Perinatal gastrointestinal pathology

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While much perinatal gastrointestinal pathology is concerned with confirmation of diagnoses, such as necrotizing enterocolitis, meconium ileus and intestinal atresia in surgically resected specimens, there are two clinical situations in the neonatal period in which the primary diagnosis rests with the histopathologist. The first is constipation (which usually presents as delayed passage of meconium) in which a rectal suction biopsy is required to confirm or exclude the diagnosis of Hirschsprung’s disease. The second is congenital diarrhea, in which a small bowel biopsy is performed to confirm or rule out a number of specific disease entities, chief among which are microvillus inclusion disease, tufting enteropathy and autoimmune enteropathy.

Hirschsprung’s disease

Hirschsprung’s disease (HD) is characterized by the absence of ganglion cells in the distal portion of the intestine and by their presence in the proximal portion. It can be categorized according to the length of the aganglionic segment. The incidence is 1 in 5,000 live births. Approximately 80% of patients are male but this ratio decreases as the length of the aganglionic segment increases, such that the sex incidence is equal in total colonic aganglionosis. The genetic basis is complex with an increased incidence of Down’s syndrome in HD. Mutations on chromosome 10q11.2 involving the receptor tyrosine kinase gene RET are present in approximately 20% of patients.

Rectal mucosal biopsy obtained by suction biopsy machine is the established method for diagnosing HD. Two biopsies are preferred, at 2 and 5 cm above the pectinate line. The biopsies should be kept moist on saline-soaked filter paper and transported to the laboratory within 15 min. One biopsy is formalin-fixed and paraffin-embedded. Sixty serial sections in a plane horizontal to the mucosal surface are stained with hematoxylin and eosin, and examined for the presence of ganglion cells. In HD there is an absence of ganglion cells and an increase in large nerve trunks.

The second biopsy is stained for acetylcholinesterase (AChE) activity. There is a well-attested body of literature, both in journals and standard textbooks, supporting the value of AChE in confirming and excluding the diagnosis of HD. The biopsy is orientated on a block of animal liver and frozen for cryostat sections in a plane perpendicular to the mucosal surface. Sections are taken at 5 mm for H&E and at 10 mm for AChE staining. In HD, there is a marked increase in coarse AChE-positive fibers in the muscularis mucosae and lamina propria. Numerous large nerve trunks are present in the submucosa.

The following provisos apply: i) An adequate biopsy must include enough submucosa to provide a reasonable chance of finding ganglion cells. ii) The biopsy must be taken from above the pectinate line. It is important that the pathologist be able to recognize a low biopsy, which is normally anatomically hypoganglionic. iii) In the neonate, ganglion cells are small and immature and look like neuroblasts. The AChE pattern is less dramatic than in the older child with only the muscularis mucosae showing increased nerve fibers. iv) In total colonic aganglionosis, there may be no increase in prominent submucosal nerves and the AChE pattern may be normal. v) With regard to interpretation of AChE staining, we routinely stain all rectal suction biopsies for AChE, even those that are clearly ganglionic. The reason for this is to keep the eye in for the range of normality as well as for the abnormal. Uniformity of staining from case to case in setting a “departmental standard” of normal and abnormal patterns is of importance and we have found that the preparation of batches of incubation media and storing in preprepared aliquots at −20 °C is useful.

At Great Ormond Street, some 250 rectal suction biopsies are currently performed annually, of which approximately 30 will prove to be HD. Two-to-three cases per year produce an “equivocal” pattern. These would all be aganglionic samples but showing only a slight abnormality in the pattern of AChE not amounting to that seen in classical HD. In these circumstances, we suggest either rebiopsy or defunctioning colostomy with intraoperative biopsy, as clinically indicated.

Microvillus inclusion disease

Microvillus inclusion disease (MID) is a rare autosomal recessive enteropathy in which infants present in the first few days of life with severe watery diarrhea. Jejunal biopsy shows severe partial villous atrophy without crypt hyperplasia and without inflammation of the lamina propria. Surface enterocytes lack a well-defined brush border. Periodic acid Schiff (PAS) staining and staining for alkaline phosphatase show distinctive inclusions in the apical cytoplasm in surface and midcrypt enterocytes. Electron microscopy (EM) of surface enterocytes shows intracellular microvillus inclusions with a lack of, or shortened, irregular microvilli on the surface. Jejunal biopsy is optimal for the histological diagnosis but peroral biopsy is only obtainable in infants of 6 weeks or older. Endoscopic small bowel biopsy can be procured in younger infants. A rectal biopsy that can be performed in the perinatal period does not exhibit PAS and alkaline phosphatase positivity but scattered intracellular inclusions may be seen by EM in occasional enterocytes in MID. An absence of inclusions in a rectal biopsy, however, does not exclude the diagnosis. An acquired form of MID is recognized and we have seen the disease in a neonate in whom sequential jejunal biopsies over a 3-week period revealed a progression from an almost normal brush border to fully developed MID. The prognosis for patients with MID is invariably fatal. Long-term parenteral nutrition used to be the only treatment but bowel transplantation is now considered the treatment of choice.
Tufting enteropathy

First described in 1994, tufting enteropathy is a clinicopathological entity in which infants present with watery diarrhea in the first month of life. The pattern of inheritance appears to be autosomal recessive. Jejunal biopsies show partial or subtotal villous atrophy with normal or hyperplastic crypts. The lamina propria is not inflamed and intraepithelial lymphocytes are not increased. The most striking finding is of epithelial cell tufts, that is, closely packed enterocytes with rounded apical plasma membranes. While this appearance may be seen focally in other enteropathies, in tufting enteropathy between 80% and 90% of the surface exhibits the change. Treatment at present is with total parenteral nutrition.

