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ORIGINAL ARTICLE

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New insights into the pathogenesis of Hirschsprung's associated enterocolitis

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Abstract The management of Hirschsprung's disease (HD) has made dramatic strides over the last 20 years. Research into the embryological development and migration of ganglion cell has enabled a greater understanding of the pathogenesis of the disease. Coupled with new techniques in surgery, such as laparoscopy-assisted pull-through and the transanal pull-through, this knowledge has led to improved outcomes for children with HD. However, although our appreciation of Hirschsprung's associated enterocolitis and its aetiology has increased, there are continued references in the literature to a multitude of theories of pathogenesis. The purpose of this review is to delineate the theories and demonstrate the evidence supporting or otherwise contradicting each other.

Keywords Hirschsprung's disease · Enterocolitis · Mucin · Immunity · Infection

Introduction

In 1886 Harold Hirschsprung was the first to describe the disease that later bore his name and its most complicated course of Hirschsprung's associated enterocolitis (HAEC). His initial paper [1] on the condition depicts the key pathological features of HAEC with 'deep ulcerations that penetrate to the serosa...an abscess under the mucosa...mottled spaces that can be seen in the submucosa containing pus'. Enterocolitis is defined as a clinical condition with diarrhoea, abdominal distension, pyrexia, colicky abdominal pain, lethargy and the passage of bloodstained stools [2].

Enterocolitis is a significant complication of HD both in the pre- and post-operative periods [3]. HAEC can occur at any time from the neonatal period onwards to adulthood and can be independent of the medical management and surgical procedure performed. The incidence of enterocolitis ranges from 20 to 58%. (Table 1). The medical management of HAEC is costly by a factor of 2.5 as opposed to those with just HD [12]. Fortunately the mortality rate has declined over the last 30 years from 30 to 1% [4, 11, 13–15]. Japanese results demonstrate a decline in mortality from 1978 to 1998 by 6.5–0.7% [11]. This decrease in mortality is related to earlier diagnosis of HD and enterocolitis, rectal decompression, appropriate vigorous resuscitation and antibiotic therapy [2, 16].

Despite multiple investigations and studies, a complete understanding of the aetiology of HAEC is still unavailable. Numerous theories have been put forward to explain its occurrence including a physical dilatation of the proximal bowel, variations in the mucin components and production, rotavirus, *clostridium difficile*, increased prostaglandin E1 activity, mucosal immunity defects, a Schwartzman-type reaction, disordered motility associated with protein sensitisation and sucrase-isomatatase deficiency. Other studies argue that histological and immunological studies have suggested that some patients are prone to recurrent HAEC due to persistent inflammation within the bowel, or an immune deficiency either local or systemic with defective white cell function [17–19]. Some of these theories have more scientific evidence than others but they are frequently presented all together in a list without further clarification. In this review we will discuss these theories and present a synopsis of the pathogenesis.

Theories of pathogenesis

Mechanical obstruction

Bill and Chapman [20] argued in 1962 that partial mechanical obstruction was pathogenetic for HAEC causing mechanical dilatation of the proximal bowel

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Table 1 Incidence of Hirschsprung's associated enterocolitis

Study	Incidence (%)	Mortality (%)
Carcassonne et al. [4]	38	0
Minford et al. [5]	35	3
Elhalaby et al. [6]	34	0
Carneiro et al. [2]	32	4
Surana et al. [7]	30	10
Marty et al. [8]	27	4
Jung [9]	24	3
Sherman et al. [10]	23	1
Suita et al. [11]	17	0.7

leading to faecal loading and stasis resulting in further dilatation thus mucosal ischaemia and bacterial invasion which was cured by colostomy. This suggests that enterocolitis only occurs in dilated ganglionic proximal bowel [20, 21]. However, this theory does not explain the enterocolitis that occurs in distal colon with a defunctioning proximal stoma and the occurrence of enterocolitis in post-operative patients or histological evidence of enterocolitis in aganglionic bowel [10, 11]. In discussing Bill and Chapman theory, it is important to note that the length of the aganglionic segment has been identified as a possible risk factor for HAEC. Studies including our own have shown that the longer segment aganglionosis have a higher risk of HAEC [6, 7, 23]. It is postulated that the increased length of aganglionic bowel implies a greater proximal obstruction with greater intraluminal pressure, increased bacterial stasis and proximal dilation. However, other studies on this condition report no difference regarding the length of the aganglionic bowel and occurence of enterocolitis [15, 20, 24].

