Immune Activation and Gut Microbes in Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a highly prevalent disorder that is characterized by chronic abdominal pain and altered bowel habit. The diagnosis of IBS has traditionally been made by matching the complaints of the patient with established clinical criteria, since the underlying pathophysiology was not known. Various new findings have recently been reported in IBS patients that challenge our concept of IBS as a syndrome with no explanation. While the florid inflammation characteristic of inflammatory bowel disease is absent in IBS, changes suggesting immune activation are present in nearly all IBS patients. Is IBS an autoimmune disease, or is the immune activation responding to a trigger? In this review we present evidence that points to a state of immune activation in IBS and show data that suggest that small intestinal bacterial overgrowth triggers immune activation in IBS. (*Gut and Liver* 2009;3:14-19)

Key Words: Functional bowel disorders; Small intestinal bacterial overgrowth; Dysbiosis; Immune response

INTRODUCTION

Irritable bowel syndrome (IBS) is a highly prevalent but poorly understood gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits.¹ Pathogenesis responsible for the development of IBS has been extensively investigated with multiple theories proposed.² While the exact cause of IBS is still in debate, there is mounting evidence of immune activation in this condition.³ In this article, we will review this evidence and the potential contributing role of small intestinal bacterial overgrowth.

POST-INFECTIOUS IBS

A subgroup of IBS patients describes the onset of their symptoms following an episode of acute infectious gastroenteritis.⁴ This has been termed post-infectious IBS.⁵ Both retrospective and prospective studies have reported this association and confirmed a significant relative risk of developing IBS following a single episode of bacterial gastroenteritis.^{4,6,7} Post-infectious IBS may account for 20 to 30% of IBS patient. These patients, in general, appear to have a better prognosis.⁸ In one prospectively conducted study, up to 25% of patients with microbiologically confirmed bacterial gastroenteritis continued to experience abnormal bowel habits 6 months after their initial illness.⁴ The term "post-infectious IBS" is then used when GI symptoms persist after acute gastroenteritis and symptoms meet the clinical criteria for IBS. The reported percentages of individuals with acute infectious diarrhea who develop IBS as a sequela vary in different studies. Some factors that may contribute to this variability include the time interval after the infection, the nature of the pathogen, and the criteria used to define IBS.

HISTOLOGIC EVIDENCE OF IMMUNE ACTIVATION IN POST-INFECTIOUS IBS

In a number of reports of post-infectious IBS patients, intestinal biopsies were collected for examination. Although conventional histology was generally unremarkable, subtle evidence of immune activation abound.^{9,10} Rectal biopsy specimens from patients complaining of IBS-like symptoms 3 months following acute infectious enteritis showed a greater number of chronic inflammatory

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(mononuclear) cells than patients who had no symptoms 3 months past their acute illness.¹⁰ Spiller *et al.*⁵ showed early increase in T lymphocytes counts (CD4 or CD8 positive) in the lamina propria and intraepithelial lymphocytes in biopsies obtained from patients with post-infectious IBS compared with controls. However, there was a decline in those counts with time, suggesting a slow resolution of the inflammatory process. This also correlated with the fact that many of those patients experienced improvement of their symptoms with time. Those patients with symptoms persistent for at least one year following the acute infection continued to show increased counts of enterochromaffin (EC) cells and intraepithelial lymphocytes in rectal mucosa biopsies.

