Modern Pathology (2014) 27, 543–553

Pediatric autoimmune enteropathy: an entity frequently associated with immunodeficiency disorders

Aatur D Singhi[1], Alka Goyal[2], Jon M Davison[1], Miguel D Regueiro[3], Robyn L Roche[1] and Sarangarajan Ranganathan[1]

[1]Department of Pathology, The University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [2]Department of Pediatrics, The University of Pittsburgh Medical Center, Pittsburgh, PA, USA and [3]Department of Medicine, The University of Pittsburgh Medical Center, Pittsburgh, PA, USA

The term pediatric autoimmune enteropathy was originally applied to a form of intractable diarrhea seen in children under the age of 6 months and characterized by male predominance, concurrent autoimmune-associated disorders, circulating gut autoantibodies, a lack of severe immunodeficiency and small bowel atrophy with prominent crypt apoptosis. However, recent studies have cast doubt over the specific clinicopathologic findings associated with this entity. We, therefore, collected 178 gastrointestinal biopsies from 14 patients and examined their clinical, serologic and pathologic findings. Patients at presentation ranged in age from birth to 15.9 years (median, 5.5 months; mean, 4.1 years) and included six males and eight females. All children suffered from chronic watery diarrhea and malnutrition. Concomitant-associated disorders were noted in 11 (79%) cases and included 10 (71%) with an immunodeficiency disorder and/or another autoimmune-related disease. Eleven patients (79%) were positive for anti-enterocyte antibodies. The salient findings of autoimmune enteropathy were most prominent in the small intestines and the majority (79%) of patients demonstrated villous blunting, crypt hyperplasia, mononuclear cell inflammatory expansion of the lamina propria and crypt apoptosis. The remaining (21%) patients showed marked intraepithelial lymphocytosis reminiscent of celiac disease. Further, acute cryptitis and crypt abscesses were seen in seven (50%) patients obscuring the presence of apoptosis. The absence of Paneth cells, goblet cells or both was noted in seven (50%) patients. Follow-up information was available for all patients with 13 (93%) receiving immunosuppressant therapy and demonstrating partial-to-complete response. In total, three patients died from continued diarrhea and sepsis with one decedent before treatment could be initiated. In summary, autoimmune enteropathy in children is a heterogenous disease with protean clinical and pathologic findings. Although anti-enterocyte antibodies were identified in the majority of the cases, their presence was variable and insensitive. In addition, pediatric autoimmune enteropathy was frequently encountered in the setting of immunodeficiency disorders.
Modern Pathology (2014) **27,** 543–553; doi:10.1038/modpathol.2013.150; published online 20 September 2013

Keywords: autoimmune enteropathy; celiac disease; crypt apoptosis; intractable diarrhea

Intractable diarrhea of infancy was originally described by Avery *et al* in 1968 as a syndrome characterized by chronic unexplained diarrhea in children younger than 3 months.[1] In current practice, the term 'intractable diarrhea' has been extended beyond the age of infancy and represents several chronic pediatric disorders. Autoimmune enteropathy is the most frequent diagnosis in children with intractable diarrhea and a prevalence of up to 29% in epidemiological studies.[2,3] Moreover, it is a life-threatening condition preferentially affecting male infants and characterized by immune-mediated damage to the intestinal mucosa, resulting in severe malabsorption and diarrhea.[4] Patients typically require both nutritional support and immunosuppressive therapies. The histopathology of pediatric autoimmune enteropathy primarily involves the small intestines, which demonstrates partial-to-complete villous blunting, lymphoplasmacytic expansion of the lamina propria and

Correspondence: Dr AD Singhi, MD, PhD Department of Pathology, University of Pittsburgh Medical Center, Presbyterian University Hospital, 200 Lothrop Street, Room A616.2, Pittsburgh, PA 15213, USA.
E-mail: singhiad@upmc.edu
Received 20 May 2013; revised 22 June 2013; accepted 27 June 2013; published online 20 September 2013

prominent crypt apoptosis. Children also often present with other autoimmune-related disorders. But, the hallmark feature of autoimmune enteropathy is the lack of immunodeficiencies and the presence of circulating autoantibodies to gut enterocytes.[5,6]

While studies have increased our understanding of the clinical and pathologic findings of this entity, more recent reports have been focused on autoimmune enteropathy occurring in adults. Akram *et al*[7] published the largest series of adult patients with autoimmune enteropathy consisting of 15 cases. The authors identified a broader spectrum of clinical features and pathologic changes than previously recognized and traditionally described for autoimmune enteropathy in children. For example, in some cases, small bowel biopsies lacked classic features of autoimmune enteropathy and instead revealed celiac-like changes. In addition, three of their patients had associated immunodeficiencies, classically considered outside the definition of autoimmune enteropathy. To expand our knowledge and to better define this clinicopathologic entity in children, we have collected 14 cases of pediatric autoimmune enteropathy and describe in detail their clinical presentation, family history, serologic findings, histopathology within the entire gastrointestinal tract and treatment including histopathologic follow-up.

Materials and methods

Study approval was obtained from the University of Pittsburgh Institutional Review Board. Patients were identified through a computer search of the University of Pittsburgh Medical Center electronic medical records for a diagnosis of autoimmune enteropathy in pediatric patients based on the clinical symptomatology of severe and intractable diarrhea unresponsive to dietary restriction and associated with multiple, correlative gastrointestinal tract mucosal biopsies, consistent with the diagnosis of autoimmune enteropathy and in which other clinicopathologic entities were ruled out. From this list, only patients with documented positive anti-enterocyte antibodies and/or clinical response to immunosuppressants were included within this study (Table 1). In two cases, patients were also diagnosed with celiac disease (positive for serologic markers), but unresponsive to a gluten-free diet. Both patients underwent gene testing and found to harbor the human leukocyte antigen (HLA)-DQ2 haplotype. In addition, both patients had positive anti-enterocyte antibodies. Three patients were known to have common variable immunodeficiency or hypogammaglobulinemia and presented with chronic diarrhea unresponsive to intravenous immunoglobulin replacement therapy. All three patients were also found to have positive anti-enterocyte serologies. Patients were seen at Children's Hospital of Pittsburgh and Presbyterian Hospital of the University of Pittsburgh Medical Center between 1996 and 2013. Demographic data including patient age, sex, onset of symptoms, time to diagnosis including delays in diagnosis, treatment regimens, treatment response and follow-up were recorded.

In total, there were 178 gastrointestinal specimens from 14 patients. All specimens were mucosal biopsies with 38 obtained from the esophagus, 48 from the stomach, 52 from the small bowel and 40 from the colon. For the purposes of this study, because small intestinal biopsies appeared to be similar from site to site (eg, duodenum *versus* terminal ileum), each set of small intestinal biopsies was regarded as a single specimen. Colonic biopsies were treated similarly.

