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Histopathological alterations in irritable bowel syndrome

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Irritable bowel syndrome is a common disorder defined by a symptom complex including abdominal pain and altered bowel habit. The etiopathogenesis appears to be multifactorial and to involve altered gastrointestinal motor function, enhanced perception of visceral stimuli and psychosocial factors. More recently a role for mucosal immune activation has been suggested. Routine histologic examination reveals no mucosal abnormality in the majority of cases but quantitative histological, immunohistochemical and ultrastructural analyses reveal subtle morphologic changes involving lymphocytes, mast cells, enterochromaffin cells and enteric nerves. The recent appreciation of these changes has led to new hypotheses linking central and enteric nervous systems to immune processes. This review highlights the spectrum of morphologic changes that occur in irritable bowel syndrome, examines their relationship to the pathophysiology of irritable bowel syndrome and considers their relevance to daily pathology practice.
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Irritable bowel syndrome (IBS) is the most common gastrointestinal disorder encountered in general and gastroenterology practice.[1,2] It affects 10–20% of adolescents and adults in western societies[3] and is the commonest cause of recurrent abdominal pain in children.[4] IBS is defined by a symptom complex which includes abdominal pain and altered bowel function in the form of diarrhea, constipation, a sensation of fullness following evacuation, bloating or the passage of mucus per rectum. There are no physical or laboratory findings which are specific for IBS and the diagnosis is therefore based on symptomatology. To standardize the diagnosis of IBS, several symptom-based diagnostic criteria have been established over the last two decades including the Manning, Rome I, Rome II and, most recently, Rome III criteria.[5] The Rome criteria have shown a reasonable sensitivity and specificity for IBS. In patients without atypical symptoms (such as loss of weight, fever, rectal bleeding and nocturnal awakening due to pain) the Rome criteria have a positive predictive value of approximately 98% for IBS, that is additional investigations will yield an alternative diagnosis in only 2% of cases.[1,6]

IBS has been subclassified into diarrhea predominant, constipation predominant and mixed subtypes. In addition, a subset of patients have associated extraintestinal symptoms including fatigue, fibromyalgia, urinary frequency and headache.[6] A small but significant proportion of IBS of patients report onset of IBS symptoms following an episode of acute gastroenteritis (post-infectious IBS, PI-IBS).[7–9] PI-IBS has been reported following *Shigella*,[8] *Salmonella*,[9,10] and *Campylobacter jejuni*[7,11] infection. A unifying hypothesis to explain the pathogenesis of IBS remains elusive. Alterations in gastrointestinal motor function, enhanced visceral perception of painful stimuli and psychosocial factors are considered key contributors to symptom generation in IBS.[12,13] Alterations in gastrointestinal motor function, enhanced visceral perception of painful stimuli and psychosocial factors are considered key contributors to symptom generation in IBS.[12,13] More recently recognized factors include reduced ability to expel intestinal gas, altered central processing of afferent signals and intestinal inflammation.[13] While routine histologic examination reveals no significant colonic mucosal abnormality in the majority of these patients, recent quantitative histological, immunohistochemical and ultrastructural analyses provide evidence of

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subtle morphologic changes in these patients. The recognition of these changes has led to new hypotheses linking central and enteric nervous systems to immune processes. This review highlights the spectrum of histologic changes that occur in IBS, examines their relationship to the pathophysiology of IBS and considers their relevance, if any, to daily pathology practice.

Colonoscopy in IBS

The American Gastroenterological Association recommends colonoscopy only in those patients with presumed IBS who are over 50 years of age or who have symptoms raising the possibility of another disease, in particular diarrhea predominance and/or weight loss.[14] Therefore, in practice, only a small minority of patients with IBS will undergo colonoscopy and biopsy. Owing of its high prevalence, however, IBS still accounts for the majority of colonoscopic biopsies seen by many gastrointestinal pathologists.[15] Most of these biopsies will be either normal or near to normal on routine histological examination. From a clinical standpoint, a normal pathology result therefore provides valuable information to the physician who is suspecting a diagnosis of IBS. It is thus important for pathologists to be aware of variations of normal as well as of artifacts that may result from bowel preparations or the biopsy procedure, and not to report these as abnormal. These changes will therefore be highlighted prior to reviewing the subtle morphologic changes recently reported in IBS.

