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Both Preoperative pANCA and Anti-CBir1 Expression in Ulcerative Colitis Patients Influence Pouchitis Development after Ileal Pouch-Anal Anastomosis

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Abstract

BACKGROUND AND AIMS—Acute pouchitis (AP) and chronic pouchitis (CP) are common after ileal pouch-anal anastomosis for ulcerative colitis. The aim of this study was to assess associations of preoperative pANCA (perinuclear antineutrophil cytoplasmic antibody) and anti-CBir1 flagellin on AP or CP development.

METHODS—Patients were prospectively assessed for clinically and endoscopically proven AP (antibiotic responsive) or CP (antibiotic dependent or refractory to antibiotic therapy). Sera from 238 patients were analyzed for ANCA and anti-CBir1 using ELISA. pANCA⁺ patients were substratified into high-level (>100 EU/ml) and low-level (<100 EU/ml) groups.

RESULTS—After a median followup of 47 months, 72 patients (30%) developed pouchitis. Pouchitis developed in 36% of pANCA⁺ patients vs. 16% of pANCA⁻ patients (p=0.005), 46% of anti-CBir1⁺ patients vs. 26% of anti-CBir1⁻ patients (p=0.02), and 54% of 35 pANCA⁺/anti-CBir1⁺ patients vs. 31% of 136 pANCA⁺/anti-CBir1⁻ patients (p=0.02). AP developed in 37 pANCA⁺ patients (22%) vs. 6 pANCA⁻ patients (9%) (p=0.02), and 12 anti-CBir1⁺ patients (26%) vs. 31 anti-CBir1⁻ patients (16%) (p=0.1). Although AP was not influenced by pANCA level, AP was seen in 38% of low-level pANCA⁺/anti-CBir1⁺ patients vs. 18% low-level pANCA⁺/anti-CBir1⁻ patients (p=0.03). CP was seen in 29% of high-level pANCA⁺ patients vs. 11% of low-level pANCA⁺ patients (p=0.03).

CONCLUSION—Both pANCA and anti-CBir1 expression are associated with pouchitis after IPAA. Anti-CBir1 increases the incidence of AP only in patients who have low-level pANCA expression, and increases the incidence of CP only in patients who have high-level pANCA expression. Diverse patterns of reactivity to microbial antigens may manifest as different forms of pouchitis after IPAA.

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Keywords

Pouchitis; Ileal pouch-anal anastomosis; Perinuclear anticytoplasmic antibody; Flagellin; Serology

Introduction

Ileal pouch-anal anastomosis (IPAA) has become the standard operative approach for patients requiring colectomy because of familial polyposis, ulcerative colitis (UC) or in selected patients with indeterminate colitis (IC). Despite excellent functional results and high patient satisfaction, pouchitis remains a significant clinical problem (1,2). Although its etiology is not fully understood, a number of clinical observations have begun to unravel the pathogenesis of pouchitis. Bacterial overgrowth occurs universally in pelvic pouches (3,4) yet patients with familial polyposis patients almost never develop pouchitis (1). Selected patients who develop pouchitis can be successfully treated with antibiotics, probiotics or even immune modulating agents (5-8). Finally, diversion of the fecal stream in patients with refractory pouchitis is almost always successful (9). Taken together, these findings strongly imply that pouchitis arises from a dysregulated mucosal immune response to intestinal microbes.

A number of antibacterial antibodies have been identified in the serum of UC patients. These antibodies have been regarded as abnormal responses to commensal bacterial antigens as they do not usually exist in the healthy population (10). Anti-neutrophil cytoplasmic antibody with perinuclear staining (pANCA) has been the most extensively studied seromarker in UC patients. This autoantibody has cross-reactivity to a number of commensal bacterial antigens (11), the target antigen appears to reside within the neutrophil nucleus (12), and pANCA is expressed in a majority of UC patients (13). Anti-CBir1 is a newly described antibody which reacts to flagellin of *Clostridium* species (14). Although expressed in 50% of CD patients, it has also been found in the sera of a small proportion (6%) of UC patients (15).

In this study, we examined whether there was any relationship between preoperative expression of the two bacterial reactive antibodies pANCA and anti-CBir1 and the incidence of pouchitis after IPAA. Our study has shown that expression of both pANCA and anti-CBir1 (individually and in combination) is associated with an increased overall incidence of pouchitis. Anti-CBir1 increases the incidence of acute pouchitis (AP) only in patients who have low-level pANCA expression, and increases the incidence of chronic pouchitis (CP) only in patients who have high-level pANCA expression.

