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A Little O₂ May Go a Long Way in Structuring the GI Microbiome



See "Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota," by Albenberg L, Esipova TV, Judge CP, et al, on page 1055.

The mammalian gastrointestinal (GI) tract houses hundreds of species of microbes from all domains of life—Archaea, Bacteria, and Eukarya. As in many environments, bacteria are the most abundant members of this complex microbial community. Hundreds of metabolites

attributed to the gut microbial community circulate in the mammalian bloodstream,¹ extending the reach of the microbiome to every host cell. It is no wonder that the GI tract has been described as the body's largest endocrine organ.² Given the role, contributions, and potential of the gut microbiome, it would be valuable to know how environmental conditions in the gut influence the composition and function of its microbiota. In this issue, Albenberg et al³ unveiled new evidence supporting a dynamic mammalian microbiome by exploring the potential for molecular oxygen (O₂) to shape the structure of the gut microbiome and offer a potential mechanism of the benefit of hyperbaric O₂ treatment in intestinal diseases.^{4,5}

O₂ in the environment has a dramatic influence on the evolution of life⁶ and its availability remains an important driver of organismal physiology and ecology, particularly in the microbial world. As a potent electron acceptor, microbes that can utilize O₂ for respiration are at a tremendous selective advantage in environments containing O₂. The consequences of this selective force are particularly strong in environments rich in organic compounds but with few alternative electron acceptors for microbial respiration, that is, any place where fermentation is the primary, energy-yielding form of metabolism. The digestive tracts of most animals have regions that fit this description precisely: A regular input of food supporting dense communities of fermentative microbes.

The availability of electron acceptors—in particular nitrate and tetrathionate—in the anoxic environment of mammalian colon leads to blooms of bacteria able to respire these compounds. Nitrate (NO₃⁻) is generated as a metabolic byproduct of the host inflammatory response and fuels blooms of *Escherichia coli* in the inflamed colons of mice.⁷ Similarly, populations of *Salmonella enterica* thrive in the inflamed gut by triggering a cascade of events that leads to the production of tetrathionate (S₄O₆²⁻), another potent electron acceptor that can be respired by populations of *Salmonella*.⁸ It has been suggested that infusion of O₂ after gastric bypass surgery⁹ may lead to the observed enrichments of Proteobacteria in the colon associated with this procedure.

Another consequence of O₂ in the gut was revealed in a recent study of gene expression in *Shigella flexneri*. Coordinated expression of virulence determinants, including genes involved in the type III secretion systems, were observed in response to a zone of oxygenation adjacent to the mucosa of the GI tract.¹⁰ This microoxic zone was attributed to diffusion of O₂ from the capillary network at the tips of villi and extended approximately 70 μm into the lumen.¹⁰

One limitation that we have in understanding the full impact of O₂ on the structure and function of the microbiota is the lack of accurate measurements O₂ concentrations that microbes experience. As reported in this issue of *Gastroenterology*, Albenberg et al met this challenge with the development of a phosphorescent probe, OxyphorMicro, that was distributed throughout the GI tract of mice by mixing the probe with mouse chow. Phosphorescence of the probes, which is quenched proportionately with the concentration of O₂, was measured with a specially designed fiberoptic detector. The instrument was inserted after laparotomy to measure phosphorescence of the probe in the organs of interest. OxyphorMicro is a particularly appropriate probe to

use because it is insensitive to the presence of solutes in the aqueous environment of the intestines, thus providing sensitive and accurate measurements of O₂.

In agreement with previous measurements in the cecum,¹¹ luminal concentrations of O₂ were approximately 40 mmHg. That concentration increased after inhalation of pure O₂, reflecting diffusion of O₂ from the tissue into the cecum lumen. Based on the compilation of O₂ concentrations throughout experimental manipulations, the authors conclude that there is a steep, radial gradient of O₂ in the intestines (Figure 1). O₂ diffusing into the lumen is consumed by mucosal-associated microbes resulting in extremely low concentrations of O₂ in the intestinal lumen (< 1 mmHg). The microbiota in the mucosa are clearly important to the health of the host, and until now we know little about this community of microbes and how it is structured by the chemical features of the environment. Albenberg et al showed that mucosa-associated bacteria are not static under physiologic conditions and are highly influenced by the O₂ tension in mucosa layer. The dynamic shifts in the microbiome in turn may affect the functional status of the mucosal layer contributing to mucosal homeostasis or pathology.

To examine the potential for O₂ to shape the composition of the gut microbiome, mice were exposed to hyperbaric oxygen therapy. After 9 days of hyperbaric oxygen therapy, tissue oxygenation increased 5-fold and the microbial community surveyed in fecal samples also shifted in composition. These findings were extended to the human microbiome through molecular surveys of microbial communities generated from biopsies of the rectal mucosa. The relative abundance of aerotolerant bacteria, particularly members of the Proteobacteria and Actinobacteria phyla, were enriched in the mucosa compared with fecal samples, again suggesting that O₂ is shaping this region of the gut microbiota.