The spectrum of normal in colonic biopsies

Variations exist in the normal histology of the right and left colon and these reflect the different functions of absorption and lubrication respectively.[16] The crypts of the cecum and right colon show a predominance of absorptive cells over goblet cells which should not be mistaken for mucin depletion. The lamina propria of this region is more cellular than the left colon and rectum and includes lymphocytes, plasma cells (which may be basal) and eosinophils. Lamina propria cellularity in the left colon is much lower with fewer eosinophils and only superficial plasma cells. An age related decline in lymphocytes and mast cells has recently been reported in normal large bowel mucosa.[16] Paneth cells are normally present in the mucosa of the cecum and right colon, but when seen in the left colon represent a metaplasia, usually in response to chronic inflammation.[15,17] Lymphoid aggregates are a normal component of colorectal mucosa and are frequently associated with branching of overlying crypts.[17] Aggregates of muciphages are commonly observed in colorectal mucosa, being present in 40% of normal rectal biopsies.[18] They probably are a result of clinically insignificant damage to the crypt epithelium which may occur during stool passage or subclinical infection. Bowel preparations may induce a number of histologic changes. Hypertonic enemas, frequently administered prior to sigmoidoscopy, can induce surface epithelial injury including mucin depletion, mild neutrophilic infiltration and loss of surface epithelium as well as edema and hemorrhage in the lamina propria.[19] Sodium phosphate preparations, the preferred preparations for full colonoscopy in adults, can induce aphthous-like lesions which histologically are associated with large lymphoid aggregates or less commonly edema, hemorrhage or acute inflammation.[20–22] These preparations may also induce foci of neutrophilic cryptitis (focal active colitis) which is thought to occur in approximately 3% of patients.[22] Tissue trauma induced by biopsy forceps can result in mucosal hemorrhage or extraction of epithelium from crypts. If this involves a large area, it may be mistaken for ischemic injury.[15]

Histological changes in IBS

The subtle morphologic changes reported in the intestinal mucosa in IBS involve chronic inflammatory cells, mast cells, enteroendocrine cells and enteric nerves, each of which is discussed below and summarized in Table 1.

Chronic Inflammatory Cells

Several investigators have demonstrated increased numbers of chronic inflammatory cells in the colonic mucosa of patients with IBS.[11,12,23–27] In most studies, quantitative immunohistochemical analyses have been required to unmask these increases[11,23–27] which are not usually apparent on routine histological evaluation. Both the lamina propria[23–25] and surface and crypt epithelium[11,23,27] have been shown to contain increased numbers of T-lymphocytes in IBS. Such increases have been reported to occur in both PI-IBS[11,12,24,25] and non-PI-IBS.[23,25] The diarrhea predominant form IBS is reported to be associated with a greater increase in mucosal T-lymphocytes than the constipation predominant form.[23] Reported increases in mucosal lymphocytes range from 20 to 100% and 80 to 250%, for lamina propria and epithelial lymphocytes, respectively. Such variations are likely to reflect differences in subpopulations of IBS patients studied and differences in the sites of biopsy. A marked increase in lamina propria inflammatory cells expressing CD25, a component the interleukin 2 receptor and a marker of immune activation, has recently been reported in colonic biopsies from patients with IBS.[23] This was present in almost 90% of patients and was not dependent on the dominant symptom profile of IBS patients or a preceding episode of gastroenteritis.

