A Controlled Study of Colonic Immune Activity and β7+ Blood T Lymphocytes in Patients With Irritable Bowel Syndrome

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Background & Aims: **The mechanisms behind irritable bowel syndrome (IBS) are incompletely understood. Recently several studies have suggested a low-grade colonic inflammation as initiator of the gut dysfunctions recorded in this patient group. The aim of this study was to characterize the phenotype and homing properties of colonic and peripheral blood lymphocytes in patients with IBS.** ***Methods:*** **Patients with IBS (n = 33), defined by the Rome II criteria, were compared with UC patients (n = 23) and control subjects (n = 15) without gastrointestinal symptoms. Colonic and peripheral blood lymphocytes were analyzed by flow cytometry. Secretion of IFN-γ from intestinal biopsies was determined by enzyme-linked immunosorbent assay, and immunohistochemical staining of colonic biopsies was performed.** ***Results:*** **IBS patients displayed an increased frequency of peripheral blood $CD4^+$ and $CD8^+$ T cells expressing the gut homing integrin β7. Accordingly, IBS and UC patients had an augmented frequency of lamina propria $CD8^+$ T cells in the ascending colon as compared with control subjects. The frequency of intestinal T cells expressing integrin $\beta7^+$ was unaltered in IBS and UC patients, although the expression of mucosal addressin cell adhesion molecule–1^+ endothelium, the ligand for integrin β7, was increased in the ascending colon of IBS and UC patients as compared with control subjects.** ***Conclusions:*** **Patients with IBS exhibit an enhanced immune activity in the gut and an increased frequency of integrin $\beta7^+$ T lymphocytes in the peripheral blood. Our data further support the hypothesis of IBS being at least partially an inflammatory disorder.**

Irritable bowel syndrome (IBS) is a common bowel disorder in Western society[1] and is defined according to the Rome II criteria.[2] Patients suffer from chronic abdominal pain, bloating, and altered bowel habits, despite the absence of identifiable organic disease explaining the sometimes severe symptoms. No ubiquitous etiologic mechanisms have so far been identified, although abnormal gastrointestinal motility,[3,4] visceral hypersensitivity,[5,6] altered colonic fermentation,[7] abnormal gastrointestinal responses to stress,[8] and nutrients[9] are factors that might contribute to symptom generation.

Recently, it has been proposed that a low-grade inflammatory process in various compartments of the small and the large bowel is associated with gut dysfunction in at least a subpopulation of IBS patients.[10,11] Different factors such as genetic susceptibility, altered gut flora, unrecognized food reactions, and infectious enteritis have all been suggested as possible mediators of an inflammatory response. IBS patients have been found to be genetically predisposed to produce low amounts of interleukin-10[12] and have increased levels of the proinflammatory cytokine interleukin-1β mRNA after infectious gastroenteritis when compared with non-IBS gastroenteritis patients.[13] Studies have also shown that patients with postinfectious IBS have an activated intestinal immune response, as defined by increased numbers of T cells and macrophages in the colonic mucosa.[14,15] Moreover, one of the most frequently recognized immunologic features of IBS is increased numbers of mast cells in colonic lamina propria (LP) and muscularis propria.[16–19] Indeed, high numbers of activated mast cells have been found in close proximity to nerves in the colonic mucosa, with a numeric correlation to abdominal pain perception.[16] Food reactions might also contribute to the immune activation, because some patients with IBS symptoms benefit from specific elimination diets.[20]

During intestinal inflammatory conditions, increased numbers of lymphocytes are recruited to the intestinal mucosa, partly by binding of selectins and integrins on the cell surface to addressins expressed on endothelial

Abbreviations used in this paper: ELISA, enzyme-linked immunosorbent assay; IBS, irritable bowel syndrome; IFN-γ, interferon-gamma; LP, lamina propria; LPL A, lamina propria lymphocytes of the ascending colon; LPL S, lamina propria lymphocytes of the sigmoid colon; MAdCAM-1, mucosal addressin cell adhesion molecule–1; PBLs, peripheral blood lymphocytes; UCa, ulcerative colitis in exacerbation; UCr, ulcerative colitis in remission.

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tissues. Homing of circulating blood lymphocytes to the gut mucosa is largely mediated by the expression of the $\alpha 4\beta 7$ integrin that binds to its ligand, mucosal addressin cell adhesion molecule–1 (MAdCAM-1), which is constitutively expressed by intestinal LP venules and Peyer's patch high endothelial venules.[21,22] UC patients have been reported to display an altered frequency of integrin $\beta 7^+$ T cells in blood and rectal mucosa,[23] but the gut homing pattern of lymphocytes in IBS has not previously been studied.

