SPECIAL ARTICLE

Pathogenic Escherichia coli in inflammatory bowel diseases

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Abstract

Several different groups have recently reported the presence of pathogenic E. coli associated with ileal and/or colonic mucosa of patients with inflammatory bowel diseases. Given the important role of the gut microflora and enteropathogens in the initiation and perpetuation of intestinal inflammation, important issues now arise. Are these IBD associated E. coli pathogenic? Have they evolved from commensal bacteria? What is their reservoir? Are these bacteria sufficient to drive IBD pathogenesis? Which immunological defects may predispose to colonization by such bacteria? In June 2007, clinicians and basic scientists met in Lille, France with the goal of exchanging ideas and materials on this emerging topic. State-of-the-art lectures given by widely recognized international experts were associated with debates, case discussions and expert opinions. This paper summarizes most data that were exchanged during this day and represents an update on the potential role of pathogenic E. coli in inflammatory bowel diseases.

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1. Introduction

The pathogenesis of Crohn’s disease (CD) and ulcerative colitis (UC) involves a complex interplay between the host genetic profile, immune dysfunction, and microbial or environmental factors. Several lines of evidence implicate the intestinal microbiota in the pathogenesis of IBD. Clinical improvement occurs in CD patients administered prolonged courses of antibiotics.1 In addition, post-surgical exposure of the terminal ileum to the luminal contents is associated with increased...
inflammation, and diversion of the fecal stream is associated with improvement of CD. Generalized or localized dysbiosis is observed in IBD, corresponding to the presence of low numbers of usual bacteria, high numbers of unusual bacteria, and in some instances, a reduction in biodiversity. Dysbiosis induces a disequilibrium between putatively "protective" and "harmful" intestinal bacteria, and may promote inflammation.

Escherichia coli is the predominant facultatively anaerobic Gram-negative bacterial species of the normal intestinal flora, in which it plays important roles in promoting the stability of the luminal microbial flora and in maintaining normal intestinal homeostasis. E. coli colonizes the gastrointestinal tract of human infants within a few hours of birth. As a commensal, E. coli and its mammalian host co-exist in harmony in the mucosal layer of the colon, with mutual benefit. E. coli competes with anaerobes and other facultative anaerobes of the intestinal microflora. Commensal E. coli strains rarely cause disease, except in immunocompromised hosts or when the normal gastrointestinal barriers are breached. However, Dr. Escherich, in the earliest description of this bacterium in 1885, suggested that certain E. coli strains are associated with disease, in that E. coli could be involved in infections of the intestinal and urinary tracts of humans. Some E. coli strains have acquired specific virulence factors via horizontal transfer of DNA by transposons, plasmids, bacteriophages, and pathogenicity islands. These virulence factors enhance the abilities of the bacteria to adapt to new niches and cause a broad spectrum of diseases. Among the E. coli strains that cause intestinal disease in humans, there are at least six well-characterized classes or pathotypes: enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC), and diffusely adherent E. coli (DAEC). All these pathogenic E. coli utilize a broad range of virulence factors that can affect a wide variety of critical host cell processes, including protein synthesis, signal transduction, cytoskeletal function, cell division, ion secretion, transcription, apoptosis, and mitochondrial function. All these pathogens express, in addition to toxins and bacterial effectors that influence eukaryotic processes, numerous fitness and colonization factors, which allow the bacteria to adhere to and/or invade host cells. On the basis of the pathogenic traits exhibited by CD-associated E. coli, a new potentially pathogenic group of E. coli was designated as AIEC (for Adherent-Invasive E. coli). The criteria for inclusion in the AIEC group are: (i) ability to adhere to and invade intestinal epithelial cells using a macropinocytosis-like process of entry that is dependent upon actin microfilament and microtubule recruitment; (ii) ability to survive and replicate extensively in large vacuoles within macrophages without triggering host cell death; and (iii) ability to induce the release of large amounts of TNF-α from infected macrophages. At least four independent studies have now reported the presence in IBD patients of intramucosal E. coli or mucosa-associated E. coli with invasive properties.

With the identification of host genetic defects in mucosal barrier function, innate bacterial killing, and immunoregulation, the presence of invasive bacteria is speculated to be a major trigger in the onset and/or perpetuation of IBD. The present report highlights some of the presentations from the 1st International Meeting on E. coli and IBD, which brought together clinicians and basic scientists with a common interest in this topic.

