical site infections, anastomotic leaks, sepsis, and a lack of understanding of why they continue to occur.

The past 100 years has seen colorectal surgery transformed from a uniformly deadly endeavor to an intervention that can be performed with minimally invasive techniques. Yet, despite these advances, the incidence of surgical site infections and anastomotic leaks persists and plague clinical practice. While surgical site infections following gastrointestinal surgery are most certainly attributed to exposure to the intestinal microbiota, this mechanism for anastomotic leak is less well accepted despite compelling data supporting it (vide infra). The complications of surgical site infection and anastomotic leak carry a substantial mortality risk.¹⁹⁻²¹ The relative risk of death is increased by a factor of 2.2 and the average length of stay is increased by five days.²² The role of mechanical bowel preparation in conjunction with oral antibiotics remains to be fully elucidated. Further characterization of the microbiome and its interactions with the host can potentially lead to novel targets to further decrease the surgical site infection rate. Yet this approach will require a more genomic understanding of the community structure, taxa, and function of the microbes we need to preserve and those that we need to eliminate. More importantly is realizing when, in terms relative to surgery, and where, meaning orally or locoregionally, to do this.

III. Emerging importance of human microbiome in health and disease

The emerging role and importance of the microbiome in human health and disease is being discovered across various medical and surgical problems. Subtle perturbations in the microbiome are now being found to influence diseases, their progression and resolution. Technological advances in genomics, proteomics and metabolomics have begun to reveal the immense impact that our microbiome has on our health and development of disease and its complex relationship with the host.

Our coexistence with the gut microbiota is a dynamic and mutually beneficial relationship. Commensal bacteria are critical in regulation of digestion. The bacteria of the gut regulate host energy metabolism and body mass index. In this way they have been linked to obesity. For example, gut flora aid in digestion and degradation of nondigestible plant polysaccharides and fiber in our diet¹. Enzymes capable of digestion of nutrients otherwise inaccessible have been found in the genomes of gut bacterial species (eg, *Bacteroides thetaiotamicron*). Gut bacteria have also been found to impact the storage of fat. Normal

flora have been shown to stimulate triglyceride storage by inhibiting Fia1 (fasting-induced adipocyte factor), a protein inhibitor of lipoprotein lipase^{1,23}. It has been hypothesized that altered gut flora may limit the ability of lipoprotein lipase to adequately process triglycerides and thereby lead to obesity. Recent studies have shown that when the cecal microflora of obese mice are transferred to lean mice, there is a significant weight gain in the previously lean mice^{24,25}. Similarly, germ-free mice are leaner than conventionally raised mice despite increased food intake^{26,27}. They must consume 30% greater calories in order to maintain the same weight as their counterparts.²⁸ If these germ free mice then receive a microbiota transplant from obese mice, they develop a 60% increase in body fat.²⁷ Studies have then looked at human subjects and found that when following a fat-restricted or carbohydrate restricted diet, *Bacteroides* species increases overtime relative to the Firmicutes.²⁹ Importantly for surgeons, patients who had a Roux-en-Y gastric bypass were found to have a decreased abundance of Firmicutes in their gut as compared to their obese counterparts.^{25,30}

Recent studies have shown the commensal bacteria and their products are vital in regulating the development, homeostasis and function of the host immune system. It has been shown that in mice who have their microbiome removed via antibiotics, that the reduction in MyD88-mediated innate immune signals leaves the mucosa more permeable to commensal bacteria and less capable of postinjury repair.³¹ The intestinal commensals also have been found to stimulate the expression of antimicrobial factors such as RegIII γ which protects the epithelium from invasion by bacteria and controls the balance of members of the microbial community.³² The microbiota also stimulate the production of cytokines that strengthen the immune system (IL-10, IL-22) and facilitate the clearance of pathogens.³² The normal microbiota play a clear and definite role in shaping and directing both the local and systemic immune system in order to maintain homeostasis and defend itself against invading pathogens.

IV. Genomics, metagenomics, proteomics, and metabolomics – the future of surgical research

Our microbial companions perform a number of metabolic reactions that are not encoded in the human genome, but are necessary for human health. Consequently, when considering the “human genome” there should be an inclusion of human genes and those of our microbes. As a result of innovations in high-throughput sequencing technologies, it is now possible to study the microbiome on a massive scale that was not possible years ago. The majority of microbial species present in and on the human body have never been isolated, cultured or sequenced, largely due to the inability to reproduce necessary growth conditions in the lab³³. Fortunately, advances in technology and the understanding of bacteria growth have allowed for the recreation of such conditions. There are numerous microbial processes waiting to be discovered.