IMMUNE ACTIVATION IS SEEN IN IBS WITHOUT AN ACUTE ONSET

In a study by Chadwick *et al.*¹¹ of 77 IBS patients, some with an acute history but others without, all of the IBS patients had some degree of immune activation regardless of the presence or absence of a history of acute onset (preceded by an acute gastroenteritis) or the pattern of bowel change (constipation, diarrhea or alternating). This study supports the idea that immune activation in IBS does not depend on acute gastroenteritis. These investigators also identified 3 distinct groups of patients based on histological examination of biopsy specimens obtained from different parts of the colon. The first group (38/77) had normal conventional histology but increased intraepithelial lymphocytes, lamina propria T lymphocytes, and CD25⁺ lymphocytes (indicative of T cell activation) on immunohistology when compared with asymptomatic controls. There was, however, no difference in the number of neutrophils or mast cells. The second group (31/77) had nonspecific microscopic inflammation by conventional histology and on immunohistology, an increase in the lymphocyte populations as well as an increase in the number of neutrophils and mast cells. The third group (8/77) fulfilled conventional histological and immunohistologic criteria for classic lymphocytic colitis.

Although the study by Chadwick *et al.* showed increased CD25⁺ regulatory T cells in their mucosal biopsies, another study showed that the immune activation in patients with IBS was associated with normal tissue concentration of CD25⁺ regulatory T cells.¹² Whether there is a normal or increased number of regulatory T cells in IBS, the contrast that may be most important is that regulatory T cells is significantly reduced in the intestinal mucosa of patients with active Crohn's disease.¹³ Interestingly, the number of regulatory T cell

counts was increased by anti-TNF- α treatments.

In a rare full-thickness biopsy study from patients with severe IBS-like symptoms,¹⁴ biopsy specimens obtained from proximal jejunum during laparoscopy showed a low-grade infiltration of T lymphocytes in the myenteric plexus with neuronal degeneration found in the majority of those patients. In this study, 4 out of 10 patients had a significant increase in the number of intraepithelial lymphocytes.

The presence of activated lymphocytes may be important to our understanding of the link between immune activation and IBS symptoms. Activated lymphocytes with their inflammatory mediators such as interleukin-11 β may be responsible for diarrhea through their known pro-secretory effect.¹⁵ Similar effects have been reported for other inflammatory products such as nitric oxide¹⁶ and prostaglandins.¹⁷

Langhorst *et al.* measured the fecal concentration of human β -defensin-2 (HBD-2).¹⁸ HBD-2 is a natural anti-microbial peptide secreted by intestinal epithelial cells. Both patients with active ulcerative colitis and irritable bowel syndrome had increased fecal concentration of HBD-2 compared to healthy controls. No significant difference in HBD-2 concentration between patients with ulcerative colitis and patients with IBS was noted. These results suggest that the activation of the mucosal innate immune defensive response to microbes may be a central part of the pathophysiology of IBS, even in the absence of macroscopic signs of inflammation.

CYTOKINE EVIDENCE FOR IMMUNE ACTIVATION IN IBS

Dinan *et al.* reported that the serum concentrations of the pro-inflammatory cytokines IL-6 and IL-8 were elevated in all IBS subgroups (diarrhea predominant, constipated, and alternating).¹⁹ However, there was not a difference in the serum concentration of the pro-inflammatory cytokine TNF- α or the anti-inflammatory cytokine IL-10. Sensitivity of the tests used to measure specific cytokines might have influenced the results but nonetheless, their findings suggested immune activation in IBS. IL-6 is crucial cytokine, released from macrophages and T-lymphocytes in response to IL-1 β and TNF- α . Its functions range from key roles in the induction of acute-phase proteins and enhancement of innate immunity to stimulation of B- and T-cell growth and differentiation.²⁰ IL-8 is a chemokine secreted by any cells expressing toll-like receptors that are involved in the innate immune response. The primary function of IL-8 is to recruit neutrophils to phagocytose the microbial

antigen that triggers the antigen pattern recognition toll-like receptor. The finding of elevated concentrations of IL-6 and IL-8 in IBS further suggest the possibility that microbes may be the trigger of immune activation in these patients.