Overall, a low inflammatory response in the intestinal mucosa is acknowledged in IBS, although the underlying mechanisms and various cell populations driving this immunologic response need to be further clarified. Therefore, the aim of this study was to determine and compare phenotype and homing properties of lymphocytes isolated from ascending and sigmoid colonic mucosa and peripheral blood in patients with IBS, UC patients, and control subjects.

Materials and Methods

Subjects

This study was approved by the Human Research Ethical Committee of the Medical Faculty, Göteborg University, and was performed after receiving written informed consent from all subjects. Study subjects were recruited among patients referred for colonoscopy at the Sahlgren's University Hospital, Göteborg, Sweden. Individuals with known celiac disease or food allergy were excluded from the study. Venous blood samples and 8 biopsies from the ascending (5 cm distal to the ileocecal valve) and the sigmoid colon, respectively, were assessed in each patient. IBS patients were defined according to the Rome II criteria[2] and underwent routine diagnostic colonoscopy to exclude organic bowel disease. Biopsies were defined as noninflammatory by routine histology. Specially, no evidence for collagenous or lymphocytic colitis was detected on the basis of standard criteria.[24] Thirty-three IBS patients were included (19 women; mean age, 42 ± 12 years; body mass index, 25.7 ± 5.2 kg/m^2). Twenty patients had diarrhea-predominant IBS, 4 had constipation-predominant IBS, and 9 had alternating bowel habits.[2] One of the IBS patients had less than 1-year duration of symptoms, 16 of the patients had a symptom duration of 1–5 years, and 16 of the patients included reported IBS symptoms for more than 5 years. Acute onset after supposed infectious diarrhea was described by 4 diarrhea-predominant IBS patients and 1 constipation-dominant IBS patient. Twenty-three UC patients, all with a well-defined disease (10 women; mean age, 42 ± 11 years; body mass index, 24.7 ± 3.2 kg/m^2), underwent colonoscopy for evaluation of disease activity and dysplasia surveillance. Nine of the UC patients had an active inflammation, of which 5 had total colitis, and 4 had left-sided colitis. Four of the UC patients had less than 1-year disease duration, 4 of the patients had a duration time of 1–5 years, whereas 15 of the patients reported disease symptoms for more than 5 years. Patients with active UC were included as positive inflammatory controls, and UC patients in remission were also studied because these patients often report IBS-like symptoms.[25]

As control subjects, we included 15 individuals (7 women; mean age, 53 ± 8 years; body mass index, 23.4 ± 2.6 kg/m^2) who underwent colonoscopy for investigation of anemia, rectal bleeding, or polyp surveillance. These subjects were free from gastrointestinal symptoms, and the colonoscopy was normal, both macroscopically and microscopically. The control subjects were significantly older than patients with IBS ($P = .008$) and UC ($P = .008$), but age did not differ significantly between the patient groups.

Isolation of Intestinal and Peripheral Blood Lymphocytes

LP lymphocytes were isolated as previously described in detail,[26] and peripheral blood lymphocytes (PBLs) were isolated from venous blood by density-gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden).

Flow Cytometric Analysis

Freshly isolated cells, 1×10^5 cells/sample, were stained for flow cytometry analysis of various surface markers by using combinations of the following antibodies: anti-CD4-PerCP, anti-CD8-APC, anti-CD45RA-FITC, anti-Integrinβ7-PE (BD Pharmingen, San Diego, CA). All cells were fixed in cellfix (BD Pharmingen) before fluorescence-activated cell sorter analysis, which was performed by using an LSR II (BD Pharmingen). At least 10,000 live lymphocytes per sample were analyzed, as defined by forward and side scatter. The data were analyzed by using Flow Jo software (Treestar Inc, Ashland, OR). Samples from 19 IBS patients, 13 UC patients, and 8 control subjects were used for fluorescence-activated cell sorter analysis.

Immunohistochemical Analysis

Endothelial cells were detected by staining against factor VIII (DakoCytomation; Glostrup, Denmark), and the endothelial expression of the addressin MAdCAM-1[21] was detected by using a specific monoclonal antibody (Bender Medsystems, Vienna, Austria). Serial tissue sections were stained with either factor VIII or MAdCAM-1. After blocking of endogenous peroxidase activity with .3% H_2O_2 in PBS, cryo-cut sections from biopsies were incubated with monoclonal antibodies against factor VIII or MAdCAM-1 in PBS with 5% human serum for 3 hours at room temperature. The reaction was developed by stepwise addition of biotinylated rat-anti-mouse immunoglobulin G antibody (BD Pharmingen) and an avidin–biotin–horseradish peroxidase complex (DakoCytomation) according to the manufacturer's instructions. Tissue sections were developed with chromogen 3-amino-9-ethylcarbazole containing H_2O_2(AEC substrate chromogen) (DakoCytomation) and counterstained with Mayer's hematoxylin (Histolab, Göteborg, Sweden). Slides were dehy-