Low-grade inflammation plays a pivotal role in gastrointestinal dysfunction in irritable bowel syndrome

Hirotada Akiho, Eikichi Ihara, Kazuhiko Nakamura

Akiho H, Ihara E, Nakamura K. Low-grade inflammation plays a pivotal role in gastrointestinal dysfunction in irritable bowel syndrome. World J Gastrointest Pathophysiol 2010; 1(3): 97-105 Available from: URL: http://www.wjgnet.com/2150-5330/full/v1/i3/97.htm DOI: http://dx.doi.org/10.4291/wjgp.v1.i3.97

INTRODUCTION

Irritable bowel syndrome (IBS) is seen throughout the world with estimated prevalences ranging from 9%-23%[1], representing an important clinical problem. It is accompanied by a poor quality of life[2]. Its symptoms include abdominal pain or discomfort associated with changes in bowel habits for which no obvious cause can be found on routine investigations[3] and its diagnosis is commonly dependent on the symptom-based Rome criteria (Table 1).

The pathogenesis is considered to be multifactorial and includes psychosocial factors, gastrointestinal (GI) dysmotility, enhanced perception of sensory stimuli conveyed from the gut wall to the central nervous system, stress, corticotrophin-releasing factor, infection, microbiota, genetics and gut wall immune activation[4]. It is now well recognized that an episode of gastroenteritis can trigger IBS symptoms, known as post-infective IBS (PI-IBS). Low-grade inflammation and immune activation are evident in biopsies both from patients with IBS[5] and PI-IBS[6]. It is becoming clear that low grade inflammation in the mucosal compartment of the gut could alter function in the underlying neuromuscular tissues from animal studies. IBS represents a clinical entity largely diffused which may heavily affect the patient’s quality of life and a strong need of oriented therapeutic interventions could be available.

This review describes the evidence for low-grade inflammation in patients with IBS, explores its mechanism
with particular focus on the inflammation-induced GI motility and highlights its implications for understanding the pathophysiology of IBS.

EVIDENCE OF INFLAMMATION IN IRRITABLE BOWEL SYNDROME

Cytokines and immune cells
Several reports have described increased numbers of T cells in various lymphoid compartments of the small or large intestine in IBS patients[5,7,8]. Prolinflammatory cytkines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in peripheral blood mononuclear cells[9] and IL-6 and IL-8 in serum[10] were reported to be increased in IBS patients. Thus, low-grade inflammation can be detected through biopsies from intestine and blood.

Subsequent studies in IBS patients revealed increased numbers of mast cells in the lamina propria of the terminal ileum[11] and mucosa of the colon[12,13]. It is becoming clear that mast cells may affect the sensorimotor function and contribute to IBS symptoms[13,14]. Barbara et al[14] showed that activated mast cells released significant amounts of various mediators including tryptase and histamine. It has been reported that mast cell tryptase elicits neuronal hyperexcitability[13,15], while histamine activates visceral sensory nerves via histamine-1 and -2 receptors[17], indicating that tryptase and histamine are candidate mediators for the gut sensorimotor dysfunction in IBS[16].

PI-IRRITABLE BOWEL SYNDROME

Epidemiological studies have indicated that 6%-17% of patients with acute gastroenteritis develop IBS[19]. Low-grade inflammation and immune activation are evident in biopsies from patients with PI-IBS and there is also evidence of increased intestinal permeability[20].

Neurotransmitters
Serotonin: Serotonin (5-hydroxytryptamine, 5-HT) is found in the GI tract and central nervous system and functions as a neurotransmitter[20]. 5-HT is the most studied neurotransmitter in IBS. About 95% of the body’s 5-HT is localized in the GI tract and 5% is present in the brain. In the GI tract, 5-HT is synthesized in serotonergic neurons in the enteric nervous system as well as in enterochromaffin (EC) cells of the GI mucosa.

EC cells produce and secrete far more 5-HT than central or peripheral serotonergic neurons and it reaches the GI lumen and blood[21]. Overflowing 5-HT from EC cells, taken up and concentrated in platelets, is virtually the sole source of blood 5-HT. 5-HT exerts its actions by binding to its receptors (5-HT1-7) which are present on intrinsic and extrinsic primary afferent neurons. The large range of effects of 5-HT mainly results from the presence of multiple receptor subtypes on enteric neurons, EC cells, gastrointestinal smooth muscle cells, enterocytes and immune tissues. Seven families and multiple subtypes of 5-HT receptors have now been identified[22]. The 5-HT1A and 5-HT3 receptors are known to affect gut motor functions[23-26]. 5-HT is well known to increase in various GI disorders such as carcinoid syndrome, celiac disease, acute bacterial enteritis and inflammatory bowel disease (IBD)[27,28]. Several studies have shown that plasma 5-HT is increased in patients with IBS[29,30]. Furthermore, the effectiveness of 5-HT3 antagonists for IBS with diarrhea (IBS-D) has been demonstrated[31] and 5-HT3 antagonists are currently widely used for IBS-D in various countries. PI-IBS has been associated with increased numbers of EC cells[32]. Although IBS with constipation (IBS-C) patients showed decreased plasma 5-HT[33,34], colonic[35,36] and duodenal[37] mucosal 5-HT appeared to be increased. Opiate-induced constipation does not alter the 5-HT content or mucosal serotonin transporter (SERT) level in humans, suggesting that the changes in 5-HT metabolism in IBS-C are primary[38].

5-HT released in the mucosa is rapidly taken up by serotonin transporters in nerve terminals or mucosal enterocytes and vascular endothelial cells[33]. The associations between SERT transcription levels, polymorphisms and IBS phenotypes have been investigated. The SERT gene-linked polymorphic region (SERT-LPR), an area 12 kb upstream of the SERT exon that has short (s) and long (l) alleles, is thought to influence the level of transcription[39].