Methods

Study Population

As part of a prospective study to examine clinical, serologic and genetic markers with clinical phenotypes in IBD, 238 consecutive UC or IC patients requiring colectomy for medically unresponsive disease or dysplasia were studied. All research related activities were approved by the Cedars-Sinai Medical Center Institutional Review Board (IRB # 3358). Complete mucosectomy was performed in all patients by one surgeon (PRF). In addition, all patients had a temporary diverting ileostomy constructed at the time of pouch creation. Patients were seen for follow up examinations (including pouchoscopy) every three months for the first year after stoma closure and yearly afterwards. Only patients followed for a minimum of 3 months after ileostomy closure were included in this study.

Assessment of Clinical Characteristics

Detailed clinical profiles assessing demographic information and characteristics of the disease and its treatment were prospectively generated by one investigator (PRF) using chart review and patient interview. Demographic information assessed included patient age at surgery, gender, disease features and length of followup after surgery. Disease characteristics examined included disease duration and extent, preoperative medication use, presence of extraintestinal manifestations (EIM), backwash ileitis, family history of inflammatory bowel disease (IBD), type of colitis (UC versus IC), and indication for surgical intervention. Anatomic location of disease was grouped into categories of pancolitis, left-sided colitis, and proctitis. EIMs included primary sclerosing cholangitis, skin lesions (pyoderma gangrenosum, erythema nodosum), bone/joint disease (arthritis, ankylosing spondylitis, sacroileitis) or eye disease (uveitis, episcleritis) considered by the investigators and the patients' physicians to be manifestations of IBD. Backwash ileitis was defined by the presence of macroscopic or histological evidence of inflammation restricted to the distal 3 cm of the extreme terminal ileum not thought to be related to CD. Treatment characteristics included the nature of medical therapy before colectomy (steroids alone vs. the use of other immunomodulators) and the indications for surgery (medically-refractory disease vs. dysplasia/cancer).

Diagnosis of Ulcerative and Indeterminate Colitis

Clinical, endoscopic and pathologic criteria were reviewed in all patients to determine if they were UC or IC. Clinically, UC patients had no perianal disease, and endoscopic features included continuous macroscopic disease extending varying distances from the dentate line. Radiologic evaluation revealed the distinct absence of either a colonic stricture or small-bowel disease. Histologic patterns of continuous microscopic inflammation and the absence of noncaseating granulomas were mandatory to rule out CD. Additionally, these features were also identified after review of intraoperative findings and pathologic evaluation of the resected specimen. Patients were classified as having IC when they had clinical features of UC with some features suggestive but not diagnostic of CD. Preoperative features of IC included skip lesions possibly related to medical therapy, anal fistula or ulcer, or small bowel inflammation more than 3 cm proximal to the ileocecal valve. Postoperative features of IC included gross or microscopic transmural colonic inflammation or discontinuous histopathologic involvement of the colon possibly related to medical therapy. All patients were classified as either UC or IC in the immediate postoperative period. Patients with granulomatous inflammation on histopathologic examination were deemed to have CD and were excluded from study analysis.

Diagnosis of Pouchitis

Pouchitis was defined as a clinical syndrome characterized by the onset of increased stool frequency often with bloody diarrhea, pelvic discomfort, urgency, malaise, and fever. The diagnosis of pouchitis was confirmed in all cases by endoscopy with afferent ileal limb intubation. Endoscopic findings in cases of pouchitis included diffuse mucosal inflammation, typically involving the entire pouch, characterized by exudate, ulceration, erythema, and sparing of the afferent ileal segment. Histopathologic evaluation of the pouch was not routinely performed. Acute pouchitis was defined as antibiotic responsive flares occurring at least four months apart during which time the patient was completely asymptomatic and had returned to his/her usual bowel pattern. Chronic pouchitis required continuous antibiotic treatment for symptom relief and also included those patients who were refractory to antibiotic treatment. Stool studies were obtained when conventional antibiotic therapy was unsuccessful or in patients with CP. However, pouchoscopy with biopsies was performed in all CP patients looking for granulomatous inflammation or cytomegalovirus inclusion bodies (16). For patients with persistent symptoms, studies were done to exclude mechanical complications of surgery such as an anal stricture or partial small bowel obstruction. Patients in whom the

ileostomy was not closed, those who did not consent to have blood drawn for research purposes and those using nonsteroidal anti-inflammatory drugs (17) were excluded from analysis. CD after IPAA was diagnosed when mucosal inflammation involved the small bowel mucosa proximal to the ileal pouch or when a pouch fistula or other perianal complication developed more than 3 months after ileostomy closure. Histologic evidence of granulomatous inflammation in pouch or small bowel biopsies was not necessary for the diagnosis of CD. As the focus of this study was on AP and CP, patients who developed CD after IPAA were included in the no pouchitis patient group. Time to diagnosis of pouchitis was defined as the time period from ileostomy closure.

