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# Vasoactive Intestinal Polypeptide and Mast Cells Regulate Increased Passage of Colonic Bacteria in Patients With Irritable Bowel Syndrome

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**BACKGROUND & AIMS:** Irritable bowel syndrome (IBS) is associated with intestinal dysbiosis and symptoms of IBS develop following gastroenteritis. We aimed to study the passage of live bacteria through the colonic epithelium, and determine the role of mast cells (MCs) and vasoactive intestinal polypeptide (VIP) in barrier regulation in IBS and healthy individuals. **METHODS:** Colon biopsies from 32 women with IBS and 15 age-matched healthy women (controls) were mounted in Ussing chambers; we measured numbers of fluorescently labeled *Escherichia coli* HS and *Salmonella typhimurium* that passed through from the mucosal side to the serosal side of the tissue. Some biopsies were exposed to agents that block the VIP receptors (VPAC1 and VPAC2) or MCs. Levels of VIP and tryptase were measured in plasma and biopsy lysates. Number of MCs and MCs that express VIP or VIP receptors were quantified by immunofluorescence. Biopsies from an additional 5 patients with IBS and 4 controls were mounted in chambers and Salmonella were added; we studied passage routes through the epithelium by transmission electron microscopy and expression of tight junctions by confocal microscopy. **RESULTS:** In colon biopsies from patients with IBS, larger numbers of *E coli* HS and *S typhimurium* passed through the epithelium than in biopsies from controls ($P < .0005$). In transmission electron microscopy analyses, bacteria were found to cross the epithelium via only the transcellular route. Bacterial passage was reduced in biopsies from patients with IBS and controls after addition of antibodies against VPACs or ketotifen, which inhibits MCs. Plasma samples from patients with IBS had higher levels of VIP than plasma samples from controls. Biopsies from patients with IBS had higher levels of tryptase, larger numbers of MCs, and a higher percentage of MCs that express VPAC1 than biopsies from controls. In biopsies from patients with IBS, addition of Salmonella significantly reduced levels of occludin; subsequent addition of ketotifen significantly reversed this effect. **CONCLUSIONS:** We found that colonic epithelium tissues from patients with IBS have increased translocation of commensal and pathogenic live bacteria compared with controls. The mechanisms of increased translocation include MCs and VIP.

*Keywords:* Intestinal Permeability; Bacteria; Ketotifen; Inflammation.

Irritable bowel syndrome (IBS) is characterized by chronically recurring abdominal pain and disturbed bowel habits and affects 10%–15% of the general population in industrialized countries, with a 2:1 female predominance.[1] Even though the pathophysiology is not completely understood, increasing evidence supports the concept of altered brain-gut microbiome interactions in IBS etiology.[2,3]

For many years it has been known that IBS-like symptoms develop following an acute gastroenteritis in about 10% of affected individuals.[4,5] Specifically, it has been confirmed in several studies that enteric infection with *Salmonella*, such as the foodborne pathogenic gram-negative *Salmonella typhimurium,* is a significant risk factor for the development of IBS.[6,7] Evidence also suggests that IBS can be associated with an altered gut microbiota composition or dysbiosis,[8] including the presence of *Escherichia coli.*[9,10] Some of the observed changes in species richness or community composition may reflect alterations in gastrointestinal transit.[11] There is increasing evidence demonstrating enhanced intestinal permeability and altered tight junction patterns[12–14] both in the colon and small bowel of IBS.[15–19]

In intestinal barrier function studies, IBS patients typically have been divided into subgroups based on predominant bowel habit (IBS-diarrhea [IBS-D], IBS-constipation [IBS-C], IBS-mixed [IBS-M]),[20] but the association between bowel habit subtype and mucosal barrier function remains unclear.[15,21] There is some evidence that visceral hypersensitivity, a common feature in IBS, may be linked to disturbances in barrier function,[22] and such changes have been observed in an animal model of gastrointestinal infection.[23]

***Abbreviations used in this paper:*** **$^{51}$Cr-EDTA, $^{51}$chromium (Cr)-EDTA; *Escherichia coli*, *E coli*; HCs, healthy controls; IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with mixed stool consistency; $I_{sc}$, short-circuit current; MCs, mast cells; *Salmonella typhimurium*, *S typhimurium*; SEM, scanning electron microscopy; TER, transepithelial resistance; TEM, transmission electron microscopy; VIP, vasoactive intestinal polypeptide; VPAC1, VIP receptor type 1; VPAC2, VIP receptor type 2.**

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**EDITOR'S NOTES**

BACKGROUND AND CONTEXT

Irritable bowel syndrome (IBS) is associated with intestinal dysbiosis and IBS symptoms can develop following gastroenteritis. The role of intestinal barrier function in the pathophysiology of IBS remains unclear.

NEW FINDINGS

Colonic biopsies from patients with IBS have increased translocation of commensal and pathogenic live bacteria compared with healthy controls. This barrier dysfunction appears to involve mast cells and vasoactive intestinal polypeptide.

LIMITATIONS

Since the IBS patients in the present study had mainly severe symptoms, results are inconclusive for patients with milder disease.

IMPACT

The findings of an enhanced translocation of live bacteria and mucosal immune activation in colonic biopsies provide evidence for a dysfunctional intestinal barrier in IBS.

