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REVIEW

Escherichia coli Pathobionts Associated with Inflammatory Bowel Disease

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SUMMARY Gut bacteria play a key role in initiating and maintaining the inflammatory process in the gut tissues of inflammatory bowel disease (IBD) patients, by supplying antigens or other stimulatory factors that trigger immune cell activation. Changes in the composition of the intestinal microbiota in IBD patients compared to that in healthy controls and a reduced diversity of intestinal microbial species are linked to the pathogenesis of IBD. Adherent invasive *Escherichia coli* (AIEC) has been linked to Crohn's disease (CD) patients, while diffusely adherent *E. coli* (DAEC) has been associated with ulcerative colitis (UC). Bacteriological analysis of intestinal biopsy specimens and fecal samples from IBD patients shows an increased number of *E. coli* strains belonging to the B2 phylogenetic group, which are typically known as extraintestinal pathogenic *E. coli* (ExPEC). Results from studies of both cell cultures and animal models reveal pathogenic features of these *E. coli* pathobionts, which may link them to IBD pathogenesis. This suggests that IBD-associated *E. coli* strains play a facilitative role during IBD flares. In this review, we explain IBD-associated *E. coli* and its role in IBD pathogenesis.

KEYWORDS carcinoembryonic antigen-related cell adhesion molecules 6, Crohn's disease, diet, *Escherichia coli*, tight junction, ulcerative colitis, inflammatory bowel disease, interleukins, probiotics, tumor necrosis factor receptors

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INTRODUCTION

Inflammatory bowel disease (IBD) is a family of chronic inflammatory diseases of the gastrointestinal tract. IBD has traditionally been divided into Crohn's disease (CD) and ulcerative colitis (UC) (1). Crohn's disease and ulcerative colitis are differentiated by their clinical manifestations and hypothesized pathogenic mechanisms. UC is a relapsing, nontransmural, chronic inflammatory disease that is restricted to the colon and during flares is characterized by bloody diarrhea. CD is a chronic, segmental, localized granulomatous disease that can affect all parts along the entire gastrointestinal tract, from the mouth to the anus. The clinical presentation depends on disease location and may include diarrhea, abdominal pain, fever, clinical signs of bowel obstruction, and anal passage of blood, mucus, or both (1).

Ulcerative colitis and Crohn's disease may appear at any age, but most patients are diagnosed in their third decade of life (2). The prevalence of ulcerative colitis and Crohn's disease is highest in the industrialized Western countries and has increased 2- to 3-fold in Europe and the United States since the early 1970s (2, 3). The prevalence of IBD in Northern Europe varies from 35 to 50 cases per 100,000 inhabitants for ulcerative colitis and from 30 to 100 cases per 100,000 inhabitants for Crohn's disease (3). The prevalence of IBD in the United States was recently estimated to be as high as 241.3 and 263 cases of CD and UC per 100,000 people, respectively (4).

The exact etiology of IBD is still unclear, but studies indicate several possible links to genetics (5), immunology (6–8), nutrition (9, 10), bacteria (11, 12), viruses (13, 14), and other environmental factors (15, 16). Animal model studies suggest that inflammation in IBD patients most likely arises as a result of either exaggerated effector T-cell function or poor regulatory T-cell function, leading to the overproduction of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-12 (IL-12), and/or the impaired production or function of known regulatory/immunosuppressive cytokines, such as IL-10 (6, 17). The gut microbiota is necessary to initiate or maintain the intestinal inflammatory process by providing antigens or other stimulatory factors, which can trigger inflammatory defenses that prove maladaptive in IBD (6). However, so far, there is no specific pathogenic microorganism directly linked to IBD (6). Experimental models have repeatedly revealed that the intestinal microbiota plays a role in IBD, while placebo-controlled studies of antibiotic treatment show some benefit in promoting remission of IBD. However, most of the clinical trials of antibiotic treatment of IBD published so far have involved limited numbers of patients treated for only a short time. To define the effect of antibiotics in the management of IBD, more randomized controlled trials (RCTs) of antibiotics need to be carried out (18–20). Studies have also noted an aberrant fecal microbiota in IBD patients compared to that found in healthy controls, while a reduced diversity of the conventional intestinal microbiota has been linked to IBD (21–23). Interestingly, bacteriological analyses of biopsy specimens and fecal samples from IBD patients show increased numbers of *Escherichia coli* isolates belonging to the B2 phylogenetic group that harbors extraintestinal pathogenic *E. coli* (ExPEC) genes (24, 25). In this review, we define IBD-associated *E. coli* and evaluate its potential role in IBD pathogenesis, disease relapses, and remission. For this purpose, the focus is on both pathogenic *E. coli* strains known to cause clinical disease and *E. coli* strains that are better described as pathobionts, which are bacteria linked to immune-mediated diseases and depending on genetic defects or other environmental factors causing disease.