Serum pANCA and anti-CBir1 Determination

Serum ANCA expression and ANCA subtype characterization were assessed using ELISA and indirect immunofluorescence, as previously described (13). Sera with circulating anti-neutrophil cytoplasmic IgG antibody exceeding the normal reference range value were termed “ANCA+.” Numeric values that were below the normal reference range were termed “ANCA-”. ANCA+ sera were further subtyped via indirect immunofluorescence (IIF) staining to determine the ANCA neutrophil binding pattern. Sera exhibiting perinuclear highlighting which then lost this characteristic staining pattern with DNase treatment were termed “pANCA+”. Our group has previously reported that patients having sera with high pANCA levels (greater than 100 ELISA units per milliliter) are predisposed to the development of chronic pouchitis but not acute pouchitis (18). These data suggest that high-level pANCA expression may identify a subset of patients with UC who may have a distinct type of mucosal inflammation representing an exquisite sensitivity to the presence of bacterial antigens. This observation stimulated our analysis of patients not only on pANCA positivity or negativity but also on the magnitude of antibody expression, namely antibody level. pANCA+ patients were thus stratified into 2 groups based on antibody level. High-level pANCA+ (HL-pANCA) patients had levels equal to and above 100 ELISA units per milliliter. Low-level pANCA+ (LL-pANCA) patients had levels below 100 ELISA units per milliliter.

Anti-CBir1 was assessed using ELISA to the NH₂-terminal fragment of CBir1 (147 amino acids), as previously described (14,15). Briefly, enzyme-linked immunosorbent assay plates were coated overnight with 100 ng/well of CBir1 and then blocked with 1% bovine serum albumin in phosphate-buffered saline for 2 hours. Plates were washed and serum added at a 1:200 dilution in 1% bovine serum albumin-phosphate-buffered saline and incubated for 30 minutes. After washing, horseradish-peroxidase-conjugated anti-human IgG at a 1:10,000 dilution was added and incubated for 30 minutes. After another wash, the plates were incubated with tetramethylbenzidine substrate for 15 minutes. The reaction was stopped with 1N sulfuric acid and read at 450 nm. Patients were deemed to be anti-CBir1+ when measured level was greater than 2 standard deviations above the mean of healthy controls.

Both pANCA and anti-CBir1 seroassays were performed blindly without knowledge of patient clinical characteristics. Similarly, clinical course after IPAA was assessed without knowledge of the patient’s seromarker profile.

Statistical Analysis

All data were entered into a standardized database computer program (Microsoft® Excel, Seattle, Washington). Statistical analyses were performed with statistical analysis software (version 8.02; SAS Institute, Inc., Cary, NC). To evaluate the relationship between serum pANCA and anti-CBir1 with baseline clinical features, linear regression analysis was performed by specifying antibody levels as dependent variables and clinical characteristics as independent variables, using a stepwise procedure. For continuous covariates, medians were compared with the use of Wilcoxon nonparametric tests. Categorical variables were compared

with the use of Fishers' exact test. The Cochran-Armitage test for trend was used to test if there was a linear trend in the proportion of patients developing pouchitis as the number of antibody responses increased. The incidence of AP and CP over time was plotted using the Kaplan-Meier method. Finally, the log-rank test was used to compare these incidence curves to assess the role of serologic risk factors in the cumulative incidence of AP or CP over time. All hypothesis testing was two-sided with p value of less than 0.05 considered statistically significant. Due to the hypothesis-generating nature of this study, a Bonferroni correction was not used.

Results

Patient Demographics and Clinical Characteristics

Patient demographic and clinical characteristics of the 238 study patients are shown in Table I. A majority of the patients were male. Of the 199 patients with medically unresponsive disease, 34 (17%) were steroid-dependent and 165 (83%) were refractory to other immunomodulatory therapy, including thiopurines (n=145), cyclosporin (n=96) and/or infliximab (n=30). Forty patients (17%) had EIMs associated with their disease, including arthritis (n=26), skin disease (n=7), PSC (n=5) or uveitis (n=1). Twenty-four of the 174 patients (14%) with pancolitis had backwash ileitis. Within the group of 70 patients with IC were 41 patients (59%) with transmural inflammation on the colectomy specimen, 23 patients (33%) with discontinuous inflammation (n=11) or involvement of the small bowel (n=12), and 6 patients (9%) with anal disease. Although the majority of patients had surgery for medically-refractory disease, IPAA was performed in 26 patients for dysplasia and 13 patients for an established carcinoma.

Pouchitis Development

After a median followup time after ileostomy closure of 47 months (range, 3 to 142 months), 72 patients developed pouchitis, representing an overall incidence of 30%. Median time to diagnosis of pouchitis was 7 months (range, 3 to 116 months). Acute pouchitis was seen in 43 patients (18%). Median time to the diagnosis of AP was 9 months (range, 3-116 months). The median number of acute pouchitis episodes per patient was 2 (range, 1 to 5).