Presentation 1: The significance of E. coli in the adherent flora of the ileal mucosa in Crohn’s disease patients. (Christel Neut. Laboratoire de Bactériologie and INSERM U795, Faculté de Pharmacie de Lille, Lille, France)

Both commensal and enteropathogenic strains of E. coli can inhabit the human intestinal tract. Ileal biopsy samples, which are obtained during surgery or endoscopy from CD patients who have undergone ileocolonectomy to remove affected intestinal sections, provide a valuable tool for the identification of links between bacterial colonization of the ileum and CD progression. Following surgery, the previously healthy ileum of the CD patient can become colonized with bacteria of colonic origin, and there is a high frequency of endoscopically assessed recurrence of CD within the first year post-surgery.

The present study focused on the bacteria that adhere to the ileal mucosa, since adherence is a crucial feature of pathogenicity. Ileal biopsy samples, which were obtained both at the time of surgery and during subsequent examinations, were compared with samples from cancer patients who had undergone similar surgery. In both the CD and cancer patients, the counts of adherent aerobic and anaerobic bacteria were increased 1000-fold at 3 months and 1 year post-ileocolonectomy, which indicates that colonic bacteria invaded the ileum via the anastomosis. However, there were distinct differences between the CD and cancer patients with respect to the predominant bacterial flora. The Gram-positive anaerobes Bifidobacterium and Ruminococcus were rarely observed in the CD patients, but were abundant in cancer patients. In contrast, the E. coli counts were at least 10-fold higher in the CD patients. In CD patients with endoscopic evidence of recurrence, the E. coli counts were 30-fold higher than in CD patients without recurrence. These findings suggested the need for careful characterization of the associated E. coli strains, and prompted the investigation with Darfeuille-Michaud’s group of the newly identified pathovar AIEC.


1.1. Bacterial virulence factors

The virulence of AIEC strains, which are abnormally predominant in CD patients, is complex. AIEC can adhere to the epithelium, invade intestinal epithelial cells, and survive and replicate within macrophages. These bacteria deploy an arsenal of virulence factors for both adhesion-invasion of intestinal epithelial cells and survival-replication within macrophages (Fig. 1).

Inside macrophages, AIEC-containing phagosomes transist along the classical endocytic pathway, and the bacteria replicate within a mature and highly acidic phagolysosome that contains active cathepsin D. Replication of AIEC strain LF82 within macrophages requires an acidic phagolysosomal environment. The periplasmic stress protein HtrA and the oxidoreductase DsbA are crucial for AIEC replication in macrophages. Under stress conditions encountered by bacteria within macrophages, the expression of genes that encode HtrA and DsbA is up-regulated. Interestingly, when
cultured in an acidic and nutrient-poor medium, mimicking the environment of the phagosome, high increased expression of the htrA gene was observed for AIEC strain LF82, but not for non-pathogenic E. coli K-12.

The outer membrane protein OmpC, the expression of which is increased under osmolarity conditions similar to those found in the gastrointestinal tract, is involved in adhesion to and invasion of intestinal epithelial cells, by regulating the expression of type 1 pili and flagella. The role of OmpC in AIEC strain LF82 is linked to activation of the sigma(E) regulatory pathway. Interestingly, for non-pathogenic E. coli K-12, increased expression of OmpC leads to decreased expression of flagella and type 1 pili. In contrast, for AIEC, increased expression of OmpC leads to overexpression of flagella and type 1 pili, thereby enhancing the ability of AIEC to colonize the intestinal mucosa.

Thus, AIEC virulence genes that are implicated in bacterial replication within macrophages and in adhesion to and invasion of intestinal epithelial cells are present in both non-pathogenic E. coli K-12 and AIEC strains. However, these genes show differential expression patterns between non-pathogenic E. coli and AIEC strains.1.2. Host susceptibility factor and AIEC colonization

AIEC strains are intimately linked to the etiopathogenesis of ileal CD in genetically predisposed patients. CD-associated AIEC adhere to the brush borders of primary enterocytes isolated from CD patients, but not those isolated from control subjects without IBD, which suggests that specific modifications to the ileal epithelial cells of CD patients allow enhanced attachment of AIEC. This adhesion is dependent upon the expression of a type 1 pili variant on the AIEC surface, and fluorescence studies using FITC-conjugated Concanavalin A, which binds to mannose, demonstrate high-level expression of mannosylated molecule(s) on the apical surface of the primary ileal enterocytes isolated from CD patients.