Review of paraffin-embedded, hematoxylin and eosin-stained sections was performed with the particular attention to inflammatory infiltrate and composition, intraepithelial lymphocytosis, villous blunting, prominence of apoptosis, small intestinal villous and crypt architecture, cryptitis and crypt abscesses. Villous blunting was graded based on the villous height-to-crypt length ratio. A normal ratio was defined as between 3:1 and 5:1. Mild, moderate and severe villous blunting were defined by a villous-to-crypt ratio between 2:1 and 3:1, 1:1 and 2:1, and <1:1, respectively. Apoptosis was graded in a semiquantitative manner such that one (or more) apoptotic body per esophageal or gastric biopsy was considered as abnormal, whereas in the small bowel and colon, greater than 1 apoptotic body per 10–15 crypts was sought.[8,9] When available, immunohistochemical stains for *Helicobacter* and cytomegalovirus were also reviewed.

Results

Clinical and Serologic Findings

The main clinical features for each case of pediatric autoimmune enteropathy are summarized in Table 1. Patients at presentation ranged in age from birth to 15.9 years (median, 5.5 months; mean, 4.1 years) and included six males and eight females. All children suffered from chronic watery diarrhea and malnutrition. The duration of symptoms before clinical presentation varied from 1 week to 3 months (median, 1 month; mean, 1.1 months). Ten of fourteen (71%) patients were diagnosed with failure to thrive and required parenteral nutritional support. The time interval from presentation to diagnosis varied from 1 month to 1.2 years (median, 4.2 months; mean 6.3 months). Before a definitive diagnosis was rendered, the clinical differential included milk allergy, infectious etiologies and celiac disease. In four cases, inflammatory bowel disease was also within the differential diagnosis. Delays in diagnosis were often due to initially

Table 1 Clinical characteristics of 14 pediatric patients with autoimmune enteropathy

Case No.	*Age at presentation*	*Time to diagnosis*	*Sex*	*Clinical symptoms*	*Gut epithelial antibodies*	*Associated disorders*	*Family history*	*Follow-up (years from diagnosis)*
1	Birth	8 months	F	Chronic diarrhea and failure to thrive	Negative	None	Older brother has similar symptoms	Responsive to FK506 (2.5)
2	Birth	1 year	M	Chronic diarrhea and failure to thrive	Negative	PI	Parents are second cousins & both have DM	Responsive to FK506 (2.2)
3	Birth	1.5 years	F	Chronic diarrhea and failure to thrive	AE IgA +	Pyloric atresia, intestinal malrotation and factor V Leiden	Twin brother with CLD and cardiomegaly	Partial response to FK506; deceased due to sepsis (5.3)
4	1 month	10 months	F	Chronic diarrhea and failure to thrive	First screen was negative; AE IgA +	None	None	Partial response to FK506; deceased due to sepsis (3.7)
5	2 months	1 month	M	Chronic diarrhea and failure to thrive	AE IgG, IgM and IgA +; repeat weak AE IgM +	IPEX, food allergies and asthma	None	Responsive to FK506 (15.3)
6	2.5 months	3.5 months	F	Chronic diarrhea and failure to thrive	AE IgM +	HG, autoimmune thyroiditis, PI and hepatomegaly	None	Deceased due to sepsis (0.5)
7	3 months	1 month	M	Chronic diarrhea and failure to thrive	AE IgM +	Hepatomegaly, Hashimoto's thyroiditis, anemia and chronic lung disease, ITCH deficiency	Multiple members with AIE	Responsive to FK506, however developed renal injury (2); responsive to rapamycin (5.5)
8	7 months	2 months	F	Chronic diarrhea and failure to thrive	AE IgM +	Juvenile rheumatoid arthritis, celiac disease (HLA-DQ2 +) and VSD	Mother with Crohn's	Responsive to 6-MP, however developed pulmonary lymphomatoid granulomatosis (5); responsive to MTX and B (6)
9	2 years	2 months	M	Chronic diarrhea and failure to thrive	AE IgM +	APECED, AH and PI	Mother with Crohn's and father with hypothyroidism	Responsive to FK506, however developed renal injury (16.2)
10	5.5 years	4.8 months	F	Chronic diarrhea	AE IgG +	Kabuki syndrome, PI and VSD	Mother with PI	Responsive to FK506 (9.5)
11	9.8 years	2.4 months	M	Chronic diarrhea and failure to thrive	AE IgG +	CVID and PI	None	Responsive to IVIG and FK506 (6.3)
12	10.2 years	7.2 months	M	Chronic diarrhea	AE IgA +	Juvenile rheumatoid arthritis and celiac disease (HLA-DQ2 +)	Father with celiac disease	Responsive to FK506 (8.75)
13	13 years	1.2 years	F	Chronic diarrhea	Negative (tested twice)	None	Sisters with autoimmune diseases, mother with Grave's and arthritis, father with Crohn's and DM, and paternal grandfather with AIE	Responsive to 6-MP and B (13.6)
14	15.9 years	2.4 months	F	Chronic diarrhea and 30 lb weight loss	AE IgM +	CVID and AG	None	Partial response to IVIG and 6-MP (1.1)

Abbreviations: 6-MP, 6-mercaptopurine; AE, anti-enterocyte; AG, autoimmune gastritis; AH, autoimmune hepatitis; AIE, autoimmune enteropathy; APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; B, budenoside; CLD, chronic lung disease; CVID, common variable immunodeficiency; DM, diabetes mellitus; F, female; FK506, tacrolimus; HG, hypogammaglobulinemia; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome; M, male; MTX, methotrexate; PI, pancreatic insufficiency; VSD, ventricular septal defect.

negative anti-enterocyte serologies (cases 1, 2, 4 and 13), consideration that symptoms may be due to congenital anomalies (cases 3 and 10) and/or the lack of classic histopathologic features of autoimmune enteropathy (case 12).

At clinical presentation, concomitant-associated disorders were noted in 11 of 14 (79%) cases and included 10 (71%) with an immunodeficiency disorder and/or another autoimmune-related disease. Six (43%) patients had a history of immunodeficiency disorders, which consisted of common variable immunodeficiency ($n=2$), hypogammaglobulinemia ($n=1$), immunodysregulation polyendocrinopathy enteropathy X-linked syndrome ($n=1$), autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy ($n=1$) and *ITCH* E3 ubiquitin ligase deficiency ($n=1$). Both patients with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy were confirmed to harbor mutations in the genes forkhead box P3 (*FOXP3*) and autoimmune regulatory (*AIRE)*, respectively. In addition, the patient demonstrating *ITCH* deficiency was reported previously by Lohr *et al.*[10] The patient belongs to large Amish kindred with a homozygous single base-pair insertion in exon 6 of the *ITCH* gene. Concurrent autoimmune-related diseases included juvenile rheumatoid arthritis ($n=2$), autoimmune thyroiditis ($n=2$), celiac disease ($n=2$), autoimmune atrophic gastritis ($n=1$), autoimmune hepatitis ($n=1$) and asthma ($n=1$). Pancreatic insufficiency was also reported in four children; while cardiac ventricular septal defects were identified in two. Of the two patients with ventricular septal defects, case 10 had a history of Kabuki syndrome, which consisted of multiple congenital anomalies, and confirmed with a loss-of-function mutation in the *MLL2* gene. A family history of autoimmune diseases was noted in 5 of 14 (36%) cases with 2 reporting a history of autoimmune enteropathy.