Chronic pouchitis developed in 29 patients (12%). Twenty-two patients with CP were maintained on long-term antibiotic therapy. Three patients required a diverting ileostomy to control symptoms, two patients required immunomodulator therapy, and two patients were controlled using probiotic therapy alone. The median time to CP development was 6 months (range, 3 to 28 months). There was no significant difference in median time to pouchitis diagnosis between patients with acute pouchitis or chronic pouchitis. Sixteen patients (7%) developed CD of the pouch after IPAA.

Serologic Marker Expression

Serologic responses in this patient cohort are shown in Figure 1. Serum ANCA with perinuclear staining pattern (pANCA) was detected in 171 patients (72%) in samples taken before colectomy. Seroreactivity to CBir was found in 46 patients, representing an overall incidence of 19%. Apart from a higher incidence of males in the anti-CBir1⁺ patient group versus the anti-CBir1⁻ patient group, there were no other significant differences in patient demographics or disease characteristics noted between the antibody positive and negative subgroups (Table II).

Median antibody level in the anti-CBir1 patient group was 60 EU/ml (range, 33 to 255 EU/ml). Median antibody level in the pANCA⁺ patient group was 51 EU/ml. However, there was a wide range of preoperative pANCA levels noted in this patient group, ranging from 10 to

170 EU/ml (Figure 1). There were 21 patients (12%) in the HL-pANCA⁺ (>100 EU/ml) group and 150 patients (88%) in the LL-pANCA⁺ (<100 EU/ml) group (see methods). There was no significant difference in clinical features between the HL-pANCA⁺ and LL-pANCA⁺ patient groups (data not shown).

Relationship between Serologic Marker Expression and Overall Pouchitis Development

The association between overall pouchitis development and preoperative marker expression is shown in Figure 2. Both pANCA and anti-CBir1 positivity were associated with a higher overall incidence of pouchitis. In an attempt to assess whether there was any significant cumulative influence of antibody responses on overall pouchitis development, we combined the positive and negative pANCA and anti-CBir1 patients into 4 distinct serologic subgroups. Within the 67 pANCA⁻ patients, 56 patients were anti-CBir1⁻ and 11 patients were anti-CBir1⁺. Of the 171 pANCA⁺ patients, 136 patients were anti-CBir1⁻ and 35 patients were anti-CBir1⁺. As demonstrated in Figure 3, the overall incidence of pouchitis rose significantly with increasing seromarker positivity ($p=0.0002$). However, there was no significant difference in overall pouchitis between pANCA-negative patients who were also anti-CBir1⁻ (16%) versus those pANCA-negative pts who were also anti-CBir1⁺ (18%). These observations suggest that anti-CBir1 affects overall pouchitis development, but only in patients who also are pANCA⁺.

Relationship between Serologic Marker Expression and Acute or Chronic Pouchitis Development

As we noted a higher incidence of overall pouchitis in patients with increasing seromarker expression, we then examined whether these trends would also be seen in patients with either AP or CP (Figure 3). There was a trend towards increased acute pouchitis development with increasing seromarker positivity ($p \text{ trend}=0.007$). Again observed is the enhancing effect of anti-CBir1 expression on acute pouchitis but only in patients who are also pANCA seroreactive. We could not find a similar relationship for patients with chronic pouchitis. There was no significant association towards increased chronic pouchitis development with increasing seromarker positivity ($p=0.05$).

Influence of pANCA Level and Anti-CBir1 Expression and Pouchitis

The association between pouchitis and the HL-pANCA⁺ and LL-pANCA⁺ patient groups is shown in Figure 4. Although there was no significant difference in overall pouchitis, there was a much higher incidence of chronic pouchitis in the HL-pANCA⁺ patient group versus the LL-pANCA⁺ patient group ($p=0.03$). There was no significant difference in the incidence of acute pouchitis between the two different pANCA⁺ level groups.

Within the 150 LL-pANCA⁺ patients, 121 patients were anti-CBir1⁻ and 29 patients were anti-CBir1⁺. Of the 21 HL-pANCA⁺ patients, 15 patients were anti-CBir1⁻ and 6 patients were anti-CBir1⁺. The association of pANCA level/anti-CBir1 expression and the incidence of AP and CP is shown in Table III. Anti-CBir1 positivity increased the incidence of AP in patients with LL-pANCA expression yet had no effect in patients with HL-pANCA positivity. Within the group of patients with high-level pANCA positivity, there was a significantly ($p \text{ trend}=0.02$) increased incidence of CP in patients who were also anti-CBir1⁺ (50%) versus those that were also anti-CBir1⁻ (20%). Anti-CBir1 seems to influence expression of CP but only in patients who express high-level pANCA.

