The Role of Bacterial Infection in the Pathogenesis of Inflammatory Bowel Disease

Toshifumi Ohkusa, Tetsuya Nomura and Nobuhiro Sato

Abstract

In the last decade, the dogma that no bacteria could grow in the acid milieu of the stomach has been destroyed by evidence that the infective agent, H. pylori, is responsible for gastric and duodenal disease. Studies on H. pylori infection suggest that some strains of intestinal bacteria may be responsible for intestinal ulceration and inflammation concomitant with inflammatory bowel diseases (IBD), i.e., ulcerative colitis and Crohn’s disease. Evidence for pathophysiological roles for certain strains of luminal bacteria result from a number of IBD animal models. Recent studies on innate immunity, including toll-like receptors and NOD isoforms, suggest that bacterial infections may contribute to intestinal inflammation in genetically susceptible hosts. This brief review focuses on the bacterial pathogenesis and the role of innate immunity in the etiology of IBD’s.

(Key words: bacteria, innate immunity, inflammatory bowel disease, ulcerative colitis, Crohn’s disease)

Introduction

Studies suggest that the sequence of immune events that culminates in the inflammatory response observed in IBD is initiated by a sensitization to luminal antigens, e.g., bacterial, viral or food, facilitated by undefined genetic influences (Fig. 1). Antigen presenting cells, e.g., dendritic cells, are the first to respond and direct the differentiation of naive CD4+ Th 0 lymphocytes into Th 1 and Th 2 cells. Subsequently, activated Th 1 cells secrete cytokines, such as TGF-α, IL-2 and IFN-γ. IFN-γ, in turn, activates macrophages causing them to secrete excess proinflammatory cytokines. Activated macrophages contribute to epithelial injury by secreting TNF-α and reactive oxygen species and by recruiting neutrophils, which also produce free radicals. Neutrophils are also recruited by IL-8 secreted by epithelial cells following activation or injury by bacteria, such as H. pylori. Neutrophils release reactive oxygen species, and oxygen species injury the epithelial cells. Together, the macrophages and neutrophils produce prostaglandin E2 and leukotriene B4 (LTB4) that contribute to the vasodilation and enhanced vascular permeability characteristic of IBD. Data from recent studies using a number of animal models suggest that indigenous luminal bacteria play an important role in the pathogenesis of mucosal inflammation (1–4). Interestingly, normal luminal bacteria appear to contribute to spontaneous colitis, but this condition fails to develop in germ-free, knockout mice (5–8). Recent studies on critical constituents in the innate immune response, such as toll-like receptors (TLR) and NOD isoforms, also suggest that bacterial infections may contribute to intestinal inflammation. This brief review focuses on the roles of intestinal bacteria and innate immunity in the pathogenesis and etiology of IBD.

Bacterial Agents in Ulcerative Colitis

Historically, numerous bacterial species have been suspected as being major contributors to the etiology of ulcerative colitis (UC) (Table 1; 9–11, 13, 15, 16, 21–23). Jex-Blake and Higgs first suggested that Bacillus coli, B. proteus, B. pyocyanea, B. lactis aerogenes, and Streptococci may be potential agents (9). Later, Bassler included B. coli communis as a possible contributing factor (10). However, the absence of bacteriological confirmation precludes definitive conclusions as to the role(s) of these organisms in the pathogenesis of these diseases.

Bargen reported bloody diarrhea in rabbits injected intravenously with bacterial cultures isolated from the feces of UC patients (11). Massive colonic hemorrhages and superficial ulcers yielding diplostrepotocacci upon culture were observed at necropsy. However, Hurst dismissed the diplostrepotocacci as normal enterococci present in all stools and often found in healthy subjects (12). Subsequent studies failed to confirm Bargen’s observations and this hypothesis lost credibility (13). In subsequent years, other bacteria, e.g.,

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Figure 1. The first cell population involved is the antigen presenting cells, such as dendritic cells, which direct the differentiation of naive CD4+ Th 0 lymphocytes to Th 1 and Th 2 cells. The activated Th 1 cells secrete cytokines such as TGF-α, IL-2 and IFN-γ, and IFN-γ activates macrophages to secrete excess proinflammatory cytokines (IL-1, TNF-α). Activated macrophages contribute to epithelial injury by (a) producing TNF-α and reactive oxygen species and (b) recruiting neutrophils, which also produce free radicals. Neutrophils are also recruited to the inflammatory site by IL-8 released from epithelial cells which have been activated by bacteria, such as H. pylori. Both macrophages and neutrophils produce prostaglandin E2 and leukotriene B4 (LTB4), which cause vasodilation and enhanced vascular permeability characteristic of IBD.

