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Role of infection in irritable bowel syndrome

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Infection by pathogenic organisms leads to mucosal damage and disruption of the gut's extensive commensal flora, factors which may lead to prolonged bowel dysfunction. Six to 17% of unselected irritable bowel syndrome (IBS) patients believe their symptoms began with an infection, which is supported by prospective studies showing a 4%–31% incidence of postinfectious IBS-(PI) sfollowing bacterial gastroenteritis. The wide range of incidence can be accounted for by differences in risk factors, which include in order of magnitude; severity of initial illness > bacterial toxigenicity > hypochondriasis, depression and neuroticism, and adverse life events in the previous 3 months. PI-IBS has been reported after *Campylobacter*, *Salmonella*, and *Shigella* infections. Serial biopsies after *Campylobacter jejuni* gastroenteritis show an initial inflammatory infiltrate, with an increase in enterochromaffin (EC) cells, which in most cases subsides over the next 6 months. Those who go on to develop IBS show increased numbers of EC and lymphocyte cell counts at 3 months compared with those who do not develop IBS. Interleukin-1β mRNA levels are increased in the mucosa of those who develop PI-IBS, who also show increased gut permeability. Recover can be slow, with approximately 50% still having symptoms at 5 years. Recent studies suggest an increase in peripheral blood mononuclear cell cytokine production in unselected IBS, an abnormality that may be ameliorated by probiotic treatment. The role of small-bowel bacterial overgrowth in IBS is controversial, but broad-spectrum antibiotics do have a temporary benefit in some patients. More acceptable long-term treatments altering gut flora are awaited with interest.

Key words: irritable bowel syndrome, infection, inflammation

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Introduction

Normal flora and host defenses

The normal gut is host to several kilograms of bacteria, most of which reside in the colon. Bacterial counts in the stomach number 10^{1-2} colony-forming units (cfu)/ml, while in the jejunum 10^{3-4} cfu/ml, largely of pharyngeal organisms, is normal. As one progresses toward the ileocecal valve, counts rise, and within the ileum 10^{5-6} is normal. However, at the ileocecal valve, there is an abrupt increase in numbers, which rise to 10^{10-13} cfu/ml, with a marked change in the nature of organisms, which become anaerobic/facultative anaerobic, with coliforms and anaerobes predominating. Throughout the gut, there are extensive innate defensive mechanisms, which include both gastric acid, bile, lysozyme, and defensins in the upper gut and tight intraepithelial junctions and mucus layers in the lower gut. There is also an acquired immunity based on immunoglobulin secretion (mainly IgA) and cell-mediated responses. Breaches of these defenses by pathogenic organisms produce acute inflammation with release of cytokines and recruitment of circulating inflammatory cells. In addition to breaches in the epithelium, there is also extensive damage to the lamina propria and submucosa together with the associated nerves. During the process of healing, metaplastic changes occur with increases in enteroendocrine cells, Paneth cells, and chronic inflammatory cells.[1] Furthermore, remodeled nerves often show aberrant expression of receptors and neuropeptides.[2]

Symptoms of postinfectious irritable bowel syndrome

While most patients rapidly recover from infectious gastroenteritis, about a quarter show persistent disturbance of bowel habit at 6 months, most commonly increased stool frequency.[3] A much smaller number,

Table 1. Incidence of postinfectious irritable bowel syndrome

Prospective studies on bacterial gastroenteritis	PI-IBS (%)	Follow-up (months)	*n*	Organism	Location
McKendrick, 1994	31	12	38	*Salmonella*	Sheffield, UK
Gwee et al., 1996[14]	29	3	75	Mixed	Sheffield, UK
Neal et al., 1997[3]	7	6	390	Bacterial	Nottingham, UK
Thornley et al., 2001[8]	9	6	180	*C. jejuni*	Nottingham, UK
Dunlop et al., 2003[9]	13	6	747	*C. jejuni*	Nottingham, UK
Wang et al., 2004[7]	8	12–24	295	*Shigella*	Beijing, China
Ji, 2005	7	6	101	*Shigella*	Seoul, Korea
Mearin, 2005	12	12	677	*Salmonella*	Catalonia, Spain

PI-IBS, postinfectious irritable bowel syndrome; *C. jejuni*, *Campylobacter jejuni*

about 10% on average, develop persistent symptoms that meet the Rome II criteria for the diagnosis of irritable bowel syndrome (IBS), namely abdominal pain and discomfort that is relieved by defecation and/or associated with a change in the stool form or consistency.[4] Such patients often undergo extensive investigations to exclude disorders of malabsorption and inflammation. Although there are quantitative changes in mucosal immunocytes, these still lie within the normal range and would be judged normal by most experienced histopathologists.

