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Review

The impact of intestinal inflammation on the nutritional environment of the gut microbiota

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ABSTRACT

The intestinal epithelium is a single cell barrier separating a sterile mucosal tissue from a large microbial community dominated by obligate anaerobic bacteria, which inhabit the gut lumen. To maintain mucosal integrity, any breach in the epithelial barrier needs to be met with an inflammatory host response designed to repel microbial intruders from the tissue, protect the mucosal surface and repair injuries to the epithelium. In addition, inflammation induces mechanisms of nutritional immunity, which limit the availability of metals in the intestinal lumen, thereby imposing new selective forces on microbial growth. However, the inflammatory host response also has important side effects. A by-product of producing reactive oxygen and nitrogen species aimed at eradicating microbial intruders is the luminal generation of exogenous electron acceptors. The presence of these electron acceptors creates a new metabolic niche that is filled by facultative anaerobic bacteria. Here we review the changes in microbial nutrient utilization that accompany intestinal inflammation and the consequent changes in the composition of gut-associated microbial communities.

1. Introduction

During intestinal inflammation the epithelium plays an important role in mounting responses that are aimed at clearing the mucosal surface from microbes. For example, production of IFN-γ during inflammation results in the activation of DUOX2 (dual function NADPH oxidase 2) [1], NOX1 (NADPH oxidase 1) [2] and iNOS (inducible nitric oxide synthase) [3] in epithelial cells. Reactive oxygen species (ROS) produced by DUOX2 and NOX1 and reactive nitrogen species (RNS) generated by iNOS create a hostile environment in close proximity to the mucosal surface. Furthermore, the pro-inflammatory cytokine interleukin (IL)-22 induces the luminal release of the antimicrobial proteins lipocalin-2, calprotectin, RegIIIβ (regenerating islet-derived 3 beta) and RegIIIγ from epithelial cells [4–6].

These epithelial defenses can be augmented by the transmigration of neutrophils into the intestinal lumen as the severity of intestinal inflammation increases. Upon transmigration, the phagocyte NADPH oxidase (PHOX), superoxide dismutase (SOD) and myeloperoxidase (MPO) of neutrophils generate additional ROS in the gut lumen. Subsequent lysis of neutrophils in the intestinal lumen releases calprotectin, which constitutes approximately 40% of their cytoplasmic content [7]. As a result, neutrophils are the main sources of luminal calprotectin during severe intestinal inflammation [8].

Some of the antimicrobials released into the intestinal lumen are bacteriocidal, thereby protecting the mucosa from infection. For instance, release of the C-type lectin RegIIIγ contributes to luminal clearance of opportunistic pathogens, such as *Listeria monocytogenes* or vancomycin-resistant *Enterococcus feacium*, which are both members of the class Bacilli within the phylum Firmicutes [9,10]. Chronic granulomatous disease, an illness caused by PHOX-deficiency, illustrates that the generation of ROS by phagocytes is essential for preventing recurrent bacterial infections [11–13]. It is thus likely that upon transmigration into the lumen, the respiratory burst of neutrophils aids in clearing bacteria from the vicinity of the mucosal surface. However, recent evidence suggests that in addition to its bacteriocidal effects, the inflammatory host response has also a profound impact on the nutritional environment in the gut lumen, which can lead to alterations in the composition of gut-associated microbial communities (microbiota). Here

Abbreviations: DUOX2, dual function NADPH oxidase 2; IFN-γ, gamma interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NOX1, NADPH oxidase 1; PHOX, phagocyte NADPH oxidase; RegIIIβ, regenerating islet-derived 3 beta; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, tricarboxylic acid; TMA, trimethylamine.

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we review these novel hypotheses and the underlying mechanisms.

2. Nutritional immunity changes the rules for microbial contestants

One subset of antimicrobial proteins released into the intestinal lumen during inflammation functions in limiting the availability of trace elements required for bacterial growth, such as iron and zinc, a host defense mechanism known as nutritional immunity. Bacteria acquire ferric iron (Fe^{3+}) by releasing high-affinity iron chelators, termed siderophores (reviewed in [14]). Enterobactin, a cyclic trimer of N-(2,3-dihydroxybenzoyl)-L-serine, is the siderophore produced by most members of the Enterobacteriaceae, a family of facultative anaerobic bacteria belonging to the class Gammaproteobacteria within the phylum Proteobacteria [15–17]. After chelating iron, the Fe^{3+}-enterobactin complex is transported actively by an energy-coupled outer membrane receptor protein into the periplasm. The energy required for transporting the Fe^{3+}-enterobactin complex across the outer membrane is provided by the proton motive force of the cytoplasmic membrane, which is transmitted to the outer membrane via the TonB protein (reviewed in Ref. [14]).

