Journal of Crohn's and Colitis (2013) 7, e522–e532

Available online at www.sciencedirect.com

ScienceDirect

Antibodies against glycoprotein 2 are novel markers of intestinal inflammation in patients with an ileal pouch

Lael Werner [a,b], Andreas Sturm [c], Dirk Roggenbuck [d,e], Lior Yahav [a,b], Tanir Zion [a], Elhanan Meirowithz [a], Amos Ofer [a,b], Hanan Guzner-Gur [b,f], Hagit Tulchinsky [b,g,1], Iris Dotan [a,b,*,1]

[a] *IBD Center, Department of Gastroenterology and Liver Diseases, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel*
[b] *Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel*
[c] *Department of Internal Medicine and Gastroenterology, Krankenhaus Waldfriede, Berlin, Germany*
[d] *Medipan GmbH, Dahlewitz, Berlin, Germany*
[e] *Faculty of Natural Sciences, Lausitz University of Applied Sciences, Senftenberg, Germany*
[f] *Department of Internal Medicine B, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel*
[g] *Department of Surgery, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel*

Received 12 November 2012; received in revised form 2 March 2013; accepted 20 March 2013

KEYWORDS:
Pouchitis
Glycoprotein 2
Anti-GP2
Serological markers
IPAA

Abstract

Background and aims: The Crohn's disease (CD)-specific pancreatic auto-antibodies (PAB), have been recently identified to target glycoprotein 2 (GP2). Pouchitis is an inflammation of the small bowel developing in up to 60% of ulcerative colitis patients undergoing proctocolectomy and ileal pouch anal anastomosis. Occurrence of CD-specific antibodies was reported to be a predictor of pouchitis. We aimed to assess the prevalence of anti-GP2 antibodies (anti-GP2) in the serum and feces of pouch patients and to correlate them with clinical parameters. Furthermore, we examined mucosal expression of the GP2 protein in the pouch.
Methods: Pouch patients were prospectively recruited and checked for clinical, endoscopic, and laboratory markers of inflammation. IgG and IgA anti-GP2 levels in serum and fecal samples were determined using ELISA. GP2 protein was assessed by immunohistochemistry.
Results: Anti-GP2 was elevated in both serum and fecal samples of patients with inflamed compared to those with non-inflamed pouches and patients with familial-adenomatous polyposis after surgery ($p < 0.05$, respectively). Moreover, patients with CD-like complications exhibited

Abbreviations: ACCA, anti-chitobioside carbohydrate antibodies; ALCA, anti-laminaribioside carbohydrate antibodies; AMCA, anti-mannobioside carbohydrate antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; CD, Crohn's disease; CRP, C-reactive protein; FAP, familial adenomatous polyposis; GP2, glycoprotein 2; IBD, inflammatory bowel diseases; IPAA, ileal pouch anal anastomosis; PAB, pancreatic (auto)-antibody; PDAI, pouchitis disease activity index; UC, ulcerative colitis.

* Corresponding author at: 6 Weizmann St., Tel Aviv, 64239, Israel. Tel.: +972 3 6947305; fax: +972 3 6974184.
E-mail addresses: wernerlael@gmail.com (L. Werner), a.sturm@waldfriede.de (A. Sturm), dirk.roggenbuck@hs-lausitz.de (D. Roggenbuck), liory@tasmc.health.gov.il (L. Yahav), tanirz@tasmc.health.gov.il (T. Zion), elhanan.me@gmail.com (E. Meirowithz), amos.ofer@gmail.com (A. Ofer), hanang@tasmc.health.gov.il (H. Guzner-Gur), hagitt@tasmc.health.gov.il (H. Tulchinsky), irisd@tasmc.health.gov.il (I. Dotan).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.crohns.2013.03.009

significantly higher anti-GP2 titers than those without CD-like complications ($p \leq 0.01$). High levels of anti-GP2 correlated with more frequent bowel movements per day and with the presence of at least one anti-glycan antibody ($p \leq 0.05$). GP2 itself was more abundant in the mucosa of patients with chronic pouchitis.

Conclusions: Anti-GP2 exists in the serum and feces of pouch patients and correlates with pouch inflammation, and presence of other serological markers. Thus, anti-GP2 may contribute to better stratification of pouchitis, more-so when the inflammation exhibits CD-like complications.