Sucrase deficiency

In 1973 Ament and Bill [25] presented a case of a 6-year-old boy with chronic enterocolitis following surgery for HD. Clinical investigations revealed the presence of a sucrase-isomaltase deficiency and the child recovered on a low-sucrose diet. This led to the postulation that non-obstructed HAEC was caused by an inborn error of metabolism [25]. It is important to note that this has not been replicated and Ament acknowledged that the boy was an Eskimo and that 10% of Greenland Eskimos are sucrase-intolerant.

Shwartzman reaction

Berry and Fraser [26] in 1968 suggested that HAEC was initiated by a sensitivity reaction similar to a Shwartzman reaction caused by the invasion of intraluminal organisms invading the submucosa. They injected endotoxin directly into the exteriorized rabbit bowel proximal to an obstruction and produced enterocolitis in six of nine animals.

Prostaglandins

A single case was reported by Lloyd-Still and Demers [27] regarding Hirschsprung's enterocolitis with fulminant unresponsive diarrhoea which revealed high PgE1 levels. In response to cholestyramine, a 12-fold decrease in prostaglandin E (PgE) levels in the colostomy fluid was detected. It was postulated that increased PgE activity, enterotoxin, and bile acid malabsorption might be involved in the enterocolitis of HD [27].

Defective white cell function

In 1988 Wilson-Storey et al. [28] postulated that defective white cell function might be a predisposing factor for HAEC. White cell counts were analysed in nine patients with HD of which five developed HAEC and ten age-matched controls. Their data showed a statistical difference between the neutrophils count (2.0, 3.6, 8.6) in those with HAEC, HD and controls, respectively. This relative neutropenia worsened in three patients during and after an episode of HAEC. They also postulated that white cells in HAEC patients were 'sluggish' in response to inflammation.

Immature mucosa

Blood group-associated antigen Le^b is normally present in foetal colon and absent in a normal ganglionated bowel [29]. Fujimoto [18] demonstrated that the strong expression of Le^b which was uniformly present along the entire length of the crypts of the aganglionic bowel. This expression in aganglionic bowel could indicate a proliferation of the immature crypt cells or that the colonic mucosa has not matured and hence the mucosa persists in a foetal stage. Thus, it is postulated that there is an underlying abnormality of the epithelium lining found in HAEC which may be causative rather than related to the effect.

Mucin

Other theories focus further on the role of increased and altered intestinal mucin/mucus. Clinically, the voluminous amount of mucus produced during HAEC is quite obvious and dramatic. Needless to say this has led to a speculation that the mucus is a pathogenic factor in this condition. The pre-epithelial mucus or mucin consists of glycoproteins and secretory immunoglobulins (IgA) and acts as the first line of defence by binding and inactivating organisms. In the normal bowel most of the mucin is silated or sulphated, thus, there is relatively a little neutral mucin present. The neutral mucin is present in the upper half of the crypts and the acid mucin in the lower [18]. The colonic mucin is kept in a stable ratio by the rapid removal of epithelial cells in the crypts and the routine desulphation of the mucin by the bacteria [21].

In 1981 Akkary et al. [30] performed rectal biopsies from ten patients with HD after the formation of a colostomy, and from six controls with normally ganglionated bowel, and reported abnormal mucin composition in patients with HD. They found a 'marked increase' in the volume of sulphated mucin and that most of the goblet cells contained less mucin especially in cases with severe diarrhoea [30]. They postulated that increased bacterial stimulation leads to both decreased mucosal cell renewal and increased sulphatization of the mucin causing abnormalities of the mucin ratio. This alteration of the ratio leads to an increased adherence of enteropathogenic organisms to enterocytes. Changes in the mucin may lead to altered susceptibility to bacterial degradation [18]. Increased amounts of neutral mucin and a decrease in the acidic sulphated mucins were also detected in the resected enterocolitic bowel using PAS–AB staining [18, 31, 32].

Teitelbaum et al. [32] in Ann Arbor proposed in 1989 that the presence of enterocolitis in HD implies an alteration in the mucins of the large bowel with associated mucin retention and crypt dilatation. Teitelbaum et al. [32] proposed a histological grading system ranging from normal to gross abnormality using both histological features and the feature of mucin retention which is unique to HD (Table 2). They demonstrated that 88% of patients with HAEC had grade III or higher while 83% of those without HAEC had grade II or lower [32]. These histological changes demonstrate that how the mucosa has become susceptible to enterocyte adherent organisms which released toxins. The toxins cause both local (crypt abscesses, ulceration and perforation) and systematic (sepsis and coagulopathy) inflammatory responses.

