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The pathogenesis of Hirschsprung's disease-associated enterocolitis

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Hirschsprung's disease-associated enterocolitis (HAEC) remains the most life-threatening complication in Hirschsprung disease (HD) patients. The pathogenesis of HAEC has not been determined and many hypotheses regarding the etiology of HAEC have been proposed. These include a possible causal relationship between the abnormal enteric nervous system development in HD and the development of enterocolitis. Based on the complex genetic causes of HD that have been discovered and the resultant heterogeneous group of patients that exists, the causes of HAEC are likely multiple. New insights regarding the relationship of the role of the enteric nervous system and its interaction between intestinal barrier function, innate host immunity, and commensal microflora have been discovered, which may shed light on this perplexing problem. This review presents current known risk factors of HAEC and the proposed theories and supporting evidence for the potential etiologies of HAEC.

Over the past 20 years, significant advances have been made in our understanding of the etiology and management of Hirschsprung disease (HD); however, the pathogenesis of Hirschsprung's disease-associated enterocolitis (HAEC), which is responsible for the most serious morbidity and mortality from HD, remains poorly understood. Further understanding of the causes of HAEC will continue to lead to improvements in recognition, early intervention, management, and perhaps even prevention of this morbid complication.

A major difficulty contributing to our lack of understanding of the etiology of this problem is the variable diagnostic criteria used for reporting its incidence. The symptoms exhibited in HAEC are often nonspecific, and a high index of suspicion is often required for diagnosis. HAEC has been defined as a clinical condition characterized by explosive diarrhea, abdominal distension, colicky abdominal pain, lethargy, and fever.[1-4] The spectrum of its presentation can range from mild abdominal distension, with loose stools and perianal excoriation, to life-threatening toxic megacolon with explosive diarrhea, vomiting, rectal bleeding, lethargy, and impending shock.[5] Patients may experience one bout of HAEC or exhibit chronic and relapsed inflammation of the gut. The use of a standardized definition for HAEC may contribute to more consistent reporting and will permit a more accurate comparison between studies, which can aid in our understanding of the pathophysiology of HAEC.[5]

Evaluation of the pathologic changes seen in the intestine of patients with HAEC has provided clues regarding the pathogenesis of this disease. Histologically, the intestinal wall exhibits inflammation and neutrophil infiltration into the crypts (cryptitis), with associated crypt dilatation and retained mucus within the crypts, in the milder stages of the disease.[6] In more advanced disease, there is progression microscopically to the presence of crypt abscesses, intraluminal fibrinopurulent debris, ulceration of mucosal epithe-

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lium, transmural necrosis, or intestinal perforation.[6,7] Paneth cell metaplasia within the colon has also been described in specimens with HAEC.[8] In one series, pseudomembranes were found on pathologic analysis in up to 35% of patients with clinical HAEC undergoing biopsy.[9] Histopathologic changes of HAEC may also be seen in both ganglionic and aganglionic segments of the HD bowel.[7-11] Late stages of HAEC often pathologically resemble the intestinal inflammation seen in ulcerative colitis. Ischemic enterocolitis has also been described in neonates in septic shock because of the escalation of systemic dysfunction from HAEC, suggesting that inadequate tissue perfusion and bowel distension can further contribute to end-organ disease in severe cases.[12]

Epidemiology

The reported incidence of HAEC varies significantly in published series and ranges from 17% to 50%, with the mean incidence of 28.5%.[1,4,7,10,11,13-31] Patients may suffer from HAEC either preoperatively or postoperatively after diversion or definitive resection of the aganglionic bowel. This suggests that the etiology is more complicated than the mere absence or presence of ganglia alone.

Preoperative HAEC has been reported to occur in 5.7%-50% of patients.[1,4,7,30,31] Enterocolitis is the clinical presenting feature of HD in up to 24% of infants and is thought to be most severe in this group.[3] Whether the severe clinical manifestations seen in patients presenting with enterocolitis is a primary issue unique to the disease or due to a delay in diagnosis and treatment is unknown.

