Genes of intestinal *Escherichia coli* and their relation to the inflammatory activity in patients with ulcerative colitis and Crohn’s disease

Laura Gombošová · I. Lazúrová · M. Zakuciová · K. Čurová · M. Kmeťová · D. Petrášová · L. Siegfried

Received: 28 December 2010 / Accepted: 19 May 2011 / Published online: 30 August 2011

© The Author(s) 2011. This article is published with open access at Springerlink.com

**Abstract** *Escherichia coli* gene *fimA* was the most frequent gene that occurred in the intestine of all investigated groups. All subjects with *fimA* gene had significantly higher values of tumor necrosis factor alpha (TNF-α) and CRP than those with other *E. coli* genes. There was also a tendency to increased serum interleukin (IL)-6 levels in patients carrying the *fimA* gene; however, no relation was observed to serum IL-8 and IL-10. Patients with Crohn’s disease had significantly higher IL-6 than those with ulcerative colitis (UC) and controls. The highest levels of TNF-α were detected in the UC group. There were no significant differences in serum IL-8 and IL-10 between all three groups. The presence of *E. coli* gene *fimA* in the large bowel of patients with IBD is related to the immunological activity of the disease which may be important from the aspect of therapeutical strategy.

Inflammatory bowel diseases (IBD) comprising two forms, Crohn’s disease (CD) and ulcerative colitis (UC), are chronically remitting disorders characterized by inflammation of the gastrointestinal tract. Currently, the etiopathogenesis of these disorders is not completely understood, although chronic relapsing inflammation is considered to be a result from a dysregulated, aberrant immune response to intestinal flora in a context of genetic predisposition (Sanchez-Muñoz et al. 2008; Chamberli and Naser 2006).

Many studies documented an increase in proinflammatory cytokine expression from T lymphocytes, neutrophils, macrophages, and epithelial cells in patients with IBD; however, the results about their clinical significance and relation to clinical activity of CD and UC are controversial (Fantini et al. 2007; Scaldaferri and Fiocchi 2007). Tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 seem to be most closely associated with clinical and laboratory activity of the disease (Irving et al. 2005).

There is strong evidence that environmental factors implicated in the pathogenesis of IBD are bacteria and their components. Both CD and UC primarily affect intestinal areas with high bacterial counts. Further evidence supporting a role of enteric flora in the pathogenesis of IBD is that treatment with antibiotics and probiotics was found to be beneficial and could be used to maintain the remission of the disease (Dignass et al. 2004; Macfarlane et al. 2009).

The most prevalent bacteria in patients with IBD are *Enterobacteriaceae*, especially *Escherichia coli* (Guarner and Malagelada 2003). Recently, novel genetic classes of *E. coli* have been considered to be associated with CD and UC. Many genes of *E. coli* were described to be present in the small and large bowel in healthy humans and patients with IBD; however, only some of them are supposed to play a role in the pathogenesis of inflammation. Currently, there are no data about the clinical significance of these genes in the pathogenesis of CD and UC as well as their prevalence in the large bowel of patients with IBD (Dogan and Simpson 2008).
The aim of this study was to determine the occurrence of pathogenic \textit{E. coli} genes in the large bowel of patients with CD and UC in comparison with control subjects and to assess their relation to clinical as well as laboratory activity of the disease.

\textbf{Subjects and methods}

\textbf{Subjects}

Group of patients consisted of 68 subjects with the diagnosis of inflammatory bowel disease, i.e., CD or UC (36 men, 32 women), with mean age of 34.5 years (range, 19–72). Thirty-two patients were diagnosed to have ulcerative colitis (14 men, 18 women; mean age, 37.8 years; range, 21–72) and 36 had Crohn’s disease (22 men, 14 women; mean age, 31.1 years; range, 19–63).

The diagnosis of IBD was based on clinical symptoms, laboratory evaluations, and endoscopic finding confirmed by histological evaluation. None of the patients were in complete endoscopic remission. Sixteen patients with CD and 12 patients with UC were treated by antibiotics (quinolones or metronidazole). The clinical activity of CD and UC in comparison with control subjects and to assess their relation to clinical as well as laboratory activity of the disease.