The gut barrier function is controlled by several physiological mechanisms. Vasoactive intestinal polypeptide (VIP) is a neuropeptide found in both immune cells, including lymphocytes[24] and mast cells (MCs),[25] and in enteric neurons of the gastrointestinal tract. These neurons innervate the intestinal epithelium and regulate ion and fluid secretion, as well as epithelial homeostasis.[26,27] A few studies have also implicated VIP in the regulation of intestinal permeability.[28–30] Our group previously showed that MCs and VIP regulate the ileal barrier of healthy humans and during stress in rats via VIP-receptors (VPAC1/VPAC2) on MCs.[25] Increased plasma levels of VIP have been reported in IBS patients[31] and in an IBS-animal model,[32] and there appears to be a positive correlation between an increased intestinal transcellular permeability and amount of mucosal MCs in IBS-D.[33] In addition, increased MC numbers and tryptase levels have been found in biopsies of caecum[34] and jejunum[35] of IBS patients, and increased paracellular permeability and higher MC numbers were demonstrated in colon of IBS-D patients.[18] To our knowledge there are no reports exploring the intestinal epithelial response to live bacteria and its regulation by VIP and MCs in the colonic mucosa of IBS subjects.

The aims of the study were to investigate (1) the passage of live commensal and pathogenic microbes through the colonic mucosa of women with IBS; (2) the role of MCs and VIP in the regulation of bacterial passage; and (3) the distribution of MCs, VIP/VPACs in the colon of IBS subjects. We present evidence of increased bacterial passage through the colonic mucosa in IBS, with MCs and VIP involved as modulating factors. Our findings indicate a potential link between altered intestinal barrier function and the increased transepithelial passage of bacteria in IBS, and suggest an involvement of VIP and MCs in this mechanism.

# Material and Methods

## Subjects and Endoscopy

**Subjects.** Thirty-seven women with IBS, mean age 32.2 years (range, 19–55 ears), meeting Rome III criteria were recruited from the Gastroenterology Department, University Hospital, Linköping, Sweden. IBS patients had a moderate-severe IBS with mean symptom severity score of 347 (range, 167–480).[36] Twenty healthy age-matched women, mean age 29.9 years (range, 20–48), without medical history of gastrointestinal symptoms or complaints, were recruited as healthy controls (HCs). IBS subjects were classified according to predominant bowel habit into IBS-M (n=21), IBS-C (n=8), and IBS-D (n=8). Exclusion criteria for both groups included self-reported allergy, organic gastrointestinal disease, metabolic or neurologic disorders, and nicotine or NSAID intake. The committee of human ethics, Linköping, approved the study and all subjects gave their written informed consent.

**IBS subgroups.** IBS patients were subgrouped based on the predominant stool consistency according to the Rome III questionnaire.[20]

**Sigmoidoscopy.** A flexible sigmoidoscopy was performed after 8 hours of fasting without sedation and with scope insertion approximately 30–40 cm orally from linea dentata. Colonic biopsies were taken with a biopsy forceps without a central lance and placed directly into ice-cold oxygenated Krebs buffer.[37] Prior to sigmoidoscopy, venous blood samples were collected for measurements of VIP concentrations.

## Ussing Chamber Experiments

**Barrier function studies.** Colonic biopsies from 32 IBS patients and 15 HCs were mounted in Ussing chambers[38] as previously described.[37] After 20 minutes biopsies were treated with 1 $\mu$mol/L anti-VPAC, 1 $\mu$mol/L of the MC-blocker ketotifen, or vehicle (Krebs). After another 20 minutes, 34 $\mu$Ci/mL of the paracellular probe $^{51}$chromium (Cr) was added to the mucosal side of each chamber. Live green fluorescent protein-labelled *E coli* HS or *S typhimurium*, prepared as previously described,[37] was added to the mucosal sides of separate chambers at a final concentration of $10^8$ CFU/mL. Serosal samples were collected and bacterial and $^{51}$Cr-EDTA passage was measured. The transepithelial potential difference, short-circuit current ($I_{sc}$), and the transepithelial resistance (TER) across the tissues were monitored throughout the experiments to ensure tissue viability.

**Electron microscopy.** To identify bacterial translocation and MC ultrastructure, colonic biopsies from 5 IBS patients and 4 HCs were mounted in Ussing chambers and exposed to bacteria. Anti-VPACs or ketotifen was added as described earlier, followed by addition of green fluorescent protein–labelled *S typhimurium*. Krebs was added to 1 biopsy as control to evaluate the eventual effects on tissue structure during incubation. After 30 minutes, biopsies were fixed and processed following standard protocols for scanning and transmission electron microscopy (SEM, TEM). MC-degranulation was also quantified in basal biopsies, not mounted in Ussing chambers, from 20 IBS patients and 8 HCs.

**Quantification of tight junctions.** To study the effects of *S typhimurium*, anti-VPACs and ketotifen on tight junctions, biopsies from 4 IBS patients and 4 HCs were mounted in Ussing chambers and added anti-VPACs, ketotifen and *S typhimurium*. After 30 minutes, biopsies were fixed in the chambers and prepared for immunofluorescence of tight junctions (see below).