Table 1. Infectious Bacteria Suggested to Cause Ulcerative Colitis

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Bacteria</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jex-Blake &amp; Higgs (9)</td>
<td>1909</td>
<td><em>Bacillus coli, B. proteus, B. pyocyanea, B. lactis aerogenes, Streptococci</em></td>
<td>stool</td>
</tr>
<tr>
<td>Bargen (11)</td>
<td>1924</td>
<td><em>Diplostreptococcus</em></td>
<td>stool</td>
</tr>
<tr>
<td>Paulson (13)</td>
<td>1928</td>
<td><em>Chlamydia</em></td>
<td>stool</td>
</tr>
<tr>
<td>Bassler (10)</td>
<td>1933</td>
<td><em>B. coli communis</em></td>
<td>stool</td>
</tr>
<tr>
<td>Dragstedt (15)</td>
<td>1941</td>
<td><em>Bacteroides necrophorum</em></td>
<td>mucosa</td>
</tr>
<tr>
<td>Burke (16)</td>
<td>1988</td>
<td><em>Adhesive E. coli</em></td>
<td>stool</td>
</tr>
<tr>
<td>Matsuda (21)</td>
<td>2000</td>
<td><em>Bacteroides vulgatus</em></td>
<td>mucosa</td>
</tr>
<tr>
<td>Ohkusa (22, 23)</td>
<td>2002</td>
<td><em>Fusobacterium varians</em></td>
<td>mucosa</td>
</tr>
</tbody>
</table>
**Table 2. Infectious Bacteria Suggested to Cause Crohn’s Disease**

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Bacteria</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent (31)</td>
<td>1978</td>
<td><em>Pseudomonas maltophilia</em></td>
<td>mucosa, lymph node</td>
</tr>
<tr>
<td>Burnham (29)</td>
<td>1978</td>
<td><em>Mycobacterium kansasii</em></td>
<td>lymph node</td>
</tr>
<tr>
<td>Chiodini (30)</td>
<td>1984</td>
<td><em>Mycobacterium paratuberculosis</em></td>
<td>mucosa, lymph node</td>
</tr>
<tr>
<td>Liu (32)</td>
<td>1995</td>
<td><em>Listeria monocytogenes</em></td>
<td>mucosa, lymph node</td>
</tr>
</tbody>
</table>

*Clamydia, Bacteroides necrophorum, Entamoeba histolytica,* were implicated and then discarded owing to the lack of conclusive evidence (14, 15).

Adhesive *Escherichia coli* has been implicated in the pathogenesis of UC (16). In two separate studies, verotoxin-producing *E. coli* was isolated from the stools and rectal biopsies of patients during UC relapse or remission (17, 18). In another study, however, stools from 34 patients diagnosed with active UC were negative for the bacteria (19).

Although most studies have examined luminal microflora, the mucosal microflora may play a more important role in the pathogenesis of these diseases since infections are usually initiated by the adherence of the microorganism to host cells (20). While there has been revived interest in intestinal microbes as contributing factors in IBD pathogenesis, studies have focused on endogenous commensal flora rather than on specific pathogens (e.g., Shigella, hemorrhagic *E. coli*, Salmonella, Amebiasis, Gonorrhea, Chlamydia, *Clostridium difficile*). A recent study reported the presence of *Bacteroides vulgatus* in the colonic mucosa of some UC patients (21). Our own study revealed another species of commensal bacteria, *Fusobacterium varium*, in the inflamed mucosa in UC patients (22). In this cohort study that included patients with active UC, Crohn’s disease, ischemic colitis, colon adenomas and healthy controls, the detection rate and ELISA titer of antibodies to *F. varium* were significantly higher in patients with UC than in the other subjects or controls. Furthermore, immunohistochemical detection of *F. varium* in the colonic mucosa was significantly higher in patients with UC than in the other subjects. In addition, we showed that butyric acid fermented by *F. varium* induced mucosal cell apoptosis and caused UC-like lesions in mice (23), suggesting that this microorganism may be a pathogenic factor in UC. The toxicity of our butyric-acid containing supernatants is consistent with reports of the *in vitro* toxicity of butyric and propionic acids from dental plaque (24), butyric acid in culture filtrates of two *Bacteroides* species (25, 26), and the *in vivo* toxicity of high butyrate concentrations in neonatal necrotizing enterocolitis (27, 28).