Emerging biomarkers
The identification of reliable biomarkers represents a major step forward in the management of disease. The physiological changes accompanying IBS have been shown to be reflected in changes in the expression levels of biomarkers[35-38]. The reliable serum biomarker for IBS is expected to reduce an unnecessary colonoscopy caused by symp tom-based criteria.

Lembo et al[40] investigated blood-based diagnostic tests to differentiate IBS from non-IBS using the Smart Diagnostic Algorithm and complex patterns of the serum concentrations among 10 biomarkers, including IL-1β, growth-related oncogene-α, brain-derived neurotrophic factor, anti-Saccharomyces cerevisiae IgA antibodies, anti-CBIR1 antibodies, anti-human tissue transglutaminase antibodies, TNF-like weak inducer of apoptosis (TWEAK), anti-neutrophil cytoplasmatic antibodies, tissue inhibitor of metalloproteinase-1 and neutrophil gelatinase-associated lipocalin. They demonstrated that the positive predictive value was 81% and the negative predictive value was 64% at 50% IBS prevalence in the validation cohort[41]. Therefore, the pathophysiology of IBS is heterogeneous and the identification of multiple biomarkers is more reliable than detection of a single biomarker for IBS.

Microbiota
The intestinal microbiota influences a broad array of host
organs including the gut and brain and is an important determinant of normal function in these systems. Disruption of the delicate balance between the host and the intestinal microbiota (termed dysbiosis) results in changes in the mucosal immune system that range from overt inflammation, as seen in Crohn’s disease, to low-grade inflammation without tissue injury, as seen in a subset of IBS patients. The dysbiosis induced by infection, diet or antibiotics can produce the low-grade inflammation seen in IBS[41]. Malinen et al[42] showed that Lactobacillus species were decreased in IBS-D patients while Veillonella species were increased in IBS-C patients.

Other groups have obtained evidence that the intestinal microflora of patients with IBS differs from that of healthy subjects[43,44]. It has recently been suggested that IBS symptoms are partly caused by a process designated small intestinal bacterial overgrowth (SIBO)[45,46]. Further therapeutic manipulation of the gut flora with antibiotics[47] or probiotics[48] improves the symptoms of IBS but is controversial. We consider SIBO as a subtype of IBS more than a distinct entity.

These lines of evidence provide proof of the concept that the intestinal microbiota can induce the persistent gut dysfunction seen in IBS.

GI MOTILITY AND SMOOTH MUSCLE

GI motility is defined by the movements of the digestive system including two fundamental patterns of motility, propulsion and mixing. Several players including central nerves, enteric nerves, interstitial cells of Cajal (ICC) and smooth muscles contribute to a coordinated regulation of GI motility. Among them, smooth muscle probably plays the most important role in GI motility since the patterns of motility observed in gut are characteristic of smooth muscle which has different properties from skeletal muscle. The contractile properties of smooth muscle are mainly regulated by the phosphorylation of regulatory light chains of myosin II (MLC)[49] which is driven by the balance between myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) activities. MLCK depends on Ca\(^{2+}\)-calmodulin. Intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]) is the primary determinant of smooth muscle contraction. However, MLCP functions independently of Ca\(^{2+}\)-calmodulin and is regulated by G protein-coupled signaling pathways. Inhibition of MLCP leads to an increase in both MLC phosphorylation and Ca\(^{2+}\) sensitization possibly via regulation of MLCP[50].

Phosphatase inhibitor protein-17 kDa (CPI-17). In addition, it has been shown that mitogen activated protein kinase pathways also contribute to intestinal smooth muscle contraction and Ca\(^{2+}\) sensitization possibly via regulation of MLCP[51].

GUT MOTOR FUNCTION IN INFLAMMATION

Conditions ranging from infective acute enteritis or colitis to IBD and functional disorders such as IBS are accompanied by altered GI motility[52] which can be a reflection of altered function of enteric nerves, ICC or smooth muscles. Alterations in GI motility with resultant changes in transit contribute to the abdominal pain, intestinal cramping and diarrhea. Colonic mast cell infiltration and mediator release in the proximity of mucosal innervations may contribute to abdominal pain perception in patients with IBS[53]. Patients with IBD in remission often generate IBS symptoms, termed IBD/IBS, and have pain and diarrhea similar to those in IBS patients in association with minimal or no evident intestinal inflammation[54].

These findings suggest that low-grade inflammation contributes to the GI motor dysfunction and abdominal symptoms in patients with functional GI disorders and IBD in remission. Since we cannot use whole human intestinal tissue to investigate the pathophysiology of IBS, we have tried to establish bench-to-bedside animal models for the development of new therapies.

Which immune cells and mediators and how do they affect gastrointestinal motility?

Macrophages: Macrophages perform a key role in innate defense against foreign invaders and produce a number of cytokines such as IL-1β, IL-6 and TNF-α. In animal experiments, macrophages infiltrate the gut wall including the neuromuscular layers during nematode infection in mice. It was reported that macrophages were not critical for the change in muscle contraction in Trichinella spiralis-infected mice[55] although another group reported that alternative activated macrophages affected the muscle hypercontractility in Nippostrongylus brasiliensis-infected mice[56].

T lymphocytes (Th1/Th2): T lymphocytes are crucial for many immune responses, including those associated with animal models such as dextran sulfate sodium colitis[57,58], 2,4,6-trinitrobenzenesulfonic acid colitis[59], nematicode infection[57,60] and anti-CD3 antibody-induced enteropathy[61]. Antigen-presenting cells present antigens to CD4\(^+\) T helper (Th) cells. Th cell-dependent immune responses are generally divided into two major subsets, Th1 and Th2[62]. Th1 cells predominantly produce interferon (IFN) γ and IL-2 while Th2 cells produce IL-4, IL-5, IL-9 and IL-13. Th1 and Th2 cells cross-regulate one another. While few generalizations can be made, it appears that contractile dysfunction depends on the specific inflammatory environment. Recent accumulated data from animal...
models have shown that Th1 and Th2 immune response was associated with hypocontractility or hypercontractility of inflamed intestinal smooth muscle respectively.