The mammalian intestinal tract has traditionally been considered to be an anoxic environment that harbors an array of obligately and facultatively anaerobic bacteria, but potential roles of O₂ in this ecosystem are being reexamined. We know that the capacity to harvest the low levels of O₂ found in these environments, which is conferred by cytochrome oxidases with a high-affinity for O₂, are encoded in phylogenetically diverse microbes.¹² Furthermore, an estimated 30% of the microbes from the mammalian GI tract were found to have the metabolic potential to use low, even nanomolar concentrations of O₂.¹²

Clinical literature has suggested potential benefits of hyperbaric O₂ therapy in chronic intestinal disorders, such as radiation enteritis and inflammatory bowel disease.^{4,5} The mechanism is believed to be primarily an increased delivery of O₂ up to the injured intestinal tissue to promote faster healing, enhance immunity, and prevent colonization of harmful bacteria.¹³ Albenberg et al demonstrated convincingly that hyperbaric O₂ therapy also alters the host mucosal bacterial communities and may provide further protection against pathogenesis by potential pathobionts in the gut. More research is needed to assess the functional role of hyperbaric O₂-induced microbiota and whether this therapy may be beneficial in the co-management of patients with chronic intestinal inflammation or injury.

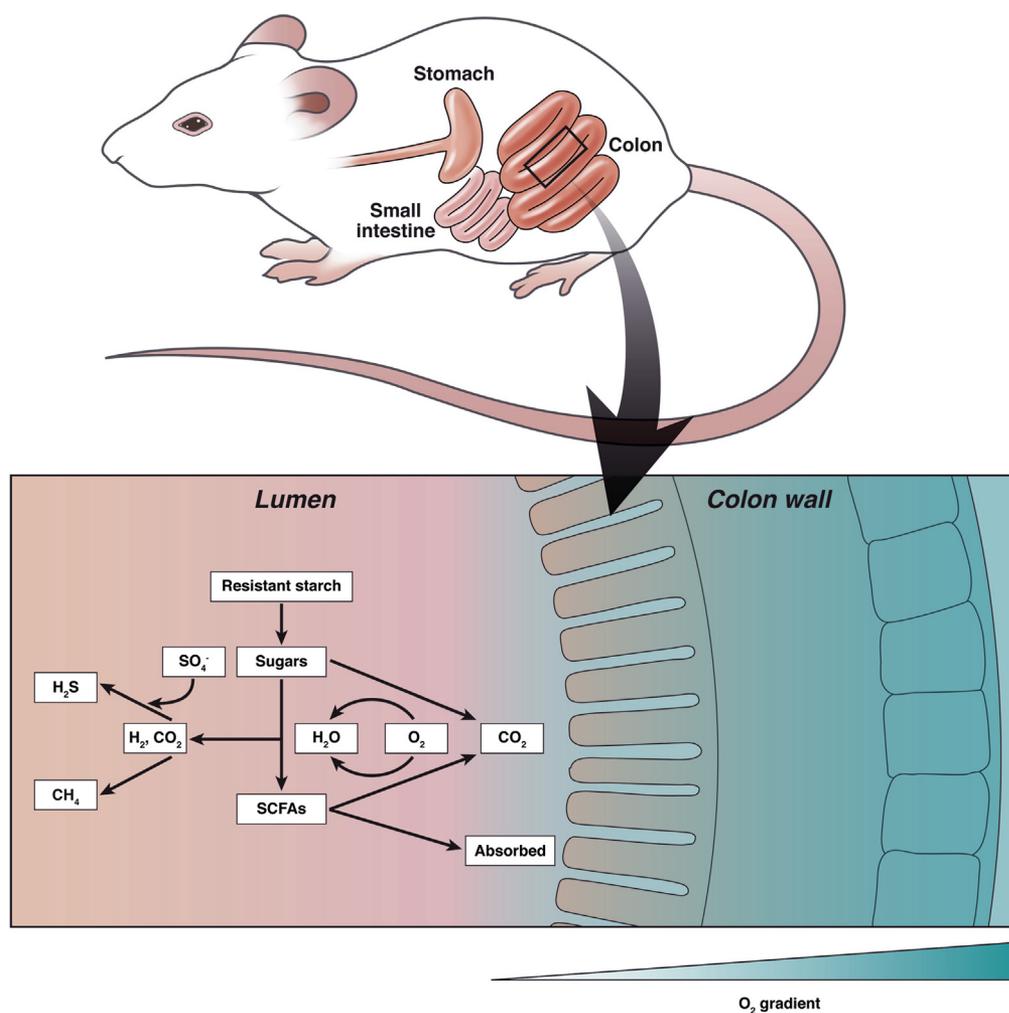


Figure 1. The radial O_2 gradient in the mouse colon and an overview of metabolic pathways that could be altered by the presence of O_2 .