Kaplan-Meier analysis was undertaken to assess the cumulative incidence of AP or CP among patients with different pANCA levels and CBir1 expression before colectomy. There was a significant association between the risk of developing AP among patients with both LL-pANCA and anti-CBir1 expression (Figure 5). This effect was statistically significant only

when comparing the LL-pANCA⁺/anti-CBir1⁺ patients versus the LL pANCA⁺/anti-CBir1⁻ patients ($p=0.04$), further demonstrating the influence of anti-CBir1 expression on AP development in patients with LL-pANCA positivity. The risk of developing CP after IPAA among patients with HL-pANCA before colectomy was significantly higher than in patients with LL-pANCA ($p=0.01$). In addition, CBir1 expression further increases the incidence of CP, but only in patients who have HL-pANCA expression (Figure 6).

DISCUSSION

Pouchitis is the most common complication after IPAA for UC (1,2). Acute pouchitis responds rapidly to oral antibiotic treatment. In a smaller number of patients, CP can develop and require long-term therapy with antibiotics or other agents (9). Acute pouchitis and CP share many clinical features. Both diseases are seen almost exclusively in IBD patients and rarely observed in patients with familial adenomatous polyposis (1,19,20). In addition, both AP and CP are significantly influenced by enteric bacteria as evidenced by clinical improvement in AP and most patients with CP after antibiotic or probiotic therapy or fecal stream diversion (5-7,9). However, there are several lines of evidence to suggest that AP and CP are distinct disease processes: 1) fecal stasis with bacteria overgrowth occurs in the pouch after IPAA (4,21) yet not every patient with an IPAA develops AP or CP; 2) CP usually appears much sooner after stoma closure than AP (22), a finding not confirmed in the current study; and 3) on a conceptual level, patients with AP appear to transiently react to endoluminal antigens while CP patients seem to have persistent inflammation to these same bacterial products. In the present study, we have shown that although both pANCA and anti-CBir1 expression are associated with pouchitis after IPAA, anti-CBir1 increases the incidence of AP only in patients who have low-level pANCA expression. On the other hand, anti-CBir1 increases the incidence of CP only in patients who have high-level pANCA expression. These findings demonstrate that AP and CP have different serologic risk factors for their development, reinforcing the concept that pouchitis is likely a heterogeneous inflammatory disorder based on different immunopathogenetic mechanisms. A better understanding of these processes may help gain insight into the etiology of pouchitis and even IBD itself, and perhaps provide possible therapeutic options for their treatment.

A number of studies have examined clinical risk factors for the development of pouchitis in UC patients. UC patients with EIMs, in particular primary sclerosing cholangitis, are clearly at risk for pouchitis after surgery (22-24). Some studies (25,26), but not all (27,28), suggest that patients with IC are also at a higher risk for pouchitis. Serologic factors have also been associated with pouchitis. In an early study of 95 patients, our group showed a strong association between patients with HL-pANCA expression before colectomy and the likelihood of developing CP after surgery (18). In the current study of over 200 patients, we again found a higher incidence of chronic pouchitis in the HL-pANCA patient group versus the LL-pANCA patient group, further validating the significant association between pANCA level and CP after IPAA.

Although antibody responses to microbial antigens have been associated with anatomic location and disease expression in patients with CD (29-31), little is known regarding the influence of these seromarkers on disease phenotype in patients with UC. A recent report from our group showed a higher incidence of antibody expression to the outer membrane porin C of *Escherichia coli* (anti-OmpC) in UC patients with a family history of CD versus those that have a family history of UC (32). The current study further unravels the interaction of these immune responses and disease expression in UC. The 19% incidence of anti-CBir1 positivity noted in the surgical UC cohort described herein is much higher than the 6% incidence of anti-CBir1 positivity noted in an unselected UC patient cohort (15). The underlying reasons underlying this observation are unclear. Perhaps anti-CBir1⁺ in UC patients is a marker for the

need for colectomy. The incidence of anti-CBir1⁺ was similar in UC compared to IC patients, suggesting that the relatively high frequency of anti-CBir1 is not related to an underlying misdiagnosis of CD. Phenotypic associations with immune response patterns need to be determined in a large cohort of unselected patients to fully assess the significance of anti-CBir1 expression in UC.

Flagellins are important molecular components of complex structures on bacterial surfaces involved with both adhesion and motility properties (33). CBir1 flagellin interacts with its toll-like receptor (TLR5) leading to activation of nuclear factor kappa-beta and the subsequent transcriptional induction of many proinflammatory cytokines (34,35). The detection of antibodies to CBir1 in patients with pouchitis should not be unexpected as there appears to be an association between both TLR allele carriage and mucosal TLR expression in patients with pouchitis (36,37).