Serologic studies for anti-enterocyte antibodies were performed in all patients with 10 of 14 initially positive. Of the remaining four negative cases, repeat testing was performed for two patients (cases 4 and 12). For case 4, 8 months after initial testing with continuation of symptoms, anti-enterocyte serologies were positive. In contrast, despite repeated symptomatic flares 1 year after presentation, case 12 remained negative. In total, anti-enterocyte antibodies were identified in 11 of 14 (79%) children. While the majority of patients harbored a single anti-enterocyte immunoglobulin (5 IgM, 3 IgA and 2 IgG), one patient with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome was found to have all three. Repeat testing of this patient, 6 months after treatment, identified only weak anti-enterocyte IgM antibodies. Further analysis on the identification of anti-goblet cell antibodies was not performed.

Histologic Findings

Esophagus. Esophageal biopsies were taken from 13 (93%) patients. In the majority of cases (9 of 13, 69%), the squamous mucosa showed mild reactive epithelial changes, including focal ballooning cytoplasm and increased lymphocytic infiltrate. No prominence of apoptosis was seen.

Stomach. Gastric biopsies were obtained in all cases. Ten of fourteen (71%) samples showed a mild-to-moderate chronic gastritis with increased lamina propria lymphocytes and, except for one case, plasma cells forming a superficial band within the mucous neck region (Figure 1a). For case 14, the mononuclear cell infiltrate also extended into the deep mucosa and accompanied by partial parietal cell loss, superficial intestinal metaplasia and enterochromaffin-like cell hyperplasia compatible with autoimmune atrophic gastritis (Figure 1c). Of note, consistent with the patient's history of common variable immunodeficiency, plasma cells were distinctly absent. Multiple apoptotic bodies were identified within the deep glandular mucosa in four (29%) patients (Figure 1d). Three (21%) patients had a lymphocytic gastritis pattern (Figure 1b). Patchy intraepithelial neutrophils were found in specimens from three (21%) patients. However, in all 14 cases, no infectious organisms were identified (eg, *H. pylori* and cytomegalovirus).

Small intestines. Small bowel specimens were reviewed for all 14 cases. In 11 (79%) patients, the biopsies demonstrated the prototypical histomorphologic findings of autoimmune enteropathy including villous blunting, crypt hyperplasia, lymphoplasmacytic expansion of the lamina propria and crypt apoptosis. However, case 13 showed only a mild inflammatory infiltrate within the lamina propria, despite presenting with chronic watery diarrhea and a significant weight loss. Subsequent biopsies, 7 months later, displayed the characteristic findings of autoimmune enteropathy (Figure 2). Villous blunting was present in all biopsies and ranged from mild ($n=3$) to moderate ($n=4$) to severe ($n=4$). Gastric mucin-cell metaplasia was seen in two specimens. In four cases, a neutrophilic infiltrate was also present within the deep mucosa and involved both the epithelium and lamina propria. Crypt abscesses were also found in three cases. Of note, in two specimens, intraepithelial neutrophils obscured the presence of crypt apoptosis and only on closer evaluation the latter were identified (Figures 3a and b). Four of eleven cases demonstrated loss of Paneth and/or goblet cells. The complete absence of both Paneth and goblet cells was seen in case 10. While only Paneth cells were lost in cases 7 and 11; and goblet cells in case 13.

As was described in adult patients,[7] small bowel biopsies from the remaining three (21%) cases demonstrated a combination of both autoimmune