**Bacterial Agents in Crohn’s Disease**

Many bacteria have been implicated in the pathogenesis of Crohn’s disease (CD), including tubercle bacillus, mycobacteria (*Mycobacterium kansasii, M. paratuberculosis*), *Pseudomonas maltophilia*, and *Listeria monocytogenes*, however, none have achieved etiologic status (Table 2; 29–32). The gross and histological similarities between CD and tuberculosis have been noted since the original description of the disease by Dalziel, and the possibility that CD is a mycobacterial disease has never been dismissed (33). In 1978, Burnham et al isolated (a) *Mycobacterium kansasii* from the lymph node of a patient with CD and (b) pleomorphic material, indicative of cell wall-deficient organisms, from 80% of CD patients and 54% of UC patients (29). However, the hypothesis that *M. kansasii* plays an etiologic role in both CD and UC was short lived. This organism is an opportunistic pathogen that exacerbates existing chronic diseases and is not a primary pathogen in healthy individuals.

Subsequent studies revived the interest in the role of mycobacteria in CD. In a series of studies, Chiodini et al reported the isolation of two strains of a *M. paratuberculosis*-like organism from 11 patients with CD, but not from those diagnosed with UC or other bowel diseases (30). These isolates were pathogenic for mice via intravenous or intraperitoneal routes, and produced hepatic and splenic granulomas containing acid-fast mycobacteria. Oral inoculation of one of the strains into a newborn goat produced a granulomatous ileocolitis after 5 months, and the authors concluded that the isolates were strains of *M. paratuberculosis* or a biovariant that plays an etiologic role in some cases of CD. Interestingly, *M. paratuberculosis* causes Johne’s disease, an intestinal disorder in ruminants such as cattle, horse and goat that resembles CD both clinically and histologically (34). Other studies reported increased titers of antibodies to *M. paratuberculosis* antigens in CD patients (35–37). Nevertheless, neither clinical nor immunohistochemical studies have provided convincing evidence for a role for mycobacteria in CD (36, 38, 39).

Studies using PCR methodology and primers recognizing the *M. paratuberculosis*-specific IS900 gene, have detected mycobacterial sequences in the intestinal tissue, but the presence of this gene sequence is not specific for CD (36, 39). However, in a recent study using laser capture microdissection, the *M. paratuberculosis*-specific IS900 gene was detected in 40% of microdissected CD granulomas in comparison to 0% in control granulomas (40).

Several recent Japanese studies observed somewhat conflicting results. One study reported that the IS900 sequence was detected in both CD patients and in healthy control subjects (41), whereas other studies were unable to detect IS900 in CD patients (42, 43). Suenaga et al reported that the anti-
body-positive prevalence rate and mean serum IgG titers to *M. paratuberculosis* were significantly higher in patients with CD in comparison to healthy subjects (44).

**Inflammatory Mediators and the Role of Bacteria in Innate Immunity**

Toll-like receptors (TLR) play an important role in the innate immune system and several homologues of TLR’s and their ligands have recently been discovered (45–49) (Table 3). 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 membrane of target cells (50, 51). Peptidoglycan, a complex amino sugar, although far less abundant in Gram-negative than in Gram-positive bacteria, is very similar in composition in both groups. Lipoproteins in the outer membranes of Gram-negative and 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 (52–54). TLR-5 recognizes bacterial flagellin, which is a protein monomer obtained from bacterial flagella (55), whereas the TLR-9 plays an essential role in the cellular response to nonmethylated bacterial DNA (CpG DNA) (56). Most TLRs initiate signal transduction via the sequential recruitment of the cytoplasmic adaptor molecule MyD88 and the IL-1 receptor-associated kinase (IRAK), which results in the activation of NF-κB and its translocation to the nucleus (57). The subsequent binding of the NF-κB complex to κB DNA-binding sites in the promoter induces transcription and the production of proinflammatory cytokines, adhesion molecules, and MHC class II molecules relevant to IBD (58). 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 4 mRNA expressions and protein synthesis in intestinal macrophages of patients with IBD is inflammation dependent (59).

