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Review article

Intestinal microbiota and ulcerative colitis

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ABSTRACT

There is a close relationship between the human host and the intestinal microbiota, which is an assortment of microorganisms, protecting the intestine against colonization by exogenous pathogens. Moreover, the intestinal microbiota play a critical role in providing nutrition and the modulation of host immune homeostasis. Recent reports indicate that some strains of intestinal bacteria are responsible for intestinal ulceration and chronic inflammation in inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD). Understanding the interaction of the intestinal microbiota with pathogens and the human host might provide new strategies treating patients with IBD. This review focuses on the important role that the intestinal microbiota plays in maintaining innate immunity in the pathogenesis and etiology of UC and discusses new antibiotic therapies targeting the intestinal microbiota.

1. Introduction

Past studies have called attention to bacteria as an etiologic agent of inflammatory bowel disease (IBD), which is composed of both ulcerative colitis (UC) and Crohn's disease (CD). The search for a causative pathogen has been undertaken in many previous reports, but without a decisive discovery, the theory that bacteria are responsible for IBD was no longer regarded as important. However, recent discovery of toll-like receptors in the innate immune system provided insight into the bacterial infection theory in IBD. Data from studies using rodent models of spontaneous colitis suggest that indigenous luminal bacteria play an important role in the pathogenesis of mucosal inflammation [1–3]. Interestingly, commensal bacteria appear to contribute to spontaneous colitis, but this condition fails to develop in germ-free, knockout mice [4–7]. Therefore, commensal bacteria and innate immunity play a major role in the development of IBD. The onset and reactivation of disease are triggered by several factors including intestinal microbiota in patients with IBD. For example, the intestinal microbiota provide antigens and adjuvants all of which stimulate T cell immune responses [1]. Moreover, environmental triggers are necessary to initiate or reactivate disease expression [1].

2. Microbial influence in UC

2.1. The role of commensal bacteria in innate immunity

Toll-like receptors (TLR) play an important role in the innate immune system, and several TLR homologues and their ligands have recently been discovered. For example, lipopolysaccharide (LPS, endotoxin), a major component of the outer membrane of Gram-negative bacteria, activates several immunologic activities by signal transduction via the TLR-4 on the surface membranes of target cells [8,9]. Peptidoglycan, a complex amino sugar that is also a TLR ligand, is far less abundant in Gram-negative bacteria than in Gram-positive bacteria, but it can still activate the innate immune system. Lipoproteins in the outer membranes of Gram-negative and Gram-positive bacteria and lipoteichoic acids in Gram-positive bacteria serve as TLR ligands. Peptidoglycan, lipoproteins and lipoteichoic acids bind to the TLR-2/TLR-6 complex [10–12]. TLR-5 recognizes bacterial flagellin, which is a protein monomer obtained from bacterial flagella [13]. TLR-9 plays an essential role in the cellular response to nonmethylated bacterial DNA (CpG DNA) [14]. Most TLRs initiate signal transduction via the sequential recruitment of the cytoplasmic adaptor molecule MyD88 and the interleukin 1 receptor (IL-1R)-associated kinase (IRAK), which results in the activation of NF-κB and its translocation to the nucleus [15]. The subsequent binding of the NF-κB complex to κB DNA-binding sites in promoter regions induces the transcription and production of proinflammatory cytokines, adhesion molecules, and

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MHC class II molecules relevant to IBD [16]. Therefore, the ability of TLRs to recognize bacteria or constituents of the bacterial wall and to activate proinflammatory mechanisms may be critical to immune reactions in the intestinal mucosa. Bacteria may constitute a most important inducer of mucosal inflammation because the induction of TLR-2 and TLR-4 mRNA expression and protein synthesis in the intestinal macrophages of IBD patients is dependent upon inflammation [17].

2.2. Increased levels of mucosal-associated bacteria in UC

Bacterial culture and fluorescence in situ hybridization (FISH) studies demonstrated dramatically increased mucosal-associated bacteria in UC [18,19]. Before these reports, we considered whether bacteria were present in the mucous membrane lesions present during UC using acridine-orange staining. As a result, a large number of bacteria attached to lesion mucous membranes, and some bacteria invaded the mucosa, as reported in a 1993 study [20]. It is hypothesized that the healthy mucosa is capable of confining fecal bacteria and that this function is profoundly disturbed in patients with UC [18].

Variants of the multidrug resistance gene MDR1 have been associated with UC [21]. MDR1 encodes P-glycoprotein 170, a transporter that governs the efflux of drugs and possibly xenobiotic compounds from cells. P-glycoprotein 170 might also function as a 'flippase' that moves amphipathic substrates from the inner to the outer leaflet of the cell membrane. It has been suggested that these gene variants indicate defective microbial clearance in UC.