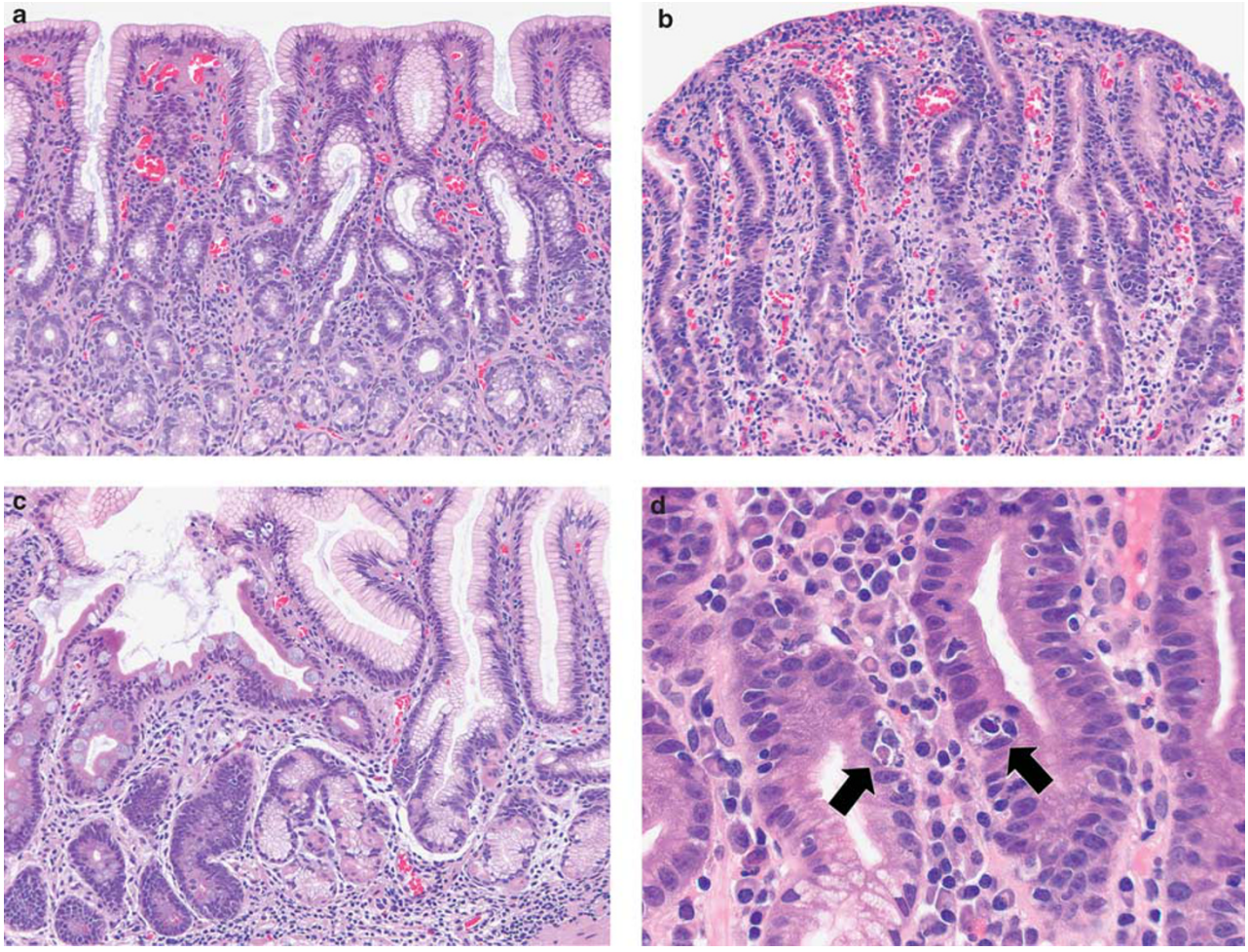

Figure 1 Gastric mucosal biopsies from children with autoimmune enteropathy often showed features of (**a**) chronic gastritis with increased lamina propria lymphoplasmacytic inflammation forming a superficial band within the mucous neck region. (**b**) In some cases, prominent intraepithelial lymphocytosis was identified within the surface, foveolar epithelium consistent with lymphocytic gastritis. (**c**) Concurrent autoimmune-related disorders were a common finding in patients with autoimmune enteropathy. Gastric biopsies taken from the fundus of case 14 demonstrated the features of autoimmune atrophic gastritis with partial parietal cell loss and superficial intestinal metaplasia. (**d**) In a subset of patients, multiple apoptotic bodies (arrows) were identified within the deep glandular mucosa.

enteropathy and sprue-like histologic findings. These specimens were characterized by severe villous blunting, marked intraepithelial lymphocytosis of >40 lymphocytes per 100 epithelial cells, diffuse mononuclear infiltrate involving the lamina propria and deep crypts, and prominent crypt apoptosis (Figures 3c and d). Patchy acute inflammation within the lamina propria and cryptitis was also present. Of note, two of three patients had concomitant celiac disease (cases 8 and 12). In addition, one patient showed lymphocytic gastritis in the corresponding stomach biopsies (case 12). In all three cases, patients were positive for anti-enterocyte antibodies and demonstrated an absence of Paneth cells (case 14), goblet cells (case 8), or both (case 12). Similar to the patient's stomach biopsies, case 14 also lacked plasma cells. Neither the classic autoimmune enteropathy nor the sprue-like morphology showed loss of enterochromaffin cells.

Colon. Concomitant colonic biopsies were taken for each patient. Similar to the small intestines, all specimens were characterized by increased crypt apoptosis. For case 13, although the small intestines showed minimal histologic findings, patchy crypt apoptosis was identified within the patient's initial colonic biopsies (Figures 4a and b). In addition, all biopsies demonstrated a mononuclear inflammatory expansion of the lamina propria with associated deep lymphoid aggregates found in five cases. In seven (50%) patients, patchy acute colitis including cryptitis was observed. Only case 8 had the lymphocytic colitis pattern and characterized by increased surface intraepithelial lymphocytes of >20 per 100 epithelial cells (Figure 4c). Despite the absence of goblet cells in small bowel biopsies from four patients, two cases (seen on repeat biopsy) lacked goblet cells within the colon. In these patients, specimens also showed dilated colonic

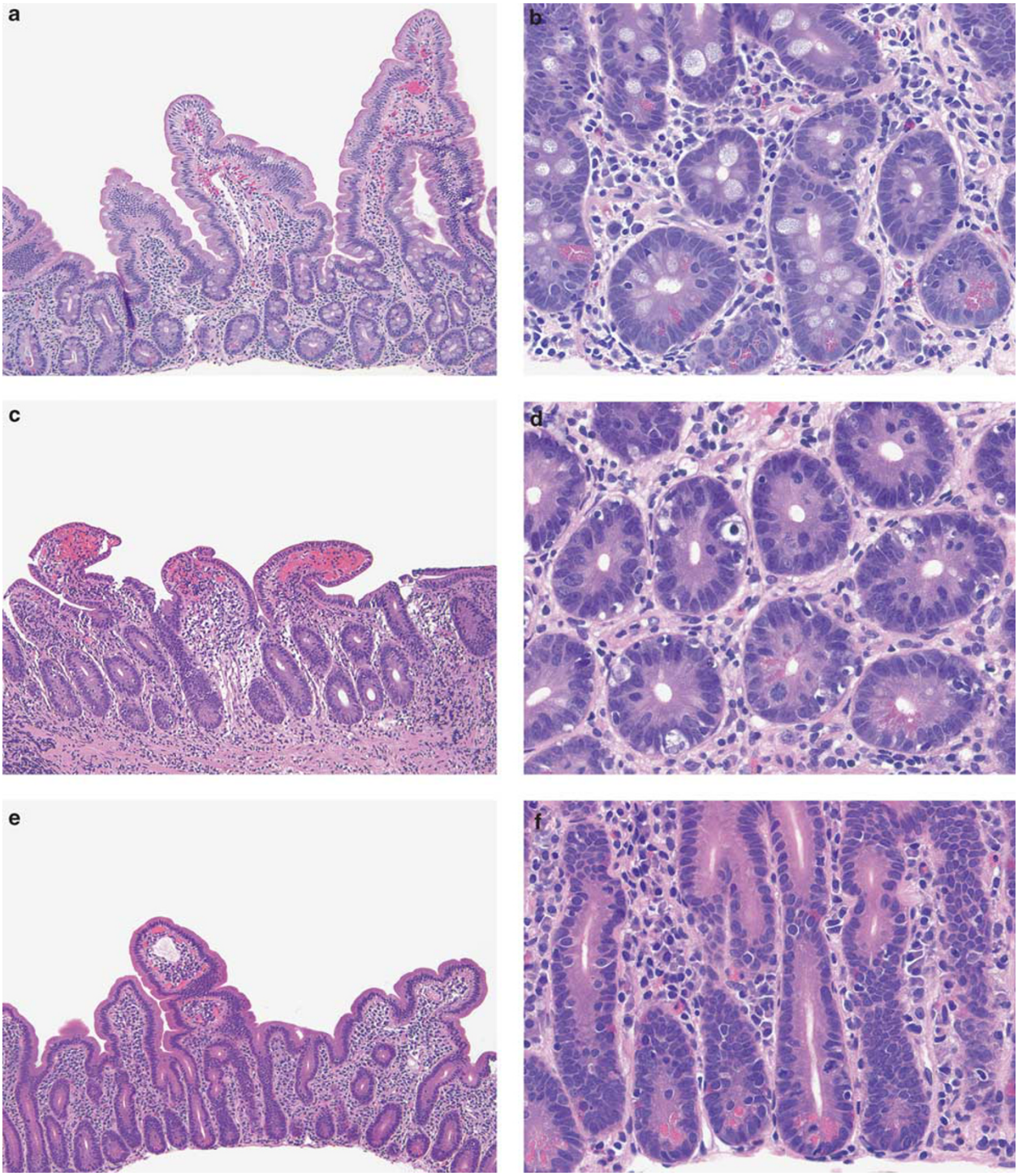

Figure 2 Although presenting with clinical symptoms of autoimmune enteropathy (**a** and **b**, low and high magnifications, respectively) initial small intestinal biopsies from case 13 showed a intact villous architecture, no prominence of crypt apoptosis and only mild lamina propria chronic inflammation. (**c**, **d**) The patient continued to present with diarrhea and weight loss, but on repeat biopsies showed classic findings of autoimmune enteropathy with moderate villous blunting, lamina propria expansion by lymphoplasmacytic inflammation, prominent crypt apoptosis and marked loss of goblet cells. (**e**, **f**) Subsequent to immunosuppressant therapy and resolution of the patient's diarrhea, villi remained mildly blunted with increased chronic inflammation and rare crypt apoptosis (not shown). However, goblet cells remained absent.

