Abnormal CEACAM6 expression in Crohn disease patients favors gut colonization and inflammation by adherent-invasive E. coli

Nicolas Barnich* and Arlette Darfeuille-Michaud*

Clermont Université; Université d’Auvergne; Clermont-Ferrand; Institut Universitaire de Technologie en Génie Biologique; Aubière, France; Pathogénie Bactérienne Intestinale; CBRV; Clermont-Ferrand, France

Abnormal expression of CEACAM6 is observed at the apical surface of the ileal epithelium in Crohn disease (CD) patients, and CD ileal lesions are colonized by pathogenic adherent-invasive Escherichia coli (AIEC). The paper of Carvalho et al. recently reported that CD-associated AIEC colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM6 acting as a receptor for type 1 pili produced by AIEC bacteria. AIEC also induce CEACAM6 expression by intestinal epithelial cells directly by adhering to host cells and indirectly via increased secretion of TNFα from AIEC-infected macrophages. Patients expressing a basal level of CEACAM6 in ileum could be predisposed to develop ileal CD and blocking interaction between type 1 pili and CEACAM6 might serve as a specific means of disrupting the colonization and the subsequent inflammatory amplification loop.

The intestinal epithelium is an efficient guard against microbial invasion. However, through a process of coevolution, potential harmful enteric microorganisms have evolved counter strategies to hijack the cellular molecules and signalling pathways of the host to become potentially pathogenic. This is well exemplified by the ability of adherent-invasive E. coli to colonize the ileal epithelium in Crohn disease (CD).

In patients with inflammatory bowel diseases, such as Crohn disease or ulcerative colitis, there are increased numbers of mucosa-associated E. coli forming a biofilm on the surface of the gut mucosa.1-7 The identification of virulence properties of E. coli strains colonizing the ileal epithelium of CD patients showed that they were able to adhere to and to invade intestinal epithelial cells (IEC) and to survive and to replicate within macrophages. Such pathogenic strains, called adherent-invasive E. coli (AIEC), are achieving increasing relevance since various studies performed in several countries (France,8 the United Kingdom,4 Spain,9 and the USA10-12 have reported them to be more prevalent in CD patients than in controls. The presence of AIEC in healthy subjects suggests that AIEC are facultative pathogens that cause disease in susceptible hosts. Bacterial adhesion to IEC is the first step in the pathogenicity of many bacteria involved in infectious diseases of the gut. Adhesion enables the bacteria to colonize the gut, thus limiting clearance from the intestine. As an initial step in the infection process, certain enteric pathogens target specific epithelial cell structures, including glycoproteins and glycolipids, which serve as receptors for bacterial attachment. AIEC strains were found to be highly associated with ileal mucosa in CD patients,2-13 suggesting that there are specific alterations to the ileal epithelial cells in patients with CD that allow AIEC to adhere to a greater extent. This was confirmed by ex-vivo experiments using primary ileal enterocytes isolated from CD patients and controls without inflammatory bowel disease in which we observed that CD-associated AIEC adhere only to the brush border of CD enterocytes.14 The receptor involved in AIEC adhesion and...
abnormally expressed in CD patients was characterized as being the carcinoma-embryonic antigen-related cell adhesion molecule (CEACAM6). In-vitro studies showed increased CEACAM6 expression in cultured intestinal epithelial cells after IFN or TNF stimulation and after infection with AIEC bacteria, which indicates that AIEC can promote their own colonization in CD patients.

The involvement of CEACAM6 in the colonization of the gut by AIEC and in subsequent chronic inflammation was recently demonstrated in CEABAC10 transgenic mice expressing human CEACAM6. In this mouse model, AIEC virulent bacteria, but not nonpathogenic E. coli K-12, were able to persist in the gut and to induce severe colitis with reduced survival rate, marked weight loss, increased rectal bleeding, presence of erosive lesions, mucosal inflammation, and increased proinflammatory cytokine expression. In-vitro experiments with cultured or primary intestinal epithelial cells have indicated that AIEC adhesion is dependent on type 1 pili variant expression on the bacterial surface. In the study of Carvalho et al., we demonstrated that the colitis depended on type 1 pili expression by AIEC bacteria and on intestinal CEACAM6 expression since no sign of colitis was observed in transgenic CEABAC10 mice infected with type 1 pili-negative isogenic mutant or infected with AIEC LF82 bacteria concomitantly with intraperitoneal administration of anti-CEACAM6 monoclonal antibody.

The new light thrown upon the etiology of ileal CD by our findings suggests that ileal CEACAM6 expression in IBD patients could lead to the development of specific therapies to treat patients with ileal involvement of CD by targeting AIEC bacterial adhesion to the gut mucosa. AIEC adhesion involving type 1 pili is mediated by recognition of mannose residues by the FimH adhesion site located at the tip of the pili and crystal structure determination of FimH complexed with oligomannose-3 showed the feasibility of using natural and engineered mannose antagonists to block bacterial invasion. Several strategies can be used to block AIEC adhesion and hence colonization. Adhesin-based vaccines may be effective in the prevention of CD, as previously reported for the prevention of recurrent and acute infections of the urogenital mucosa. Another strategy could be to interrupt pilus assembly and thereby block pilus-mediated adhesion using pilicides, which are pilus inhibitors that target chaperone function by inhibiting pilus biogenesis.