Of the various glycosylated receptors reported to bind type 1 pili, carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) showed strong expression in both uninvolved and involved areas of the ileal mucosa of 35% of CD patients. Adhesion of AIEC to the brush borders of CD primary enterocytes was blocked by anti-CEACAM6 antibodies. The expression of CEACAM6 on intestinal epithelial cells was markedly up-regulated in vitro when intestinal epithelial cells were treated with TNF-α or IFN-γ or infected with AIEC, which indicates that AIEC can promote their own colonization. Thus, the presence of AIEC and the secretion of proinflammatory cytokines should lead to an amplification loop of increased colonization and inflammation. In conclusion, patients who have a high risk of developing severe ileal CD in response to AIEC appear to: (i) express a variant of the NOD2 intracytoplasmic receptor, thereby lacking the ability to control intracellular bacterial replication; and (ii) abnormally express the CEACAM6 receptor at the ileal mucosal surface (Fig. 1).

Presentation 3: Spatial organization of the mucosal flora in inflammatory bowel disease. (Alexander Swidsinski. University Hospital Charité of the Humboldt University, Berlin, Germany)

Intestinal bacterial flora is highly organized spatially with local concentrations of single bacterial groups ranging from undetectable to more than 10^11 bacteria/ml. The biosstructure of the fecal flora is disease specific and allows to diagnose Crohn’s disease and ulcerative colitis from samples of punched fecal cylinders. In vitro and in vivo experiments on bacterial movements within gels of varying viscosity indicate, that the viscosity gradient within the mucus layer is important for the spatial structure of fecal microbiota and for separation of pathogens from contact with the colonic wall.
polymicrobial disease, characterized by a sustained interrupted or disturbed mucus barrier with subsequent bacterial migration towards the mucosa and the proliferation of complex bacterial biofilms on the epithelial surface, cytopathologic effects and infiltration of the submucosa. The restitution of the mucus barrier is important to cure IBD. This might be either via eradication of pathogens such as AIEC (antibiotics), selective control of mucus secretion and dehydration (glucocorticosteroids), inducing a higher grade of differentiation of the epithelial layer (anti TNF), restitution of the anatomic architecture of the inflamed mucosa (immunosupression), suppression of adherent biofilms (5 ASA), reduction of emulsifiers and detergents in foods, or stimulation of innate immunity with, for example, probiotics or GM-CSF.19,20

Presentation 4: Interactions of colonic IBD- and cancer-associated mucosal E. coli with epithelial cells, M cells, and macrophages.

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Patients with colon cancer and those with UC share specific abnormalities in the synthesis of mucosal glycoproteins.21 We have recently shown that in the unaffected monozygotic twins of IBD patients, abnormal mucosal glycosylation correlates with NF-κB activation,22 which suggests abnormal interactions between bacteria and the surface epithelial cells of these subjects before disease presentation.

Colonoscopy samples from CD and UC patients contained markedly higher levels of aerobic bacteria than those from control subjects. Mucosa-associated E. coli were more common in CD patients than in noninflamed controls, possessed adhesins, had higher hemagglutination activities, and resembled uropathogenic E. coli strains.23

The early lesions of CD appeared in the Peyer’s patches.24 M cells, which are specialized enterocytes overlaying the Peyer’s patches and colonic lymphoid follicles, are the main entry point for a number of enterotoxigenic bacteria, such as Salmonella, Shigella, and enteropathogenic E. coli. Therefore, we hypothesized that the intestinal portal of entry of bacteria in CD patients is the M cells. Using an in vitro model of M cells, we showed that E. coli strains from colonoscopy samples of CD patients were better at translocating across these M cells than a reference E. coli strain.

Thus, phagocytic dysfunction in CD patients, which results from either genetic defects or environmental stimuli, may result in inadequate clearance of bacteria, such as E. coli, within macrophages, leading to granuloma, abscess, and fistula formation. Antibiotics that kill bacteria within macrophages, such as ciprofloxacin, may represent a potent treatment for CD.