INFLAMMATORY BOWEL DISEASE AND THE GUT MICROBIOTA

Because IBD is an inflammatory disease of the gastrointestinal tract, it has been speculated that luminal factors are involved. Therefore, gastrointestinal bacteria are frequently suspected as the cause of IBD relapses. Some IBD patients experience clinical improvement when they receive antibiotics, such as ciprofloxacin or rifaximin (26). Microbiological findings for IBD patients with active disease show a reduction of the resident aerobic and anaerobic microbiota, such as *Faecalibacterium prausnitzii*, belonging to clostridial cluster IV (27) (compared to that in healthy controls), and an

increase in potentially pathogenic microorganisms, such as *Klebsiella*, *Enterobacter*, *Proteus*, and fungi (19). Studies also show a significant reduction in lactobacilli and bifidobacteria as well as an increase in *Bacteroides* in the intestines of IBD patients (22). The decreased prevalence of lactobacilli and bifidobacteria might play an important role in the etiology of IBD, since these bacteria have immunoregulatory effects and therefore contribute to intestinal host defenses through their interactions with the immune system (28, 29). The reduced prevalence of butyrate-producing bacteria (such as those belonging to the clostridial group) in the guts of IBD patients with active disease leads to reduced levels of butyrate. This may worsen IBD, since butyrate normally serves as an inhibitor of proinflammatory cytokine expression in the intestinal mucosa as well as a stimulator of mucin and antimicrobial peptide production and a strengthener of epithelial barrier integrity by increasing the expression of tight junction (TJ) proteins (30).

Several microorganisms have been suggested to play a role in the pathogenesis of IBD. *Mycoplasma* spp. (31), *Mycobacterium* spp. (32), *Clostridioides difficile* (33), *Salmonella* spp. (34), *Listeria monocytogenes* (35), *Aeromonas hydrophila* (36), *Proteus* spp. (37), *E. coli* (38, 39), and some viruses (40) have all been linked to IBD and are suspected to play a causal role in disease relapses. On looking at these possible IBD pathogenesis links, it is currently unresolved whether the major part is played by specific microorganisms or by the reduced diversity of the microbiota as such. Interestingly, a recent paper described both reduced microbiota diversity and an increased frequency of virulence markers primarily linked to *E. coli* as being associated with both ulcerative colitis and Crohn's disease (41).

E. COLI IN INTESTINAL DISORDERS

E. coli is a predominantly facultative anaerobic Gram-negative bacterium which colonizes the intestinal tract of human infants immediately after birth and helps to maintain normal intestinal homeostasis (42). *E. coli* strains are classified—on the basis of genetic and clinical criteria—into the following three major groups: (i) commensal strains found in the human and animal gut (lacking specialized virulence factors), (ii) intestinal pathogenic strains (diarrheagenic), and (iii) extraintestinal pathogenic *E. coli* (ExPEC) (43). While the diarrheagenic *E. coli* strains have not been linked to IBD, they have been clearly shown to promote intestinal inflammation and pathophysiology. Six well-known intestinal pathogenic *E. coli* types are enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (44). These *E. coli* strains cause gastrointestinal diseases ranging from self-limiting diarrhea to hemorrhagic colitis (44).

EPEC

EPEC was the first pathotype of *E. coli* described (in 1945) and was isolated from the intestines of infants with diarrhea in the United Kingdom (44, 45). EPEC adheres to epithelial cells via the BFP type IV pilus (46, 47) followed by activation of its type III secretion system. As a result of this activation, protein kinase C, protein tyrosine kinase(s), phospholipase Cγ, myosin light chain kinase, and mitogen-activated protein (MAP) accumulate under the attached bacteria (46). Thereafter, various effector proteins—including EspF, Map, EspG, EspH, and Tir—are translocated into the infected host cell, which increases intracellular calcium (Ca^{2+}) and triggers depolymerization of the microvillus actin, leading to the formation of the characteristic pedestal complex (48, 49). This leads to increased permeability within the intestinal epithelium due to loosened tight junctions and the activation of NF-κB, followed by production of IL-8 and the transmigration of polymorphonuclear leukocytes (PMNs) across the epithelium and into the intestinal lumen (44). Diarrhea usually results from increased ion secretion, increased intestinal permeability, intestinal inflammation, and a loss of absorptive surface area resulting from microvillus effacement (44).

ETEC

ETEC is primarily associated with high mortality in children under 5 years of age, as well as being a frequent cause of diarrhea in tourists visiting developing countries (50). The reasons for ETEC infections occurring predominantly in countries with warm climates are still unknown, but it is likely that water contaminated by human or animal sewage is an important means of spreading the infection (50). ETEC strains produce a heat-stable enterotoxin (ST) and a heat-labile cholera toxin-like enterotoxin (LT) (44). The heat-labile toxin consists of an A subunit and five identical B subunits. The B subunits promote binding of the holotoxin to the cell surface gangliosides GM1 and GD1b, while the A subunit promotes the enzymatic activity of the toxin (44, 51). The heat-stable enterotoxin STb has also been associated with human disease (44). It stimulates the intestinal brush border guanylate cyclase C (GC-C) receptor, which increases levels of the intracellular messenger cyclic GMP (50). Cyclic GMP mediates reduced absorption of sodium and chloride ions and increased secretion of bicarbonate and chloride ions, ultimately resulting in watery diarrhea (50). Colonization factor CFA/I also plays an important role in the pathogenesis of STh-ETEC diarrhea by facilitating microbial adherence to the intestinal mucosa.