Schwartz et al.[66] showed that surgical manipulation suppressed jejunal contractions with upregulation of IL-6, TNF-α, cyclooxygenase-2 and inducible nitric oxide synthase. It has been shown that both TNF-α[67] and IL-1β[68] were associated with hypocontractility of inflamed intestinal smooth muscle. Furthermore, we have shown that incubation of IFNγ with intestinal smooth muscle decreased carbachol-induced smooth muscle cell contraction[68]. It has also been shown how these Th1-related cytokines cause hypocontractility of inflamed intestinal smooth muscle. TNF-α and IL-1β inhibited carbachol-induced contraction via down-regulation of CPI-17[69] and L-type Ca2+ channels[70] respectively.

On the other hand, the Th2 cytokines IL-4 and IL-13 acting via Stat6 mediate the development of nematode infection-induced intestinal muscle hypercontractility which contributes to worm expulsion[71,72]. Other studies supported our finding that Th2 responses mediated muscle contractility in nematode N. brasiliensis-infected mice[73,74]. Although it remains to be investigated how these Th2-related cytokines mediate hypercontractility of inflamed intestinal smooth muscle, Ibara et al[81] showed that mitogen-activated protein kinase pathways played crucial roles in the Th2 cytokine-mediated Ca2+ sensitization and hypercontractility observed in inflamed colonic circular smooth muscle from dextran sulfate sodium-treated mice.

**Th1/Th2 balance:** To evaluate the role of Th1/Th2 in infection-induced alterations of enteric muscle function, Khan et al.[77] investigated the effects of IL-12 overexpression on intestinal muscle contractility and worm expulsion in T. spiralis-infected mice. IL-12 gene transfer via a single injection of a recombinant adenovirus vector expressing IL-12 (Ad5IL-12) in T. spiralis-infected mice effectively inhibited the development of infection-induced intestinal muscle hypercontractility and prolonged worm survival in the gut. A shift to a Th1 response after overexpression of IL-12 significantly altered the intestinal muscle hypercontractility in this Th2-based enteric infection.

Furthermore, we evaluated the association of 5-HT with Th1/Th2 responses. 5-HT influences intestinal homeostasis by altering the gut physiology and has been implicated in the pathophysiology of various GI disorders such as IBD, IBS and GI infection[35,36,76,77]. In a colonic parasitic infection with *Trichuris muris*, resistant strains (BALB/c, C57BL/6 and NIH Swiss) expelled the parasites through the generation of a Th2 response whereas susceptible strains (AKR and B10.BR) developed a chronic infection with activation of a Th1 response[78].

We used *Trichuris muris*-infected AKR (susceptible to infection with generation of a Th1 response), BALB/c (resistant to infection with generation of a Th2 response), Stat4-deficient (impaired in Th1 responses) and Stat6-deficient (impaired in Th2 responses) mice to explore the mechanism of the EC cell and 5-HT responses in Th1/Th2-dominant environments[79]. We found that the EC cell and 5-HT responses to the same infectious agent were influenced by Th1 or Th2 cytokine predominance, suggesting that the immunological profile of the inflammatory response is important in the regulation of EC cell biology in the gut. Furthermore, we evaluated the 5-HT response and intestinal motility in an IBD/IBS model using T cell-induced enteropathy in Th1/Th2-dominant environments[80]. In BALB/c mice, carbachol-induced intestinal smooth muscle cell contraction was significantly increased at d 7 after anti-CD3 antibody injection when the tissue damage returned to the normal histological appearance. We also observed that 5-HT protein in the intestine was significantly increased at d 7. On the other hand, in AKR mice, carbachol-induced muscle cell contraction was significantly decreased at d 7. 5-HT protein in the intestine was also decreased at d 7. We showed that Th1 and Th2 cytokines had opposing effects on intestinal muscle contraction via 5-HT signaling in the post-inflammation phase in this model.

**Th17:** Previous concepts regarding the roles of Th cells in chronic inflammatory and autoimmune diseases have been challenged by the description of a novel T-cell subset characterized by the production of IL-17[82]. Several disorders originally considered to be Th1-mediated have been reclassified as Th17-mediated inflammation[82,83]. Th17 cells produce IL-17, IL-17F and IL-22, thereby inducing a massive tissue reaction owing to the broad distribution of the IL-17 and IL-22 receptors. Th17 cells also secrete IL-21 to communicate with the cells of the immune system. The differentiation factors (TGF-beta plus IL-6 or IL-21), the growth and stabilization factor (IL-23) and the transcription factors (STAT3, RORgammat and RORalpha) involved in the development of Th17 cells have just been identified[84]. IL-17 is a proinflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokines, chemokines and cell adhesion molecules. This cytokine is augmented in the sera and/or tissues of patients with contact dermatitis, asthma and rheumatoid arthritis[85]. Although the Th1/Th17 balance in human IBD remains unclear, IBD seems to have a relationship with Th17 cells[86]. Low-grade inflammation in the mucosa is considered to be a factor involved in the pathophysiology of IBS and further investigations of Th17 cells in the intestinal mucosa of patients with IBS should therefore be carried out[86]. A recent study showed that Th17 cells were increased during acute infection with *T. spiralis* and that jejunal smooth muscle strips cultured with IL-17 showed enhanced contractions elicited by acetylcholine in a concentration-dependent manner[87]. We found that IL-17 protein in the small intestine was upregulated in mice injected with an anti-CD3 antibody[88] and that IL-17 incubation with smooth muscle cells enhanced carbachol-induced smooth muscle cell contraction (unpublished observation). Further investigations are required using IL-17/-/- mouse and IL-17 antagonist to confirm the role of IL-17-induced muscle hypercontractility (Table 2).
VISCERAL HYPERSENSITIVITY