Although there was a significant association between the risk of developing pouchitis among patients with both pANCA and anti-CBir1 expression, the most striking finding in this study was that different forms of pouchitis developed based on different pANCA and anti-CBir1 seroreactivity. The influence of anti-CBir1 expression on AP development was only observed in patients having LL-pANCA positivity. On the other hand, anti-CBir1 expression influenced the onset of CP only in patients having HL-pANCA expression. It is unclear why anti-CBir1 expression results in different disease expression (i.e., acute or chronic pouchitis) after IPAA, however the different immunologic responses to fecal bacteria in patients with LL-pANCA⁺ expression vs. HL-pANCA⁺ expression are most likely responsible. Experimental data and clinical observations suggest that fecal stasis and immune stimulation by stool bacteria play an important role in the development of pouchitis (38). Although bacteriologic studies have shown qualitative or quantitative differences in the stools of pouches with and without pouchitis (4,21), it is unknown if there are any differences in fecal bacteria between patients with AP and CP. Much work remains to further define the complex interactions between fecal pouch bacteria and the innate and adaptive immune systems.

It is possible that the hypothesis generating results of this study may reflect type 1 errors and must be independently validated in future studies. Despite this limitation, in the present study we have shown that patients who have distinct serologic patterns have different forms of pouch inflammation following IPAA. This finding again reinforces the theory that exaggerated immune responses to enteric bacterial antigens may be responsible for the development of pouchitis in IBD patients following IPAA. In addition, the immunopathogenetic mechanisms underpinning the pathogenesis of acute and chronic pouchitis may well be different.

References

1. Fazio VW, Ziv Y, Church JM, et al. Ileal pouch-anal anastomoses complications and function in 1005 patients. *Ann Surg* 1995;222:120–7. [PubMed: 7639579]
2. Meagher AP, Farouk R, Dozois RR, Kelly KA, Pemberton JH. J ileal pouch-anal anastomosis for chronic ulcerative colitis: complications and long-term outcome in 1310 patients. *Br J Surg* 1998;85:800–3. [PubMed: 9667712]
3. Shepherd NA, Hulten L, Tytgat GNJ, et al. Workshop: Pouchitis. *Int J Colorectal Dis* 1989;4:205–29. [PubMed: 2693561]
4. Kuisma J, Mentula S, Luukkonen P, et al. Factors associated with ileal mucosal morphology and inflammation in patients with ileal pouch-anal anastomosis for ulcerative colitis. *Dis Colon Rectum* 2003;46:1476–1483. [PubMed: 14605565]
5. Madden MV, McIntyre AS, Nicholls RJ. Double-blind crossover trial of metronidazole versus placebo in chronic unremitting pouchitis. *Dig Dis Sci* 1994;39:1193–96. [PubMed: 8200250]
6. Shen B, Achkar JP, Lashner BA, et al. A randomized trial of ciprofloxacin and metronidazole in treating acute pouchitis. *Inflamm Bowel Dis* 2001;7:301–5. [PubMed: 11720319]