The incidence of postoperative enterocolitis has been reported to occur in 2%-35% of patients.[1,4,7,30,31] This range may represent changes in how the disease is diagnosed or may reflect different populations at varying risk. Postoperative HAEC can occur >18 months after definitive surgery[4,18]; however, most have their last episode of significant HAEC within 2 years of their pull-through procedure.

Several studies have shown that some patients who develop HAEC are prone to recurrent episodes.[4,13,14] The incidence of recurrent HAEC varies from 5.2% to 56%[1,4,19] and may be attributed to the persistence of mucosal histopathologic changes.[6-8] Chronic symptoms are commonly seen in the subset of HD patients with trisomy 21.[30] Recent studies involving long-term follow-up of patients who have had an episode of HAEC have found that the majority of these patients continued to have disturbances of bowel function many years after definitive surgery for HD.[28] This information clearly highlights the significant morbidity of this problem and implores future study for improved treatment and prevention of this disease.

Risk factors

Many risk factors for HAEC have been identified, which may contribute to theories of pathogenesis. Perhaps the most confirmed risk factor is that of trisomy 21.[1,15,19,32-37] The incidence of HAEC in Down syndrome patients has been reported to occur almost twice as frequently as in all other children with HD.[1,15] Identified risk factors such as the presence of trisomy 21, and other genetic syndromes, such as cartilage-hair hypoplasia, family history of HD, and female sex, point to a possible genetic contribution to the etiology of HAEC.[14,15,32-39]

Other risk factors include delay in diagnosis of HD beyond 1 week of age, with 16 days of life being the average age of neonate presenting with enterocolitis.[15,19,40] The presence of major associated congenital anomalies involving either cardiac, gastrointestinal (GI), genitourinary, or central nervous system has also been reported as a risk factor.[1,7] Several studies report that HAEC seems to be more common in children with longer-segment disease,[17,22] although it is unclear whether this tendency is independent of genetic predisposition, which is also associated with long-segment disease.

Historically, the type of surgical pull-through was implicated as a possible contributor to HAEC; however, the majority of recent series found the incidence of post–pull-through HAEC to be unrelated to the type and timing of definitive surgery.[1,19,24,25,41] Postoperative complications such as stricture or obstruction, however, have been found to be associated with an increased incidence of enterocolitis.[25]

Focusing on patients with increased risk for HAEC has enabled us to make observations and identify specific abnormalities, which have led to additional proposed etiologies of HAEC and debunked others.

Abnormal enteric nervous system development and its significance to intestinal homeostasis

Although a specific cause for HAEC is unknown, it is known that the heterogeneous group of patients with HD universally has an abnormally developed enteric nervous system (ENS) with the congenital absence of the parasympathetic ganglia in the distal bowel. It seems logical to speculate that HAEC is possibly secondary to specific abnormalities of the ENS, which may negatively impact intestinal homeostasis. New insight regarding the relationship between the role of the ENS and its interaction between intestinal barrier function, innate host immunity, and commensal microflora has been discovered, which may shed light on this perplexing problem.

It is well established that the ENS participates in almost all aspects of GI function, including epithelial transport, motility, mucosal immune defense, release of gut peptides from enteroendocrine cells, and mucosal blood flow. The myenteric plexus principally regulates intestinal motility, whereas the submucosal plexus together with nerve fibers in the lamina propria are involved in regulating epithelial transport functions.[42,43]

Normal intestinal function is, therefore, dependent on a functional ENS, which aids in regulating epithelial barrier integrity. When the ENS is compromised, the epithelial barrier integrity may be compromised, abnormal immune responses may be present, and/or abnormal responses to the intestinal microbiome could occur and pathologic intestinal inflammation may develop such as that seen in HAEC. Similarly, intestinal inflammatory conditions can contribute to loss of epithelial barrier function, and further inflammation may develop, initiating a vicious cycle of perpetual inflammation.[43] This intestinal inflammation may contribute to even more abnormalities of the ENS, compounding the problem. Understanding the influence of the ENS on intestinal barrier function and homeostasis may shed light on the pathogenesis of HAEC. Many related findings have recently emerged that greatly enhance our understanding of how the ENS participates in the regulation of the gut immune system.