2.3. Defective mucosal barrier function and bacterial invasion

Intestinal epithelial cells are physical barriers against excessive entry of bacteria and other antigens from the intestinal lumen into the circulation. Mucosal barrier function can be compromised in UC patients [22]. Johansson et al. reported that the inner mucous layer is densely packed, firmly attached to the epithelium, and devoid of bacteria [23]. In contrast, the outer layer is flexible, has an expanded volume due to proteolytic cleavage of Muc2 mucin that is a major mucin produced in the small and large intestine and is colonized by bacteria. Muc2-deficient mice exhibit bacteria that are in direct contact with epithelial cells deep in the crypts, explaining the development of inflammation and cancer observed in these animals. These findings demonstrate that Muc2 mucin can build a mucous barrier that separates bacteria from the colon epithelia and suggest that defects in this mucus can cause colon inflammation. In addition, these authors examined murine colitis models, such as mice deficient in Muc2 mucin or IL-10 together with dextran sodium sulfate-treated mice was immunostained for Muc2 expression. Bacterial localization in the mucus of these animals was analyzed [24], and bacteria and beads appeared to penetrate the inner mucus layer. Humans with active UC and some patients with UC in remission have an inner mucus layer that is penetrable. Moreover, a variant in a genomic region that includes the gene encoding MUC19 has been associated with CD and UC [25,26]. It is known that certain bacterial species, such as *Akkermansia muciniphila* and *Enterorhabdus mucosicola*, degrade mucus and can thrive on the mucus layer [27]. Therefore, inherited alterations in mucosal composition and degradation of mucus by harmful luminal bacteria may reduce barrier function and trigger inflammation and ulceration.

2.4. Dysbiosis in UC (Table 1)

Many studies have identified dysbioses, which are defined as quantitative and qualitative microbial imbalance in the gut compared to normal controls, in cases of UC. Culture-dependent analysis of mucosal-associated bacteria revealed that UC patients have higher numbers of mucosa-associated bacteria than do healthy controls. Swidsinski et al. reported that *Bacteroides* and *Enterobacteriaceae* (primarily *Escherichia coli*) in UC were significantly higher in concentration than they were in healthy controls [18].

The remarkable improvements in DNA sequencing technology and analysis over the past decade have set the stage for investigations of the UC microbiome [19–38]. The studies were conducted using various materials and methods. Various materials included stool, mucosa or mucosal lavage. Various methods were terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis analysis (DGGE), FISH, temporal temperature gradient electrophoresis (TTGE), metagenome analysis and/or real time PCR. All but one of these technologies [33] analyzed PCR-amplified 16S rRNA genes. Although the materials and methods differ among these studies, all except one article showed gut dysbiosis in UC patients [19–24,26–38]. The reduction in bacterial diversity and richness, especially that of Firmicutes, including the clostridial clusters such as *Clostridium coccoides*, *Clostridium leptum* clusters, *Fecalibacterium prausnitzii*, *Roseburia, Ruminococcus, Enterococcus*, and *Lactobacillis*, was reported in 10 articles [21,23,26,30,31,33–35,37,38]. Increased proteobacteria populations, including *Escherichia* sp., *Helicobacter* sp., and *Campylobacter* sp., were reported in seven papers that investigated patients with active UC [19,20,24,27,28,31,36]. Bacteroidetes increased in the inflamed mucosa of active UC as reported in one paper [26]; however, Bacteroidetes concentrations were decreased in UC patients compared with healthy controls [19,21,29]. Actinobacteria, including *Bifidobacterium*, increased in active UC patients as reported in two papers [27,32,38], but it has been reported that *Bifidobacterium* decreased in UC patients in a separate article [33]. Fusobacteria were detected in only UC patients [20] and increased in UC patients [31]. These studies include small patient cohorts and differ in the sources of gut microbiota sampling and analytic methods. More detailed full metagenomics and large-scale studies are need in the future.

2.5. Bacterial agents increased in UC (Table 2)

Adherent-invasive *E. coli* (*E. coli*, AIEC) has been implicated in the pathogenesis of UC [39]. In two separate studies, verotoxin-producing *E. coli* was isolated from the stools and rectal biopsies of patients during UC relapse or remission [40,41]. In another study, however, stools from 34 patients diagnosed with active UC were negative for the bacteria [42]. *E. coli* were detected at a higher prevalence in UC patients compared to controls by real-time quantitative PCR [43,44], FISH [45] and 16S rRNA gene sequence analysis [25]. *E. coli* strains belonging to the B2 and D phylogenetic groups are suggested to have a significant relationship with adhesins and serine protease autotransporter proteins and display higher-adhesion indices in comparison to pathogenic *E. coli* strains from normal controls [46]. These *E. coli* were more prevalent in patients with UC than in controls and were also isolated from pediatric UC patients [46]. In another study of pediatric UC, no major changes in the fecal microbiota, including changes in *E. coli* strains, were observed in UC patients by real-time PCR [47].

Detection of all Campylobacter DNA by PCR and sequencing analyses was significantly higher in cases of UC compared to in controls [48]. Nested PCR for *Campylobacter concisus* DNA was detected significantly higher (33.3%) in biopsy samples from UC comared to controls (10.8%). Moreover, sequencing of the remaining *Campylobacter*-positive samples revealed that *Campylobacter ureolyticus* was detected significantly higher (21.7%) in samples