Abdominal pain is an essential symptom of IBS and visceral hypersensitivity is the most widely accepted mechanism\(^9\). Visceral sensitivity is regulated at the level of the peripheral (mucosa/submucosa), spinal cord and central nervous system. Non-inflammatory mediators such as stress, glycerol and glutamate, as well as inflammatory mediators have the potential to trigger visceral pain\(^8\). Inflammatory mediators such as prostaglandin E\(_2\) from inflammatory cells, or chemical mediators such as ATP, bradykinin, 5-HT, substance P and calcitonin gene-related peptide, directly activate nerve endings and trigger the release of algicogenic mediators, histamine, 5-HT, nerve growth factor and prostanoids from other cells and afferent nerves, resulting in an increasing response of pain\(^9\). Nerve fibers expressing the capsaicin receptor, transient receptor potential vanilloid type-1, were increased in colonic mucosa from IBS patients and may contribute to the visceral hypersensitivity and pain in IBS\(^9\). Recent animal studies showed that 5-HT\(_{3}\) receptor antagonists\(^9,92\), melatonin\(^9\), corticotrophin-releasing hormone receptor 1 antagonists\(^9\) and protease-activated receptor-4\(^9\) inhibited visceral hypersensitivity. These molecules are candidates for novel therapies against the visceral hypersensitivity in IBS.

SECRETOMOTOR DYSREGULATION

Secretomotor neurons

Disordered defecation in IBS is directly related to the physiology of the enteric secretomotor neurons. Secretomotor neurons are excitatory motor neurons in the submucosal plexus of the enteric nervous system which innervate and stimulate secretion from the intestinal crypts of Lieberkuhn, Brunner’s glands and goblet cells.

Secretomotor neurons have receptors that receive excitatory and inhibitory synaptic inputs from other neurons in the integrative circuitry of the enteric nervous system and from sympathetic postganglionic neurons. They are also influenced by paracrine chemical messages from non-neural cell types in the mucosa and submucosa such as EC cells and immune/inflammatory cells\(^17\).\(^9\)

Activation of the excitatory receptors on secretomotor neurons stimulates the neurons to fire and release their transmitters at neuroepithelial junctions in the crypts. Secretomotor neurons express excitatory receptors for acetylcholine, 5-HT and histamine. The overall result of the activation of the excitatory receptors and associated increase in secretomotor neuronal firing is stimulation of the secretion of H\(_2\)O, electrolytes and mucus from the crypts into the intestinal lumen\(^9\).

Knowledge of the cellular neurobiology of submucosal secretomotor neurons is key to understanding the pathophysiology of secretory diarrhea and constipation. Suppression of secretomotor firing by antidiarrheal agents such as opiates is manifested as harder-drier stools. On the contrary, stimulation by chemical mediators such as acetylcholine, 5-HT and histamine is manifested as more liquid stools\(^9\). The proinflammatory cytokines IL-1\(\beta\) and TNF-\(\alpha\) increased epithelial tight junction permeability \textit{in vitro} in Caco-2 cells in a dose- and time-dependent manner\(^9\).\(^9\). This effect was mediated by an increase in myosin L chain kinase expression and activity. IFN\(\gamma\) is well known to increase tight junction permeability in the T84 cell line accompanied by activation of the PI3-kinase pathway\(^9\). Green tea and probiotics\(^10\) are candidates for reducing the mucosal hyperpermeability seen in IBS.

EFFECTIVE TREATMENTS

Several emerging clinical trials for IBS are ongoing that target visceral hypersensitivity, motility, neurotransmitters, microbiota and immune systems acting peripherally and/or centrally. Thus far, 5-HT\(_{3}\) agents have been the most effective for IBS\(^10,10\).\(^2\). A systematic review showed that the 5-HT\(_{3}\) antagonist alosetron and 5-HT\(_{4}\) agonist tegaserod were more effective than placebos and that the 5-HT\(_{3}\) antagonists showed to have more efficacy than tegaserod due to a potential risk of adverse events, although the Food and Drug Administration announced discontinued marketing of tegaserod due to a potential risk of adverse events, cerebrovascular and cardiovascular ischemic events in 2007\(^10,10\).\(^2\).

One of the excitatory receptors on secretomotor neurons belongs to the 5-HT\(_{3}\) serotonergic receptor subtype\(^2\).\(^10,10\).\(^4\). The observed efficacy of blockade of 5-HT\(_{3}\) receptors by a 5-HT\(_{3}\) antagonist in the treatment of diarrhea in diarrhea-predominant IBS suggests that over-stimulation of secretomotor neurons by 5-HT is a significant pathophysiological factor in this form of IBS\(^10,10\).\(^8\). On the contrary, 5-HT\(_{4}\) agonists stimulate GI motility and intestinal secretion and have demonstrated efficacy in improving bowel habits for constipation-predominant IBS\(^10,10\).\(^8\).

Three 5-HT\(_{4}\) agonists, prucalopride, ATI-7505 and TD-5108, in development are reported to have greater selectivity for 5-HT\(_{4}\) over other receptors and have advanced to human trial. 5-HT\(_{4}\) receptor antagonist, ramose-
IMPLICATIONS

It is becoming increasingly clear that inflammation of the intestinal mucosa and nerves causes the altered GI dysfunction seen in IBS. Several studies have been performed to detect robust and reliable biomarkers for IBS. However, IBS contains many different conditions with different underlying causes and different responses to therapy and different mechanisms, biomarkers and therapies therefore need to be identified in each IBS subgroup. Understanding the immunological basis of the altered GI motor dysfunction in IBS by considering the role of the Th1/Th2 balance or Th17 cytokines may ultimately lead to new therapeutic strategies for IBS.