Similarly, Liebrechts *et al.* showed that peripheral blood mononuclear cells obtained from patients with IBS demonstrated greater TNF- α , IL-1 β and IL-6 production compared to cells obtained from healthy volunteers.²¹ This difference was seen particularly in diarrhea-predominant IBS patients. Thus, immune activation may be detected both in the gut and peripherally, in blood. A genetic predisposition favoring greater production of TNF- α has also been reported in a subgroup of IBS patients.²²

Aerssens *et al.* studied gene expression in mucosal biopsy specimens between patients with IBS and controls;²³ they found differentially expressed genes involved in immune response, providing further evidence of functional alterations of several components of the mucosal immune response in IBS.

MAST CELL ACTIVATION IN IBS

Mast cells release potent mediators that alter the function of enteric nerves and smooth muscles and may contribute to the pathogenesis of irritable bowel syndrome. The results are somewhat dependent on whether activated mast cells were specifically examined and on the biopsy site. Weston *et al.* observed an increase in the number of mast cells in the terminal ileal mucosa of patients with IBS.²⁴ Talley *et al.* showed that mast cell infiltration and degranulation occur in the sigmoid colon in both IBS patients as well as healthy controls without a significant difference.²⁵ O'Sullivan *et al.* investigated mast cell infiltration in different parts of the colon, and found that the only significant difference in mast cell density between IBS patients and healthy controls was in the cecum.²⁶

Proteases are generally known as degradative enzymes (e.g., trypsin). Certain proteases produced by microbes and mast cells, however, function as signaling molecules acting on protease-activated receptors (PAR2).²⁷ Trypsin is a specific protease that is released by mast cells. The potential role of proteases and mast cells in IBS was shown by Barbara *et al.*²⁸ who reported that 1) The number of trypsin⁺ mast cells in the colonic mucosa of IBS patients was greater when compared to healthy controls, 2) The release of trypsin from colonic tissue obtained from IBS patients was greater when compared to controls, and 3) The severity of abdominal pain correlated with the number of mast cells in close proximity to colonic nerves. Fecal bacterial serine protease concentration

is also elevated in IBS.²⁹ Animal data shed light on the origin of this protease. In mice, protease release can be suppressed by antibiotics supporting the role of gut microbes as the trigger of its release.³⁰ Could all these findings in IBS patients be explained as a part of an immune response to gut microbes?

ENTEROCHROMAFFIN CELL EVIDENCE OF IMMUNE ACTIVATION IN IBS

Kyosola *et al.* found an increased number of enterochromaffin (EC) cells in rectal mucosa of patients with IBS compared with control.³¹ This finding was also supported by Spiller *et al.* who demonstrated that there was a significantly higher number of EC cells in rectal mucosa of patients with a clinical course consistent with post-infectious IBS.⁵ Dunlop *et al.* showed that an increased number of EC cells on rectal biopsy specimen predicted the development of IBS 3 months following an episode of acute infectious colitis.³² EC cells play a pivotal part in the control of gut motility and secretions.³³ Their microvilli project into the lumen, enabling them to sense intraluminal events, to which they respond by releasing 5-hydroxytryptamine (5-HT). Receptors to 5-HT on nerve endings in the lamina propria allow EC cells to transduce chemical and physical stimuli within the gut lumen to neural impulses. An excess of EC cells in the trinitrobenzene sulfonic acid-induced experimental colitis model is associated with increased 5-HT release.³⁴ Greater release of 5-HT may lead to increased intestinal peristalsis and secretion. A human study of 5-HT release after a meal also suggest this connection between an excess of 5-HT and the diarrhea symptom of IBS.³⁵

HOW CAN INFLAMMATION LEAD TO SYMPTOMS OF IBS?

A significant body of research is available supporting a modulatory role of the immune system on enteric neuromuscular functions²² as well as the neurons of the brain³⁶ accounting for motility disturbances seen in IBS. Similar to the effects of the immune response to a flu or a cold virus, immune mediators may also contribute to the fatigue, aches and pains, depression and foggy headedness that are common in IBS.³⁷ In addition, an altered hypothalamic-pituitary-adrenal axis has been reported in IBS.³⁸ Rather than a primary defect, an alternate possibility is that these findings could all be a part of the overall systemic immune response to a microbial challenge. Additionally, by triggering the defensive, "fight or flight" response, immuneactivated sympathetic drive^{36,39} could

explain the anxiety, disturbed sleep and cold hand and feet (shift of blood flow) reported frequently by patients with IBS.