7. Laake KO, Bjornekleit A, Aamodt G, et al. Outcome of four weeks' intervention with probiotics on symptoms and endoscopic appearance after surgical reconstruction with a J-configured ileal-pouch-anal-anastomosis in ulcerative colitis. *Scand J Gastroenterol* 2005;40:43–51. [PubMed: 15841713]
8. Kooros K, Katz AJ. Infliximab therapy in pediatric Crohn's pouchitis. *Inflamm Bowel Dis* 2004;10(4):417–20. [PubMed: 15475750]
9. Sandborn WJ. Pouchitis following ileal pouch-anal anastomosis: definition, pathogenesis, and treatment. *Gastroenterology* 1994;107:1856–60. [PubMed: 7958702]
10. Beaven SW, Abreu MT. Biomarkers in inflammatory bowel disease. *Curr Opin Gastroenterol* 2004;20:318–327. [PubMed: 15703659]
11. Seibold F, Brandwein S, Simpson S, Terhorst C, Elson CO. pANCA represents a cross-reactivity to enteric bacterial antigens. *J Clin Immunol* 1998;18:153–8. [PubMed: 9533659]
12. Eggena M, Cohavy O, Parseghian MH, et al. Identification of histone H1 as a cognate antigen of the ulcerative colitis-associated marker antibody pANCA. *J Autoimmun* 2000;14(1):83–97. [PubMed: 10648119]
13. Saxon A, Shanahan F, Landers C, et al. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *J Allergy Clin Immunol* 1990;86:202–210. [PubMed: 2200820]
14. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004;113:1296–1306. [PubMed: 15124021]
15. Targan SR, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, Vasilias E, Elson CO, Hershberg RM. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005;128:2020–2028. [PubMed: 15940634]
16. Munoz-Juarez M, Pemberton JH, Sandborn WJ, Tremaine WJ, Dozois RR. Misdiagnosis of specific cytomegalovirus infection of the ileoanal pouch as refractory idiopathic chronic pouchitis. *Dis Colon Rectum* 1999;42:117–20. [PubMed: 10211531]
17. Shen B, Fazio VW, Remzi FH, et al. Comprehensive evaluation of inflammatory and noninflammatory sequelae of ileal pouch-anal anastomosis. *Am J Gastroenterol* 2005;100:93–101. [PubMed: 15654787]
18. Fleshner PR, Vasilias E, Kam LY, et al. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. *Gut* 2001;49:671–7. [PubMed: 11600470]
19. Mahadevan U, Sandborn WJ. Diagnosis and management of pouchitis. *Gastroenterology* 2003;124:1636–50. [PubMed: 12761722]
20. Dayton MT, Faught WE, Becker JM, Burt R. Superior results of ileoanal pullthrough (IAPT) in polyposis coli vs ulcerative colitis patients. *J Surg Res* 1992;52:131–4. [PubMed: 1311047]
21. Shepherd NA, Hulten L, Tytgat GNJ, et al. Workshop: Pouchitis. *Int J Colorectal Dis* 1989;4:205–29. [PubMed: 2693561]
22. Achkar JP, Al-Haddad M, Lashner B, et al. Differentiating risk factors for acute and chronic pouchitis. *Clin Gastroenterol Hepatol* 2005;3:60–6. [PubMed: 15645406]
23. Penna C, Dozis R, Tremaine W, et al. Pouchitis after ileal pouch-anal anastomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. *Gut* 1996;38:234–9. [PubMed: 8801203]
24. Lohmuller JL, Pemberton JH, Dozois RR, Ilstrup D, van Heerden J. Pouchitis and extraintestinal manifestations of inflammatory bowel disease after ileal pouch-anal anastomosis. *Ann Surg* 1990;211:622–7. [PubMed: 2339922]
25. Atkinson KG, Owen DA, Wankling G. Restorative proctocolectomy and indeterminate colitis. *Am J Surg* 1994;167:516–8. [PubMed: 8185040]
26. Bodzin JH, Klein SN, Priest SG. Ileoproctostomy is preferred over ileoanal pull-through in patients with indeterminate colitis. *Am Surg* 1995;61:590–3. [PubMed: 7793739]
27. Yu CS, Pemberton JH, Larson D. Ileal pouch-anal anastomosis in patients with indeterminate colitis: long-term results. *Dis Colon Rectum* 2000;43:1487–96. [PubMed: 11089581]

28. Rudolph WG, Uthoff SM, McAuliffe TL, Goode ET, Petras RE, Galandiuk S. Indeterminate colitis: the real story. *Dis Colon Rectum* 2002;45:1528–34. [PubMed: 12432302]
29. Vasilias EA, Kam LY, Karp LC, et al. Marker antibody expression stratifies Crohn's disease into immunologically homogeneous subgroups with distinct clinical characteristics. *Gut* 2000;47:487–96. [PubMed: 10986208]
30. Mow WS, Vasilias EA, Lin YC, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004;126:414–424. [PubMed: 14762777]
31. Papadakis KA, Yang H, Ippoliti A, et al. Anti-flagellin (CBir1) phenotypic and genetic Crohn's disease associations. *Inflamm Bowel Dis* 2007;13:524–530. [PubMed: 17260364]
32. Ling M, Targan SR, Landers CJ, et al. Familial expression of anti-*Escherichia coli* outer membrane porin C in relatives of patients with Crohn's disease. *Gastroenterology* 2006;130:1078–1085. [PubMed: 16618402]
33. Ramos HC, Rumbo M, Sirard JC. Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. *Trends in Microbiology* 2004;12:509–17. [PubMed: 15488392]
34. Hayashi F, Smith KD, Ozinsky A, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001;410:1099–103. [PubMed: 11323673]
35. Means TK, Hayashi F, Smith KD, et al. The Toll-like receptor 5 stimulus bacterial flagellin induces maturation and chemokine production in human dendritic cells. *J Immunol* 2003;170:5165–75. [PubMed: 12734364]
36. Lammers KM, Ouburg S, Morre SA, et al. Combined carriership of TLR9-1237C and CD-14-260T alleles enhances the risk of developing chronic relapsing pouchitis. *W J Gastroenterol* 2005;11:7323–29.
37. Toiyama Y, Araki T, Yoshiyama S, et al. The expression patterns of toll-like receptors in the ileal pouch mucosa of postoperative ulcerative colitis patients. *Surg Today* 2006;36:287–290. [PubMed: 16493544]
38. Sartor RB, Rath HC, Sellon RK. Microbial factors in chronic intestinal inflammation. *Curr Opin Gastroenterol* 1996;12:327–333.

Figure 1.