\textbf{Methods}

All patients underwent colonoscopic examination after preparation using 3–4 L of the mineral water (Šaratica, Slovakia) with high magnesium content. During the colonoscopy (performed with the colonoscopic device Olympus Q 145 L), specimens of the colon mucosa were collected for histological and microbiological evaluation.

\textbf{Microbiological evaluation}

Two mucosal specimens were obtained during colonoscopy from the surrounding of maximal ulcerative mucosal lesions. Samples were immediately inserted into a 0.9% NaCl solution and processed within 1 h after sampling. Bacteria were isolated after washing, and the biopsy specimens were homogenized and inoculated into MacConkey agar and blood agar and cultivated for 24 h at 37°C. Identification of strains was done based on biochemical characteristics using Enterotest 16, Entero-Rapid 24 (Lachema, Czech Republic), and some strains were confirmed by using identification kits BBL Crystal (Becton Dickinson, USA). The virulence of \textit{E. coli} strains was tested by the phenotype expressions and genes detection. Examined genes coded factors of virulence in bacterial genome of tested strains. Polymerase chain reaction (PCR) was used to detect \textit{E. coli} genes. One gene was detected by simplex PCR, while occurrence of other genes was detected by multiplex PCR.

\textit{Preparation of template DNA for PCR} Bacteria were inoculated into LB broth (5 mL), incubated for 24 h at 37°C, and then centrifuged (1,000×g, 10 min). Sediment was resuspended in 200 μL of sterile distilled water, incubated for 10 min in water bath at 100°C, and cooled to laboratory temperature. After an additional 10 min, fragments of lysed cells were removed by centrifugation from the lysate. Supernatant was used as a template DNA for PCR.

\textbf{PCR detection of genes encoding virulence factors} For demonstration of virulence genes’ (bfpA, \(\alpha\)-hly, cnf1, sfa, pap, iai, ipaH, eaeA, fimA, iucC, aer) characteristic for enterovirulent \textit{E. coli}, the protocol according to Kuhnert et al. (1997), Yamamoto et al. (1995), and Liptakova et al. (2001) was used; appropriate bacteria encoding each of the virulence genes were included as PCR positive controls. \textit{E. coli} C600 Rif was used as a negative control.

\textbf{Detection of PCR products} Specific gene products were detected using electrophoresis. The reaction mixture was analyzed by electrophoresis on 2.0% agarose gels, and the reaction products were visualized by staining with ethidium bromide under UV light.

According to the presence of genes coding for virulence factors, we have included examined \textit{E. coli} into enter-
ovirulent strains. 

\textit{E. coli} strains without genes of virulence were included into nonpathogenic strains of commensal enteric flora.

\textit{E. coli} strains were not detected in mucosal samples from nine patients. Some other strains, such as \textit{Shigella} sp., \textit{Streptococcus} sp., \textit{Klebsiella} sp., \textit{Morganella} sp., \textit{Serratia} sp., were also found. No bacteria were detected in mucosal samples of 11 patients (Table 1).

\textit{Laboratory evaluations} All patients as well as controls underwent blood sample collection before endoscopy. Laboratory investigation included the measurement of serum cytokines, namely IL-6, IL-8, IL-10 and TNF-\(\alpha\) and serum C-reactive protein (CRP). All cytokines were measured by ELISA method (ELISA kits, Thermo Scientific; Pierce Biotechnology, USA). CRP was measured by standard biochemical methods.

\textit{Statistical analysis} Data are presented as means±SD. An unpaired \(t\) test was used for the demonstration of differences between two groups, and \(\chi^2\) test was used to assess differences in the prevalence of evaluated parameters.

\section*{Results}

\textbf{Immunological evaluations}

Patients with CD had significantly higher serum level of IL-6 than those with UC (\(p<0.01\)) and mildly higher IL-6 than control subjects that reached a borderline significance (\(p=0.05\); Table 2). There were no significant differences in serum levels of IL-8 and IL-10 between all three groups. Serum TNF-\(\alpha\) was the highest in the UC group (\(p<0.01\)). We also detected a tendency to higher TNF-\(\alpha\) levels in CD group; however, it did not reach the statistical significance in comparison with the control group. Both CD and UC groups had significantly higher CRP values than controls with the highest levels in UC group.