Understanding the role of O_2 in shaping the structure and function of microbial communities, and thus the interaction of these communities with the mucosa, will advance our ability to build predictive models of interactions between the host and microbiota. Such an understanding will provide information important not only in the GI tract, but at all mucosal surfaces where gradients of O_2 exist.

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HCV NS5A Inhibitors Disrupt Replication Factory Formation: A Novel Mechanism of Antiviral Action



See “Daclatasvir-like inhibitors of NS5A block early biogenesis of hepatitis C virus-induced membranous replication factories, independent of RNA replication,” by Berger C, Romero-Brey I, Radujkovic D, et al, on page 1094.

In recent years, progress in the development of effective antivirals to treat chronic hepatitis C virus (HCV) infection has accelerated enormously. For more than a decade the standard of care therapy for HCV was a combination of pegylated interferon- α and ribavirin (pegIFN- α /RBV) for 24–48 weeks. Unfortunately this treatment regimen is associated with only moderate efficacy (50%–80% sustained virologic response rates) and severe side effects. However, as a result of the efforts of academic and industry research groups and advances in our understanding of the HCV life cycle, made possible by reliable cell culture systems, numerous promising direct-acting antivirals are in advanced stages of clinical development or have already been approved. The addition of first-generation NS3/4A protease inhibitors, telaprevir and boceprevir, to pegIFN- α /RBV therapy significantly improved sustained virologic response rates for genotype 1 infections.^{1,2} Likewise, the recent approvals of the second-wave NS3/4A protease inhibitor simeprevir,³ and the highly effective nucleotide analog inhibitor of the viral NS5B polymerase, sofosbuvir,⁴ brings closer the goal of a safe, effective, all-oral, and IFN-free direct-acting antiviral combination therapy in the near future. Along with molecules that target NS3/4A and NS5B, potent inhibitors of the viral NS5A phosphoprotein will likely be important components of future direct-acting antiviral combination therapies. Indeed, the first-in-class NS5A inhibitor daclatasvir (DCV) and structurally related NS5A inhibitors ledipasvir and ombitasvir are in the final stages of clinical development for use in various combinations, and a number of second-generation NS5A inhibitors with higher genetic barriers to resistance (eg, ACH-3102, MK-5172, and GS-5816) are in earlier stages of clinical development. NS5A has no known enzymatic activity and to date the exact

mechanism(s) of action of these inhibitors and indeed the exact functions of NS5A remain unclear. In this issue of *Gastroenterology*, Berger et al⁵ report that NS5A inhibitors interact with NS5A and block formation of the “membranous web” (MW) that houses HCV RNA replication, independent of effects on HCV RNA replication. Furthermore, the authors present evidence that DCV derivatives interact with NS5A dimers and moderately impair functional interaction of NS5A with phosphatidylinositol-4 kinase III α (PI4KIII α) that stimulates local accumulation of PI4-phosphate (PI4P) at sites of HCV RNA replication. Together this study sheds new light on the mechanisms of action of this unique and extraordinarily potent class of antivirals.

Given its essential roles in multiple aspects of the HCV life cycle, NS5A is an attractive and unique target of antiviral therapy for chronic HCV infection. Since the identification of DCV (formerly BMS-790052) as a potent, pangenotypic inhibitor of HCV RNA replication,⁶ a number of studies have investigated the potential mechanism(s) of action of DCV and structurally related NS5A inhibitors. Collectively, these studies have identified several inhibitor properties that may explain their efficacy (Figure 1B; reviewed in^{7–9}). First, their remarkable potency (picomolar to low nanomolar median effective concentration values) suggests that these inhibitors may synergistically disrupt multiple functions of NS5A in the HCV life cycle and/or target essential events in establishment of replication sites that in time will prevent continued HCV RNA replication. Second, the location of resistance mutations in domain I of NS5A (namely substitutions at L31 and Y93; Figure 1A) indicate that domain I-associated functions are specifically targeted. In this context, the class-defining resistance site Y93 is located at opposing, membrane-proximal surfaces of the dimer interface for both “back-to-back” and “clam-like” alternative domain I (genotype 1b) crystal structures.^{10,11} Third, biotin-tagged DCV derivatives enable precipitation of NS5A from pretreated HCV replicon-harboring cells,^{6,12} although interestingly fail to precipitate NS5A from replicon lysates or pretreated NS5A-overexpressing cells.¹² In this context, it is noteworthy that preformed replication complexes (RCs) are refractory to inhibition of HCV RNA replication by NS5A