Table 1 Low-grade inflammation in IBS: summary findings of a literature review

Investigators	*Clinical subtype*	*Number of patients*	*Site of biopsy*	*Layer of intestine*	*Chronic inflammation, NOS*	*T-lymphocytes*	*Mast cells*	*Enterochromaffin cells*	*Inflammatory cells in proximity to nerves*
Gwee *et al*[12]	PI-IBS	10	Rectum	Mucosa	+	NA	–	NA	NA
Spiller *et al*[11]	PI-IBS	10	Rectum	Mucosa	NA	++	–	+++	NA
Dunlop *et al*[24]	PI-IBS	30	Rectum	Mucosa	NA	+	–	+	NA
Dunlop *et al*[25]	PI-IBS	23	Rectum	Mucosa	NA	+	–	+	NA
Dunlop *et al*[25]	Non-PI-IBS	52	Rectum	Mucosa	NA	+	+	–	NA
Weston *et al*[32]	D-IBS	20	Terminal ileum	Mucosa	NA	+	+++	NA	NA
O'Sullivan *et al*[31]	D-IBS	14	Cecum	Mucosa	–	NA	++	NA	NA
Wang *et al*[8]	D-IBS	56	Terminal ileum	Mucosa	NA	NA	++	NA	+
Park *et al*[29]	D-IBS	14	Cecum, rectum	Mucosa	NA	NA	++	NA	++ (Mast cells)
Barbara *et al*[30]	IBS	44	Left colon	Mucosa	NA	NA	+++	NA	++ (Mast cells)
Salzmann *et al*[26]	IBS	51	Colon	Mucosa	+	NA	NA	NA	NA
Tornblom *et al*[27]	IBS	10	Jejunum	Myenteric plexus	Lymphocytes	++	NA	NA	+ (Lymphocytes)
Hiatt and Katz[28]	'Spastic colitis'	4	Colon	Muscularis externa	NA	NA	+	NA	NA

PI-IBS, postinfectious IBS; Non-PI-IBS, non-postinfectious IBS; D-IBS, diarrhea predominant IBS; NA, not assessed.

The histologic changes of PI-IBS following *C. jejuni* gastroenteritis have been well described.[11] At 2 weeks postinfection, the mucosa in most cases has returned to normal by both macroscopic and conventional histologic assessment. However, quantitative histology reveals evidence of ongoing inflammation, which gradually decreases over the following 3 months. Inflammatory changes persist at 1 year in a small subgroup of these patients who also have clinical features fulfilling the Rome II criteria for IBS. These patients exhibit greater IL-1β mRNA expression, both during and after the infection, compared with individuals who do not develop IBS after an episode of gastroenteritis.[12] In addition, patients with PI-IBS have recently been shown to have greater IL-1β mRNA expression than patients with non-PI-IBS,[8] consistent with immune activation in this subset of patients.

Mast Cells

In 1962, Hiatt and Katz[28] reported increased numbers of mast cells in the muscularis propria of four patients with 'spastic colitis'. These findings were not pursued by other investigators, perhaps due to the unavailability of full thickness colonic specimens in IBS patients. It is only the last decade that has seen a renewed interest in the association of mast cells in IBS.[8,25,29–32] In 1993, Weston *et al*[32] demonstrated a marked increase in mast cell density in terminal ileum biopsies from patients with IBS compared to controls, a finding later confirmed by others.[8] This increase was most marked in the diarrhea predominant subgroup[32] but was present both in PI- and non-PI-IBS.[8] Subsequent studies have demonstrated increased mast cell density in the cecal mucosa of IBS patients.[29,31] While several investigators have failed to demonstrate increased numbers of mast cells in colorectal biopsies from patients with IBS,[8,24,31,33,34] recent studies using sensitive techniques, such as electron microscopy and/or immunohistochemistry combined with computer assisted morphometry, have demonstrated an increased mast cell density in the descending colon,[30] caecum and rectum.[29] Immunoenzymatic studies have demonstrated increased mucosal mast cell tryptase content as well as increased spontaneous release of tryptase and histamine in IBS patients compared to controls.[30] Ultrastructural studies have shown increased numbers of degranulating mast cells in IBS patients compared to controls[29,30] as well as increased numbers of mast cells in proximity to enteric nerves in the rectum,[29] descending colon,[30] caecum[29] and terminal ileum.[8]