EIEC

Molecular studies and phenotyping results have shown that *E. coli* strains are closely related (sister species) to *Shigella* spp. (52). In fact, enteroinvasive *E. coli* (EIEC) shares many properties with *Shigella*, including virulence mechanisms (53). They both possess a large invasion plasmid encoding the Mxi-Spa type III secretion system and invasion plasmid antigen (Ipa) effectors, which enable bacterial invasion of eukaryotic cells (53). The plasmid also encodes IcsA, which enables bacteria to spread to other cells *in vivo* while avoiding the immune system (53). EIEC/*Shigella* pathogenesis mechanisms initially involve epithelial cell penetration followed by lysis of the endocytic vacuole, intracellular replication, directional movement through the cytoplasm, extension into/invasion of adjacent epithelial cells (54), and, finally, the induction of apoptosis in infected macrophages and the release of IL-1β (55). EIEC may on occasion cause inflammatory colitis, dysentery, and watery diarrhea (55).

EAEC

EAEC is associated with chronic diarrheal disease in developing countries and in immunocompromised patients (45, 55). EAEC was first defined in 1987 (56) as an *E. coli* strain with the ability to adhere to Hep-2 cells in a stacked-brick-like configuration (57). EAEC pathogenic mechanisms initially involve bacterial adherence to the intestinal mucosa via aggregative adherence fimbriae (AAFs). EAEC surface structures along with its release of flagellin cause inflammation by inducing the release of the chemokine IL-8, stimulating neutrophil transmigration across the epithelium and, as a result, tissue damage (58). The *aggR* gene in EAEC regulates expression of multiple virulence factors, such as AAFs, as well as the ability to form biofilms (e.g., see reference 59). The toxins associated with EAEC are Pic, an autotransporter protease with mucinase activity (60); EAST1, a homologue of STa toxin which can cause watery diarrhea (61); and Pet, an autotransporter with enterotoxic activity which causes cytoskeletal changes (62).

STEC/Verocytotoxigenic *E. coli* (VTEC)

STEC is best known as the cause of hemorrhagic colitis and hemolytic-uremic syndrome (HUS), for which contaminated food is an important source of infection (63). *Escherichia coli* producing a toxin similar to Shiga toxin (Stx; produced by *Shigella dysenteriae*) is a common cause of HUS in children and often manifests with bloody diarrhea and acute renal failure (64, 65). STEC was first discovered in 1982, when Karmali et al. (66) found increased Stx activity in the fecal filtrates of children with HUS who were infected with *E. coli* serotype O157:H7. Later, additional STEC serotypes were discovered, such as serotypes O145 and O121 (64, 67). The pathogenic mechanisms of STEC start when Stx-*E. coli* is ingested and then adheres to gastrointestinal epithelial cells via the bacterial outer membrane protein

intimin (68), similar to the adherence mechanisms of EPEC. The STEC toxin is then transported into the kidney via blood or by transmigration of neutrophils (PMNs) (69), or it binds to blood platelets (68) and erythrocytes (70).

ExPEC

ExPEC causes diseases outside the gut, such as in the urinary tract, and also causes infections of the central nervous system, the circulatory system, and the respiratory tract (43, 71, 72). Clermont et al. (73) divided *E. coli* into four main phylogenetic groups, A, B1, B2, and D, based on the following three genes: *chuA* (heme transport gene), *yjaA* (unknown function), and TspE4.C2 (anonymous DNA fragments). The most virulent extraintestinal *E. coli* strains belong to phylogenetic group B2 (harboring *chuA* and *yjaA*). The *E. coli* strains displaying less virulence during extraintestinal infections belong to phylogenetic group D (harboring *yjaA*), and finally, the various commensal strains of *E. coli* belong to groups A (harboring none of the above-mentioned genes) and B1 (harboring TspE4.C2) (73). Recently, *E. coli* phylogenetic groups E (harboring the *arpA* [unknown function], *chuA*, and TspE4.C2 genes) and F (harboring *chuA*), known as sister groups to phylogenetic group B2, and phylogenetic group C (harboring the *arpA* and *yjaA* genes), closely related to phylogenetic group B1, were described (74).

Johnson et al. (75) defined *E. coli* isolates harboring at least two of the following virulence genes as ExPEC strains (76): *sfa/foc* (S and FIC fimbria subunit), *papA* or *papC* (P fimbriae), *afa/dra* (Dr-antigen-binding adhesins), *iutA* (aerobactin; iron acquisition system), and *kpsMT*II (capsule; host defense avoidance mechanisms). Virulence markers such as *hly* (toxin; hemolysin) and/or *ompT* (outer membrane protease T subunit) have also been linked to ExPEC strains (77).

E. COLI STRAINS ASSOCIATED WITH CROHN'S DISEASE

Since the 1970s, *E. coli* has been suspected as a possible reason for the onset of disease in IBD patients (78). Several studies have found increased numbers of *E. coli* strains with virulence properties isolated from IBD patients compared to those from healthy controls, especially when focusing on IBD patients during disease relapses (78, 79). In 1978, Keighley et al. (80) observed a modification of luminal bacterial concentrations in CD patients, with evidence of a dramatic increase in *E. coli* strains. Burke and Axon (78) showed a significantly larger proportion of adhesive *E. coli* strains present in active CD (CDA) patients than in a control group (79). Ilnyckyj et al. (81) published a case report in which infection with *E. coli* O157:H7 (82) mimicked right-sided colonic CD. In 1998, Darfeuille-Michaud et al. (83) showed a high prevalence of *E. coli* isolated from ileal biopsy specimens from CD patients, i.e., 100% prevalence in early lesions and 65% prevalence in chronic lesions. These findings suggested that *E. coli* might participate in initiation as well as acting as a chronic promoter of the inflammatory processes in CD.