In Bristol, Aslam et al. [33] demonstrated that the total mucin turnover was significantly reduced in Hirschsprung's patients as opposed to age-matched normal controls. Although ganglionated colon demonstrated similar mucin turnover alterations, the changes were more significant in the aganglionic bowel. This signifies an abnormal mucus defensive barrier in the colon of HD patients, even in the histologically normal bowel. The same team [34] also studied the colonic mucins of the proximal ganglionated bowel in nine HD patients at the time of pull-through. Radioactive precursors, 35s-sulphate and 3H-glucosamine, were added to the mucins of the remaining intact mucosa and the patients were followed for a mean duration of 30 months. They [34] found that patients without enterocolitis ($n=4$) had a turnover rate of six times higher than those with HAEC. This reduced turnover of mucins will give rise to a defective mucus-defensive barrier, hence, allowing enterocyte adhesion and toxin release [34]. In 1999 Aslam et al. [35] demonstrated that the mucin glycoproteins in children with HD, although quantitatively deficient, showed no qualitative histological or immunological differences from normal controls. The mucin gene expression and the quality of mucins were also similar to normal controls [35]. Yet, those patients who developed HAEC had mucin turnover rates that were seven times lower than those without enterocolitis [35]. Gork et al. [36] showed mucin-inhibited bacterial translocation in vitro across both foetal and adult-cultured intact enterocyte monolayers. Also in this study, they demonstrated that the inhibitory effect on translocation was lower on the foetal cells compared to the adult cells.

MUC-2 has recently been shown to be the predominant mucin gene expressed in human bowel [37]. Mattar et al. [38] showed in 2003 that MUC-2 protein expression was found to be significantly lower in patients with HD when compared to controls (19.8 ± 15 vs 121 ± 47) and not detectable during active enterocolitis. The decline in MUC-2 expression in patients with no inflammatory response implies an intrinsic problem which could allow bacterial adherence and translocation. The authors [38] suggest the use of probiotics prophylactically, such as Lactobaccillus GG casei, in order to increase the epithelial expression of MUC-2 and possibly decrease bacterial translocation.

Overall, the evidence has not proven that the mucin alteration is either due to the underlying aganglionic condition or due to a result of the enterocolitis, however, the balance of data supports the concept that the mucin variations are the expression of an altered mucosal barrier and the underlying aganglionic process itself [38].

Table 2 Description of histological grading system (according to Teitelbaum)

Grade	Histopathology
0	No abnormalities
I	Crypt dilatation and mucin retention
II	Cryptitis or two crypt abscesses
III	Multiple crypt abscesses
IV	Fibrinopurulent debris and mucosal ulceration
V	Transluminal necrosis or perforation

Intestinal wall defence

Secretory IgA immunoglobulin provides a major immunological barrier in the gastrointestinal tract. IgA is the predominate immunoglobulin at all levels in the intestinal tract both in the lumen and within the wall. Albanese et al. [39] showed that secreted IgA binds to bacteria and prevents bacterial translocation across an intact segment of viable intestinal tissue.

Piebald mice have a congenital megacolon with the absence of distal ganglion cells, hence, they are the excellent models for HD [40–42]. A number of studies were performed in our centre with a breeding colony of piebald mice in order to investigate the model and establish mucosal secretory function in HAEC [43, 44]. Two distinct patterns of mortality occurred with the majority of mice (64%) being characterized by becoming unwell acutely with the evidence of acute enterocolitis at 3–4 weeks and then dying quickly while the second group died between 9 and 11 weeks due to ileus with

findings of massive abdominal distension and megacolon [43]. Interestingly, two different immunological responses were evident. Those with a more acute history had acute splenitis and a severe diffuse lymphocytic response in the intestinal submucosa and lamina propria with a significantly raised level of IgA as opposed to controls and late death group. In contrast, the late death group had increased plasma cell distribution within the deep layer of the lamina propria only. This increased level of plasma cell infiltration into the ganglionic segment of the colon in the early death group implies that the local antigenic stimulation was the principal pathological event [43].

Wilson-Storey et al. [17] postulated that there was a marked deficiency in the transfer of IgA across the intestinal mucosal membrane in patients with HAEC. They implied this by the absence of secretory IgA in the buccal mucosa in patients with HAEC. Five out of six patients with HD had no detectable secretory IgA within their saliva. These patients also had an increased amount of IgA within their buccal mucosal tissue.

Imamura et al. [45] demonstrated the similar results in colonic resection specimens including elevated levels of IgM and J chain plasma cells in the bowel of those with enterocolitis. Multiple factors, such as CD68-positive monocytes/macrophages and $CD45RO^{+}$ and CD57-positive natural killer, were elevated in those with enterocolitis. A marked increase in IgA plasma cells in the lamina propia was found and yet there was a distinct reduction in the luminal IgA in four of the five patients with HAEC. Normal luminal and epithelial IgA was present in the ganglionated bowel.