Mast cells may be central in the strong association between interstitial cystitis (IC) and IBS, both of which are exacerbated by stress. Bladder and colon biopsies from a patient with both IC and IBS were showed an increase in both bladder and colonic mast cells, the latter mostly close to substance P-positive nerves.[35]

Enterochromaffin Cells

Increased numbers of enterochromaffin cells (EC) have been reported in rectal biopsies from patients with PI-IBS[11,24,25] but not in non-PI-IBS.[25] A fivefold increase in synaptophysin positive EC cells has been reported in rectal biopsies 2 weeks following *C. jejuni* infection.[11] In these patients, EC numbers decreased gradually in biopsies taken at 6 and 12 weeks.[11] However, in a small subset of patients who remained symptomatic at 1 year postinfection, rectal biopsies showed persistently elevated EC levels in the range seen at 2 weeks postinfection.[7,11] These levels were similar to those seen in a group of PI-IBS patients recruited from an outpatient clinic.[11] The profile of secretory granules also changed significantly postinfection, being 5-hydroxytryptamine (5-HT, serotonin) predominant 3 months following *Campylobacter* infection compared to peptide YY (PYY) predominant in normal controls.[7] However, EC cells from patients who developed PI-IBS showed similar 5-HT/PYY ratios to those of non-PI-IBS patients and normal controls.[25]

Enteric Nerves

Increased numbers of nerve fibres staining positively for neurone specific enolase, substance P and 5-HT (but not calcitonin gene-related peptide) have been demonstrated in biopsies from the terminal ileum and rectosigmoid in patients with both PI- and non-PI-IBS.[8] In addition, positively stained nerve fibres around mast cells are reported to be significantly increased in density in IBS patients compared to controls.[8] The distance between axonal fibres of the enteric nervous system and inflammatory cells, including mast cells[8,29,30] and lymphocytes,[36,37] is reported to be decreased in patients with IBS compared to controls. A study in evaluating full thickness jejunal biopsy specimens in 10 patients with severe IBS, reported striking neuronal changes including lymphocytic infiltration of the myenteric plexus (9/10 patients) and neuronal degeneration (7/10 patients).[27] Mast cells were not identified in the vicinity of the myenteric plexus. An editorial expressed caution in the interpretation of these findings due to the small patient numbers, inclusion of only severe cases of IBS, suboptimal controls and the use of a scoring system for myenteric plexus associated lymphocytes that might exaggerate any differences present.[38]

Morphologic changes and pathophysiology of IBS

The recently documented low-grade inflammatory changes outlined above suggest that immune activation may, at least in part, play a role in the pathogenesis of IBS. The reported increases in inflammatory cells, although modest, could potentially be of pathophysiological significance if accompanied by similar increases in cytokine production. The recent documentation of increased numbers of immune cells expressing CD25, a marker of immune cell activation,[23] provides functional evidence of immune activation in IBS. CD25 + cells are considered important regulators of intestinal inflammation, and it has been speculated that they may play a role in downregulating the inflammatory process in IBS.[39] Further evidence for immune cell activation in IBS includes upregulation of IL-1-β mRNA which has been documented in patients with PI-IBS[8,40] but not in non-PI-IBS.[8] This may reflect different pathogenetic mechanisms in these two subsets of patients.

The role of psychological stress is well recognized in the etiopathogenesis of IBS and there is growing evidence of interplay between immune and central nervous systems. Animal studies suggest that stress may enhance responsiveness to inflammatory stimuli in the gut, while inflammatory processes in the gut may influence behavior and brain function.[41–44] The close proximity of chronic inflammatory cells to enteric nerves in the mucosa[36,37] and muscularis externa[27] of patients with IBS, provides an interface for direct interaction between cells and the enteric nervous system. Potential mechanisms of immune activation in IBS that have been suggested include a previous episode of gastroenteritis, alterations in intestinal microflora, undiagnosed food allergies and genetic factors.[45]