References
1. Conte MP, Schippa S, Zamboni I, Penta M, Chiarini F, Seganti L, et al. Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. Gut 2006; 55:1760-7.
2. Darfeuille-Michaud A, Neut C, Barnich N, Lederman E, Di Martino P, Desreuxaux P, et al. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn’s disease. Gastroenterology 1998; 115:1405-15.
3. Kordowska R, Bernstein CN, Sephner S, Krause DO. High prevalence of Escherichia coli adhering to the B2 + D phylogenetic group in inflammatory bowel disease. Gut 2007; 56:669-75.
4. Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singhi R, et al. Enhanced Escherichia coli adherence and invasion in Crohn’s disease and colon cancer. Gastroenterology 2004; 127:80-93.
5. Mylonaki M, Raymond NB, Rampton DS, Hudspith BN, Brostoff J. Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. Inflamm Bowel Dis 2005; 11:481-7.
6. Neut C, Bulou P, Desreuxaux P, Membrel JM, Lederman E, Gambier L, et al. Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn’s disease. Am J Gastroenterol 2002; 97:999-46.
7. Swidsinski A, Ladhoff A, Perntzhaler A, Swidsinski S, Loening-Bauke V, Oertner M, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology 2002; 122:44-54.
8. Darfeuille-Michaud A, Boudeau J, Bulou P, Neut C, Glasser AL, Barnich N, et al. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn’s disease. Gastroenterology 2004; 127:411-21.
9. Martinez-Medina M, Aldriger X, Lopez-Siles M, González-Huix F, López-Oliu C, Dabhi G, et al. Molecular diversity of Escherichia coli in the human gut: new ecological evidence supporting the role of adherent-invasive E. coli (AIEC) in Crohn’s disease. Inflamm Bowel Dis 2009; 15:872-82.
10. Baumberg M, Dogan B, Kishnaw M, Weirzmann G, Bosworth B, Yarranton R, et al. Culture-independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridia in Crohn’s disease involving the ileum. Isme J 2007; 1:403-18.
11. Eaves-Pyles T, Allen CA, Taormina J, Swidsinski A, Turt CR, Jezek GE, et al. Escherichia coli isolated from a Crohn’s disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. Int J Med Microbiol 2008; 298:397-409.
12. Sasaki M, Sitaraman SV, Babbin BA, Germer-Smidt P, Ribot EM, Garrett N, et al. Intravesical Escherichia coli are a feature of Crohn’s disease. Lab Invest 2007; 87:1042-54.
13. Boudeau J, Glaser AL, Mayser E, Joly B, Darfeuille-Michaud A. Invasive ability of an Escherichia coli strain isolated from the ileal mucosa of a patient with Crohn’s disease. Infect Immun 1999; 67:4499-509.
14. Barnich N, Carvalho FA, Glaser AL, Darcha C, Jantschke P, Allez M, et al. CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. J Clin Invest 2007; 117:1566-74.
15. Carvalho FA, Barnich N, Sivignon A, Darcha C, Chan CH, Stanners CP, et al. Crohn’s disease adherent-invasive Escherichia coli colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM. J Exp Med 2009; 206:2379-89.
16. Boudeau J, Barnich N, Michaud AD. Type 1 pilus-mediated adherence of Escherichia coli strain LF82 isolated from Crohn’s disease is involved in bacterial invasion of intestinal epithelial cells. Mol Microbiol 2001; 39:1272-84.
17. Otte I, Hasty DL, Abraham SN, Sharon N. Role of bacterial lectins in urinary tract infections. Molecular mechanisms for diversification of bacterial surface lectins. Adv Exp Med Biol 2009; 485:183-92.
18. Wellens A, Garofalo C, Nguyen H, Van Gerven N, Slattegard B, Hernalsteens JP, et al. Intervening with urinary tract infections using anti-adservatives based on the crystal structure of the FimH-oligomannose-3 complex. PLoS One 2008; 3:2040.
19. Langermann S, Palazyonsky S, Barnhart M, Auguste G, Pinkner JS, Burlein J, et al. Prevention of mucosal Escherichia coli infection by FimbH-adhesin-based systemic vaccination. Science 1997; 276:607-11.
20. Svensson A, Larsson A, Emtehsis H, Hedenstrom M, Fex T, Hultgren SJ, et al. Design and evaluation of pilicides: potential novel antibacterial agents directed against uropathogenic Escherichia coli. ChemBiochem 2001; 2:915-8.
21. Larsson A, Johansson SM, Pinkner JS, Hultgren SJ, Almqvist F, Kihlborg J, et al. Multivariate design, synthesis and biophysical evaluation of peptide inhibitors of FimC/FimH protein-protein interactions in uropathogenic Escherichia coli. J Med Chem 2005; 48:935-45.