The interplay between the ENS and its potential abnormalities in HD and the intestinal barrier, intestinal immunity, and intestinal microbiota is illustrated in Figure 1. Alterations in the ENS such as those seen in HD could participate in the initiation/perpetuation of HAEC. Recently, it has become clear that the defect in the ENS in HD is more than simply the absence of distal ganglia. There is evidence that neurons within the ganglionated bowel of HD patients may be dysfunctional qualitatively, as they may exhibit markers of immaturity compared with control subjects.[44] Other investigators have also discovered that mucosal nerves were also severely deficient in patients with HD, even in segments that contained ganglia.[45] Theoretically, these abnormalities may contribute to intestinal dysfunction. As the ENS abnormalities precede gut disease in HAEC, it is possible that the alterations seen in the ENS in HD patients may be the "upstream" effectors of many downstream pathways contributing to HAEC, although this remains to be determined.

Evaluating the following theories proposed regarding the causes of HAEC, in light of emerging information pertaining to the possible role the ENS plays on intestinal homeostasis, may assist with solving this complex problem.

Proposed etiologies of HAEC

The many proposed potential etiologies of HAEC can be classified broadly into 1 of 3 main potential abnormalities of intestinal homeostasis: (1) intestinal barrier dysfunction, (2) an abnormal innate immune response, and/or (3) an abnormal microbiota.

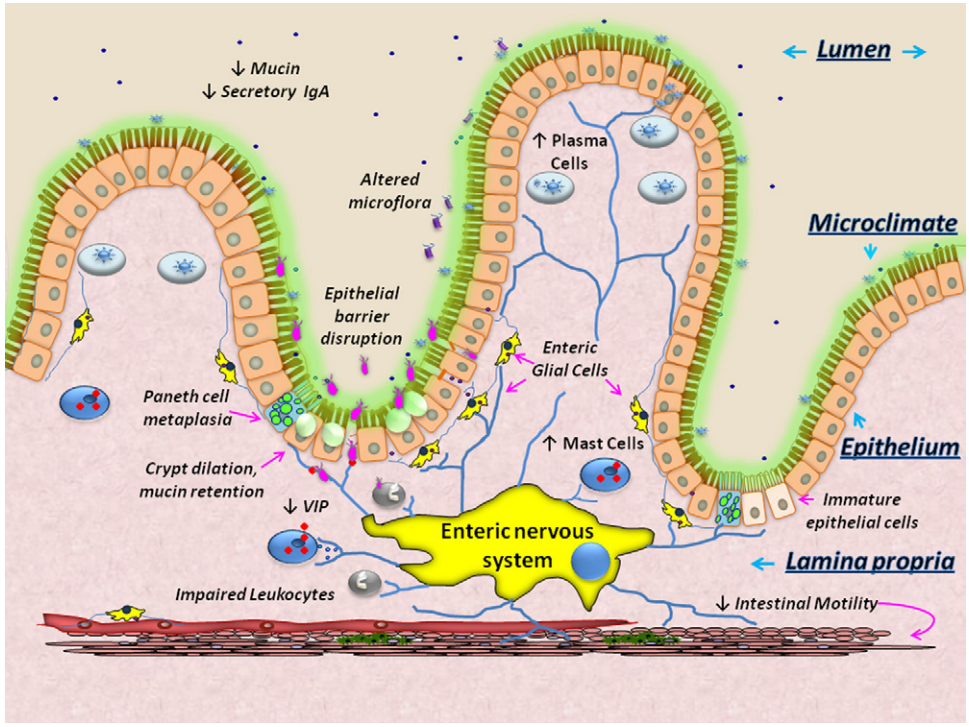

Figure 1 The Enteric Nervous System regulates intestinal homeostasis through its interaction with the intestinal barrier, the innate and adaptive immune system, and the intestinal microbiota via the 4 main regions of intestinal defense: the lumen, the microclimate, the epithelium and the lamina propria. Possible abnormalities in intestinal homeostasis contributing to epithelial barrier disruption and the development of HAEC include decreased intestinal motility; decreased vasoactive intestinal peptide (VIP) potentially contributing to epithelial tight junction disruption, decreased mucin secretion, and/or effects on intestinal motility; crypt dilation and mucin retention, with decreased secreted mucin within the microclimate; increased lamina propria plasma cells and decreased secretory immunoglobulin A (IgA); impaired leukocyte function; increased lamina propria mast cells; decreased submucosal glial cells, potentially contributing to tight junction instability and epithelial barrier disruption; immature epithelial cells; Paneth cell metaplasia; and altered intestinal microbiota.