Reports of increased mast cells numbers,[8,25,29–32] increased mast cell degranulation,[29,30] increased spontaneous release of mast cell tryptase and histamine[30] and increased proximity of MC to enteric nerves[8,29,30] in IBS suggest a role for mast cells in the disturbed sensorimotor function characteristic of this condition. The proximity of mast cells to enteric nerves suggests that mast cell mediators have increased potential to activate enteric neurons. Indeed, the latter has been reported to correlate with the severity or frequency of abdominal pain or discomfort in patients with IBS.[30] Mast cell mediators such as tryptase and histamine are known to activate enteric neurons leading to abnormal secretomotor function and visceral hypersensitivity.[46,47] Conversely, substance P in low concentrations has been shown to alter mast cell excitability and can directly modulate mast cell function following release from nerves.[48] The cause of mast cell infiltration and degranulation in IBS remains uncertain, but past episodes of infectious enteritis,[7,11] undiagnosed food allergies[49] and stress[50] may contribute.

The modest increase in EC cell numbers reported in PI-IBS[7,11,24,25] may have pathophysiologic significance. EC cells constitute the bulk of the body's 5-HT stores[13] and there is evidence of increased 5-HT release in patients with IBS.[51,52] Enteric nerves and sensory afferents contain a number of receptors for 5HT[53,54] and the prokinetic and secretory effects of

5HT may underlie the diarrhea and loose stools in IBS.[25,54] In addition to its prokinetic effects, 5-HT may exert pro-inflammatory effects via 5-HT(2) receptors on vascular endothelial cells facilitating recruitment of additional T-lymphocytes.[25] The mechanism underlying the increase in EC cells in IBS has not been elucidated although lymphocyte-derived cytokines or prostaglandins have been shown to induce increased EC numbers in animals.[54] A decrease in serotonin transporter has been reported in association with EC hyperplasia in a mouse model of PI-IBS and this may serve to further enhance mucosal 5-HT availability.[55] Anti5-HT(3) receptor antagonists have proved useful in the treatment of diarrhea predominant IBS, while the constipation predominant form may respond to 5-HT(4) receptor agonists.[56,57] In rare cases 5-HT(3) receptor antagonist has been associated with colon ischemia.[58] Interestingly, the rate of colon ischemia among patients carrying a diagnosis of IBS is over three times higher than that of the general population.[59] This raises the question of whether 5-HT(3) receptor antagonists may really potentiate pre-existing ischemia or cause it *de novo*.

The reported increase in mucosal nerve fibres,[8] increased proximity between nerve fibres and mast cells[8,13,29] and lymphocytes[36,37] and lymphocytic infiltration and neuronal degeneration of the myenteric plexus[27] in IBS provide a morphologic basis for a neuro-immune interaction. This close association between inflammatory cells and nerves may, at least in part, reflect plasticity of intestinal mucosal nerves[60] as regenerating nerves are reported to contact mast cells more frequently.[61] Such nerve plasticity is well documented in the setting of intestinal inflammation.[60] The ability of mucosal inflammation to alter enteric nerve and smooth muscle function is well established.[62] The potential mechanisms facilitating these neuro-immune interactions, including both inflammatory and neural mediators have been discussed.

Finally, it should be emphasized that the precise relationship between the reported histopathologic changes in IBS and its pathogenesis remains to be defined. While a number of hypotheses link reported histologic changes to the pathogenesis of IBS, it remains possible that many such changes are unrelated to the pathogenesis of IBS and could be due to other factors such as associated motility disturbances (which may be particularly relevant in the constipation predominant form of IBS).

Normal and minimally inflamed mucosal biopsies: more than meets the eye?