Since 1976 the question, whether the decrease of luminal IgA reflects a primary deficiency in transfer of IgA out of the cells onto the luminal surface or whether it is due to inflammatory change, has been asked [46]. Turnock et al. [47] attempted to answer the question of whether or not there is a pre-morbid deficiency of the intestinal immune response in patients whom develop HAEC. They examined the rectal suction biopsies of 20 patients with HD of which 8 developed HAEC. They [47] found no evidence of a significant deficiency or difference in population in the IgA, IgM and IgG plasma cells in the lamina propria in patients with HD, HAEC or the normal controls. Overall, there is evidence that the IgA function and formation is normal within the cells but that there is a deficiency in the transfer of the immunoglobulin into the lumen to assist the mucin in its role in the front line of immunological response, however, this hypothesis has not been proven conclusively.

Mucosal neuroendocrine cells (NE) mediate intestinal function through synthesis and storage of neuroendocrine, neuropeptides and biogenic amines which act as chemical messengers [48, 49]. Soeda et al. [50] demonstrated in 1992 that NE cells were increased in the aganglionic segment of bowel in HD as opposed to the ganglionated bowel or the normal controls. In 1993 they noted a marked reduction in the NE cells in the ganglionated bowel with HAEC as opposed to those without HAEC. These diminished NE cells may represent an impaired immune response or a deficiency which may facilitate the initialization of inflammation [51]. This impaired immune response theory is echoed in trisomy 21. The combination of HD and trisomy 21 is associated with a higher incidence of enterocolitis with 50% of patients with Trisomy 21 and HD developing into HAEC as opposed to 29% in the normal population [2]. Infants with Trisomy 21 have an intrinsic immunity deficiency due to both decreased cytotoxic T-lymphocytes and derangement in humoral function which may explain their increased risk of developing HAEC [52–54].

Histological evidence of enterocolitis comprises a number of features including crypt abscesses, leucocyte aggregates, ulceration and Paneth cell metaplasia [31]. Paneth cells are normally present in the small bowel and secrete lysozymes which digest the bacterial wall membranes. Their presence in HAEC colon suggests an attempt on the reinforcement of the mucosal immunity [31]. ICAM-1 is a cell surface inter-cellular adhesion glycoprotein which is involved in leucocyte recruitment when inflammation occurs. Kobayashi et al. [55] demonstrated that ICAM-1 had increased expression in the endothelium of both the ganglionated and aganglionic bowel in patients with HAEC. This emphasized the importance of endothelial cell activation in HAEC pathogenesis. Elhalaby [6] postulated that the occurrence of a single episode of HAEC can alter the intrinsic intestinal immunity by a chronic change in the mucosa to an increased risk for further episodes. This would help to explain the lower but real recurrence rate of HAEC, both following a 'diversion' colostomy or a successful pull-through [6, 7].

Abnormal motility and macrophages

In Japan, Suzuki et al. [56] used endothelin receptor null rats as a model for long-segment HD, as they have a mega ileum proximal to a constricted aganglionic region. They [56] showed that the number of macrophages was increased in the tunica muscularis suggesting that macrophages may play an important role in the inflammation of tunica muscularis in rats. They postulated that the increased numbers and activation of macrophages may result in damage to the interstitial cells of Cajal networks leading to a disordered intestinal rhythmicity in the regions of the gut in which myenteric ganglia are intact. This disordered movement may encourage stasis, bacterial growth and with the abnormal mucins' increased translocation.

Microbiology

Bacteria and viruses have been linked to the enterocolitis by a number of studies. Thomas et al. [57] first reported *C. difficile* in 1982 when high titres of the toxin were