\textit{Occurrence of \textit{E. coli} genes in colonic mucosa of patients with UC, CD, and control subjects}

Gene \textit{fimA} encoding the surface properties was the most frequent gene that occurred in all the investigated groups, including the control subjects (Table 3). UC patients had significantly higher prevalence of \textit{iucC} gene encoding the aerobactin in comparison with CD patients. The invasivity gene \textit{IpaH} was not detected in both CD and UC patients. We did not detect significant differences in the prevalence of other \textit{E. coli} genes among all three groups.

All subjects with the \textit{E. coli} gene \textit{fimA} tended to have increased serum IL-6 levels (\(p=0.05\)). There were no significant differences in the serum levels of IL-8 and IL-10 in relation to the presence of various \textit{E. coli} genes. All subjects carrying the \textit{E. coli} gene \textit{fimA} had significantly higher values of TNF-\(\alpha\) (\(p<0.01\)). There were no significant differences in serum TNF-\(\alpha\) in relation to other \textit{E. coli} genes. Moreover, patients with \textit{fimA} gene had significantly higher CRP than those with other \textit{E. coli} genes (\(p<0.05\); Fig. 1).

\section*{Discussion}

The relation of inflammatory bowel diseases to the presence of some strains of \textit{E. coli} bacteria has been studied during the last years; however, there are still no data about the relation of the \textit{E. coli} genes to the clinical and laboratory activity of IBD. For that purpose, we evaluated the prevalence of some of \textit{E. coli} genes in relation to the severity of UC or CD.

Patients with CD and UC had significantly increased CRP serum level, which is in agreement with previous studies that showed that CRP serum levels are related to systemic inflammation. Nevertheless, some studies docu-
mented that CRP level has less significance in the assessment of clinical activity of IBD (Vermèire et al. 2006; Douda et al. 2006; Florin et al. 2006; Denis et al. 2007).

Bowel inflammation was shown to be characterized by mucosal infiltration with neutrophils which is associated with the release of proinflammatory cytokines (Sansonetti et al. 1999). We also found significantly higher plasma levels of IL-6 in patients with CD in comparison with those with UC and control subjects. An increase in IL-6 is considered to reflect the clinical activity of IBD because none of the patients were in clinical remission.

This is in agreement with previous studies that found an increased serum IL-6 level in patients with CD as compared to UC and controls (Mudter and Neurath 2007). On the other hand, some other authors found that lamina propria mononuclear cells produce markedly higher amounts of IL-6 in UC patients than in those with CD (Reinecker et al. 1993). Because IL-6 was identified to promote tumor growth, this finding may partially explain an increased occurrence of colonic cancer in UC. We also found higher levels of IL-6 in UC patients as compared to control subjects which is also in agreement with previous observations.

There were no significant differences in serum levels of IL-8 and IL-10 in all three groups. Our results are in agreement with Nielsen et al. (1997), who reported that IL-10 concentrations did not differ among UC, CD, and healthy control subjects. Controversially, Mitsuyama et al. (2006) found that the concentrations of serum IL-10 increased in patients with UC but not in those with active CD when compared to normal subjects. This controversial finding is difficult to explain. Values of IL-10 might be influenced by the treatment because all of the patients were treated pharmacologically and were in various stages of clinical activity. Moreover, the serum levels of IL-10 may not strictly reflect its mucosal mononuclear production, which has been demonstrated in in vitro studies to be increased (Sanchez-Muñoz et al. 2008; Scaldaferrri and Fiocchi 2007).

We also detected a significantly higher serum TNF-α concentration in patients with UC and its mild increase in patients with CD. The results are in agreement with Scaldaferrri and Fiocchi (2007) and Mitsuyama et al.