Martin et al. (84) showed increased mucosa-associated Gram-negative bacteria in colonic biopsy specimens obtained from patients with CD, of which 73% were identified as *E. coli*. A number of studies indicate that there is a link between the prevalence of *E. coli* and IBD relapses (85, 86). One of the histological characteristics of CD is the presence of epithelioid granulomatous inflammation of the intestine (87, 88). Adherent invasive *E. coli* (AIEC), which is linked to CD, causes the same histological characteristic *in vitro*, such as forming multinucleated giant cells, along with the subsequent recruitment of lymphocytes (89).

Epithelium-associated invasive *E. coli* has frequently been isolated from the ileal and colonic mucosa of CD patients and has been shown to often possess the ability to bind to intestinal epithelial cell monolayers as well as to synthesize alpha-hemolysin (90, 91). Colonic biopsy specimens from patients with CD show specific pathogenic strains of *E. coli* with the ability to infect and invade host cells, where they multiply and damage host tissues (85, 86).

In 1976, Schussler et al. (91) showed a significant elevation of antibody titers against the lipid A and O antigens of *E. coli* in CD patient groups compared to those in healthy controls as well as those in UC and acute enteritis groups and suggested that these

titers may function as a potential marker to differentiate CD and UC. *E. coli* antigens have been detected in 57% of CD patient biopsy or resection specimens (35), while polyclonal antibodies against *E. coli* were detected in macrophages within the lamina propria, in the germinal centers of mesenteric lymph nodes, and in giant cells along fissures, below ulcers, and in granulomas. Additionally, increased numbers of antibodies against the *E. coli* outer membrane protein C were detected in 37 to 55% of patients with CD (92, 93). Most of these CD studies are association studies that do not clarify if the infection with AIEC or the intestinal inflammation came first.

E. COLI ASSOCIATED WITH ULCERATIVE COLITIS

Tabaqchal et al. (94) showed that the majority of IBD patients display increased positive antibody reactions to a variety of *Escherichia coli* O antigens, such as those of the O1, O2, O6, O18 (O18ac and O18ab), and O75 serotypes, compared to those of a control group. These serotypes are mostly associated with urinary tract infections and originate from the fecal microbiota (82, 94). Serotyping of *E. coli* strains isolated from IBD patients showed that 83% of *E. coli* isolates from active UC (UCA) cases, 33% from inactive UC (UCI) cases, 50% from CDA cases, and 33% from inactive CD (CDI) cases harbor O1, O2, O6, O18 (O18ab and O18ac), or O75 genes, which are linked to urinary tract infections and thereby belong to the ExPEC group (25). However, only 22% of *E. coli* isolates from healthy controls harbored one of the above-mentioned O antigens.

In 1987, Burke and Axon (95) showed that isolated *E. coli* strains from the stool of UC patients were predominantly diffusely adherent *E. coli* (DAEC) with both enterotoxigenic (96) and enteropathogenic (97) properties, in contrast to isolates from healthy persons. Bacteriological analysis of rectal biopsy specimens or fecal samples from UCA patients showed distinct variability among the mucosal bacteria and increased numbers of *E. coli* strains of the B2 and D phylogenetic groups (24, 25). A previous study showed that UCA patients colonized with B2 *E. coli* display increased burdens of inflammation as measured by the colitis activity index (CAI) and fecal calprotectin levels (98). Mirsepasi-Lauridsen et al. (38) showed that UC-associated *E. coli* p19A, an ExPEC strain harboring alpha-hemolysin, dissolved the TJ protein occludin in cell lines and disrupted the TJ in Caco-2 cells *in vitro*, followed by increasing barrier permeability (38). Additionally, the UC-associated *E. coli* strain p19A induces cell death in dendritic cells and stimulates the release of the cytokines TNF-α, IL-6, and IL-23 (99). As is the case with AIEC and CD, the link between DAEC and/or B2 *E. coli* is associative, and no data so far reveal a causative link.

ANTIBIOTIC AND PROBIOTIC TREATMENT OF IBD

Since gut bacteria are suspected to play a central role in the pathogenesis of IBD, antibiotics have often been used as a therapeutic option (18, 100, 101). One of the antibiotic combinations used as a therapeutic option for CD is clofazimine together with clarithromycin and rifabutin, since they are effective against *Mycobacterium paratuberculosis*, which is speculated to be a possible cause of CD (102). Clofazimine together with clarithromycin and rifabutin is effective at inducing remission when used concurrently with a course of corticosteroids. Nevertheless, the combination of antibiotics used concurrently with a course of corticosteroids has several disadvantages (103), such as the masking of any infection by suppressing the symptoms and signs of inflammation as well as an increased risk of bleeding. However, a meta-analysis of the usage of broad-spectrum antibiotics in CD patients showed that metronidazole and ciprofloxacin are the most effective at promoting clinical improvement (20, 104). A 3-month follow-up of *E. coli* present in the intestine showed that 1 week of ciprofloxacin treatment in patients with UCA was not effective against *E. coli* strains of the B2 phylogenetic group (98).