Intestinal barrier dysfunction

Proper functioning of the intestinal barrier between host and microbe is critical for health. Circumstantial evidence suggests that intestinal barrier dysfunction is associated with the pathogenesis of many forms of enterocolitis.[42,43] Components of the intestinal barrier include the lumen, the microclimate or mucus-containing layer, the epithelium, and the lamina propria. Each region plays a role in maintaining the intestinal barrier, and abnormalities seen in HD may contribute to the barrier dysfunction and the development of HAEC.

Lumen

The intestinal lumen actually provides the first line of defense in normal GI function, as it is here where degradation of bacteria and antigens first occur by bile, gastric acid, and pancreatic juice.[43] Commensal bacterial are also present and may inhibit colonization of pathogens. Proper intestinal motility is required for the maintenance of this line of defense. Abnormal intestinal motility causing functional intestinal obstruction and resulting in bacterial stasis, overgrowth and translocation, and/or intestinal distension and possible ischemia was the earliest proposed etiology of HAEC.[3,4] Patients with HD typically exhibit intestinal dysmotility before surgical intervention and sometimes postoperatively if intestinal strictures, obstruction, or internal sphincter spasm develop. Support for obstruction as a cause for HAEC has been substantiated by investigators who have found increased risk for HAEC in patients with evidence of postoperative strictures or internal sphincter spasm.[25,46] This theory may play a significant role in the pathogenesis of HAEC in some patients; however, it cannot explain the occurrence of HAEC in patients with a defunctioning stoma or without evidence for obstruction.[30]

Microclimate (mucus layer)

The next line of defense of the intestinal barrier is a microclimate made up of unstirred water and mucus predominantly composed of the MUC family of glycosylated proteins and produced by goblet cells within the lining of the epithelium. Mucus serves as a scaffold for bactericidal or bacteriostatic proteins that together create a primary barrier against bacterial invasion.[42] Immunoglobulins that have been secreted via transcytosis through the epithelial layer and defensins secreted by Paneth cells make up some of the antimicrobial proteins in this layer. This layer has viscoelastic properties and acts as an adhesion site for bacterial and viral pathogens, preventing them from binding to epithelial cells.[47] It has also been found to inhibit the replication of rotaviruses.[48] An altered microclimate may lead to an increased susceptibility to infection. The maintenance of this defense is particularly important for the prevention of enterocyte adherence by the pathologic organisms. Enterocyte adherence allows organisms to invade epithelial barriers and makes the host susceptible to infection.[49] Teitelbaum et al[6] demonstrated enterocyte adherence on histology in up to 39% of patients with HAEC, with the identified organisms being *Escherichia coli*, *Clostridium difficile*, and *Cryptosporidium*.

Deficient mucin secretion may predispose the host to the adherence of enteropathogenic organisms and contribute to the development of HAEC.[8,50-52] Several studies document deficiencies in both mucin and secreted immunoglobulin within this important mucus layer in patients with HAEC, suggesting that the resulting intestinal barrier dysfunction may play a key role in the etiology of HAEC.[8,50,53-55] Abnormalities of the mucus protective barrier have been shown in the proximal ganglionated bowel after definitive surgery, which may contribute to the occurrence of post–pull-through enterocolitis and recurrent episodes of HAEC.[6]