detected in four of six patients with HAEC. In 1986 Thomas et al. [58] detected the cytopathic toxin in 7 of 13 (54%) and *C. difficile* was isolated in 77% of children with HAEC. In the control groups *C. difficile* was isolated in 18% of those with HD and 30% of children without HD. Thomas et al. [58] postulated that the toxin was pathogenic due to the incidence of toxin presence in the faeces; the magnitude of the toxin levels and the isolation rates for *C. difficile* were significantly higher in HAEC patients as opposed to those without enterocolitis or even HD. The possibility that HAEC could prevent the development of a 'benign' colonic bacterial flora and treating *C. difficile* aggressively could improve this 'benign' colonic bacterial flora development and this conception has become a very exciting theory. However, this has not been proven on subsequent investigations; 50% of patients with HD have *C. difficile* and there was no variation in incidence between those pre- and post-operative periods [59]. Wilson-Storey et al. [60] in 1990 demonstrated a broad spectrum of organisms present in the stools with no significant difference in the clostridium carriage rate between those with HAEC and without enterocolitis or normal controls. Stool samples in our centre reveal a wide range of colonic flora present during episodes of HAEC. However, after an episode of enterocolitis 70% of patients with HAEC had *C. difficile* present as opposed to 42% of non-enterocolitis patients [61]. It is postulated that after the start of the enterocolitis episode that alteration in mucosal immunity allows *C. difficile* to flourish. Although it may not be causative, it can significantly complicate the colitis. Pseudomembranous colitis with *C. difficile*-positive stools is rare and has been reported in four patients with 50% mortality despite the vancomycin therapy [62].

Bacterial adherence has been viewed as an important factor for the last 15 years being demonstrated histologically in up to 40% of pull-through specimens in patients with prior HAEC. When intestinal mucus was removed in the mouse model, there was an increased adherence of *Escherichia coli* colonic mucosal layers [63]. *E. coli*, *C. difficile* and *Cryptsporidium* were the adherent organisms found suggesting that the adherent nature of the organism is an important factor. Suzuki et al. [56] observed abnormal intestinal flora with a marked increase in gram-negative aerobes (Enterobacteriaceae) and anaerobes (Bacteroidaceae) in the distended region of the small intestine of their endothelin receptor null rats.

Imamura et al. [45] hypothesized that the diversity of the altered local response in HAEC was due to a multifactorial microbiology aetiology. They examined the entire resected colon in 12 patients with HD. CD57-positive natural killer (NK) cells which act as anti-viral agents were found to be significantly increased in the ganglionic segment of the HAEC patients while no difference was found in the non-enterocolitis group or the normal controls. This has led to the postulation that the increase in these anti-vial cells implies a viral aetiology [45].

Wilson-Storey [61] agrees that HAEC has a multifactorial infective aetiology. Rotavirus has been identified in seven of nine patients with enterocolitis [60]. Of note, there were no symptoms of vomiting in these patients which is pathognomonic of rotavirus gastroenteritis. Also, there was no evidence of contact before, during or after admission to the hospital [60]. However, these results have not been replicated.

Treatment

The theories of pathogenesis of HAEC have had an impact on our treatment of the condition. The incidence of pre-operative enterocolitis has significantly fallen in a nationwide study, in Japan, of 3,852 patients over 30 years from 29% (during 1978–1982) to 17% (during 1998–2002) [11]. Historically, Swenson recommended the use of flatus or rectal tube to enable colonic decompression. Today, commencement of an early washout programme and prompt surgery are viewed as the key features in the prevention of HAEC [64, 65]. Vancomycin and metronidazole can be given either orally or via enema when *C. difficile* is present on stool cultures. Clinical deterioration in the neonate particularly those long-segment disease may require an emergency decompression colostomy. Concerns over the incidence of mortality due to fulminate enterocolitis in the post-operative period led Marty et al. [8] suggesting that routine post-operative rectal washouts decrease both the incidence and the severity of the episodes of enterocolitis following definitive surgery. In episodes of recurrent enterocolitis, which can develop in up to 56% of patients, anal dilatations have been recommended [6, 8] However, prior to commencing a treatment regime, a contrast enema should be performed to rule out a mechanical obstruction. Patients with a normal rectal biopsy may require a sphincterotomy [6, 10]. Hence, in our centre rectal biopsies are also taken to ensure the presence of ganglionated bowel. Wildhaber et al. [66] had 59% of patients with recurrent enterocolitis of which 75% were symptom-free following a posterior myotomy/myectomy. Redo pull-through operations, when appropriate, appear to be as effective as primary procedures in terms of continence, stooling frequency and can decrease the episodes of HAEC [67]. Rintala and Lindahl [68] treated six of eight patients with recurrent HAEC successfully with a mast cell stabilizer Sodium Cromoglycate (SCG).

Conclusion

The balance of the event implies a multifactorial aetiology with abnormal mucin production, mucin ratio and turnover as the key factors. An abnormal local immune response with decreased secretion of IgA allows enterocyte adherence and bacterial translocation. Local antigenic is the principal stimulation for the pathological

event. Colonic stasis may encourage bacterial growth and allow more virulent strains of bacteria to flourish. Probiotics, such as Lactobacillus GG casei used prophylactically that can increase the epithelial expression of MUC-2, will decrease bacterial translocation.

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