REFERENCES

1. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002; 123: 2108-2131
2. Lea R, Whorwell PJ. Quality of life in irritable bowel syndrome. *Pharmacoconomics* 2001; 19: 643-653
3. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Heaton KW, Martino CA, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; 130: 1480-1491
4. Barbara G, De Giorgio R, Stanghellini V, Cremon C, Salvioli B, Corinaldesi R. New pathophysiological mechanisms in irritable bowel syndrome. *Aliment Pharmacol Ther* 2004; 20 Suppl 2: 1-9
5. Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002; 122: 1778-1783
6. Spiller RC. Postinfectious irritable bowel syndrome. *Gastroenterology* 2003; 124: 1662-1671
7. Tönshoff H, Lindberg C, Nyberg B, Veress B. Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* 2002; 123: 1972-1979
8. Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteric-endocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000; 47: 804-811
9. Liebregts T, Adam B, Bredack C, Röth A, Heinzel S, Lester S, Downie-Doyle S, Smith E, Drew P, Talley NJ, Holtmann G. Immune activation in patients with irritable bowel syndrome. *Gastroenterology* 2007; 132: 913-920
10. Dinan TG, Quigley EM, Ahmed SM, Scully P, O’Brien S, O’Mahony L, O’Mahony S, Shanahan F, Keeling PW. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology* 2006; 130: 304-311
11. 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
12. O’Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, Whorwell PJ. Increased mast cells in the irritable bowel syndrome: neuroimmunological basis. *Neurogastroenterol Motil* 2000; 12: 449-457
13. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnell NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004; 126: 693-702
14. Barbara G, Wang B, Stanghellini V, de Giorgio R, Cremon C, Di Nardo G, Trevisani M, Campi B, Geppetti P, Tonini M, Bunnell NW, Grundy D, Corinaldesi R. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 2007; 132: 26-37
15. Reed DE, Barajas-Lopez C, Cottrell G, Velazquez-Rocha S, Dery O, Grady EF, Bunnell NW, Vanner SJ. Mast cell tryptase and proteinase-activated receptor 2 induce hyperexcitability of guinea-pig submucosal neurons. *J Physiol* 2003; 547: 531-542
16. Gao C, Liu S, Hu HZ, Gao N, Kim GY, Xia Y, Wood JD. Serine proteases excite myenteric neurons through protease-activated receptors in guinea pig small intestine. *Gastroenterology* 2002; 123: 1554-1564
17. Wood JD. Enteric neuroimmunophysiology and pathophysiology. *Gastroenterology* 2004; 127: 635-657
18. De Giorgio R, Barbara G. Is irritable bowel syndrome an inflammatory disorder? *Curr Gastroenterol Rep* 2008; 10: 385-390
19. Longstreth GF, Hawkey CJ, Mayer EA, Jones RH, Naesdal J, Wilson IK, Peacock RA, Wiklund IK. Characteristics of patients with irritable bowel syndrome recruited from three sources: implications for clinical trials. *Aliment Pharmacol Ther* 2001; 15: 959-964
20. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 2007; 132: 397-414
21. Tamir H, Payette RF, Huang YL, Liu KP, Gershon MD. Human serotonin: a blood glycoprotein that binds serotonin and is associated with platelets and white blood cells. *J Cell Sci* 1985; 73: 187-206
22. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* 1999; 38: 1083-1152
23. De Ponti F, Tonini M. Irritable bowel syndrome: new agents targeting serotonin receptor subtypes. *Drugs* 2001; 61: 317-332
24. Read NW, Gwee KA. The importance of 5-hydroxytryptamine receptors in the gut. *Pharmacol Ther* 1994; 62: 159-173
25. Tonini M, Vicini R, Cervio E, De Ponti F, De Giorgio R, Barbara G, Stanghellini V, Dellabianca A, Sternini C. 5-HT17 receptors modulate peristalsis and accommodation in the guinea pig ileum. *Gastroenterology* 2005; 129: 1557-1566
26. Sikander A, Rana SV, Prasad KK. Role of serotonin in gastro-intestinal motility and irritable bowel syndrome. *Clin Chim Acta* 2009; 403: 47-55
27. Spiller R. Serotonin and GI clinical disorders. *Neuropsychopharmacology* 2008; 33: 1072-1080
28. Spiller R. Recent advances in understanding the role of serotonin in gastrointestinal motility in functional bowel disorders: alterations in 5-HT signalling and metabolism in human disease. *Neurogastroenterol Motil* 2007; 19: 25-31
29. Dunlop SP, Coleman NS, Blackshaw E, Perkins AC, Singh G, Marsden CA, Spiller RC. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2005; 3: 349-357
30. Atkinson W, Lockhart S, Whorwell PJ, Kevii B, Houghton LA. Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhoea-predominant irritable bowel syndrome. *Gastroenterology* 2006; 130: 34-43
31. Cremonini F, Delgado-Aros S, Camilleri M. Efficacy of alosetron in irritable bowel syndrome: a meta-analysis of randomized controlled trials. *Neurogastroenterol Motil* 2003; 15: 79-86
32. Dunlop SP, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; 125: 1651-1659
33. Miwa J, Echizen H, Matsuda K, Umeda N. Patients with constipation-predominant irritable bowel syndrome (IBS) may...
have elevated serotonin concentrations in colonic mucosa as compared with diarrhea-predominant patients and subjects with normal bowel habits. *Digestion* 2001; 63: 188-194.

34 *Kerchhoff AP*, Ter Linde JJ, Akkermans LM, Samson M. Trypsinogen IV, serotonin transporter transcript levels and serotonin content are increased in small intestine of irritable bowel syndrome patients. *Neurogastroenterol Motil* 2008; 20: 900-907.