Placebo-controlled studies of IBD or irritable bowel syndrome patients indicated that probiotic treatment significantly reduced small bowel permeability (lactulose/mannitol ratio) and induced remission in IBD patients (103, 105). The probiotic *E. coli* strain Nissle 1917 (19, 106) was isolated during World War I from the feces of a German soldier who seemed to be protected from infectious diarrheal disease (107). Genomic

studies of *E. coli* strain Nissle 1917 showed that in contrast to other commensal *E. coli* strains, it expresses microcins, adhesins, and at least six different iron uptake systems (including enterobactin, salmochelin, aerobactin, yersiniabactin, and EfeU) for the generation of energy through ATP. Nevertheless, it lacks prominent virulence factors, such as *hlyA* (108–112).

Studies showed that *E. coli* Nissle 1917, which interestingly belongs to the B2 phylogenetic group (ExPEC), has immunoregulatory properties, such as decreasing the number of T cells within the intestinal mucosa as well as reducing the secretion of proinflammatory cytokines, such as IL-2, gamma interferon (IFN-γ), and TNF-α, while stimulating the secretion of regulatory proteins, such as IL-10 and IL-1β (112–114). Schultz et al. demonstrated that *E. coli* Nissle 1917 is effective at preventing colitis in different murine models of colitis (115). Schlee et al. (116) showed that *E. coli* Nissle 1917 induces the expression of human β-defensin 2 (hBD-2), a human antimicrobial peptide, which helps to reinforce the intestinal mucosal barrier by limiting bacterial adherence as well as bacterial invasion of the gut mucosa. *In vivo* models have shown that *E. coli* Nissle 1917 protects against infections with *Salmonella enterica* (117) and *Candida albicans* (118). *In vitro* models using various cells have shown that *E. coli* Nissle 1917 inhibits invasion by *Salmonella enterica*, *Yersinia enterocolitica*, *Shigella flexneri*, *Listeria pneumophila*, and *L. monocytogenes* (119) and invasion of host cells by AIEC (120).

A number of clinical trials suggest that *E. coli* Nissle 1917 is as effective as mesalazine at maintaining remission in UC patients (121–125), but there are some disputed points to discuss. In a study by Kruis et al. (121), only UC patients with inactive disease or those in remission were included. In a study by Rembacken et al. (125), UC patients with active disease were treated with corticosteroids, which diminish the signs/symptoms of any infection/inflammation that might be caused by the use of *E. coli* Nissle 1917 as an add-on treatment. Yet a randomized double-blind study of *E. coli* Nissle 1917 given as an add-on treatment to patients with active UC showed that fewer patients treated with *E. coli* Nissle 1917 had symptomatic remission and that patients treated with *E. coli* Nissle 1917 often withdrew from the study (98, 126). Considering these troubling findings, larger studies are needed to confirm any potential beneficial effects of *E. coli* Nissle 1917 in IBD. Studies show that exposure to antibiotics during pregnancy significantly increases the risk of CD development in children (127). The first year of life is critical for newborn children to develop their gut commensal microbiota. Studies show that the use of antibiotics in the first year of life significantly increases the risk of developing pediatric IBD (128). In addition, antibiotic usage for CD has been shown to decrease the number of beneficial bacteria belonging to genera such as *Lactobacillus*, *Bacteroides*, and *Bifidobacterium*, creating an environment for an increased prevalence of pathogenic bacteria, such as invasive *E. coli*, to adhere to—and invade—the intestinal epithelium (129). Thus, neither antibiotic nor probiotic studies exist that convincingly clarify if modulation or eradication of *E. coli* in IBD patients will lead to control of inflammation.

ROLE OF DIET IN CONTROLLING BACTERIAL CONTRIBUTIONS TO IBD

In the last several decades, there has been significant speculation regarding the role of diet and environmental factors in IBD. Food and clean water consumption plays an important role in shaping intestinal bacterial colonization. Diet has a significant influence on the composition of the intestinal microbiota early in life. Studies show that a high daily intake of fast food, which is rich in fats (pork, beef, corn, sunflower oils, and margarines) and digestible sugar, increases the risk of IBD (130). However, diets rich in olive oil, fish, fruits (131), and nondigestible fibers, such as vegetables and whole-wheat bread, seem to be protective against IBD (132). Dietary carbohydrates, starches, and fibers are substrates for fermentation that produce short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate. The rate of SCFA production depends on the species and amount of microbiota in the colon. SCFA have anti-inflammatory effects (133) and contribute to the inhibition of *E. coli* growth in the gut (134). *In vivo* studies show that high-fat/high-sugar diets cause microbial dysbiosis, decreased mucus layer thickness, increased permeability, and increased susceptibility to colonization with