BACTERIAL OVERGROWTH IN IBS

The enteric flora has been implicated in the pathogenesis of IBS³⁷ as there is accumulating evidence that some patients with IBS may have a shift in host-gut microbial relationship exemplified by a loss of containment of the indigenous colonic microbial community or small intestinal bacterial overgrowth. In a double-blinded, randomized, placebo-controlled treatment trial, 84% of IBS patients had an abnormal pattern of excretion of microbial gases in the exhaled breath consistent with the abnormal fermentation seen in small intestinal bacterial overgrowth.⁴⁰ In this study, patients who were randomized to and successfully treated with a non-absorbable antibiotic reported a 75% improvement in their global symptoms. In another double-blinded, randomized, placebo-controlled study in IBS patients, clinical improvement was achieved and sustained for 10 weeks after completion of a 10-day course of a nonabsorbable, small bowel-targeting antibiotic.⁴¹ These treatment responses suggest that underlying cause of the symptoms of IBS is an antibiotic-sensitive, microbial explanation localized to the gut. Consistent with this important role of gut microbes in IBS is the recent finding that the antibody to the bacterial antigen flagellin is elevated in IBS.⁴²

PROBIOTICS AND IMMUNE ACTIVATION IN IBS

Probiotics are microorganisms that are consumed in the belief that they may have a potentially beneficial effect on human health. A possible mechanism by which probiotics confers such a beneficial outcome is their anti-inflammatory effect.^{43,44} The possibility that probiotics may exert this effect was tested in IBS patients by comparing the ratio of IL-10, an anti-inflammatory cytokine to IL-12, a pro-inflammatory cytokine before and after treatment with either *Bifidobacterium infantis* or *Lactobacillus salivarius* for 8 weeks.⁴⁵ The results skewed toward a T_H1 proinflammatory profile in IBS patients with a lower anti-to pro-inflammatory cytokine ratio when compared with healthy volunteers. This ratio was normalized by *Bifidobacterium infantis* but not *Lactobacillus salivarius*. Why would ingesting probiotics have this effect? Gut microbes must be involved in the immune activation in IBS.

SMALL INTESTINAL BACTERIAL OVERGROWTH AS THE TRIGGER OF IMMUNE ACTIVATION AFTER GASTROENTERITIS

A recent experiment tested the hypothesis that small intestinal bacterial overgrowth may be a sequela of acute bacterial gastroenteritis.⁴⁶ Rats were experimentally infected with *Campylobacter jejuni* to induce an acute episode of gastroenteritis. The vast majority of infected animals (97%) rapidly cleared this pathogen from their gastrointestinal tract so that their stool cultures were negative for *C. jejuni* after 2 weeks. However, mirroring the clinical course of patients with post-infectious IBS, 57% of the animals still had altered stool consistency 3 months later vs. only 7.4% in the control group. The rats were euthanized at 3 months and the number of 16S rRNA gene (a well-conserved microbial gene) counted by quantitative PCR. Pimentel and colleagues found that 27% of the *C. jejuni*-exposed rats had small intestinal bacterial overgrowth and an increase in the number of intraepithelial lymphocytes. These findings demonstrated that small intestinal bacterial overgrowth is a sequela of acute bacterial gastroenteritis and may serve as a trigger of immune activation when the original pathogen had long been cleared.

FINAL THOUGHTS

The evidence is rapidly accumulating supporting the idea that a shift in host-gut microbial relationship may be responsible for IBS. Nevertheless, many questions remain unanswered. For example, does small intestinal bacterial overgrowth follow acute gastroenteritis in patients as in rats? What are the mechanisms responsible for developing bacterial overgrowth in those patients who do not have a triggering episode of acute gastroenteritis? Does treating small intestinal bacterial overgrowth reverse the immune activation in IBS? What are the determinants accounting for the controlled immune response to gut microbes such as that seen in IBS?