35 *Camilleri M*, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE, McKinzie S, Urrutia R. Serotonin-transporter polymorphism pharmacoepidemiology in diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2002; 123: 425-432.

36 *Garvin B*, Wiley JW. The role of serotonin in irritable bowel syndrome: implications for management. *Curr Gastroenterol Rep* 2008; 10: 363-368.

37 *Clarke G*, Quigley EM, Cryan JF, Dinan TG. Irritable bowel syndrome: towards biomarker identification. *Trends Mol Med* 2009; 15: 478-489.

38 *Barbara G*, Stanghellini V. Biomarkers in IBS: when will they replace symptoms for diagnosis and management? *Gut* 2009; 58: 1571-1573.

39 *Aerssens J*, Camilleri M, Talloen W, Thielemans L, Göhmann HW, Van Den Wyngaert I, Thielemens T, De Hoogt R, Andrews CN, Bharucha AE, Carlson PJ, Busciglio I, Burton DD, Smyrk T, Urrutia R, Coulie B. Alterations in mucosal immunity identified in the colon of patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2008; 6: 194-205.

40 *Lenbo AJ*, Neri B, Tolley J, Barker D, Carroll S, Pan H. Use of serum biomarkers in a diagnostic test for irritable bowel syndrome. *Aliment Pharmacol Ther* 2009; 29: 834-842.

41 *Collins SM*, Denou E, Verdu EF, Bercik P. The putative role of the intestinal microbiota in the irritable bowel syndrome. *Dig Liver Dis* 2009; 41: 850-853.

42 *Malinen E*, Rinttilä T, Kajander K, Mattiö J, Kassinen A, Krogus L, Saarela M, Korpela R, Palva A. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005; 100: 373-382.

43 *Nobaek S*, Johansson ML, Molin G, Ahrné S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol* 2000; 95: 1231-1238.

44 *Cummings JH*, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* 1991; 70: 443-459.

45 *Pimentel M*, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 2000; 95: 3503-3506.

46 *Ghoshal UC*, Park H, Gwee KA. Bugs and irritable bowel syndrome: The good, the bad and the ugly. *J Gastroenterol Hepatol* 2010; 25: 244-251.

47 *Yang J*, Lee HR, Low K, Chatterjee S, Pimentel M. Rifaximin versus other antibiotics in the primary treatment and re-treatment of bacterial overgrowth in IBS. *Dig Dis Sci* 2008; 53: 169-174.

48 *Spiller R*. Probiotics: an ideal anti-inflammatory treatment for IBS? *Gastroenterology* 2005; 128: 783-785.

49 *Somlyo AP*, Somlyo AV. Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphorylation. *Physiol Rev* 2003; 83: 1325-1358.

50 *Harshdorne DJ*, Ito M, Erdödi F. Myosin light chain phosphorylation: subunit composition, interactions and regulation. *J Muscle Res Cell Motil* 1998; 19: 325-341.

51 *Kimura K*, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of myosin phosphorylation by Rho and Rho-associated kinase (Rho-kinase). *Science* 1996; 273: 245-248.

52 *Li L*, Eto M, Lee MR, Morita F, Yazawa M, Kitazawa T. Possible involvement of the novel CPI-17 protein in protein kinase C signal transduction of rabbit arterial smooth muscle. *J Physiol* 1998; 506 (Pt 3): 871-881.

53 *Deng JT*, Sutherland C, Brautigan DL, Eto M, Walsh MP. Phosphorylation of the myosin phosphate inhibitors, CPI-17 and PHI-1, by integrin-linked kinase. *Biochem J* 2002; 367: 517-524.

54 *Kiss E*, Marúňay C, Corstots C, Gergely P, Ito M, Hartshorne DJ, Erdödi F. Integrin-linked kinase phosphorylates the myosin phosphate target subunit at the inhibitory site in platelet cytoskeleton. *Biochem J* 2002; 365: 79-87.

55 *MacDonald JA*, Eto M, Borman MA, Brautigan DL, Haystead TA. Dual Ser and Thr phosphorylation of CPI-17, an inhibitor of myosin phosphoryse, by MYPT-associated kinase. *FEBS Lett* 2001; 493: 91-94.

56 *Ihara E*, Moffat L, Ostrander J, Walsh MP, MacDonald JA. Characterization of protein kinase pathways responsible for Ca2+ sensitization in rat ileal longitudinal smooth muscle. *Am J Physiol Gastrointest Liver Physiol* 2007; 293: G699-G710.

57 *Khan WI*, Collins SM. Gut motor function: immunological control in enteric infection and inflammation. *Clin Exp Immunol* 2006; 143: 389-397.

58 *Grover M*, Herfarth H, Drossman DA. The functional-organic dichotomy: postinfectious irritable bowel syndrome and inflammatory bowel disease-irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2009; 7: 48-53.

59 *Galeazzi F*, Haapala EM, van Rooijen N, Vallance BA, Collins SM. Inflammation-induced impairment of enteric nerve function in nematode-infected mice is macrophage dependent. *Am J Physiol Gastrointest Liver Physiol* 2000; 278: G259-G265.

60 *Zhao A*, Urban JF Jr, Anthony RM, Sun R, Stiltz J, van Rooijen N, Wynn TA, Cause WC, Shea-Donohue T, Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. *Gastroenterology* 2008; 135: 217-225.e176.

61 *Ihara E*, Beck PL, Chappellaz M, Wong J, Medlicott SA, MacDonald JA. Mitogen-activated protein kinase pathways contribute to hypercontractility and increased Ca2+ sensitization in murine experimental colitis. *Mol Pharmacol* 2009; 75: 1031-1041.