pathogenic *E. coli* (135). Modulation of the gut microbiota in IBD influences the levels of critical vitamins and minerals in IBD patients, such as the bioavailability of vitamin K (136). A decreased prevalence of butyrate-producing bacteria, such as *Clostridiales* species, in IBD patients with active disease explains the decreased amounts of SCFA (such as butyrate) in their fecal samples, which would normally contribute to inhibition of *E. coli* growth in IBD (134, 137). Butyrate serves as a major source of energy for colonic epithelial cells (138) and as an inhibitor of proinflammatory cytokine expression in the intestinal mucosa (139). One of the most common complications of IBD is anemia, caused by iron, zinc, folate, and vitamin B_{12} deficiency (140). Zinc is critically involved in DNA replication and transcription and has immunoregulatory effects (141). Iron, folate, and vitamin B_{12} are critical for hemoglobin/blood cell formation. Decreased levels of vitamin D are associated with IBD (140), since vitamin D regulates the gut barrier function by inducing E-cadherin transcripts in gut epithelial cells (142), suppresses the proliferation of T cells *in vitro* (143), and induces production of several antimicrobial peptides, such as β-defensins and cathelicidin (144). Nutritional therapy has been shown to be more effective than corticosteroids for healing the mucosa (145). The evidence indicates that nutritional therapy has some prebiotic properties, which enables the modulation of the gut microbiota and regulation of the immune defense in IBD.

IBD AND *E. COLI* IN *IN VIVO* MODELS

Overall, IBD animal models can be divided into the following five categories: antigen-induced colitis and colitis induced by microbiota (146), chemically induced forms of colitis (147), genetically modified colitis models (148), adaptive infection models (149, 150), and spontaneous colitis models (151, 152).

The most important discoveries made by use of IBD animal models are that germfree animals generally do not develop intestinal inflammation and that spontaneous gut inflammation requires a certain genetic background. T cells are involved in most IBD animal models, and interactions between T cells and dendritic cells seem to be crucial for the initiation and perpetuation of inflammation (153). Ulceration of lymphoid follicles and the involvement of Peyer's patches have been reported for CD patients (154, 155). In CD patients, inflammation in Peyer's patches might be caused by the passage of particulate matter/bacteria from the bowel lumen into the lymphoid tissue of the mucosa and thereby into the lymphatic system of the gut (156). Chassaing et al. (157) showed that AIEC cells harboring long polar fimbriae (LPF) colonize the Peyer's patches of *Nod2*$^{-/-}$ mice. Experimental animal models have repeatedly revealed that the intestinal microbiota plays a major role in IBD. In addition, genetically susceptible IBD mouse models indicate a key role for dysfunctional, unregulated, T-cell-mediated immune responses in IBD pathogenesis (158). For example, IL-10 stimulates the development of humoral Th2 cytokine-driven immune responses and prevents development of Th1 immune responses by reducing the ability of macrophages to produce IL-12, which is a key inducer of Th1 immune responses (159). *In vivo* models have shown that IL-10-deficient mice colonized with nonpathogenic *E. coli* strains develop distal colitis and produce high levels of IFN-γ and IL-4 as a result (160).

A previous study showed that a UC-associated *E. coli* strain (p19A) colonized both the intestines and extraintestinal tissues of dextran sulfate sodium-treated C57BL/6 mice, causing systemic infection (161). IBD animal models have so far given us a better understanding of IBD pathogenesis, such as the involvement of the intestinal microbiota and the importance of T-helper cells in driving gut inflammation in vulnerable hosts. In IBD mouse models, commensal *E. coli* strains do not seem to have the ability to induce and maintain chronic inflammation as IBD-associated *E. coli* pathobionts; however, more studies are needed to confirm this outcome (161).

SUGGESTED MECHANISMS OF IBD-ASSOCIATED *E. COLI* PATHOGENESIS

One of the *E. coli* types linked to CD is AIEC (162). In terms of pathogenesis, it is described that AIEC affects host cell processes such as protein synthesis, signal transduction, cell division, ion secretion, transcription, cytoskeletal function, and mitochon-