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Dr. Lin has intellectual property rights related to the topic covered by this work.

REFERENCES

1. Drossman DA, Li Z, Andruzzi E, et al. US householder

- survey of functional gastrointestinal disorders: prevalence, sociodemography, and health impact. *Dig Dis Sci* 1993;38:1569-1580.
2. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002;123:2108-2131.
  3. Collins SM. A case for immunological basis for irritable bowel syndrome. *Gastroenterology* 2002;122:2078-2080.
  4. Neal KR, Hebden J, Spiller R. Prevalence of gastrointestinal symptoms six months after bacterial gastroenteritis and risk factors for development of the irritable bowel syndrome: postal survey of patients. *BMJ* 1997;314:779-782.
  5. Spiller RC, Jenkins D, Thornley JP, et al. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter* enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000;47:804-811.
  6. McKendrick M, Read NW. Irritable bowel syndrome: post salmonella infection. *J Infect* 1994;29:1-3.
  7. Cumberland P, Sethi D, Roderick P, et al. The infectious intestinal disease study of England: a prospective evaluation of symptoms and health care use after an acute episode. *Epidemiol Infect* 2003;130:453-460.
  8. Harvey R, Mauad E, Brown A. Prognosis in the irritable bowel syndrome: a 5-year prospective study. *Lancet* 1987;1:963-965.
  9. Bercik P, Verdu EF, Collins SM. Is irritable bowel syndrome a low grade inflammatory bowel disease? *Gastroenterol Clin North Am* 2005;34:235-245.
  10. Gwee KA, Leong YL, Graham C, et al. The role of psychological and biological factors in postinfective gut dysfunction. *Gut* 1999;44:400-406.
  11. Chadwick V, Chen W, Shu D, et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002;122:1778-1783.
  12. Holmén N, Isaksson S, Simrén M, Sjövall H, Ohman L. CD4⁺CD25⁺ regulatory T cells in irritable bowel syndrome patients. *Neurogastroenterol Motil* 2007;19:119-125.
  13. Ricciardelli I, Lindley K, Londei M, Quarantino S. Anti tumour necrosis-alpha therapy increases the number of FOXP3⁺ regulatory T cells in children affected by Crohn's disease. *Immunology* 2008;125:178-183.
  14. Tornblom H, Lindberg G, Nyberg B, Veress B. Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* 2002;123:1972-1979.
  15. Plaisancié P, Barcelo A, Moro F, Claustre J, Chayvialle JA, Cuber JC. Effects of neurotransmitters, gut hormones, and inflammatory mediators on mucus discharge in rat colon. *Am J Physiol* 1998;275:G1073-1084.
  16. Kuwahara A, Kuramoto H, Kadowaki M. 5-HT activates nitric oxide-generating neurons to stimulate chloride secretion in guinea pig distal colon. *Am J Physiol* 1998;275:G829-834.
  17. Frieling T, Rupprecht C, Kroese A, Schemann M. Effects of the inflammatory mediator prostaglandin D2 on sub-mucosal neurons and secretion in guinea pig colon. *Am J Physiol* 1994;266:G132-139.
  18. Langhorst J, Wieder A, Michalsen A, Musial F, Dobos G, Rueffer A. Activated innate immune system in irritable bowel syndrome? *Gut* 2007;56:1325-1326.
  19. Dinan TG, Quigley EM, Ahmed SM, et al. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology* 2006;130:304-311.
  20. Murphy K, Travers P, Walport M. *Janeway's Immunobiology*. 7th ed. London: Garland Science, 2007.
  21. Liebrechts T, Adam B, Bredack C, et al. Immune activation in patients with irritable bowel syndrome. *Gastroenterology* 2007;132:913-920.
  22. Collins S. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. *Gastroenterology* 1996;111:1683-1699.
  23. Aerssens J, Camilleri M, Talloen W, et al. Alterations in mucosal immunity identified in the colon of patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2008;6:194-205.
  24. Weston AP, Biddle WL, Bhatia PS, Miner PB Jr. Terminal ileal mucosal mast cells in irritable bowel syndrome. *Dig Dis Sci* 1993;38:1590-1595.
  25. Talley NJ, Butterfield JH. Mast cell infiltration and degranulation in colonic mucosa in the irritable bowel syndrome. *Am J Gastroenterol* 1996;91:1675-1676.
  26. O'Sullivan M, Calyton N, Breslin NP, et al. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil* 2000;12:449-457.
  27. Cenac N, Coelho A, Nguyen C, et al. Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am J Pathol* 2002;161:1903-1915.
  28. Barbara G, Stanghellini V, De Giorgio R, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004;126:693-702.
  29. Roka R, Rosztoczy A, Leveque M, et al. A pilot study of fecal serine-protease activity: a pathophysiologic factor in diarrhea-predominant irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2007;5:550-555.
  30. Roka R, Demaude J, Cenac N, et al. Colonic luminal proteases activate colonocyte proteinase-activated receptor-2 and regulate paracellular permeability in mice. *Neurogastroenterol Motil* 2007;19:57-65.
  31. Kyosola K, Penttilä O, Salaspuro M. Rectal mucosal adrenergic innervation and enterochromaffin cells in ulcerative colitis and irritable colon. *Scand J Gastroenterol* 1977;12:363-367.
  32. Dunlop SP, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in post-infectious IBS. *Gastroenterology* 2003;125:1651-1659.
  33. Gershon M. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. *Aliment Pharmacol Ther* 1999;13(Suppl 2):15-30.
  34. Linden D, Chen J, Gershon M, Sharkey K, Mawe G. Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G207-216.
  35. Bearcroft C, Perrett D, Farthing M. Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. *Gut* 1998;42:42-46.
  36. Bosedovsky H, del Rey A, Sorkin E, Da Prada M, Burri R, Honegger C. The immune response evokes changes in