### Table 2 Immunological evaluation

| Parameter | Controls | CD     | UC     | Significance |
|-----------|----------|--------|--------|--------------|
|           |          |        |        | Controls vs. CD | Controls vs. UC | CD vs. UC |
| IL-6      | 2.98±0.17| 4.58±0.80| 2.41±0.19 | p=0.05 | ns | p<0.01 |
| IL-8      | 12.1±3.21| 9.54±1.47| 15.6±3.00 | ns | ns | ns |
| IL-10     | 7.92±2.08| 7.32±1.10| 7.06±1.40 | ns | ns | ns |
| TNF-α     | 24.5±3.25| 47.8±9.09| 75.5±19.22 | ns | p<0.01 | ns |
| CRP       | 4.40±0.84| 25.5±4.60| 41.3±7.27 | p<0.01 | p<0.01 | p<0.05 |

For numbers of CD, UC samples, and controls, see Table 1

### Table 3 Occurrence of E. coli genes in colonic mucosa of patients with UC, CD, and control subjects

| E. coli gene | Control | CD | UC | Significance |
|--------------|---------|----|----|--------------|
|              |         |    |    | Controls vs. CD | Controls vs. UC | CD vs. UC |
| ipaH         | 1       | 0  | 0  | –            | –            | –        |
| iucC         | 17      | 10 | 18 | ns           | ns           | p<0.01   |
| fimA         | 28      | 23 | 22 | ns           | ns           | ns       |
| α-hly        | 0       | 3  | 5  | ns           | ns           | ns       |
| afa          | 2       | 6  | 1  | ns           | ns           | ns       |
| aer          | 16      | 16 | 14 | ns           | ns           | ns       |
| cnf1         | 4       | 8  | 10 | ns           | ns           | ns       |
| sfa          | 7       | 9  | 4  | ns           | ns           | ns       |
| pap          | 10      | 13 | 10 | ns           | ns           | ns       |
| bfpA         | 0       | 0  | 0  | –            | –            | –        |
| eae          | 0       | 1  | 5  | –            | –            | ns       |

For numbers of CD, UC samples, and controls, see Table 1

ns not significant
(2006) who demonstrated higher levels of TNF-α in serum as well as in mucosa in patients with IBD, which reflects the clinical activity of the disease.

In our study, the most prevalent gene was the fimA encoding the superficial properties of bacteria. It is supposed to participate in initiation and persistence of chronic inflammation by uncontrolled production of proinflammatory cytokines. Our findings are supported by results of Boudeau et al. (2001) who evaluated the relationship between fimbriae I, encoded by the gene fimA, and secretion of proinflammatory cytokines; they confirmed that the adherence of fimbriae I initiates the production of proinflammatory cytokines. In healthy humans, the significance of this mechanism is unknown. Genes eae, encoding aerobactin, and iuc, encoding aerobactinsyntase, were found to occur less frequently. We also demonstrated significantly higher levels of TNF-α, IL-6, and CRP in patients with E. coli carrying the gene fimA.

E. coli is the predominant facultative aerobic inhabitant of human intestine and part of normal enteric flora. There is growing evidence that E. coli is more prevalent in patients with IBD in comparison to control subjects (e.g., Martin et al. 2004). For example, some studies demonstrated that CD is associated with invasive E. coli strains since 29% of CD patients were infected with intramucosal E. coli in comparison with 9% of control subjects (Mitsuyama et al. 2006). Sasaki et al. (2007) showed that E. coli isolated from IBD patients induced significantly higher TNF-α expression in macrophage cell cultures in vitro. This is also supported by our study; we showed that some E. coli genes,
fimA in particular, were associated with the laboratory inflammatory activity, i.e., higher levels of TNF-α, IL-6, as well as CRP.

To the best of our knowledge, this is the first clinical study evaluating the relationship between fimA gene of E. coli and laboratory markers of inflammation in patients with Crohn’s disease and ulcerative colitis, demonstrating that the presence of E. coli gene fimA is related to the laboratory activity of the disease.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Best WR, Becktel JM, Singleton JW et al (1976) Development of a Crohn’s disease activity index. National Cooperative Crohn’s Disease Study. Gastroenterology 70:439–444

Boudeau J, Barnich N, Darfeuille-Michaud A (2001) Type 1 pili-mediated adherence of E. coli strain LF82 isolated from Crohn’s disease is involved in bacterial invasion of intestinal epithelial cells. Mol Microbiol 39:1272–1284

Chamberli WM, Naser SA (2006) Integrating theories of the etiology of Crohn’s disease: questioning the hypotheses. Med Sci Monit 12:27–33