Postinfectious (PI)-IBS is a useful model for understanding the mechanisms in IBS because it appears in patients with otherwise normal bowel patterns. It is essentially a natural experiment, since infection is largely a random event. Compared with other IBS patients, there is a clearly defined start date, and symptoms tend to be more homogeneous. Compared with "non-PI-IBS," the role of psychological abnormalities is less in PI-IBS, with only 25% reporting a history of prior treatment for anxiety or depression, compared with nearly 50% in non-PI-IBS.[5]

Definition of clinical PI-IBS in clinical practice

Many patients acquire gastroenteritis while away from home, where they encounter flora they are otherwise not familiar with. Under these circumstances, it is not uncommon to lack a positive stool culture. Therefore, a useful clinical definition of PI-IBS, which we have used before,[6] is as follows:

New onset of IBS symptoms meeting the Rome criteria, developing acutely after an illness characterized by two or more of the following:

1. fever
2. vomiting
3. acute diarrhea
4. positive stool culture.

The clinical features of PI-IBS are bloating, loose and watery stools, urgency, and the passage of mucus per rectum.[3] The incidence of PI-IBS varies according to the series, from as low as 7% to as high as 31%, though the largest studies seem to agree on around 10% (Table 1). It has been reported after *Salmonella*, *Shigella*, and *Campylobacter* gastroenteritis and appears to be a nonspecific response. These studies come from Canada, Spain, Korea, China, and the United Kingdom, so PI-IBS does not appear to be unique to any specific country or race.

Risk factors for developing PI-IBS

Risk factors include both central and peripheral components. Peripheral components probably reflect the severity of the mucosal lesion, while central components reflect past and present psychosocial factors.

Severity of initial injury

One measure of severity is the duration of the initial diarrheal illness. Diarrhea lasting more than 3 weeks gives a relative risk of 11.4 [95% confidence interval (CI), 2.2–58] compared with diarrhea lasting less than 7 days,[3] while in another study a duration of >14 days gave a relative risk of 4.6 (95% CI, 2.1–9.9).[7] Specific bacterial factors cannot be readily compared between species, but within the *Campylobacter* species, the presence of an "elongating toxin," as defined by its effect on cultured cell lines, gave a relative risk of 12.8 (95% CI, 1.6–101) for the development of persistent bowel dysfunction.[8] During the healing process, changes in the epithelium are also predictive of the development of PI-IBS, with each one standard deviation increase in enterochromaffin (EC) cell counts giving a 3.2-fold (95% CI, 1.8–8.2) increase in risk of developing

postinfectious symptoms.[9] EC counts correlate closely with other inflammatory cells, including lymphocytes, which in animal models drive EC hyperplasia,[10] so it is likely that other inflammatory markers would show similar relationships.

Host factors

Host factors include female sex, which gives a two- to threefold increased risk,[3,11] and age >60, which gives a protective effect, with a relative risk of just 0.36 (95% CI, 0.1–0.9).[3] Older subjects may have acquired immunity, but they also have fewer immunocytes in their rectal mucosa[12] and may be less reactive to infection. Women show no difference in mucosal immunocyte numbers,[12] and it appears, when a multivariate analysis is done, that the sex effect is spurious, owing to confounding by psychological disturbances, which are commoner in women. When these are included in a multivariate model, sex no longer has a predictive effect.[11,9]

Psychological factors

The presence of depression, which correlates closely with anxiety, gives a relative risk of 3.2 (95% CI, 1.8–8.2)[9] for each standard deviation increase in the score on the Hospital and Anxiety Scale,[13] while hypochondriasis gives a relative risk of 2.0 (95% CI, 1.7–2.5).[14] Gwee et al.[11] also found that the presence of adverse life events in the previous 3 months doubled the risk of persistent symptoms, relative risk 2.0 (95% CI, 1.7–2.4). Thus, as with other IBSs, there is an interaction between central and peripheral components, which no doubt varies between patients and accounts for the heterogeneity between different series, both in symptoms and response to treatment.