1. Introduction

Inflammatory bowel diseases (IBD), comprising of Crohn's disease (CD) and ulcerative colitis (UC), are chronic diseases affecting millions worldwide with an increasing incidence.[1] Serological markers, such as anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibody (pANCA); as well as the more recently reported anti-glycan antibodies anti-laminaribioside, anti-chitobioside, and anti-mannobioside carbohydrate antibodies (ALCA, ACCA, and AMCA respectively)[2,3] are widely utilized for the serological diagnosis and stratification of IBD patients.[4,5] Pancreatic (auto) antibody (PAB) is another serological marker which was reported to be more prevalent in CD[6–9] more than two decades ago.[10] Its antigenic target was recently identified as glycoprotein 2 (GP2).[11] It is still unknown why anti-GP2 auto-antibodies (anti-GP2) are elevated in patients with CD and their association with CD behavior and activity is controversial.[12] However, they seem to be associated with the clinical phenotype of CD; particularly with early onset, ileocolonic location, and stricturing behavior.[13]

Approximately 25% of patients with UC will undergo large bowel resection (total proctocolectomy) due to refractory disease or severe clinical complications.[14,15] Most will undergo a restorative surgery-ileal pouch anal anastomosis (IPAA, pouch surgery), whereby a reservoir is created from the terminal ileum and connected to the anal canal to maintain bowel continuity. Up to 60% of patients after pouch surgery may develop inflammation of the small bowel within or proximal to the pouch, termed pouchitis and pre-pouch ileitis, respectively.[16] The etiology of pouchitis is presently unknown and, similar to IBD, it can be categorized according to activity and behavior (reviewed in [17]).

As pouchitis develops in the small bowel of these UC patients after pouch surgery, which by definition did not suffer from small bowel inflammation, and this pouchitis may be associated with CD-like complications, we hypothesized that pouchitis may be a model for the development of *de-novo* small bowel inflammation, similar to the one observed in CD. Occurrence of CD-specific antibodies was previously reported to be associated with pouchitis, particularly in combination with the development of CD-like complications.[18,19] However, no data regarding the prevalence of antibodies against GP2 in pouch patients have been reported yet and their potential significance in pouch inflammation is still elusive.

In the current study, we assessed the appearance of anti-GP2 in patients after pouch surgery, particularly in those with more aggressive disease phenotypes. Thus, we investigated pouch patients with different disease behavior and searched for anti-GP2 in their sera and feces. Correlation of anti-GP2 levels with inflammatory activity and clinical parameters was also examined as was the mucosal expression of the GP2 protein in the pouch.

2. Methods

2.1. Patients

Patients were prospectively recruited at the comprehensive pouch clinic at the Tel Aviv Medical Center.[20] Disease activity was defined according to the pouchitis disease activity index (PDAI) score[21,22]; and determined using a combination of clinical, endoscopic, and histological parameters assessed within one month from time of sample attainment. Pouchitis is diagnosed when the PDAI is ≥ 7. When endoscopic and histological scores were unavailable; the clinical sub-score was used to determine disease activity. Importantly, when the patients did not have a complete PDAI, we either utilized stool cultures to exclude infections, or CRP levels to support inflammatory activity. Disease behavior was stratified to normal pouches, patients with acute, recurrent acute and chronic pouchitis, based on previous definitions[17,20], as detailed in Table 1.

Briefly, patients with a normal pouch were defined as those not reporting an increase in bowel movements above their postoperative norm, or the appearance of abdominal pain, fever or rectal bleeding within the 2 years previous to recruitment, and not using any antibiotic, probiotic, or anti inflammatory therapy. Acute pouchitis was defined in patients exhibiting symptoms such as diarrhea and/or abdominal cramps, rectal bleeding, and/or fever, with a PDAI > 7, responding to a short course (up to 2 weeks) of antibiotic therapy. Recurrent acute pouchitis was diagnosed when antibiotic-responsive acute pouchitis patients had repeated episodes of flares (up to 4 per year). Chronic pouchitis was defined in patients requiring antibiotic or anti-inflammatory therapy for at least 4 weeks, or patients having more than 5 flares of pouchitis per year. In order to exclude the possibility that response to antibiotics is due to infectious diarrhea, we use two approaches. First — patients have a stool culture in at least one attack of diarrhea exacerbation. Moreover, stool samples of respective patients were subjected to 16S pyrosequencing analysis. The presence of Shigella, Salmonella or Campylobacter, was not detected in our patients, further supporting non-infectious causes for diarrhea. For reasons of stringency we used pouch endoscopy and histology data, obtained within a month of sample attainment, to define pouchitis activity based on the PDAI. These were available for