crypts with inspissated mucin (Figure 4d). For cases 8 and 12, goblet cells were still present, although among prominent crypt apoptosis. Of the five patients with loss of Paneth cells within their small intestines, right colon biopsies were available for two patients. In both patients, Paneth cells were

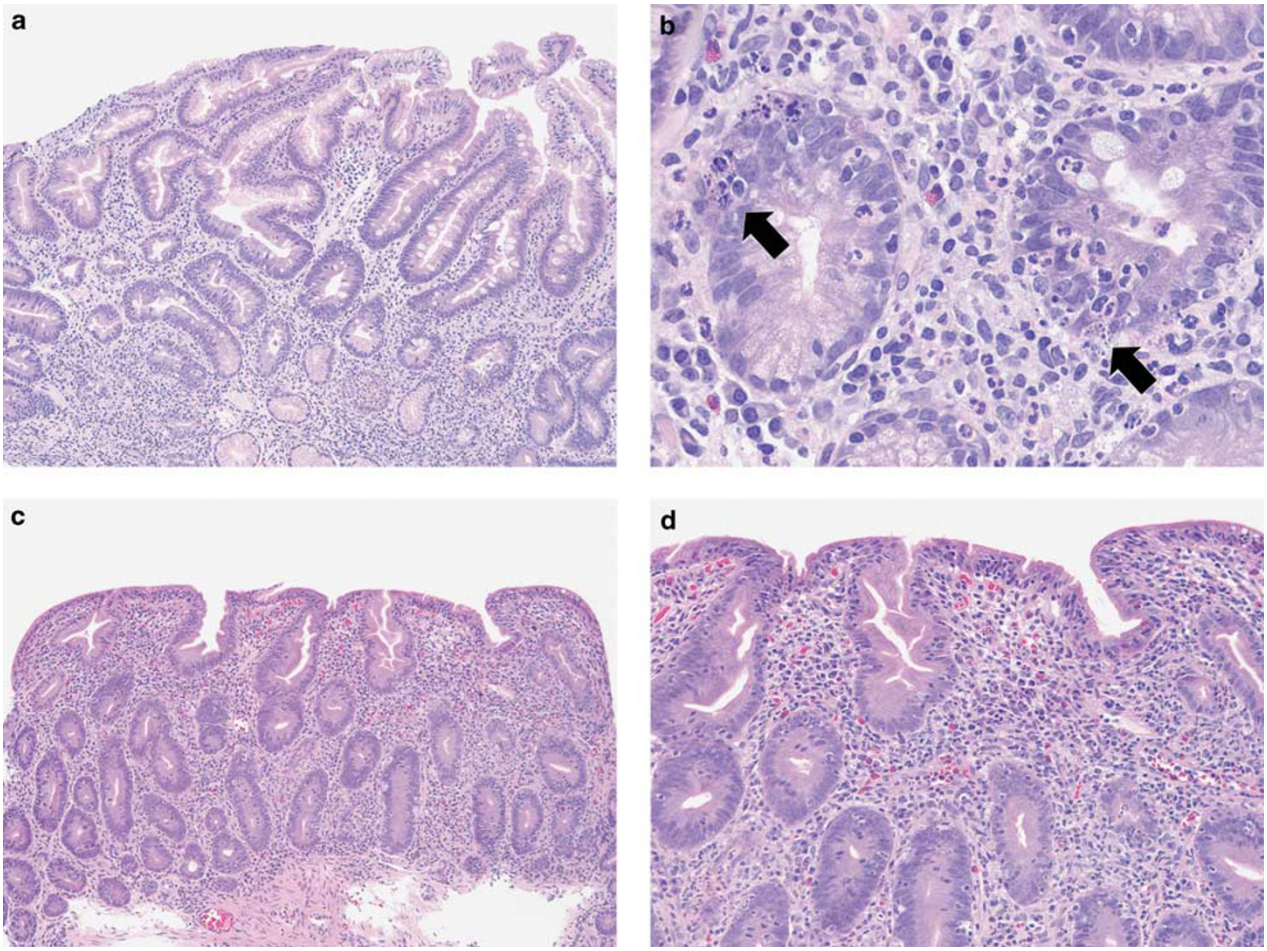

Figure 3 (**a**, **b**, low and high magnifications, respectively) In some cases of pediatric autoimmune enteropathy, the small intestinal biopsies showed cryptitis and crypt abscesses that may obscure the salient finding of autoimmune enteropathy, crypt apoptosis (arrows). In this case (case 11), there is also an absence of Paneth cells. (**c**, **d**) As described in adult patients, small intestinal biopsies can demonstrate a combination of both autoimmune enteropathy and sprue-like histologic findings, characterized by severe villous blunting, marked intraepithelial lymphocytosis, diffuse mononuclear inflammatory infiltrate and prominent crypt apoptosis. Of note, goblet cells are lacking within this specimen (case 8).

absent. Enterochromaffin cells were present in all cases.

Follow-up

Follow-up clinical data were available for all 14 patients and ranged from 6 months to 16.2 years (median, 5.75 years; mean, 6.9 years). Upon diagnosis of autoimmune enteropathy, 13 (93%) patients were treated with immunosuppressants that first included steroids and followed by maintenance of immunomodulators. All 13 patients demonstrated a partial-to-complete resolution of diarrhea. In addition to immunosuppressants, cases 11 and 14 received intravenous immunoglobulins for treatment of common variable immunodeficiency. In total, three patients died from continued diarrhea and sepsis at 6 months (case 6), 3.7 years (case 4) and 5.3 years (case 3) from diagnosis; however, case 6 died before starting therapy. Cases 3 and 4 had only partial response to treatment. In addition, case 3 had other comorbidities including a small bowel transplant. Side effects of immunosuppressant therapy included chronic renal injury in two patients, and pulmonary lymphomatoid granulomatosis in one patient.