Histological changes following infection

Serial biopsy studies show an initial inflammatory response with increases in CD3 lymphocytes, CD8 intraepithelial lymphocytes, and calprotectin-positive macrophages.[1] These changes rapidly decline in most subjects, but a small number with persistent symptoms fail to show this decline.[1] One of the most striking abnormalities is the increased numbers of serotonin-containing EC cells. These are found in the crypts, where on stimulation they release serotonin into the lamina propria. There, the serotonin acts on receptors on both nerve endings and enterocytes. Relevant stimuli include adrenergic stimuli as well as bacterial toxins such as cholera and *Escherichia coli* enterotoxins and distortion by intraluminal pressure. Recent studies using isolated enteroendocrine cells indicate the presence of numerous receptors, which allow these cells to integrate many different stimuli.[15] Patients who develop PI-IBS have a 20% increase in EC cells when assessed 3 months after infection[9] and a switch in the ratio of 5-hydroxytryptamine (5HT)/peptide YY (PYY)-containing enteroendocrine cells so that 5HT is the predominant component of enteroendocrine granules after the infection, whereas before PYY predominates.[1]

Abnormalities of serotonin metabolism in PI-IBS

As well as increased numbers, there is evidence of increased release of 5HT following a test meal, as can be ascertained from measuring 5HT in platelet-poor plasma (PPP). Compared with controls, PI-IBS patients show increased 5HT in PPP after a 520-Kcal spaghetti-on-toast meal (Fig. 1). These levels are significantly greater than in either controls or constipated IBS patients. Although this may reflect an increased release from the gut, the origin of 5HT in PPP is unclear, since 5HT is rapidly taken up by platelets and only a very small fraction of 5HT released in the gut is likely to reach peripheral blood. An alternative explanation for the elevated levels is an impairment of the serotonin transporter affecting both the gut and platelets. The action of 5HT is rapidly terminated by the active uptake of this sodium-linked transporter. In the mucosa, the enterocytes provide the largest uptake capacity, where serotonin is metabolized by intracellular monoamine oxidases to 5-hydroxyindoleacetic acid (5HIAA). This water-soluble molecule is then excreted in the urine. Several studies have shown elevation of both plasma 5HT and 5HIAA following a meal in IBS patients with diarrhea.[16] By measuring not only 5HT levels in the blood but also those in the mucosa, it is possible to estimate the relative amount of 5HT per EC cell in different conditions. When this was done, the ratio of 5HT concentration to EC cell numbers/mm^2 was found to increased in constipated IBS, in keeping with impaired release and hence increased 5HT stores in each EC cell.[17] The 5HIAA/5HT ratio in the mucosa is depressed both in constipated IBS and to a lesser extent in PI-IBS. The lowered 5HIAA levels are as expected in constipated IBS with a reduced 5HT turnover. However, the reduced ratio in PI-IBS is unexpected given the increased release and implies either an impairment of serotonin reuptake or of enterocyte monoamine oxidase activity.

Abnormalities of serotonin transporter

One seminal study has shown marked impairment of serotonin transporter (SERT) immunoreactivity in rec-