Denis MA, Reenaers C, Fontaine F et al (2007) Assessment of endoscopic activity index and biological inflammatory markers in clinically active Crohn’s disease with normal C-reactive protein serum level. Inflamm Bowel Dis 13:1100–1105

Dignass AU, Baumgart DC, Sturm A (2004) Review article: the etiopathogenesis of inflammatory bowel disease—immunology and repair mechanisms. Aliment Pharmacol Ther 20(suppl V):9–17

Dogan B, Simpson KW (2008) Microflora in Crohn’s disease: the emergence of adherent and invasive Escherichia coli. Expert Rev Clin Immunol 4:133–137

Douda T, Bureš J, Rejchrt J (2006) Mean platelet volume in Crohn’s disease patients. Cas Lek Cesk 145:870–873

Fantini MC, Monteleone G, MacDonald TT (2007) New players in the cytokine orchestra of inflammatory bowel disease. Inflamm Bowel Dis 13:1–5

Florin TH, Paterson EW, Fowler EV (2006) Clinically active Crohn’s disease in the presence of a low C reactive protein. Scand J Gastroenterol 41:306–311

Guerfer F, Malagelada JR (2003) Role of bacteria in experimental colitis. Best Pract Res Clin Gastroenterol 17:793–804

Irving PM, Pasi KJ, Rampton DS (2005) Thrombosis and inflammatory bowel disease. Clin Gastroenterol Hepatol 3:617–628

Kuhnert P, Hacker J, Muhldorfer I et al (1997) Detection system for Escherichia coli-specific virulence genes: absence of virulence determinants in B and C strains. Appl Environ Microbiol 63:703–709

Liptáková A, Sehnálková H, Sabol M et al (2001) Detection of shiga-like toxin producing Escherichia coli by multiplex PCR. Biologia Bratislava 56:65–68

Macfarlane S, Steed H, Macfarlane GT (2009) Intestinal bacteria and inflammatory bowel disease. Crit Rev Clin Lab Sci 46:25–54

Martin HM, Campbell BJ, Hart CA et al (2004) Enhanced E. coli adherence and invasion in Crohn’s disease and colon cancer. Gastroenterology 127:1649–1650

Mitsuyama K, Tomiyasu N, Takaki K (2006) IL-10 in the pathophysiology of inflammatory bowel disease: increased serum concentrations during the recovery phase. Med Inflamm 1:1–7

Mudter J, Neurath MF (2007) IL-6 signaling in inflammatory bowel disease: pathophysiology role and clinical relevance. Inflamm Bowel Dis 13:1016–1023

Nielsen OH, Rudiger N, Gaustadnes M et al (1997) Intestinal interleukin-8 concentration and gene expression in inflammatory bowel disease. Scand J Gastroenterol 32:1028–1034

Reinecker HC, Steffen M, Witthoeft T et al (1993) Enhanced secretion of tumour necrosis factor-α, IL-6, and IL-1β by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn’s disease. Clin Exp Immunol 94:174–181

Sanchez-Muñoz F, Dominguez-Lopez A, Yamamoto Furusho JK (2008) Role of cytokines in inflammatory bowel disease. World J Gastroenterol 14:4280–4288

Sanseonetti PJ, Arondel J, Huerre M (1999) IL-8 controls bacterial transepithelial translocation at cost of epithelial destruction in experimental shigellosis. Infect Immun 67:1471–1480

Sasaki M, Sitaraman SV, Babbin BA et al (2007) Invasive Escherichia coli are a feature of Crohn’s disease. Lab Invest 87:1042–1054

Scaldaferri F, Fiocchi C (2007) Inflammatory bowel disease: progress and current concepts of etiopathogenesis. J Dig Dis 8:1–9

Schroeder KW, Tremaine WJ, Istrup DM (1987) Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis: a randomised study. New Engl J Med 317:1625–1629

Vermiere S, van Assche G, Rutgeerts P (2006) Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut 55:426–431

Yamamoto S, Terai A, Yuri K et al (1995) Detection of virulence factors in Escherichia coli by multiplex polymerase chain reaction. FEMS Immunol Med Microbiol 12:85–90