NOD 2 appears to function in host signaling pathways activated by Gram-negative LPS (60). Furthermore, recent studies revealed an association between certain NOD 2 variants and CD. A two- to three-fold greater frequency of three single nucleotide polymorphisms in or near leucine-rich LPS-binding regions in the NOD 2 gene on chromosome 16 has been reported in Western Crohn’s disease patients in comparison to control subjects (61–63). NOD 2 is primarily expressed in monocytes, macrophages, and dendritic cells, it recognizes LPS and peptidoglycan from multiple species of bacteria, and it activates NF-κB (64). Truncation of the leucine-rich LPS-binding region by the Crohn’s frameshift mutation decreases NF-κB activation by LPS from Gram-negative bacteria, suggesting that defective NOD 2 activity may decrease the clearance of invading bacteria (62). These data strongly suggest that an interaction between gram-negative bacteria and the intestinal innate immune responses is a critical element in the pathogenesis of CD. Despite these observations, it has recently been reported that a population of Japanese CD patients lack the NOD 2 gene variants, suggesting the need for further study on NOD2 gene polymorphisms (65).

**Conclusion**

Current theories suggest that a dysregulated mucosal immune response to, as yet, unidentified microorganism(s) in the normal intestinal milieu in a genetically susceptible host is at the core of these diseases (66). We recently reported that culture supernatants from commensal bacteria (*e.g.*, *Fusobacterium varium*) recovered from the inflamed mucosa of UC patients, induced UC-like lesions in mice (22, 23). We have also observed that a combined antibiotic regimen directed against *F. varium* was effective in patients with active chronic UC (67). Therefore, we suggest that *F. varium* is one of the elusive pathogenic factors in the etiology of UC. While we cannot deny that other commensal bacteria may contribute to the pathogenesis of UC, there are insufficient data to confirm their role(s). Subsequent studies focused on

### Table 3. Ligands of Toll-like Family Members

<table>
<thead>
<tr>
<th>TLR</th>
<th>Main ligand</th>
<th>Pathogens</th>
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<tbody>
<tr>
<td>TLR1</td>
<td>cofactor of TLR2</td>
<td>Gram–, Gram+, mycobacteria, Spirochetes, mycoplasma</td>
</tr>
<tr>
<td>TLR2</td>
<td>peptidoglycan, lipoproteins, Glycopeptides, LPS (minor spp.)</td>
<td>Gram–, Gram+, mycobacteria, Spirochetes, mycoplasma</td>
</tr>
<tr>
<td>TLR3</td>
<td>double-strand RNA</td>
<td>virus</td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS (major spp.)</td>
<td>flagella of Gram–, Gram+</td>
</tr>
<tr>
<td>TLR5</td>
<td>flagellin</td>
<td>Gram–, Gram+, mycobacteria, Spirochetes, mycoplasma</td>
</tr>
<tr>
<td>TLR6</td>
<td>cofactor of TLR2</td>
<td>virus?</td>
</tr>
<tr>
<td>TLR7</td>
<td>imidazoquinoline</td>
<td>all bacteria</td>
</tr>
<tr>
<td>TLR8</td>
<td>unknown</td>
<td>?</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG DNA</td>
<td>?</td>
</tr>
<tr>
<td>TLR10</td>
<td>unknown</td>
<td>?</td>
</tr>
</tbody>
</table>
the cross-talk between commensal bacteria and the mucosal immune system are essential to elucidate the pathogenesis of IBD. Recent data on intestinal innate immunity provide clues to the identification of the host factors in patients with IBD. For example, polymorphisms in the NOD 2 and, perhaps, TLR genes may be causal host factors. Fortunately, the technology necessary to accomplish this goal is currently available.

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