62 *Ohama T*, Hori M, Momotani E, Ikawara Y, Guo F, Kishi H, Kobayashi S, Ozaki H. Intestinal inflammation down-regulates smooth muscle CPI-17 through induction of TNF-alpha and causes motility disorders. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: G1429-G1438.

63 *Collins SM*. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. *Gastroenterology* 1996; 111: 1683-1689.

64 *Merger M*, Viney JL, Borovjec R, Steele-Norwood D, Zhou P, Clark DA, Riddell R, Marie R, Podack ER, Croitoru K. Defining the roles of perforin, Fas/FasL, and tumour necrosis factor alpha in T cell induced mucosal damage in the mouse intestine. *Gut* 2002; 51: 155-163.

65 *Morsmann TR*, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7: 145-173.

66 *Schwarz NT*, Kalff JC, Türler A, Speidel N, Grandis JR, Billiar TR, Bauer AJ. Selective jejunal manipulation causes postoperative pan-enteric inflammation and dysmotility. *Gastroenterology* 2004; 126: 159-169.

67 *Kinoshita K*, Hori M, Fujisawa M, Sato K, Ohama T, Momotani E, Ozaki H. Role of TNF-alpha in muscularis inflammation and motility disorder in a TNBS-induced colitis model: clues from TNF-alpha-deficient mice. *Neurogastroenterol Motil* 2006; 18: 578-588.

68 *Ohama T*, Hori M, Sato K, Ozaki H, Karaki H. Chronic treatment with interleukin-1beta attenuates contractions by decreasing the activities of CPI-17 and MYPT-1 in intestinal smooth muscle. *J Biol Chem* 2003; 278: 48794-48804.

69 *Akiho H*, Khan WJ, Al-Kaab A, Blennerhassett P, Deng Y, Collins SM. Cytokine modulation of muscarinic receptors in the murine intestine. *Am J Physiol Gastrointest Liver Physiol* 2007; 293: G250-G255.
Kinosita K, Sato K, Horii M, Ozaki H, Karaki H. Decrease in activity of smooth muscle L-type Ca2+ channels and its reversal by NF-kappaB inhibitors in Crohn’s colitis model. *Am J Physiol Gastrointest Liver Physiol* 2003; 285: G483-G493.

Akiho H, Deng Y, Blennerhassett P, Knaushay H, Collins SM. Mechanisms underlying the maintenance of muscle hypercontractility in a model of postinfective gut dysfunction. *Gastroenterology* 2005; 129: 131-141.

Khan WI, Vallance BA, Blennerhassett PA, Deng Y, Verdu EF, Matthaei KJ, Collins SM. Critical role for signal transducer and activator of transcription factor 6 in mediating intestinal muscle hypercontractility and worm expulsion in Trichinella spiralis-infected mice. *Infect Immun* 2001; 69: 838-844.

Akiho H, Blennerhassett P, Deng Y, Collins SM. Role of IL-4, IL-13, and STAT6 in inflammation-induced hypercontractility of murine smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol* 2002; 282: G226-G232.

Zhao A, McDermott J, Urban JJ Jr, Gause W, Maddon KB, Yeung KA, Morris SC, Finkelman FD, She-Donohoue T. Dependence of IL-2 and IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. *J Immunol* 2003; 171: 948-954.

Khan WI, Blennerhassett PA, Deng Y, Gauldie J, Vallance BA, Collins SM. IL-12 gene transfer alters gut physiology and host immunity in nematode-infected mice. *Am J Physiol Gastrointest Liver Physiol* 2001; 281: G1102-G1110.

Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* 2004; 126: 1657-1664.

Crowell MJ, Sheteline MA, Moses PL, Mawe GM, Talley NJ. Enterochromaffin cells and 5-HT signaling in the pathophysiology of disorders of gastrointestinal function. *Curr Opin Investig Drugs* 2004; 5: 55-60.

Deschoolmeister ML, Else KJ. Cytokine and chemokine responses underlying acute and chronic Chiluris muris infection. *Int Rev Immunol* 2002; 21: 439-467.

Motomura Y, Ghia JE, Wang H, Akiho H, El-Sharkawy RT, Collins M, Wan Y, McLaughlin JH, Khan WI. Enterochromaffin cell and 5-hydroxytryptamine responses to the same infectious agent differ in Th1 and Th2 dominant environments. *Gut* 2006; 57: 475-481.

Makae S, Akiho H, Mizutani T, Yamada M, Tokunaga N, Aso A, Ogino H, Kanayama K, Sumida Y, Ibisoh Y, Itaba S, Nakamura K, Takayanagi R, Khan WI. Reciprocal modulation of smooth muscle cell contractility in Th1 and Th2 dominant environments using murine model of early post inflammatory gut dysfunction. (Abstract) T1778 DDW2009.

Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Charukova T, Zarawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedwick JD. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003; 421: 744-748.

Komiyama Y, Nakae S, Matsuki T, Nambo A, Ishigame H, Kakuta S, Sudoh K, Iwakura Y. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 2006; 177: 566-573.

Nakae S, Nambu A, Sudoh K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol* 2003; 171: 6173-6177.

Korn T, Betelli E, Owuka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; 27: 485-517.

Kobayashi T, Okamoto S, Hisamatsu T, Kamada N, Chinen H, Saito R, Kitazume MT, Nakazawa A, Sugita A, Koganei K, Isobe K, Hibi T. IL-23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn’s disease. *Gut* 2008; 57: 1682-1689.

Andoh A, Ogawa A, Bamba S, Fujimura Y. Interaction between interleukin-17-producing CD4+ T cells and colonic subepithelial myofibroblasts: what are they doing in mucosal inflammation? *J Gastroenterol* 2007; 42 Suppl 17: 29-33.

Fu Y, Wang W, Tong J, Pan Q, Long Y, Qian W, Hou X, Th17: a new participant in gut dysfunction in mice infected with Trichinella spiralis. *Mediators Inflamm* 2009; 2009: 517052.