After identifying the components of the human microbiome, it is then necessary to study their activities and functionalities in their environment by high throughput functional metagenomic approaches³⁴. Metagenomics, or the study of microbial communities, takes advantage of the advances in sequencing technology and analysis methods to comprehensively examine microbial communities directly from their natural habitats,

potentially revealing the previously unseen³³. Accordingly, the NIH has established the Human Microbiome Project (HMP) as a Roadmap Initiative³³. The HMP uses 16S rRNA and metagenomic sequencing to characterize the complexity of the human microbiome at various body sites. This has generated extraordinary amounts of data about the complexity of the human microbiome, and provides a foundation for further research into the influence of the microbiome on human health and disease. With the support of the HMP, an enormous amount of consideration has been put toward defining which microbes are “normal” and which may be associated with disease pathogenesis¹. The translational significance of microbes is relevant not only to research within every NIH institute, but certainly to every surgical discipline.

Though phylogenetic and metagenomic approaches may reveal candidate species and genes that may be important in certain conditions or diseases, they do not provide evidence for the actual involvement of these species or genes. In other words, metagenomics allows for the identification of microbial genes, but fails to provide information about downstream molecules that are actually produced in their environment. Hence, functional approaches are needed that aim to identify the active molecules and species. These functional studies also provide the basis for studying the environmental interactions between the microbe and their host. This is of significant importance as functional studies could reveal molecular mechanisms and therefore lead to developing treatment strategies for diseases associated with the human microbiota. With this concept in mind, researchers have introduced transcriptomics, proteomics, and metabolomics.^{35,36} Transcriptomic, proteomic and metabolomic approaches at the community level, also termed meta-omics, have all been applied to observe molecular activities of the human microbiota³⁷. Unfortunately, metatranscriptomic studies, analyzing bacterial mRNA, are difficult as the prokaryotic mRNA is highly unstable. Similarly, metabolomics, metabolite analysis, is also considerably difficult due to the fact that all body fluids are in contact with cells, and thus metabolites are rapidly absorbed. Moreover, metabolite analysis is rather challenging requiring a series of high throughput instruments and large databases for which many metabolites are unknown, and hence would require initial identification³⁷. In contrast, metaproteomics analyzes proteins, which are generally quite stable and can correlate directly to the genetic code, with the advantage that prokaryotes are known to have limited post-translational processing. Therefore, metaproteomics is likely to illustrate a highly reliable picture of microbial function with relation to the host environment.

V. Application of next generation sequencing technology to surgical problems

Anastomotic Leak

Although anastomotic leak is most commonly framed as a problem of surgical technique, there is compelling evidence that its etiopathogenesis is microbial. Although clinical studies as early as 1939 implicated the intestinal microbiota as causative agents in anastomotic leak by virtue of a reduction in leak rates with oral antibiotics, it was not until 1955 that Cohn demonstrated in dogs that the microbial content of the intestine causes leak.³⁸ This notion was confirmed later by a rat study in which oral versus intravenous antibiotics were tested to

prevent anastomotic leak with results that clearly showed that oral antibiotic eliminated leaks whereas intravenous antibiotics with a similar spectrum of activity did not.³⁹ These studies were largely ignored as leak rates, surgical site infection rates, and mortality following colon surgery decreased from 1970 through to 2000. As such oral antibiotics have been largely eliminated and antibiotic leaks in high risk regions. Recently several randomized prospective studies have proven that oral antibiotics decrease anastomotic leak rates, however, the microbiologic detail around these empirical observations remains undefined. Our group has recently demonstrated that intestinal microbes can cause anastomotic leak in rats via mechanisms that involve their phenotypic transformation to high collagenase producers. Despite decades of work in this area, the application of oral antibiotics in gastrointestinal surgery remains controversial and largely abandoned. There are no groups worldwide studying a microbial mechanism of anastomotic leak using new generation technology. Again, an immense opportunity to define the role of microbes in anastomotic leak is being overlooked. Furthermore, the choice and route of currently recommended antibiotics for gastrointestinal surgery is based on incomplete data that is culture based and more than 40 years old.

Gut-derived Sepsis

Normal homeostasis of the gut is maintained through complex interactions between the microbiome and its environment. Humans and their microbiome generally maintain a well-tolerated symbiotic relationship.⁴⁰ However, during prolonged critical illness, the gut is exposed to numerous stressors including antibiotics, vasopressors, hypoxia, etc. which contribute to the development of a community of virulent pathogens in the gut which we refer to as the “pathobiome.”⁴¹⁻⁴³ We hypothesize that this “pathobiome” then interacts with the gut epithelium and immune system to drive the immunopathology of sepsis.

The normal microbiome is composed of a wide variety of bacterial species. Our lab has found that the bacterial composition of the gut as measured through the feces of normal subjects is diverse with protective anaerobes predominating and very low amounts of proteobacteria. However, when the stool of an ICU patient with severe sepsis is examined, there is an almost complete loss of those protective anaerobes and diversity. These patients undergo prolonged exposure to multiple antibiotics throughout their ICU admission. Despite the treatment they received, we were still able to culture virulent and drug resistant pathogens from the stool throughout their hospital course.