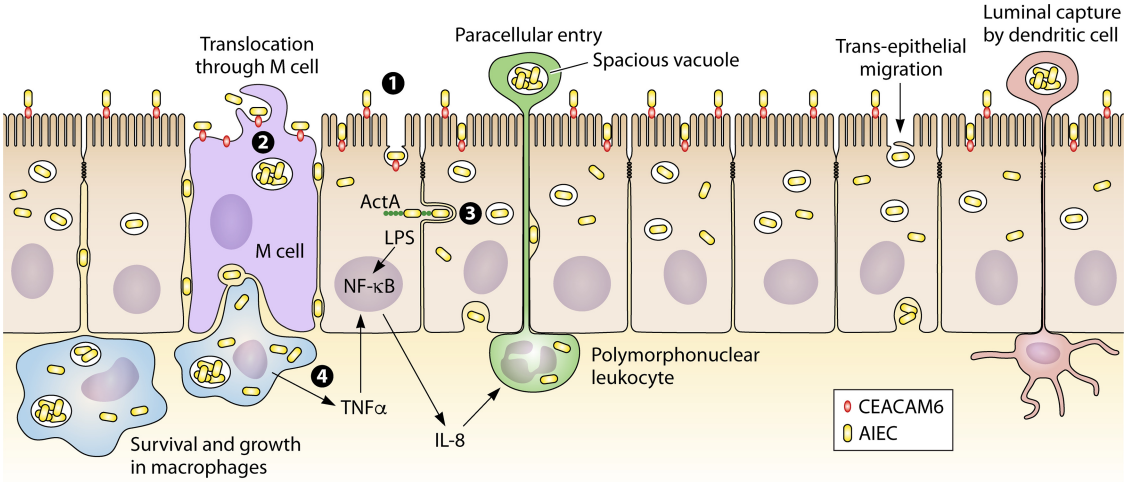

FIG 1 Invasion of host cells by AIEC in CD. Abnormal colonization of the ileal mucosa is initiated by the interaction of AIEC with intestinal epithelial cells. (1) AIEC binds to CEACAM6, which is upregulated in the CD patient's ileum. (2) By using a macropinocytosis-like process, AIEC enters, survives, and replicates inside the host cell cytoplasm after lysis of the endocytic vacuole. (3) By using its invasive ability and host cell actin microfilaments and microtubules, it crosses the intestinal barrier through intestinal epithelial cells or through M cells, invades host cells, and translocates to other cells. (4) AIEC is able to survive extensively within resident macrophages and dendritic cells and induces the secretion of large amounts of TNF-α and granulomatous inflammation.

drial function (44, 163). Studies on AIEC show that it is able to adhere to the intestinal mucosa by binding to carcinoembryonic antigen-related cell adhesion molecules 6 (CEACAM6), invade intestinal epithelial cells by using host cell actin microfilaments and microtubules, replicate intracellularly, translocate across the human intestinal barrier, and move into deeper tissues (162, 164–168) (Fig. 1). Studies also show that AIEC is able to survive within macrophages, stimulate TNF-α production, and promote a granulomatous inflammatory response (89, 168). However, at present, there is still little known about the *in vivo* pathogenesis of AIEC or any genes that are specific for AIEC, as has been described for other *E. coli* pathogens, such as ETEC and EAEC. So far, the properties/fitness characteristics linked to AIEC are also found in other *E. coli* strains and therefore do not specifically belong to AIEC. More studies are needed to characterize AIEC as well as its potential role in CD and markers that can be used to specifically identify AIEC.

Another *E. coli* type, associated with UC rather than CD, is DAEC, which is an ExPEC strain expressing afimbrial adhesins (*afa*) (25, 96, 169). The involvement of DAEC harboring Afa/Dr in diarrhea was controversially demonstrated in polarized monolayers of intestinal T84 cells (170, 171). DAEC has also been shown to adhere to the colonic mucosae of UC patients and to promote proinflammatory responses via the interaction of its bacterial adhesins with membrane-bound host receptors (171). Studies on the pathogenesis of DAEC show that DAEC initiates its interactions with fully differentiated epithelial cells through bacterial recognition of decay/accelerating factor (DAF), CEACAM1, or CEACAM6 (by Afa/Dr_{CEA} adhesins) (Fig. 2). Le Bouguenec and Servin showed that DAEC interferes with host cell signaling pathways (169), inducing the rearrangement of brush border-associated F-actin and villin cytoskeletal proteins and the loss of the epithelial cell microvilli (169). It was also shown that DAEC induces the secretion of cytokines, including IL-8, TNF-α, and IL-1β, and induces changes in the distribution of tight junction-associated proteins, which leads to increased paracellular permeability (169). However, there are only limited studies associating DAEC with UC, and there are no epidemiology reports directly linking UC with DAEC. Moreover, there are no specific genes found in DAEC strains that enable one to specifically identify DAEC in UC patients. Clearly, more studies are needed to clarify the potential role of DAEC in pathogenesis of UC.