Qualitative and quantitative changes in mucin have been discovered in patients with HD. Initially, depletion of sulfated and neutral mucin was a characteristic finding in the aganglionic bowel of patients with HD.[8] An abnormal mucin composition was identified in the pathologic specimens of HD.[50] Aslam et al[56] revealed that total mucin turnover was reduced in the colon of patients with HD. More specifically, an increase in the ratio of intracellular to secreted mucins and a reduction in mucin turnover were found in this prospective study. Patients who developed HAEC had mucin turnover rates that were 7-fold lower than HD patients in whom enterocolitis did not develop.[56] The lower the turnover rate in the residual ganglionated bowel at time of definitive surgery, the more prone the patient is to developing HAEC.[56] Also, the predominant mucin expressed in the human colon, MUC-2,[57] was found to be significantly decreased in stool samples of patients with HD compared with control subjects, and nondetectable in those patients with HAEC.[54]

Mucus secretion is known to be partially regulated by submucosal noradrenergic neurons,[58] and it has been known that neuroendocrine cells, which may be responsible for interfacing with mucus secreting goblet cells, are reduced in patients with HAEC compared with control subjects.[59] A number of in vitro and in vivo studies have indicated the vasoactive intestinal peptide (VIP) expressed in intrinsic nonadrenergic noncholinergic neurons is a potent regulator of GI motility, water absorption and ion flux, mucus secretion, and immune homeostasis. VIP-deficient mice exhibit an overall reduction in secretion of mucus from goblet cells and a major increase in thickness of the muscular layers as well as an intestinal transit defect.[60]

This supports the possibility that the inherent abnormality of the autonomic nervous system and the reduction in neuroendocrine cells of the ganglionated bowel in these patients may be the cause of the abnormal mucus barrier seen in HAEC.

Epithelial barrier and tight junctions

The next line of defense of the intestinal barrier is the epithelial layer. Under normal conditions, enterocyte tight junction (TJ) proteins allow the gut epithelium to function

as a barrier yet absorb substances in a paracellular manner. Disturbances in epithelial TJ barrier function have been found in multiple conditions of enterocolitis.[42] Proinflammatory cytokines released in inflammation and bacterial products themselves can influence TJ proteins, causing them to become dysfunctional and disrupting the intestinal barrier.[42,43] This provides another explanation for a possible cause for recurrent enterocolitis. Alteration in the anatomic functional integrity of the intestinal barrier has been suggested in the pathophysiological process of HAEC. One possible mechanism that may be proposed is related to the decrease in VIP neurons in patients with HD.[61,62] Recent studies have identified the important role that VIP plays in the nerve-mediated maintenance of intestinal barrier function by its direct effects on epithelial cell action on the TJ protein, ZO-1.[63]

Role of enteric glial cells

In addition to neurons, the ENS is also composed of enteric glial cells (EGCs), which are the most abundant cell type of the ENS.[64] These cells form a dense network throughout the GI tract, where they not only function in support of the enteric neurons but also act as regulators of vascular and epithelial integrity. Enteric glia have recently been shown to maintain intestinal epithelial barrier function primarily through the secretion of the TJ stabilizer S-nitrosoglutathione.[64,65] Furthermore, animal models of EGC ablation develop enterocolitis, stressing the important role EGCs play in regulating the intestinal immunity.[66,67] Preliminary studies have shown that mouse models of intestinal aganglionosis can have decreased EGCs in proximity to the subepithelial region of the intestine.[68] This may contribute to increased epithelial permeability. Other investigators have shown that EGCs can also influence intestinal barrier function by modulating epithelial cell proliferation.[69] The contribution of the EGCs in the pathogenesis of HAEC remains to be determined.

Role of mast cells

One of the most well studied neuroimmune signaling pathways in GI pathophysiology is nerve-mediated activation of mast cells. On activation by cholinergic pathways, such as those found in the aganglionic bowel of patients with HD, the mucosal mast cells release a range of bioactive mediators, which can have effects on both trans- and paracellular permeability.[42] This may lead to an inappropriate immune activation. Mast cells play a major role in stress-induced changes of intestinal permeablility.[42,43] They have been found in high concentration in the aganglionic and hypoganglionic regions of the bowel in HD patients.[70,71] They have also been implicated as playing a possible role in the inflammation seen in HD.[70,71] It remains unclear whether they are a primary influence or a secondary affect of HAEC.