This pathobiome that can develop in the gut of the critically ill patient can cause dysfunction within the immune system. Given the limitation of antibiotics to eliminate all potential pathogens and to suppress the mutability of microbes, other strategies to “contain” rather than eliminate pathogens is needed. It is not the mere presence of the pathogen itself that leads to lethal inflammation, but rather whether pathogens are “cued” to express virulence. Our laboratory has made the novel observation that during extreme physiologic stress, such as occurs during critical illness, host compensatory cues are released into the local gut microenvironment that are gathered, processed, and transduced by certain pathogenic species leading to triggering of their virulence circuits. As such a dynamic *in vivo* virulence activation mechanism among intestinal pathogens exists whereby pathogens are

cued by host factors to express enhanced virulence. In animal models of lethal gut derived sepsis, we have defined many of these cues and they include endogenous opioids, immune elements, and end products of ischemia.

An interesting aspect of this work was the identification by our lab that bacteria also have membrane sensors that assess the abundance of resources present in the local microenvironment. One highly conserved sensor system is the system that senses phosphate abundance. Most bacteria possess a membrane based phosphosensory phosphoregulatory two component system that is connected to their core virulence mechanism such as the quorum sensing signaling system. When phosphate is abundant, incoming cues such as opioids do not transduce virulence, however when phosphate is low, opioid responsiveness, and hence virulence is enhanced. From an evolutionary perspective, these results indicate that when microbes sense abundant resources within the local microenvironment that support their growth and proliferation (i.e phosphate); they do not waste energy expressing virulence, unless predation is sensed. As this plays out in the gut during critical illness, both host derived “cues” and depleted local resources create conditions associated with virulence activation. We tested whether embedding phosphate in the local intestinal microenvironment of mice during surgical stress would prevent pathogen virulence activation and lethal sepsis. We intestinally inoculated mice with healthcare acquired pathogens (i.e *C. albicans*, *E. faecalis*, *S. marcescens*, *K. oxytoca*) and performed a surgical injury (30% hepatectomy), a model that results in lethal gut- derived sepsis. When phosphate was embedded into the host pathogen interaction in the gut by locally applying it as a covalently bonded polymer carrier, the microbiota proliferated, the pathogens were attenuated in expressing virulence and mortality was prevented⁴⁴ Thus provision of key resources within the host pathogen interaction in the gut, using compounds that do not kill but attenuate microbes, may be an approach to consider in a clinical trial. Unlike SDD or addition of a probiotics, this approach may leverage the strength of the microbiota to suppress virulence while also suppressing virulence directly by triggering phosphosensory and phosphoregulatory pathways.

Wound Infection

During the process of investigating the human microbiome and its association with our patients, we recognized that many traditional aspects of surgical care have a detrimental impact on the state of the innate microbiome, and thus can affect clinical outcomes¹. One example of the detrimental effects on the innate microbiome is the administration of antibiotics. The administration of perioperative antibiotics is known to be standard of care in certain circumstances. In accordance with national entities such as the Surgical Care Improvement Project (SCIP), the proper timing, regimen and duration of therapy are viewed as important quality measures⁴⁵. The motivation behind the administration of prophylactic antibiotics prior to surgery is to reduce the pre-operative bacterial burden, and as a result, will decrease the rate of surgical site infections, a complication that costs an estimated \$3.5 to \$10 billion annually in the U.S⁴⁶. However, the use of prophylactic antibiotics should be further evaluated because inappropriate overuse leads to the emergence of resistant organisms, and on the other hand, inadequate antibiotic use may adversely affect patient outcomes. This has been shown to be true in patients suffering from pneumonia, bacteremia, and also surgical site infections as measured by endpoints ranging from length of stay to

death⁴⁷⁻⁴⁹. Application of genomic and metagenomic analysis to studies of wound infection has the potential to provide a more high resolution readout of the microbial community structure, taxa, and function that assembles in a wound over time and may better calibrate our use, choice, and dose of antibiotics to prevent wound infections.

Conclusion

The way we view surgical infection and our interaction with microbes both beneficial and pathogenic, has changed immensely over the past couple of decades. Technological advances have fueled these advances as the time and cost to sequence whole genomes and to measure whole preteomes and metabolomes has been significantly reduced. Understanding and demonstrating the information within these megadatasets is becoming a reality as computational scientists, mathematicians, physicists, and bioinformatics experts work together. However, there are still limitations of these advances. We must be cautious not to ascribe causality to data that are purely descriptive. Rather we must correlate this new data with disease states and inform the system's biology. Armed with new approaches to examine the microbiome and its correlation to varying states of health and disease, new treatment strategies may emerge and antibiotic use may even diminish.

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Key Points

- Colorectal surgery has evolved overtime and yet the incidence of surgical site infections and anastomotic leaks persists.
- The microbiome has an emerging role in human health and various diseases such as anastomotic leak, gut-derived sepsis, and wound infection
- Current investigations using high throughput functional metagenomics approaches have significantly advanced our knowledge or the host-microbiome interaction