Anti-CBir1 was expressed in 19% of patients. Lines indicate the median level for each group. The percentage of positive samples defined as >2 SD above the mean of the normal controls for each group is shown.

Figure 2.

Relationship between pANCA and anti-CBir1 seroreactivity and the overall incidence of pouchitis. There was a significantly higher incidence of pouchitis noted in pANCA⁺ patients versus pANCA⁻ patients (*p=0.005). Anti-CBir expression was also associated with overall pouchitis development (**p=0.02).

Figure 3.

Association between pANCA/anti-CBir1 positivity and the incidence of pouchitis. The overall incidence of pouchitis (■) rose significantly with increased seromarker positivity (p trend=0.0003). There was also a significant direct association between acute pouchitis (□) development (p trend=0.007) but not chronic pouchitis (▒) development (p trend=0.05) with increasing seromarker positivity. Zero, one or two represents number of seromarkers expressed. Patient numbers are italicized along the x axis.

Figure 4.

Relationship between pANCA level and pouchitis. There was a higher incidence of chronic pouchitis in patients with high-level pANCA expression (> 100 ELISA units/ml) compared to low-level pANCA expression (*p=0.03).

Figure 5.

Effect of anti-CBir1 expression and pANCA level on the cumulative incidence of acute pouchitis. The risk of developing acute pouchitis after IPAA among patients with low-level pANCA (LL-pANCA+) was significantly ($p=0.04$) higher in patients who were also anti-CBir1⁺ (●) versus patients who were also anti-CBir1⁻ (○). There was no effect of anti-CBir1 on the incidence of acute pouchitis in patients with high-level pANCA (HL-pANCA+) expression.

Figure 6.

Effect of anti-CBir1 expression and pANCA level on the cumulative incidence of chronic pouchitis. The risk of developing chronic pouchitis after IPAA among patients with high-level pANCA (HL-pANCA+) was significantly ($p=0.04$) higher in patients who were also anti-CBir1⁺ (●) versus patients who were also anti-CBir1⁻ (○). There was no effect of anti-CBir1 on the incidence of chronic pouchitis in patients with low-level pANCA (LL-pANCA+) expression.

Table 1

Study Patient Characteristics

Sex (M/F)	137/101
Median age (range)	38 years (8-81)
Median disease duration (range)	60 months (1-480)
Disease extent	
Pancolitis	174
Left-sided	61
Proctitis	3
Preoperative medication	
Steroids	34
Immunomodulators	165
Extraintestinal disease	40
Arthritis	26
PSC	5
Other	9
Backwash ileitis	24
Family history of IBD	57
Type of Colitis	
Ulcerative colitis	168
Indeterminate colitis	70
Indication for IPAA	
Medically unresponsive	199
Cancer/Dysplasia	39

PSC, primary sclerosing cholangitis
IPAA, ileal pouch anal anastomosis
IBD, inflammatory bowel disease

Table II
Patient Characteristics in the Antibody Positive and Negative Subgroups

	pANCA+ (n=171)	pANCA-negative (n=67)	Anti-CBir1+ (n=46)	Anti-CBir1-negative (n=192)
Sex (M/F)	97/74	40/27	34/12*	103/89
Median age (years)	38	37	38	38
Median disease duration (months)	60	60	66	60
Disease extent (%)				
Pancolitis	73	73	83	71
Left-sided	25	27	17	28
Proctitis	2	0	0	2
Preoperative medication (%)				
Steroids	14	12	9	15
Immunomodulators	69	73	74	69
Extraintestinal disease (%)				
PSC	3	0	7	1
Other	15	13	4	17
Backwash ileitis (%)	12	6	11	10
Smoking history (%)	32	27	37	29
Family history of IBD (%)	24	24	26	23
Type of Colitis (%)				
Ulcerative colitis	70	73	65	72
Indeterminate colitis	30	27	35	28
Indication for IPAA (%)				
Medically unresponsive	83	85	83	84
Cancer/Dysplasia	17	15	17	16

* p=0.03 vs anti-CBir1-negative

Table III
Association of pANCA+ Level and anti-CBirI Expression and Pouchitis Development

pANCA ⁺ Level / anti-CBirI Expression	n	Acute Pouchitis	Chronic Pouchitis ⁺
LL-pANCA ⁺ / anti-CBirI ⁻	121	22 (18) [*]	14 (12)
LL-pANCA ⁺ / anti-CBirI ⁺	29	11 (38) [*]	4 (14)
HL-pANCA ⁺ / anti-CBirI ⁻	15	3 (20)	3 (20)
HL-pANCA ⁺ / anti-CBirI ⁺	6	1 (17)	3 (50)

⁺ p trend = 0.02
^{*} p = 0.03
LL low-level (< 100 EU/ml)
HL high-level (> 100 EU/ml)
All numbers in parentheses denote percentage