Patients with autoimmune enteropathy present with diarrhea in the first year of life and may present in the neonatal period. A family history of autoimmune disease is often present. In some patients, the disorder may occur in the context of a primary immunodeficiency syndrome. Jejunal biopsies show mild, partial to subtotal villous atrophy with crypt hyperplasia. Surface enterocytes show numerous intraepithelial lymphocytes and numerous vacuolated and individually necrotic surface enterocytes as a marker of cytopathic damage. The lamina propria shows dense lymphocytic and plasma cell infiltration (although in agammaglobulinemia and hypogammaglobulinemia plasma cells are absent) and there may be crypt abscesses. Antigut antiepithelial or antigoblet cell antibodies are detected in the blood. Treatment is directed to the underlying immune defect in immunodeficient patients and immunosuppressive therapy in those who do not have a demonstrable immunodeficiency.

References


Gastric pathology in pediatric Crohn’s disease

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Systematic endoscopic evaluation of the upper gastrointestinal (GI) tract of patients with Crohn’s disease (CD) has revealed frequent endoscopic and histological abnormalities (1, 2). Recent studies have shown that there is gastric involvement in over three-fourths of patients with CD. However, in these studies, only a minority of these gastric lesions were specific cases of granulomatous gastritis. The most striking lesion was a focal acute inflammation of the stomach (3, 4). Other studies have shown that the majority of the gastritis that occurs in CD is not associated with Helicobacter pylori infection (5). The greatest challenge for understanding upper gastrointestinal involvement in CD is to define the value of this focal inflammation more precisely.

Focal inflammation may be defined as the presence of inflammatory cells surrounding at least a foveolar pit or a small group of foveolae or glands. The inflammatory cells are mononuclear cells (histiocytes and lymphocytes) and polymorphonuclear neutrophils. The neutrophils infiltrate the epithelium that shows alterations, such as discontinuities or cuboidal-shaped cells. The inflammation is often deep within the mucosa and is rarely associated with granulomas. This histological definition of focal inflammation rules out activity when the majority of crypts or glands are infiltrated by inflammatory cells or when adjacent normal mucosa cannot be found in a given biopsy specimen. Focal inflammation is characteristic of H. pylori-negative CD and is uncommon in non-CD cases. The value of focal enhanced gastritis is so great that some authors claim that this type of gastritis should encourage further investigations of patients not yet known to have CD, or that it could help to classify indeterminate colitis.

The concept that immunological changes play a key role in the pathogenesis of CD is now established (6). The cytokine production profiles in CD strongly suggest the prevalence of a Th1 response (7). In contrast, murine models of ulcerative colitis, such as the T-cell receptor alpha mutant, indicate the critical role of the Th2 cytokine in the pathogenesis of this colitis (8). The cytokine interleukin-12 (IL-12) is composed of two subunits, p40 and p35, encoded by two separate genes (9). IL-12 is produced mainly by monocytes and macrophages and plays a pivotal role in the differentiation of Th1 T-cells, as it induces naïve T-cells to produce interferon-y (10,11). Murine models of experimental granulomatous colitis mimicking CD indicate that IL-12 plays a major role in the disorder (12). Recent studies have shown greater amounts of IL-12 in the intestinal tissues of CD patients than in the normal and inflamed tissues of controls (13).

The important part played by IL-12 in CD prompted us to study its expression in the gastritis of pediatric patients with confirmed distal CD in an attempt to identify a link between cytokine production and nongranulomatous and granulomatous gastritis occurring in CD. We demonstrated in situ enhanced IL-12 production in the gastric mucosa of pediatric CD patients. The patterns of IL-12-positive cells in the small bowel and gastric mucosa were the same, with small to large foci of positive cells in the lamina propria. However, no IL-12-positive cells were found in the gastric mucosa of the patients with ileal CD and normal gastric mucosa, or in the controls and only few scattered positive cells were found in 50% of the gastric mucosa specimens from patients with H. pylori-positive chronic gastritis. Also, the amounts of IL-12 expression in the gastric biopsy specimens exhibiting granulomatous gastritis and non granulomatous gastritis were similarly enhanced. A recent study has shown that gastritis-associated CD is characterized by a focal periglandular accumulation of CD68+ histiocytes and CD3+ lymphocytes with a high content of TiA1+ cytotoxic T lymphocytes (3). As IL-12 is mainly produced by macrophages and enhances T-cell and natural killer cell cytotoxicity, with increased productions of granzyme B and perforin messenger RNA, IL-12 may play a major role in the pattern and accumulation of immune cells in gastritis-associated CD. The demonstration of focally enhanced IL-12 expression in both nongranulomatous gastritis and granulomatous gastritis, as well as in ileal specimens from CD patients, highlights the link between all these lesions in CD.

Finally, IL-12 expression is elevated at the time of CD diagnosis and remains increased for months or years during the course of CD, suggesting that IL-12 is important for both initiating and maintaining inflammation in this disease. In conclusion, IL-12 expression is focally enhanced in the gastric mucosa of pediatric patients.