Twelve of fourteen (86%) patients had follow-up upper and lower gastrointestinal tract biopsies. Multiple specimens from each of these patients were obtained and ranged 3 months to 15.1 years (median, 4.3 years; mean, 5.3 years) from diagnosis. Seven of twelve (58%) gastric biopsies continued to demonstrate a mild-to-moderate chronic gastritis; however, no epithelial apoptosis or intraepithelial neutrophils were identified. Similarly, all 12 small bowel biopsies showed the continued mononuclear inflammatory expansion of the lamina propria, but crypt apoptosis was markedly diminished in 10 of 12 (83%) cases. Two patients (cases 10 and 13) continued to demonstrate patchy crypt apoptosis and lacked goblet cells. Paneth cells were also

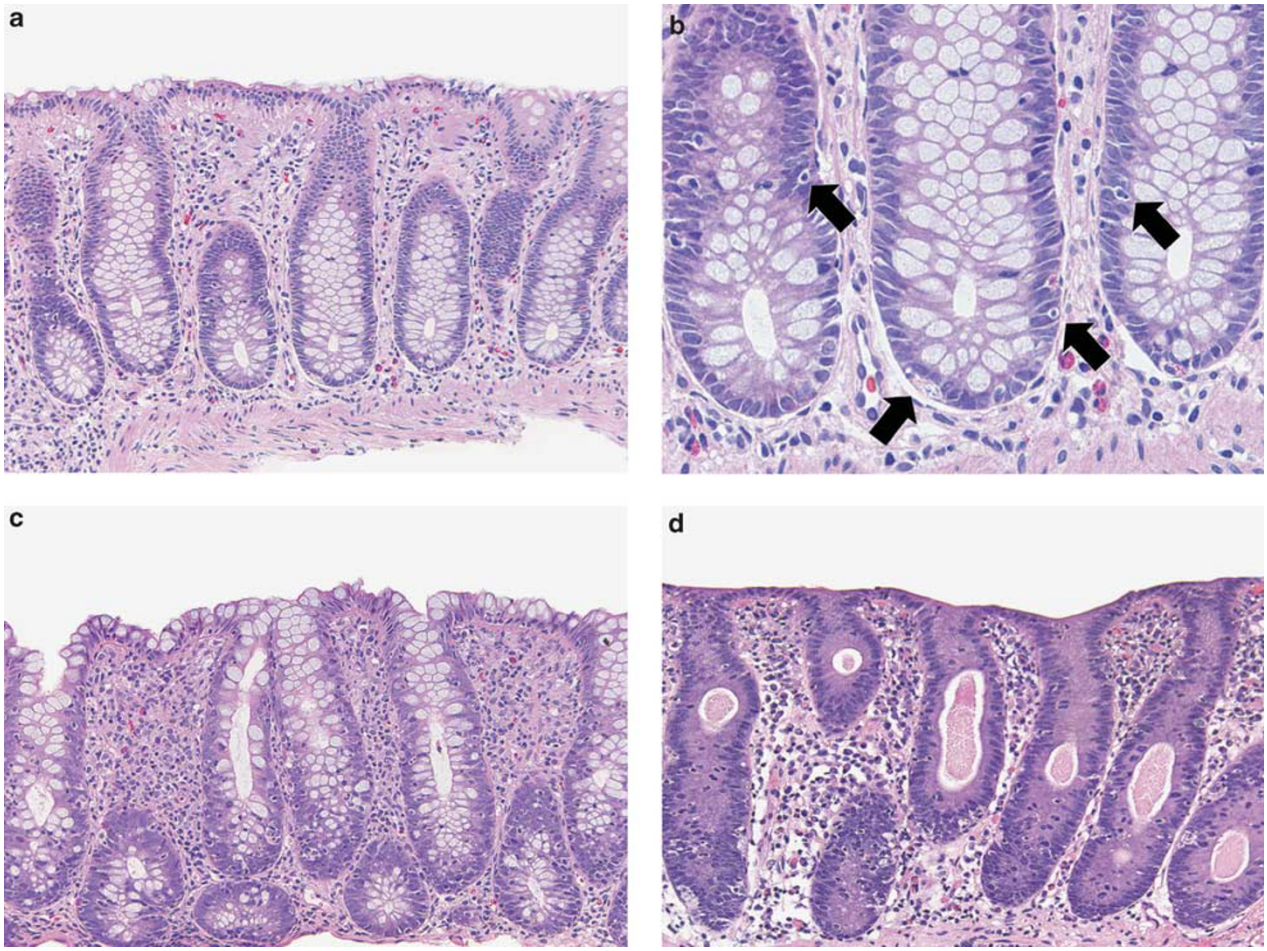

Figure 4 Corresponding colonic biopsies from patients with autoimmune enteropathy were characterized by increased crypt apoptosis (arrows) and in case 13 (**a**, **b**) was the earliest histologic finding. (**c**) In addition, to crypt apoptosis, case 8 had the lymphocytic colitis pattern with increased surface intraepithelial lymphocytes. (**d**) For some specimens where crypt apoptosis was markedly prominent, the colonic crypts were dilated and filled with inspissated mucin. These cases were associated with the absence of goblet cells.

absent in case 10. Corresponding colonic biopsies from both patients showed similar findings. Of the remaining 10 patients, no increase in apoptosis was seen within their colonic specimens with return of goblet and Paneth cells.

Discussion

The term autoimmune enteropathy was coined by Unsworth and Walker-Smith[4] to describe a subgroup of infants with (1) severe and protracted diarrhea, (2) no response to dietary restriction, (3) the presence of circulating gut autoantibodies and/or associated autoimmune diseases and (4) the lack of severe immunodeficiency. To date, less than 50 cases of pediatric autoimmune enteropathy have been reported, but largely limited to case reports and small case series.[9,11–19] To the best of our knowledge, the study herein represents the largest clinicopathologic series of pediatric autoimmune enteropathy. Analysis of these patients and those published previously suggests that pediatric autoimmune enteropathy represents a heterogenous group of disorders with protean clinical and pathologic findings rather than a discrete entity.

Classically, autoimmune enteropathy was thought to occur in children before the age of 6 months with a strong male predominance, concomitant autoimmune-related disorders and positive serologies for gut autoantibodies.[5] However, within our study cohort males and females were equally affected. Furthermore, autoimmune enteropathy can be seen in any age group from newborns to the elderly. Associated autoimmune conditions can be quite broad and include endocrine, renal, pulmonary, liver, hematologic and musculoskeletal system involvement. In addition, other autoimmune conditions involving the gastrointestinal tract have been identified. In this study, three (21%) patients presented with concurrent celiac disease or autoimmune atrophic gastritis. The importance of gut antibodies in the pathogenesis and diagnosis of autoimmune enteropathy is also debatable, as not all patients within our study had positive serologies.

Overall, 79% of children tested had identifiable anti-enterocyte antibodies; however, one patient was noted to be initially negative and on subsequent testing later found to be positive. Another child with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome was positive with three serologic heavy chain subtypes and later weakly positive for only one. And yet another child with no identifiable goblet cells on mucosal biopsies but repeatedly negative by serology. These findings are not uncommon and have been reported previously. In fact, it seems that the role of anti-enterocyte antibodies in the pathogenesis of autoimmune enteropathy is secondary as these autoantibodies seem to appear only after the onset of mucosal damage and to disappear before the restoration of normal mucosa.[20] Moreover, it seems that there is no correlation between titer and the histologic severity of the enteropathy.[12,20,21] Their presence is not pathognomonic, considering that antibodies to enterocyte components at low titers have also been found in patients affected by other gastrointestinal disorders, such as cow's milk intolerance and inflammatory bowel disease, and in adults with HIV infection.[22–26] Thus, this raises the issue of whether these antibodies are merely an epiphenomena in response to bowel injury rather than a primary event.