Immature mucosa

A recent study by Lui et al[72] suggested that the loss of mucosal integrity may be due to immature mucosa secondary to defective epithelial cell proliferation and differentiation. This group showed a reduced expression of *caudal-type homeobox gene-1 and -2 (CDX-1 and CDX-2)*, which are involved in maintaining control of proliferation and differentiation of intestinal mucosal cells. Deficient mucosal proliferation and differentiation may also impact epithelial wound healing after injury and weaken the intestinal barrier.

Additionally, the expression of blood group–associated antigen Le^b, which is a marker for undifferentiated crypt cells, was observed throughout the crypts in aganglionic bowel in HD, representing persistence of a fetal stage of development.[73] It may be postulated that this immaturity may contribute to breaches in epithelial wall barrier directly or indirectly through inadequate mucin secretion by the epithelial cells.

Abnormal innate immune response

Defective white blood cell function has been reported to predispose some patients to the development of HAEC. Affected patients have a marked deficiency in the transfer of secretory immunoglobulin (Ig)A across the GI mucosa and exhibit hypersensitivity reactions to absorbed bacterial antigens.[74] This results in a deficient microclimate and has been proposed by some investigators as a possible cause for HAEC. This theory was based on the significant decrease of secretory IgA in the saliva of patients with HD despite an increased amount of IgA in their buccal mucosal tissue.[75] Interestingly, patients with HAEC had a complete absence of secretory IgA in their buccal mucosa.[75]

These results were substantiated by Fujimoto et al,[8] who found significant elevations of IgA-, IgM-, and IgG-containing plasma cells in the lamina propria of bowel with HAEC compared with nonenterocolitis bowel. Elevated IgA-, IgM-, and IgG-containing plasma cells were also confirmed to be significantly increased in the lamina propria of bowel with HAEC compared with nonenterocolitis bowel in subsequent studies[76]; however, luminal secretory component staining of the aganglionic segment of colon from these same patients with HAEC was considerably reduced. Imamura et al[76] also observed a unique distribution pattern of CD57-positive (+) natural killer cells within the ganglionic bowel only of patients with HAEC compared with control subjects. CD68+ monocytes/macrophages and CD45RO+ leukocytes were increased in all regions in patients with HAEC.

Additional support for the possibility that primary immune abnormalities may contribute at least in part to HAEC comes from the study by Cheng et al.[77] These investigators described splenic lymphopenia in the endothelin receptor B–null mouse model of aganglionosis and enterocolitis. They also noted a relative reduction in B cells compared with T cells and a 5- to 20-fold reduction in CD19+, CD4+, and CD8+ T cells compared with control ones.

Additionally, they noted a strong inverse correlation of increased severity of colitis in the animals with the lowest CD19+ cell counts.

An intrinsic immune deficiency may also at least partially explain the increased risk of HAEC in patients with trisomy 21, as they can have both, a decrease in cytotoxic T-lymphocyte function and a derangement in humoral function.[78-80]

Abnormal microbiota

Under normal conditions, we live in a symbiotic relationship with our commensal gut microbiota, with ongoing cross talk between our GI tract and our microbiota. Disruption of this mutually beneficial relationship can occur owing to abnormalities in either the bowel or the microbiota, resulting in serious illness.[81] The role the gut microbiota plays in the pathogenesis of HAEC is unclear; however, several investigators have proposed an infectious etiology.

Although no specific organisms have been found to consistently cause HAEC, *C difficile* and rotavirus have been associated with HAEC, although the etiology is extremely variable.[82,83] One study reported that cytopathic *C difficile* toxin was present with significantly greater frequency and in consistently higher concentration in the stools of children with HAEC.[84] In that study, the presence of toxin-positive stools also correlated with the most severe clinical manifestations of HAEC. Additionally, patients with HAEC and fecal *C difficile* toxin (63% of all HAEC in this series) responded to treatment with enteral vancomycin.[1] One study showed that patients with HAEC carry *C difficile* for prolonged periods compared with control subjects.[85] Several other studies, however, report very few patients positive for *C difficile*.[19,15,86] The fact that up to 90% of healthy neonates have *C difficile* in their stools and may secrete the toxin suggests that the pathogen alone does not cause HAEC.[87]