- brain noradrenergic neurons. *Science* 1983;221:564-566.
37. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding IBS. *JAMA* 2004;292:852-858.
38. Chang L, Sundaresh S, Elliott J, et al. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in irritable bowel syndrome. *Neurogastroenterol Motil* 2008 Aug 5. [Epub ahead of print]
39. Elsenbruch S, Holtmann G, Oezcan D, et al. Are there alterations of neuroendocrine and cellular immune responses to nutrients in women with irritable bowel syndrome? *Am J Gastroenterol* 2004;99:703-710.
40. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003;98:412-419.
41. Pimentel M, Park S, Mirocha J, Kane SV, Kong Y. The effect of a nonabsorbed oral antibiotic (rifaximin) on the symptoms of the irritable bowel syndrome: a randomized trial. *Ann Intern Med* 2006;145:557-563.
42. Schoepfer AM, Schaffer T, Seibold-Schmid B, Müller S, Seibold F. Antibodies to flagellin indicate reactivity to bacterial antigens in IBS patients. *Neurogastroenterol Motil* 2008;20:1110-1118.
43. O'Mahony L, Feeney M, O'Halloran S, et al. Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice. *Aliment Pharmacol Ther* 2001;15:1219-1225.
44. McCarthy J, O'Mahony L, O'Callaghan L, et al. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. *Gut* 2003;52:975-980.
45. O'Mahony L, McCarthy J, Kelly P, et al. *Lactobacillus* and *bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005;128:541-551.
46. Pimentel M, Chatterjee S, Chang C, et al. A new rat model links two contemporary theories in irritable bowel syndrome. *Dig Dis Sci* 2008;53:982-989.