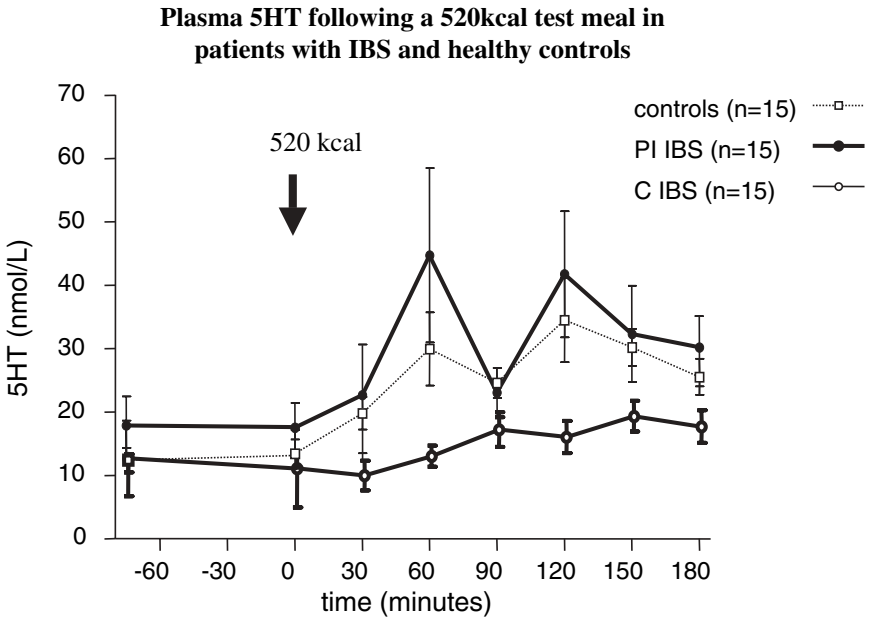

Fig. 1. Increased postprandial 5-hydroxytryptamine (*5HT*) levels in platelet-poor plasma in postinfectious irritable bowel syndrome (*PI-IBS*). *C-IBS*, constipation IBS. From Dunlop et al. Clin Gastroenterol Hepatol 2004;3:349–57.[17] Reproduced by kind permission of the editor of *Clinical Gastroenterology & Hepatology*

tal biopsies from patients with IBS of both the diarrhea (IBS-D) and constipated (C-IBS) variety. This was associated with a decrease in SERT mRNA. However,[18] these results are controversial, and recent attempts to reproduce them have failed, showing only a modest nonsignificant decrease in mRNA in IBS-D and no change in C-IBS.[19] Genetic factors are known to influence the expression of SERT throughout the body with a promoter polymorphism that predicts reduced function when assessed in an immortalized lymphoblast cell line. Possessing either one or two shortened alleles (*s*) predicts reduced serotonin uptake and has been associated with an increased susceptibility to develop depression in response to adverse life events[20] and post-traumatic stress disorder.[21] This important gene by environment interaction means that the numerous cross-sectional population studies of the association of SERT polymorphisms and psychiatric disease yield inconsistent results, since they rarely include the environmental factors. Studies of IBS have also been inconsistent, but the largest suggested an excess of the *ss* but not of the *sl* genotype in IBS-D.[22] However, a recent study did not show any relation between this SERT promoter polymorphism and mRNA for SERT in mucosal biopsies,[19] which may suggest that this is not the relevant promoter for the gut as others have suggested.[23] Any link therefore between this SERT polymorphism and IBS may not be direct but mediated by its impact on psychological factors such as neuroticism and anxiety.

Evidence of gut inflammation in IBS

Increased mucosal lymphocyte counts are associated with the development of PI-IBS.[9,11] Furthermore, as Table 2 documents, there are now numerous studies that indicate that mucosal biopsies in both PI-IBS and IBS-D show increased numbers of $CD3^+$ lymphocytes. Different researchers have looked at different classes of immunocytes, but there is broad agreement on the increase in $CD3^+$ lymphocytes. Chadwick et al.[24] also showed increases in $CD25^+$ lymphocytes, indicating the presence of interleukin (IL)-2 receptor, a marker of activated lymphocytes. Other researchers have also examined the presence of mast cells (MC), which have been increased in a number of studies (Table 2). A recent study from Bologna showed increased MC in unselected IBS, which included patients with constipation.[25] In addition, two studies have examined the presence of mRNA for IL-1β, a macrophage product that has been shown to be increased in both PI-IBS[26] and IBS-D.[7] These inflammatory cells produce cytokines, which are known to alter enteric neural function and contribute to diarrheal symptoms.[27,28]

Gut permeability, which is known to be increased by inflammatory cytokines, has also been shown to be increased by bacterial gastroenteritis[1] and in both PI-IBS and IBS-D.[29] This increase appears to be long lasting, since the Walkerton, Ontario, study of 10012 cases of infective gastroenteritis due to *E. coli* O157 and *Campylobacter jejuni* found increased permeability 2 years after the infectious event in those who developed PI-IBS.[30]

Table 2. Histological findings in mucosal biopsies in IBS of various subtypes

Author, year, type of IBS	Site	Mast cells	CD3 lymph	Macrophage	EC cells
Weston et al., 1993,[49] IBS-D	T ileum	++	+		NA
O'Sullivan et al., 2000,[50] IBS-D	Cecum	++	NA		NA
Gwee et al., 1999,[11] **PI-IBS**	Rectum	–	++	IL-1 mRNA↑	NA
Spiller et al., 2000,[1] **PI-IBS**	Rectum	–	++		++
Chadwick et al., 2002,[24] IBS-D	Colon	–	++		NA
Dunlop et al., 2003,[5] IBS-D (not PI-IBS)	Rectum	++	++		–
Dunlop et al., 2003,[9] **PI-IBS**	Rectum	–	++		++
Wang et al., 2004,[7] **PI-IBS** & IBS-D	T ileum	+	+	IL-1 mRNA↑	NA
	Colon	–	+		NA
Barbara et al., 2004,[25] C-IBS & IBS-D	Colon	++	+		NA
Park et al., 2006,[51] IBS-D	Rectum		++		–