Rotavirus has been found in up to 77% of patients with HAEC in some studies.[83] These investigators propose that immunologic defense against rotavirus is dependent on IgA in the gut wall; therefore, the rotavirus invasion seen in these patients may be secondary to the IgA secretory abnormality present in these patients.[83]

Recent advances in molecular technology have made it possible for us to better assess the intestinal microbiome. Using stool samples to analyze for bacterial DNA, Shen et al[88] tested whether the important probiotic microorganisms, bifidobacteria and lactobacilli, were present in the bowel of HD patients with HAEC. This study found that the colonization of bifidobacteria and lactobacilli were lower in patients with HD compared with control subjects and extremely scarce in patients with HAEC.

Another group recently performed a pilot study using a genomics approach for the analysis of bacterial communities residing in one patient with HAEC.[89] Here the microflora compositions of 15 stool samples from a 3-year-old boy with HD were harvested during 4 episodes of HAEC, and remission phases were analyzed using amplified ribosomal DNA restriction analysis (ARDRA). Restriction analysis showed that HAEC episodes seemed to cluster together in ARDRA analysis, suggesting a possible predisposing bacterial community for HAEC development and the need for microflora equilibrium to maintain wellness. These investigators note that the use of ARDRA may become more prominent as a rapid and inexpensive tool to assess microflora dynamics and assist with proper antibiotic selection and therapy in HAEC patients.[89]

Both recent studies support that the intestinal microbiome is in dysbiosis in patients with HAEC and suggest that the use of probiotics may be helpful in patients with HD. Additional investigation in this exciting area may also provide insight into the mechanisms underlying HAEC.

Genetic abnormalities

The genetics of HD are complex, and it is possible that a genetic predisposition to HAEC may exist. As previously described, patients with trisomy 21 may have an increased risk for HAEC, at least in part from an immunodeficiency. Moore et al[90] recently investigated the *ITGB2* (CD18) immunomodulatory gene for mutations in patients with HD to explore its correlation with HAEC. This candidate gene was chosen because abnormalities in this gene may play a role in impaired leukocyte function and regulatory T-cell regulation, predisposing patients to HAEC. They found *ITGB2* gene variations in 66% of their HD patients, and 59% of patients with the genetic variations developed HAEC. Patients with >1 variant identified in the gene had more severe symptoms.[90] Other studies also support the role of additional genetic abnormalities in contributing to the development of HAEC. For example, one study found a measurable autonomic dysfunction—in addition to aganglionosis—in a subset of HD patients with the *RET* mutation.[91] Autonomic dysfunction alone in non-HD patients may cause intestinal dysmotility and contribute to more severe disease. One case report described a patient with chronic HAEC who was ultimately found to have an additional inborn error of metabolism of sucrase–isomaltase deficiency and responded to a low-sucrose diet once diagnosed.[92]

Lacher et al[93] investigated for *NOD2* variants in patients with HD. The *NOD2* mutation is an important susceptibility gene for Crohn enterocolitis and has been linked to increased intestinal permeability and decreased anti-inflammatory interleukin-10 production in Crohn disease patients.[94] They evaluated 52 patients with HD, 7 (13%) with HAEC, but none of them were carriers of *NOD2* variants; therefore, they concluded that HAEC develops independently of *NOD2* variants.

Conclusions

HAEC remains a significantly morbid and potentially life-threatening complication of HD. Long-term follow-up after even 1 episode HAEC is consistent, with worsening intestinal dysfunction compared with HD patients who have never had HAEC.[28] The pathogenesis of HAEC remains unknown. The complexity of the genetic abnormalities contributing to HD and the heterogeneity of the patients with HD suggest multiple factors may contribute to its etiology. Continued insights into the role of the ENS in the regulation of intestinal homeostasis, mucosal barrier function, innate immunity, and the intestinal microbiome are emerging. Advances in molecular analysis are permitting us to gather improved understanding about the intestinal microbiome of these patients. Additionally, improved molecular analysis may ultimately lead to localizing additional genetic links that may improve HAEC patient selection, and lead to improvements in early intervention and management. With the identification and use of potential molecular targets, perhaps even prevention of this morbid complication will be possible.

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