Lipocalin-2 prevents bacterial iron acquisition by binding and sequestering enterobactin [18–20]. While uptake of Fe^{3+}-enterobactin is a viable strategy for obtaining iron in the non-inflamed intestine, the epithelial release of lipocalin-2 during conditions of inflammation inhibits growth of bacteria relying solely on enterobactin for iron acquisition. Thus, bacteria acquiring iron through mechanisms that are not inhibited by lipocalin-2 gain a relative luminal growth advantage in the inflamed gut. This concept was first described in *Salmonella enterica*, a member of the Enterobacteriaceae that secretes enterobactin along with a glycosylated derivative of enterobactin, termed salmochelin [21]. Salmochelin is not sequestered by lipocalin-2, thereby conferring resistance against this antimicrobial protein [22,23]. Deletion of the *iroN* gene, which encodes the TonB-dependent outer membrane siderophore receptor [24], renders *S. enterica* unable to utilize salmochelin [21]. As a result, an *S. enterica iroN* mutant solely relies on enterobactin for iron-acquisition. Compared to wild-type bacteria, growth of a *S. enterica iroN* mutant in the lumen of the mouse gut is reduced in the presence, but not in the absence of intestinal inflammation. Furthermore, *S. enterica* wild type and *iroN* mutant grow equally well in the inflamed gut of lipocalin-2-deficient mice [5]. Thus, luminal growth of lipocalin-2 resistant bacteria is favored in the inflamed gut, but not in the absence of intestinal inflammation.

A second metal that is sequestered by the host during inflammation through the release of antimicrobial proteins into the intestinal lumen is zinc. Calprotectin, a heterodimer composed of S100A8 and S100A9, inhibits bacterial growth in tissue by chelating both manganese and zinc [25]. Recent studies suggest that the transepithelial migration of neutrophils and the subsequent release of calprotectin from dead neutrophils reduce the availability of zinc in the intestinal lumen [8]. Zinc is transported across the cytoplasmic membrane of *S. enterica* by the high-affinity ABC (ATP binding cassette) transporter ZnuABC [26]. Compared to the *S. enterica* wild type, luminal growth of a *znuA* mutant is impaired in the inflamed intestine of wild type mice, but not in the inflamed intestine of S100A9-deficient mice [8]. These data support the idea that by overcoming the calprotectin-mediated host zinc sequestration, bacterial high-affinity zinc acquisition confers a luminal fitness advantage during colitis.

Above examples illustrate that the inflammatory host response can influence bacterial growth by changing the nutritional environment in the intestinal lumen. As a result, bacterial metal acquisition strategies that bestow no apparent growth benefit in the healthy gut can confer a luminal fitness advantage in the inflamed intestine. In other words, the host response can alter the contest rules that govern microbial competition for metals.

Interestingly, reducing the availability of metals brings microbes, which rely on similar iron acquisition strategies, into a contest. For example, the commensal *Escherichia coli* strain Nissle 1917, a member of the family Enterobacteriaceae, elaborates four siderophores, including enterobactin, salmochelin, aerobactin and yersiniabactin [27–29]. Of these siderophores, only enterobactin is sequestered by lipocalin-2. Co-colonization with *E. coli* Nissle 1917 reduces luminal growth of the pathogenic *S. enterica* in wild-type mice, but not in lipocalin-2-deficient mice. Furthermore, co-colonization of mice with a siderophore utilization-deficient *E. coli* Nissle 1917 *tonB* mutant does not reduce the ability of *S. enterica* to grow in the intestinal lumen [30]. These data suggest that by lowering the availability of iron in the lumen, the host inflammatory response can alter the outcome of a competition between bacterial species that utilize overlapping siderophore repertoires.

3. Microbial metabolism in the healthy large intestine

In addition to conferring nutritional immunity by lowering the availability of metals in the intestinal lumen, the host response changes the luminal environment by generating inflammation-derived nutrients as a by-product. The resulting bloom of bacterial species that can utilize inflammation-derived nutrients can alter the composition of gut-associated microbial communities. To understand how inflammation-derived nutrients alter the growth conditions in the large bowel, it is important to first grasp the nutrient acquisition and utilization strategies that characterize a balanced microbiota, which inhabits the healthy gut.

In healthy individuals, obligate anaerobic bacteria belonging to the classes Bacteroidia (phylum Bacteroidetes) and Clostridia (phylum Firmicutes) dominate microbial communities inhabiting the anaerobic environment of the lower gastrointestinal tract [31]. Since simple carbohydrates and proteins are digested and absorbed in the upper gastrointestinal tract, complex carbohydrates (e.g. fiber or mucus carbohydrates) or non-digestible proteins (e.g. gluten) are the main nutrients supporting growth of Bacteroidia and Clostridia in the large bowel. Oxygen or other exogenous electron acceptors are not available in the healthy distal gut to support respiration. Thus, microbes rely largely on fermentation of carbohydrates and amino acids to generate energy via substrate-level phosphorylation and to acquire carbon and nitrogen for the biosynthesis of proteins, carbohydrates, lipids and nucleotides.

To maintain redox balance during fermentation, electrons have to be transferred from NADH onto organic compounds, such as phosphoenolpyruvate, thereby generating metabolic end products that are released. Microbiota-derived fermentation end products that commonly accumulate in the gut lumen include formate, acetate, proprionate, butyrate, lactate and hydrogen (H_2) (Fig. 1A). Some bacteria, such as *Bacteroides fragilis*, maintain redox balance by transferring electrons onto fumarate to generate succinate, a process known as fumarate respiration (reviewed in Ref. [32]). During this process, *B. fragilis* fixes host-derived carbon dioxide (CO_2) onto phosphoenolpyruvate to generate oxaloacetate, which is converted by reversing reaction of the tricarboxylic acid (TCA) cycle into the endogenous electron acceptor fumarate [33]. Succinate is released as a metabolic end product of fumarate respiration. Thus fumarate respiration and fermentation have in common that metabolically valuable phosphoenolpyruvate is removed from anabolic reactions and converted into metabolic end products to maintain redox balance.