One of Unsworth and Walker-Smith's criteria for autoimmune enteropathy was the exclusion of underlying immunodeficiencies. However, this notion has been challenged by clinical experience and better understanding of the immunology of autoimmunity and self-tolerance. Interestingly, the first reported case of autoimmune enteropathy was by McCarthy *et al* in 1978 in a child with IgA deficiency.[27] Murch *et al*[28] published a case of autoimmune enteropathy in association with a T-cell activation deficiency. In addition, Catassi *et al*[29] reported a patient with diabetes mellitus type 1 and common variable immunodeficiency. Consistent with these findings, six (43%) patients within our study cohort had a history of immunodeficiency disorders: two with common variable immunodeficiency, one with hypogammaglobulinemia, two with autoimmune polyglandular syndromes and one with *ITCH* deficiency. Patients with common variable immunodeficiency and hypogammaglobulinemia often present with digestive symptoms; however, these are typically ameliorated by intravenous immunoglobulin replacement.[9,30,31] The patients described herein presented with intractable diarrhea and weight loss, unresponsive to immunoglobulin replacement alone until the addition of immunosuppressants. Autoimmune polyglandular syndromes are a group of inheritable monogenic disorders that consist of immunodysregulation polyendocrinopathy enteropathy X-linked syndrome and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy and characterized by the combination of multiple autoimmune diseases, including autoimmune enteropathy. Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome is caused by mutations in the *FOXP3* gene, which results in the absence or dysfunction of regulatory T cells.[32] Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy results from mutations in the *AIRE* gene, which modulates transcription of peripheral self-antigens in the thymus, presented by HLA molecules to maturing T cells.[33] *ITCH* deficiency was recently described within a large Old Order Amish kindred consisting of children presenting with organomegaly, failure to thrive, developmental delay, dysmorphic features and multisystem autoimmune diseases. Autoimmune enteropathy was a frequent finding in many members of this family.[10] Single-nucleotide polymorphism mapping found all patients affected, harbored a truncating mutation in the *ITCH* gene. *ITCH* deficiency results in abnormal T helper cell differentiation and failed T-cell anergy induction.[34] Interestingly, although common variable immunodeficiency is thought to be primarily a B-cell disorder, 20% of patients present with autoimmune syndromes, which is hypothesized to be due to reduced regulatory T cells and consequently elevated levels of activated T cells.[35] T cells are thought to have a pivotal role in chronic autoimmune diseases and their dysfunction may be responsible for autoimmune enteropathy. In fact, tacrolimus, an effective agent in the treatment of autoimmune enteropathy, exerts its immunosuppressive effects by primarily interfering with T-cell activation.[36] This certainly would explain the inconsistencies of circulating gut autoantibodies as T-cell mediated organ damage does not necessarily go through B-cell activation and antibody production. Regardless, additional studies are needed to elucidate the pathogenesis of autoimmune enteropathy.

Similar to the clinical findings, the pathologic features of pediatric autoimmune enteropathy can vary and as seen within this study involve multiple sites within the gastrointestinal tract. The salient findings of pediatric autoimmune enteropathy are typically most prominent in the small intestines and characterized by mild-to-severe villous blunting, crypt hyperplasia, mononuclear cell inflammatory expansion of the lamina propria and crypt apoptosis. Analogous to cases reported in adults, marked intraepithelial lymphocytosis reminiscent of celiac disease can present in a subset of patients. Further, acute cryptitis and crypt abscesses can also be seen that may obscure the presence of apoptosis and represent a potential diagnostic pitfall. The absence of goblet, Paneth and/or enterochromaffin cells aids in the diagnosis. Concomitant gastritis and colitis is often present in the majority of cases. Epithelial apoptosis can be found within both the stomach and the colon, the latter of which may be one of the earliest findings of

autoimmune enteropathy and should not be overlooked.

On the basis of histologic findings, the differential diagnosis of pediatric autoimmune enteropathy would include other immune-mediated disorders, such as food sensitivity enteropathies (eg cow's milk intolerance and celiac disease), Crohn's disease and graft-*versus*-host disease. In infants with intractable diarrhea, cow's milk intolerance is a common illness that should be excluded. Similar to autoimmune enteropathy, cow's milk intolerance can affect the entire gastrointestinal tract with predominant findings in the small intestines. Biopsy specimens reveal flattened villi, edema and prominent mononuclear cell infiltrate of the lamina propria. In contrast to autoimmune enteropathy, cow's milk intolerance is often characterized by an increased number of eosinophils and the absence of crypt apoptosis. Differentiating autoimmune enteropathy from celiac disease can also be difficult. Both diseases have villous blunting, intraepithelial lymphocytosis, increased lamina propria inflammation and crypt hyperplasia. Moreover, lymphocytic gastritis and colitis can be seen with both. However, the presence of crypt apoptosis can be a distinguishing feature of autoimmune enteropathy. In addition, food sensitivity enteropathies typically resolve upon elimination of the offending agent from the patient's diet. Crohn's disease can resemble autoimmune enteropathy in terms of both clinical and pathologic manifestations. But the mucosal injury seen in Crohn's disease is more often accompanied by a prominent acute inflammation rather than a lymphoplasmacytic infiltrate. In addition, the presence of granulomas favors a diagnosis of Crohn's disease. Finally, the apoptosis seen in autoimmune enteropathy is indistinguishable from graft-*versus*-host disease. In this scenario, correlation with the patient's clinical history is imperative.

Immunosuppressant therapy is the mainstay of treatment for patients with pediatric autoimmune enteropathy. However, it is not without complications as patients are at high risk of developing nephrotoxicity and predisposed to infectious complications. In fact, 39% of patients within our study cohort reported therapy-related adverse effects. Unfortunately, no therapeutic regimen has proven to be successful in all cases and relapses do occur. Furthermore, resolution of diarrhea does not seem to correlate with histologic findings as two patients continued to demonstrate patchy apoptosis and the absence of goblet and/or Paneth cells on follow-up biopsies.