We also propose a model for E. coli involvement in UC pathogenesis, whereby a genetic or acquired defect in mucosal barrier function causes loosening of the tight junctions in the intestinal epithelium, allowing access to the basolateral Toll-like receptor 5 (TLR5), which is the cellular receptor for bacterial flagella. This activates proinflammatory pathways and induces neutrophil recruitment, leading to the development of chronic colitis (Fig. 2).25

Presentation 5: Immunologic responses of monocytes from Crohn’s disease patients to adherent-invasive E. coli.
Circulating monocytes from CD patients display specific immunologic responses to AIEC. The NOD2 deficiency seen in 40% of CD patients results in defects in NF-κB activation and cytokine production, which in turn cause dysregulated mucosal hemostasis in these patients. We isolated monocytes from the blood samples of 40 CD patients, infected these cells with AIEC strain LF82, and measured the levels of various cytokines. The monocytes of patients with caspase activation and recruitment domain 15 (CARD15) polymorphisms showed reduced levels of IL-1β, IL-6, and IL-10 early after infection. These results suggest that CD patients have a genetically altered host response to disease-related bacteria. CARD15 polymorphisms disturb the monocytic response to AIEC, without affecting either monocyte viability or intracellular AIEC survival or replication. This dysregulated cytokine response to AIEC would decrease neutrophil recruitment and allow bacterial influx, with consequent induction of chronic inflammation.

We also examined the effect of metallothionein (MT) expression on IL-8 secretion by epithelial cells following challenge with different bacterial strains. MTs, which are small metal-binding proteins present in all living organisms, confer a survival advantage in situations of stress, such as infection and inflammation. CD patients with colonic involvement show significant downregulation of MT mRNA levels in both the blood and colonic biopsies. We examined the functional consequences of deficient MT expression in epithelial cells using an MT-knockdown cell line. When the MT-knockdown cells were infected with AIEC or treated with lipopolysaccharide (LPS) or peptidoglycan (PGN), they produced less IL-8 and secreted less GM-CSF than WT cells treated in the same way. Therefore, the bowel mucosa of CD patients mounts a defective early acute immune response to invading bacteria (Fig. 3).

Presentation 6: Prevalence and diversity of adherent-invasive *Escherichia coli* in Crohn’s disease patients from Girona, Spain. (Margarita Martinez-Medina1, Mireia Lopez-Siles1, Xavier Aldeguer2, Ferran Gonzalez-Huix2, Carles Lopez-
In a study of bacteria that adhere to the ileocolonic mucosa, we found differences between the microbiota in biopsy samples obtained from CD patients and healthy controls. PCR of 16S rRNA gene fragments showed that the molecular footprint of the microbiota formed a cluster for the majority of the CD patients. In addition, the patient-to-patient variability in microbiota composition was greater within the CD cluster than in the healthy/UC cluster. *Clostridium* species and *E. coli* were significantly more prevalent in the CD samples, whereas *Faecalibacterium* species were found more frequently in the control subjects.

In a recent analysis of adherent *E. coli* isolated from colonic biopsy samples, 27 strains, after analyzing approximately 100 isolates per patient, were detected in 13 individuals (six CD patients and seven control subjects). None of the *E. coli* strains were found more frequently in the CD group. The clones were unique to the individual in all but two cases. Most individuals carried at least one strain with a known *E. coli* virulence gene characteristic from extra-intestinal pathogenic *E. coli*. No Diarrheagenic *E. coli* was found in CD patients. Invasive *E. coli* strains were investigated in a further 15 CD patients and 13 control subjects. Invasive *E. coli* were more prevalent (47% versus 15%; p = 0.086) and more abundant (5% versus <1% of *E. coli* analyzed; p = 0.066) in the CD patients. These findings suggest that AIEC are well established in CD patients, and further support the association between AIEC and CD (Fig. 4).

**Presentation 7: Adherent-invasive Escherichia coli isolated from Crohn’s disease patients induce granuloma formation in vitro.** (Frédéric Altare. Laboratory of Molecular Physiology of Mycobacterial Granulomas, Department of Molecular Mechanisms of Mycobacterial Infections, Institut de Pharmacologie et Biologie Structurale, CNRS/UMR 5089, Toulouse, France)

The epithelioid granulomas found in CD patients are a feature shared with several infectious diseases in which pathogenic bacteria, such as *Mycobacterium tuberculosis*, invade and survive within host cells. We have developed an in vitro model of mycobacterial granuloma formation, in which granuloma-like cellular aggregates are induced by bacterial infection of human peripheral blood mononuclear cells (PBMCs). In this model, AIEC strain LF82 induced these cellular aggregates. Over a period of about 7 days, PBMCs incubated with AIEC strain LF82 developed into large multilayered cellular structures, which under scanning electron microscopy appeared to be composed mainly of macrophages, along with some lymphocytes (Fig. 5). In contrast, PBMCs incubated with the control *E. coli* strain DH5α formed small aggregates that disappeared by Day 5 post-infection. Cytologic comparison of the aggregates with a granuloma biopsy sample from a CD patient revealed that both contained macrophages, lymphocytes, and occasional multinucleated giant cells. The presence of multinucleated giant cells within the aggregates indicated that differentiation and fusion of macrophages was occurring, as in a classic granuloma. Epithelioid cells were not found in the aggregates, perhaps due to the short duration of incubation or the in vitro conditions used.