Tokita Y, Sato K, Nishiyama M, Ikemura R, Akiho H, Nakamura K, Murao H, Ogin H, Kanayama K, Aso A, Ibisoh Y, Itaba S, Takayanagi R, Yamamoto M. Modulation of smooth muscle cell contractility by Th1/Th2 cytokines in gut hypercontractility in a murine model of T-cell-mediated persistent gut motor dysfunction. (Abstract) W1928 DDW2010.

Buono L, Fioramonti J. Visceral perception: inflammatory and non-inflammatory mediators. *Gut* 2002; 51 Suppl 1: i19-i23.

Wood JD. Neuropathophysiology of functional gastrointestinal disorders. *World J Gastroenterol* 2007; 13: 1313-1332.

O’Mahony SM, Bulmer DC, Coelho AM, Fitzgerald P, Bongiovanni C, Lee K, Winchester W, Dinan TG, Cryan JF. 5-HT(2B) receptors modulate visceral hypersensitivity in a stress-sensitive animal model of brain-gut axis dysfunction. *Neurogastroenterol Motil* 2010; 22: 573-578, e124.

Ohashi-Doi K, Himaki D, Nagao K, Kawai M, Gale JD, Furness JB, Kurebayashi Y. A selective, high affinity 5-HT1B receptor antagonist inhibits visceral hypersensitivity in rats. *Neurogastroenterol Motil* 2009; 22: e69-e76.

Mickle A, Sood M, Zhang Z, Shahmohammadi G, Sengupta JN, Miranda A. Antiinociceptive effects of melatonin in a rat model of post-inflammatory visceral hyperalgesia: a centrally mediated process. *Pain* 2010; 149: 555-564.

Saito-Nakaya K, Hasegawa R, Nagura Y, lto H, Fukudo S. Corticotropin-releasing hormone receptor 1 antagonist blocks colonic hypersensitivity induced by a combination of inflammation and repetitive colorectal distension. *Neurogastroenterol Motil* 2008; 20: 1147-1156.

Augé C, Balz-Hara D, Steinhoff M, Vergnolle N, Cenac N. Protease-activated receptor-4 (PAR 4): a role as inhibitor of visceral pain and hypersensitivity. *Neurogastroenterol Motil* 2009; 21: 1189-e107.

Ma TY, Boivin MA, Ye D, Pedram A, Said HM. Mechanism of TNF-α/alpha modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am J Physiol Gastrointest Liver Physiol* 2005; 288: G422-G430.

Al-Sadi RM, Ma TY. IL-1beta causes an increase in intestinal epithelial tight junction permeability. *J Immunol* 2007; 178: 4641-4649.

Al-Sadi R, Boivin M, Ma T. Mechanism of cytokine modulation of epithelial tight junction barrier. *Front Biosci* 2009; 14: 2765-2778.

Watson JL, Ansari S, Cameron H, Wang A, Akhtar M, McKay DM. Green tea polyphenol (-)epigallocatechin gallate blocks epithelial barrier dysfunction provoked by IFN-gamma but not by IL-4. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G954-G961.

Ramakrishna BS. Probiotic-induced changes in the intestinal epithelium: implications in gastrointestinal disease. *Trop Gastroenterol* 2009; 30: 76-85.

Brant LJ, Chey WD, Foxx-Orenstein AE, Schiller LR, Schoenfeld PS, Spiegel BM, Talley NJ, Quigley EM. An evidence-based position statement on the management of irritable bowel syndrome. *Am J Gastroenterol* 2009; 104 Suppl 1: S1-S35.

Foord AC, Brant LJ, Young C, Chey WD, Foxx-Orenstein AE, Moayyedi P. Efficacy of 5-HT3 antagonists and 5-HT4 agonists in irritable bowel syndrome: systematic review and meta-analysis. *Am J Gastroenterol* 2009; 104: 1831-1843; quiz 1844.

Frielings J, Cooke HJ, Wood JD. Serotonin receptors on submucosal neurons in guinea pig colon. *Am J Physiol* 1991; 261: G1017-G1023.

Bearcroft CP, André EA, Farthing MJ. In vivo effects of the...
5-HT3 antagonist alosetron on basal and cholera toxin-induced secretion in the human jejunum: a segmental perfusion study. *Aliment Pharmacol Ther* 1997; **11**: 1109-1114

105 Camilleri M, Chey WY, Mayer EA, Northcutt AR, Heath A, Dukes GE, McSorley D, Mangel AM. A randomized controlled clinical trial of the serotonin type 3 receptor antagonist alosetron in women with diarrhea-predominant irritable bowel syndrome. *Arch Intern Med* 2001; **161**: 1733-1740

106 Camilleri M, Northcutt AR, Kong S, Dukes GE, McSorley D, Mangel AW. Efficacy and safety of alosetron in women with irritable bowel syndrome: a randomized, placebo-controlled trial. *Lancet* 2000; **355**: 1035-1040

107 Gale JD. The use of novel promotility and prosecretory agents for the treatment of chronic idiopathic constipation and irritable bowel syndrome with constipation. *Adv Ther* 2009; **26**: 519-530

108 Camilleri M. Review article: new receptor targets for medical therapy in irritable bowel syndrome. *Aliment Pharmacol Ther* 2010; **31**: 35-46

109 Clarke G, Quigley EM, Cryan JF, Dinan TG. Irritable bowel syndrome: towards biomarker identification. *Trends Mol Med* 2009; **15**: 478-489

110 Macsharry J, O’Mahony L, Fanning A, Bairead E, Sherlock G, Tiesman J, Fulmer A, Kiely B, Dinan TG, Shanahan F, Quigley EM. Mucosal cytokine imbalance in irritable bowel syndrome. *Scand J Gastroenterol* 2008; **43**: 1467-1476