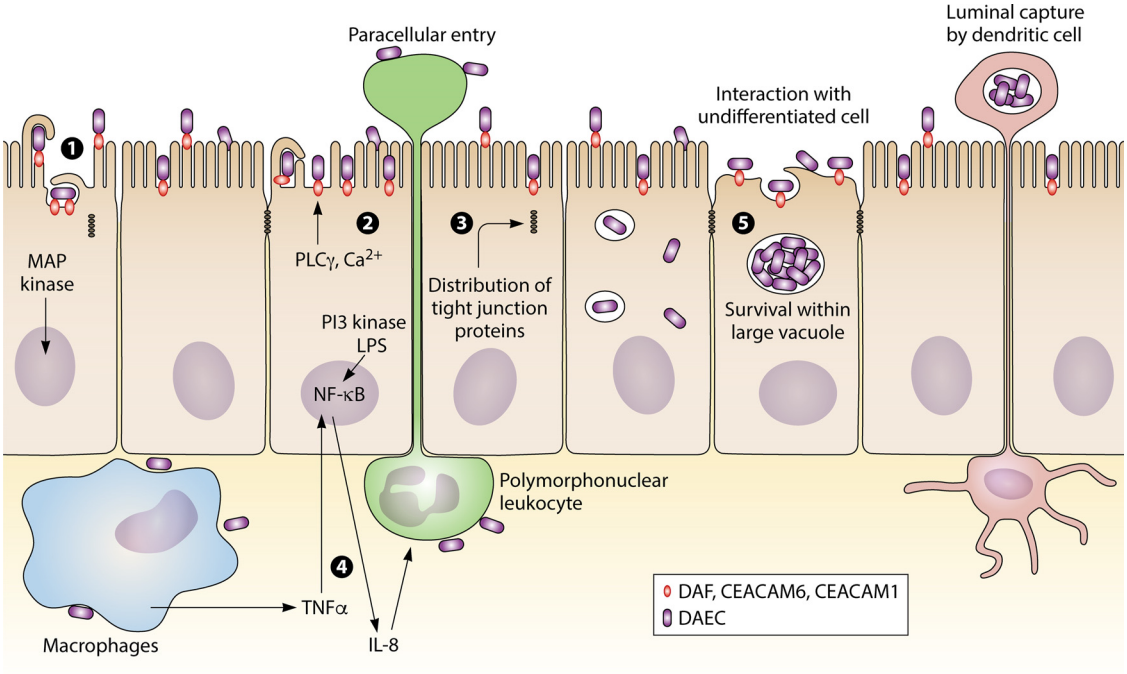

FIG 2 Infection with DAEC in UC. (1) Infection with Afa/Dr DAEC starts by bacterial interaction with fully differentiated epithelial cells via bacterial recognition of DAF, CEACAM1, or CEACAM6. (2) DAEC interferes with host cell signaling pathways involving protein tyrosine kinases(s), phospholipase Cγ, phosphatidylinositol 3-kinase (PI3 kinase), and protein kinase C, followed by an increase of Ca^{2+} in the host cell. Increased Ca^{2+} in the host cell induces rearrangements of brush border-associated F-actin and villin cytoskeletal proteins, which results in the loss of the epithelial cell microvilli. (3) Changes in the distribution of tight junction-associated proteins leads to paracellular permeability. (4) Activated MAP kinase-dependent signaling pathways induce secretion of cytokines, such as IL-8, TNF-α, and IL-1β, causing an upregulation of DAF and major histocompatibility complex (MHC) class I chain-like gene A. (5) DAEC interacts with undifferentiated cells via recognition of DAF by Afa/Dr adhesins followed by invasion of the host cell. DAEC survives within a large vacuole and spreads to other epithelial cells after host cell apoptosis.

DISCUSSION

Recently developed concepts in IBD pathogenesis include defective innate immune function resulting in diminished bacterial killing as well as functional alterations in the composition of the commensal bacteria, such as *E. coli*, which in turn leads to their enhanced adherence and invasion of epithelial cells, bacterial persistence within intestinal epithelial and phagocytic cells, and metabolic derangements that negatively influence epithelial cell function.

E. coli possesses three nitrate reductases and three nitric oxide reductases. *E. coli* strains are thus able to convert nonfermentable nutrients/nitrates to fermentable nitrates, which is a nutritional benefit possessed by only a few bacteria. During IBD or infectious gastroenteritis, the host inflammatory response in the gut generates high levels of nonfermentable nitrate, which can serve as a substrate for nitrate respiration, enabling the overgrowth of commensal *E. coli*/*Enterobacteriaceae* in the lumen of the inflamed gut (172). This might explain the reduced intestinal bacterial diversity in IBD patients compared to that in healthy controls (27, 172–174), as it may be caused by intestinal overgrowth of aggressive bacteria in IBD patients which are normally in symbiotic balance with other intestinal bacteria in healthy individuals. Whether nitrate respiration specifically benefits IBD-associated *E. coli* has yet to be studied. However, reduction of the *Enterobacteriaceae* level by treatment with tungstate (an inhibitor of molybdenum cofactor-dependent microbial respiratory pathways) reduced the severity of intestinal inflammation in murine models of colitis, suggesting that the inflammation is dysbiosis driven (175).

The potential causal role of IBD-associated *E. coli* in the pathophysiology of IBD has been linked to the ability to adhere to and invade epithelial cells and multiply within macrophages, such as that seen with the prototypical AIEC strain, LF82. Our studies

have shown that UC-associated *E. coli* (p19A) from the B2 phylogenetic group (ExPEC), harboring two alpha-hemolysin genes, induces cell death in dendritic cells, stimulates the release of the cytokines TNF-α, IL-6, and IL-23 (99), and causes rapid loss of the TJ integrity in differentiated Caco2-cell monolayers (38). The difference between p19A and strain LF82 is that LF82 does not disturb the epithelial TJ, obviously indicating that these two IBD-associated strains, both from the B2 phylogenetic group, differ in their pathogenic mechanisms. However, neither AIEC nor intestinal ExPEC has been well characterized, making it difficult to identify these strains in the course of IBD. So it is still unknown if ExPEC causes intestinal infections, and AIEC is described only by its phenotype, as not a single specific gene is linked to AIEC to enable us to identify AIEC in IBD. Additionally, there is still a lack of epidemiological studies, specifically on the prevalence of AIEC and and/or ExPEC in IBD in relation to disease onset and disease burden. What we have learned from animal models is that a combination of a genetic/immune defect and *E. coli* pathobionts plays an essential role in IBD.

Taken together, these findings suggest that IBD-associated *E. coli* might play a role in the pathogenesis of IBD and might also play a role in disease relapses in IBD patients. Additional studies of the gut microbiota and IBD-associated *E. coli* strains are needed to confirm whether IBD reflects an abnormal host response to commensal bacteria or if the acquisition of pathogenic features by specific *E. coli* strains drives the onset of IBD. However, clinical experience with treatment effects (though variable) of both immunosuppressants and antibiotics (and probiotics) suggests that the nexus of IBD pathogenesis lies in the interactions between the predisposing host genetic factors and the host immune response to intestinal bacteria. More epidemiology, animal, and treatment studies are still needed to convincingly verify the possibly important role of *E. coli* pathobionts in IBD pathogenesis.

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