IBS, irritable bowel syndrome with diarrhea; C-IBS, irritable bowel syndrome with constipation; EC, enterochromaffin; IL, interleukin; NA, not assessed

Evidence of systemic immune activation in IBS

Peripheral blood mononuclear cell cytokine production has recently been shown to be elevated in IBS by a number of researchers.[31–34] Some of these cytokine abnormalities may be driven by psychological stress, since experimental stress has been shown to increase some cytokines, including IL-6 and interferon.[35] IBS patients have also been shown to have a depressed IL-10/IL-12 ratio compared with controls, an abnormality that is corrected by treatment with a probiotic, *Bifidobacterium infantis.*[32] These cytokine abnormalities may have relevance to the extraintestinal symptoms that are often so prominent in IBS.[36]

Interaction between inflammation and impaired SERT

Several animal studies have indicated that inflammation leads to downregulation of SERT.[10,37] Whether this downregulation is specific or merely a feature of mucosal injury has not been determined. The implications are that low-grade inflammation could lead to an increase in mucosal serotonin availability, and this might account for the symptomatic improvement noted with $5HT_3$ antagonists in IBS-D.[38] Alternatively, inflammation may increase the local release of 5HT as a result of tissue injury and the release of adenosine, which is known to be capable of stimulating 5HT release from EC cells.[39]

Small-bowel bacterial overgrowth in IBS?

There have been several reports of small-bowel bacterial overgrowth (SIBO) in IBS as detected using the lactulose breath test.[40] The criteria used by the researchers were somewhat inconsistent but rely on the concept that small-bowel bacterial overgrowth will result in a double peak of hydrogen excretion as the lactulose bolus encounters successfully first small intestinal and then colonic bacteria. Unfortunately, as Riorden et al.[41] showed using a lactulose breath test combined with scintigraphy,[41] the double peak is quite unreliable. Thus, in a study of 28 patients in whom the lactulose breath hydrogen test was combined with scintigraphy and culture of jejunal aspirate, the lactulose breath tests detected only 38% of true SIBO (jejunal culture $>10^5$ cfu/ml). Furthermore, only 17% of those with positive jejunal cultures showed a double peak, and single peaks were seen in 21% of subjects with true SIBO. Finally, 50% of double peaks were seen in subjects with a sterile small bowel.

However, even when the subjects studied did not have bacterial overgrowth, a randomized control trial of neomycin given for just 10 days showed a transient improvement 1 week after treatment ceased, an effect which was greatest for those whose breath hydrogen fell the most.[40] Others have also reported transient symptomatic benefit with antibiotics such as metronidazole.[42] The mechanism of benefit is unclear, but may be similar to that seen in diets that exclude sources of poorly absorbed carbohydrate, which have been shown to lead to a reduction in 24-h hydrogen and methane gas excretion.[43] However, antibiotic treatments seem unlikely to be useful, as symptoms promptly return on stopping antibiotics and chronic antibiotic therapy is invariably associated with the development of bacterial resistance. Furthermore, other authors have suggested that the consumption of antibiotics actually increases the risk of subsequently developing IBS symptoms.[44]

Possible role for probiotics

A more attractive alternative would be to manipulate the colonic flora by the use of probiotics. These preparations have a number of potentially valuable properties, including the inhibition of other bacteria and the reduction of symptoms during acute infection. Furthermore, some probiotics are known to exert anti-inflammatory effects through downregulating systemic immunity.[45] As recent meta-analyses indicate, not all probiotics are equal,[46,47] but two recent large studies have suggested a symptomatic benefit in IBS with *Bifidobacterium infantis*,[32,48] and in one study there was an associated normalization of the immune response.[32] Whether this effect will prove useful and how to target it effectively to the right type of patient remains to be established.

Implications for treatment

Not all patients develop IBS after infection, and not all have evidence of inflammation or abnormal flora. Even the best current treatment for IBS, the 5HT3 antagonists, still have a number needed to treat of seven,[38] indicating clearly that "most treatments don't work for most patients," to quote the chairman of a famous pharmaceutical company. As we further understand the mechanisms of IBS, the aim must be to identify easily measurable objective markers that will allow classification of IBS patients into more homogenous groups in a way that current symptom-based systems do not. Only then can we expect the current poor therapeutic response to be improved as we target treatments to underlying causes.

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