In summary, autoimmune enteropathy in children, like adults, is a heterogenous disease characterized by severe and intractable diarrhea, immune-mediated damage to the gastrointestinal tract and concurrent autoimmune-associated conditions. Although circulating anti-enterocyte antibodies were identified in the majority of cases, their presence is neither diagnostic nor specific for autoimmune enteropathy. In addition, autoimmune enteropathy is frequently encountered in the setting of immunodeficiency disorders. The histologic findings of autoimmune enteropathy can vary greatly and may mimic similar immune-related disorders. Therefore, recognition of both the clinical and pathologic features of this entity is critical in establishing the diagnosis and promptly initiating appropriate therapy.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Avery GB, Villavicencio O, Lilly JR, *et al.* Intractable diarrhea in early infancy. Pediatrics 1968;41:712–722.
2 Goulet OJ, Brousse N, Canioni D, *et al.* Syndrome of intractable diarrhoea with persistent villous atrophy in early childhood: a clinicopathological survey of 47 cases. J Pediatr Gastroenterol Nutr 1998;26:151–161.
3 Catassi C, Fabiani E, Spagnuolo MI, *et al.* Severe and protracted diarrhea: results of the 3-year SIGEP multicenter survey. Working Group of the Italian Society of Pediatric Gastroenterology and Hepatology (SIGEP). J Pediatr Gastroenterol Nutr 1999;29:63–68.
4 Unsworth DJ, Walker-Smith JA. Autoimmunity in diarrhoeal disease. J Pediatr Gastroenterol Nutr 1985;4:375–380.
5 Russo PA, Brochu P, Seidman EG, *et al.* Autoimmune enteropathy. Pediatr Dev Pathol 1999;2:65–71.
6 Montalto M, D'Onofrio F, Santoro L, *et al.* Autoimmune enteropathy in children and adults. Scand J Gastroenterol 2009;44:1029–1036.
7 Akram S, Murray JA, Pardi DS, *et al.* Adult autoimmune enteropathy: Mayo Clinic Rochester experience. Clin Gastroenterol Hepatol 2007;5:1282–1290; quiz 45.
8 Washington K, Stenzel TT, Buckley RH, *et al.* Gastrointestinal pathology in patients with common variable immunodeficiency and X-linked agammaglobulinemia. Am J Surg Pathol 1996;20:1240–1252.
9 Daniels JA, Lederman HM, Maitra A, *et al.* Gastrointestinal tract pathology in patients with common variable immunodeficiency (CVID): a clinicopathologic study and review. Am J Surg Pathol 2007;31:1800–1812.
10 Lohr NJ, Molleston JP, Strauss KA, *et al.* Human ITCH E3 ubiquitin ligase deficiency causes syndromic multisystem autoimmune disease. Am J Hum Genet 2010;86:447–453.
11 Fisher SE, Boyle JT, Holtzapple P. Chronic protracted diarrhea and jejunal atrophy in an infant. Cimetidine-associated stimulation of jejunal mucosal growth. Dig Dis Sci 1981;26:181–186.
12 Unsworth J, Hutchins P, Mitchell J, *et al.* Flat small intestinal mucosa and autoantibodies against the gut epithelium. J Pediatr Gastroenterol Nutr 1982;1:503–513.

13 Hill SM, Milla PJ, Bottazzo GF, *et al.* Autoimmune enteropathy and colitis: is there a generalised autoimmune gut disorder? Gut 1991;32:36–42.
14 Mirakian R, Richardson A, Milla PJ, *et al.* Protracted diarrhoea of infancy: evidence in support of an autoimmune variant. Br Med J (Clin Res Ed) 1986;293: 1132–1136.
15 Sanderson IR, Phillips AD, Spencer J, *et al.* Response to autoimmune enteropathy to cyclosporin A therapy. Gut 1991;32:1421–1425.
16 Moore L, Xu X, Davidson G, *et al.* Autoimmune enteropathy with anti-goblet cell antibodies. Hum Pathol 1995;26:1162–1168.
17 Bousvaros A, Leichtner AM, Book L, *et al.* Treatment of pediatric autoimmune enteropathy with tacrolimus (FK506). Gastroenterology 1996;111:237–243.
18 Bishu S, Arsenescu V, Lee EY, *et al.* Autoimmune enteropathy with a CD8+ CD7- T-cell small bowel intraepithelial lymphocytosis: case report and literature review. BMC Gastroenterol 2011;11:131.
19 Oliva-Hemker MM, Loeb DM, Abraham SC, *et al.* Remission of severe autoimmune enteropathy after treatment with high-dose cyclophosphamide. J Pediatr Gastroenterol Nutr 2003;36:639–643.
20 Walker-Smith JA, Unsworth DJ, Hutchins P, *et al.* Autoantibodies against gut epithelium in child with small-intestinal enteropathy. Lancet 1982;1:566–567.
21 Savilahti E, Pelkonen P, Holmberg C, *et al.* Fatal unresponsive villous atrophy of the jejunum, connective tissue disease and diabetes in a girl with intestinal epithelial cell antibody. Acta Paediatr Scand 1985;74:472–476.
22 Mitton SG, Mirakion R, Larcher VF, *et al.* Enteropathy and renal involvement in an infant with evidence of widespread autoimmune disturbance. J Pediatr Gastroenterol Nutr 1989;8:397–400.
23 Skogh T, Heuman R, Tagesson C. Anti-brush border antibodies (ABBA) in Crohn's disease. J Clin Lab Immunol 1982;9:147–150.
24 Martin-Villa JM, Camblor S, Costa R, *et al.* Gut epithelial cell autoantibodies in AIDS pathogenesis. Lancet 1993;342:380.
25 Biagi F, Bianchi PI, Trotta L, *et al.* Anti-goblet cell antibodies for the diagnosis of autoimmune enteropathy? Am J Gastroenterol 2009;104:3112.
26 Folwaczny C, Noehl N, Tschop K, *et al.* Goblet cell autoantibodies in patients with inflammatory bowel disease and their first-degree relatives. Gastroenterology 1997;113:101–106.
27 McCarthy DM, Katz SI, Gazze L, *et al.* Selective IgA deficiency associated with total villous atrophy of the small intestine and an organ-specific anti-epithelial cell antibody. J Immunol 1978;120:932–938.
28 Murch SH, Fertleman CR, Rodrigues C, *et al.* Autoimmune enteropathy with distinct mucosal features in T-cell activation deficiency: the contribution of T cells to the mucosal lesion. J Pediatr Gastroenterol Nutr 1999;28:393–399.
29 Catassi C, Mirakian R, Natalini G, *et al.* Unresponsive enteropathy associated with circulating enterocyte autoantibodies in a boy with common variable hypogammaglobulinemia and type I diabetes. J Pediatr Gastroenterol Nutr 1988;7:608–613.
30 Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol 1999;92:34–48.
31 Deane S, Selmi C, Naguwa SM, *et al.* Common variable immunodeficiency: etiological and treatment issues. Int Arch Allergy Immunol 2009;150:311–324.
32 Patey-Mariaud de Serre N, Canioni D, Ganousse S, *et al.* Digestive histopathological presentation of IPEX syndrome. Mod Pathol 2009;22:95–102.
33 Michels AW, Gottlieb PA. Autoimmune polyglandular syndromes. Nat Rev Endocrinol 2010;6:270–277.
34 Su J, Liu YC. Foxp3 positive regulatory T cells: a functional regulation by the E3 ubiquitin ligase Itch. Semin Immunopathol 2010;32:149–156.
35 Carter CR, Aravind G, Smalle NL, *et al.* CVID patients with autoimmunity have elevated T cell expression of granzyme B and HLA-DR and reduced levels of Treg cells. J Clin Pathol 2013;66:146–150.
36 Liu J, Farmer JD Jr., Lane WS, *et al.* Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 1991;66:807–815.