We have also shown that macrophages and lymphocytes aggregate on Sepharose beads coated with lysates of LF82, but not of DH5α. Importantly, this should provide a means to identify proinflammatory proteins or lipids produced by LF82 that induce aggregation and trigger differentiation of macrophages. Previous investigations of mycobacterial antigens using this model have shown that only slight structural differences produce opposite inflammatory activities, so the distinct granulomatous responses to LF82 and DH5α may reflect a similar phenomenon. It is hoped that identification of the pathways acted upon by proinflammatory compounds will lead to the development of specific blockers of granuloma formation that could be used in CD treatment.

**Presentation 8: Alterations in the flora and characterization of *E. coli* strains from Crohn’s disease, ulcerative colitis, and control resected specimens.** (Ed Boedeker. Division of Gastroenterology/Hepatology, Department of Internal Medicine, University of New Mexico School of Medicine, Albuquerque, USA)

We have characterized *E. coli* strains cultured from intestinal specimens that were obtained from patients undergoing surgery for CD or UC. *E. coli* strains were successfully isolated from about 30% of these patients. When the clonality of the isolates was determined by restriction fragment length polymorphism/PCR analysis of their enterobacterial repeat intergenic consensus (ERIC) sequences, the mean number of ERIC types was significantly fewer in the CD patients (about one per patient) than in the UC patients or control subjects with other diseases (about three per patient). *E. coli* isolates from CD patients were also more likely to be motile and to express a single flagellar type. Only 33% of the isolates from the CD patients were adherent-invasive, which was a higher proportion than in the UC patients (23%) or control subjects (18%).

Molecular analysis of the same surgical specimens using bacterial 16S rDNA primers to identify 80 to 100 sequences per specimen revealed that the predominant phyla (for all specimens) were Firmicutes, Proteobacteria, and Bacteroides, with similar distributions in the small intestine and colon. Principal components analysis identified a cluster of samples that consisted almost entirely of samples from patients with IBD (both CD and UC). This subset of IBD samples showed marked decreases in Firmicutes and Bacteroides, and a corresponding increase in the proportion of Proteobacteria. The depleted Firmicutes included metabolically important strains that produce butyrate. *E. coli* strains were included among the Proteobacteria of various types that were enriched in the IBD subset. These findings suggest that permissive pathogens among the Proteobacteria may play roles in the pathogenesis of IBD, at least in a subgroup of individuals.
Presentation 9: Granulomatous colitis in boxer dogs: a curable enteropathy associated with adherent-invasive E. coli. (Kenneth Simpson. Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, USA)

We have identified and characterized E. coli strains in the colonic mucosa of Boxer dogs suffering from granulomatous colitis (GCB), which shares some features with human IBD. Immunosuppressants have been the mainstay of treatment since the initial description of GCB, although recent therapeutic successes with enrofloxacin have turned attention to a possible infectious basis. We have shown that bacterial invasion of the mucosa occurs in the GCB colon, but not in the normal colon. Four new E. coli strains, KD1-4, were isolated from the mucosa of affected dogs. These strains have certain properties in common with the CD-associated E. coli strain LF82, based on their abilities to invade, persist, and replicate in cultured epithelial cells. The KD1 and KD3 strains belong to the B2 phylogenetic group, as does LF82. These findings support the view that GCB involves a persistent specific infection, in combination with an underlying genetic predisposition.

We also recently compared strains of E. coli isolated from CD patients with the canine E. coli strains. Biopsy samples of the ileal mucosa were obtained from patients with CD restricted to either the ileum or the colon, and also from healthy individuals. Analysis of bacterial 16S rDNA libraries, quantitative PCR, and fluorescence in situ hybridization all indicated higher levels of E coli in the samples from patients with ileal CD. The E. coli counts were correlated with the severity of ileitis, as assessed by endoscopy and histology. PCR identification of E. coli revealed that the strains belonged to diverse phylogenetic groups A, B1, B2, D, as was the case with the GCB strains. E. coli strains with adherent and invasive behavior in cultured cells were present in healthy and diseased ilea, but were much scarcer in the healthy tissues. The virulence-associated genes hcp, ratA, and colV were detected in many of the strains. It is hoped that future knockout studies will establish whether these genes have important roles in bacterial pathogenicity.

References