The recent advances in our understanding of the immunopathology of IBS highlight the role of quantitative analyses in unmasking subtle changes in apparently normal mucosa. The concept of pathology that is subtle or unapparent on routine assessment is not unique to IBS. Quantitative analyses have uncovered subtle mucosal abnormalities in other conditions including food allergy,[63] regressive autism,[64–67] developmental disorders[64] and Brainerd diarrhea.[68] For example, there is an emerging pattern of mucosal immunopathology in a subpopulation of children with regressive autism who also have gastrointestinal symptoms.[64–67] Histologically, the changes are fairly subtle and, apart from ileal-lymphoid-nodular hyperplasia, these patients have variable, non-specific inflammatory mucosal changes which are easily overlooked on routine assessment. However, immunohistochemical studies have demonstrated significant increases in CD8 and $\gamma\delta$ T-cells in the colonic mucosa of these patients.[65] Moreover, flow cytometry of duodenal, ileal and colonic mucosal biopsies have shown increases in CD3+, CD4+, CD8+ and CD19+ intraepithelial lymphocytes as well as lamina propria CD3+ cells in these patients compared to controls.[66] A higher proportion of CD3+ cells are positive for TNFα, IL-2, IFNγ and IL-4 in these patients than in controls while a smaller proportion are IL-10 positive.[67] Interestingly, CD4+ intraepithelial lymphocytes and lamina propria CD19+ B-cells are increased compared to non-autistic controls with mucosal inflammation (including a group of inflammatory bowel disease patients), suggesting a novel pattern of mucosal immunopathology.[66] Similarly, morphometry has proved useful in children with multiple food allergies and has revealed subtle villous abnormalities which are not obvious on routine assessment.[63] Finally, subtle morphologic alterations have been described in colonic biopsies from patients with Brainerd diarrhea, a term used to describe outbreaks of chronic watery diarrhea of unknown etiology associated with an abrupt onset and a prolonged course.[68] Colonic biopsies from such patients are reported to show an intraepithelial lymphocytosis which may be subtle or can approach levels seen in lymphocytic and collagenous colitis. In contrast to the latter conditions, the surface epithelium in patients with Brainerd diarrhea retains its tall columnar appearance and there is no thickening of the subepithelial collagen plate.[68] There may be a degree of overlap between Brainerd diarrhea and PI-IBS as both conditions have a presumed infectious etiology, can persist for more than a year and are often associated with an intra-epithelial lymphocytosis. The precise relationship between these two conditions remains to be elucidated. In summary, it appears that routine histopathological assessment may provide a limited view of mucosal abnormalities and, in some instances, may underestimate the changes present. Although there is currently no rationale for the use of immune markers in the routine assessment of gastrointestinal mucosal biopsies, it is possible that this may change as new immunopathologic data emerges.

Conclusion

IBS is a symptom complex including abdominal pain/discomfort and altered bowel function and is heterogeneous with respect to aetiopathogenesis, clinical presentation and histopathology. Alterations in gastrointestinal motor function, increased visceral perception, altered central processing of afferent signals and psychosocial factors are thought to underlie symptom generation in IBS. Recent morphologic studies have seen enteric immune activation and altered neuro-immune interactions receiving increasing attention. Quantitative analyses using immunohistochemistry, morphometry or electron microscopy have shown unequivocal increases in enteric mucosal chronic inflammatory cells, mast cells and EC cells in IBS, despite apparently normal morphology on routine examination. There is also evidence of immune cell activation, mast cell degranulation and neuro-immune interactions. Such morphologic changes, although modest, may have pathophysiological significance in IBS. However, a definitive cause–effect relationship between minimal inflammation and IBS remains to be demonstrated.

Do the reported morphologic changes in IBS have applicability to daily pathology practice? Current data would indicate not. The overlap between patient and control groups, the need for appropriately matched controls, and laborious quantitative methods required to demonstrate abnormalities and non-specificity of the inflammatory changes, all pose major limitations. However, these reported changes do alter the way we view IBS and highlight the role of quantitative analyses in unmasking subtle changes in apparently normal tissue.

Finally, the value of recognizing and reporting a 'normal' or 'near normal' colonoscopic biopsy must be emphasized since this remains important in the diagnosis of the IBS in patients undergoing colonoscopic biopsy.

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