Environmental enteropathy: critical implications of a poorly understood condition

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Environmental enteropathy (also called tropical enteropathy) is a subclinical condition caused by constant fecal–oral contamination and resulting in blunting of intestinal villi and intestinal inflammation. Although these histological changes were discovered decades ago, the clinical impact of environmental enteropathy is just starting to be recognized. The failure of nutritional interventions and oral vaccines in the developing world may be attributed to environmental enteropathy, as the intestinal absorptive and immunologic functions are significantly deranged. Here we review the existing literature and examine potential mechanisms of pathogenesis for this poorly understood condition.

The deleterious effects of unsanitary living conditions

On July 28, 2010, Bolivian Ambassador Pablo Solón pleaded before the United Nations General Assembly that access to potable water and sanitation should be considered a basic human right. "The vast majority of illnesses around the world are caused by fecal matter", he proclaimed, "3.5 million people die of waterborne illness each year" [1]. Although the Ambassador's appeal was met with great support, in actuality he had grossly understated the impact of hygiene and sanitation on the health of impoverished people worldwide; he failed to recognize the effect of an insidious condition called environmental enteropathy (EE).

EE is a subclinical disorder that occurs among inhabitants of environments with poor sanitation and hygiene, such as those often found in developing countries. Chronic exposure to fecal pathogens is hypothesized to cause inflammation and structural changes in the small bowel, which ultimately result in functional changes. EE is marked by increased intestinal permeability, impaired gut immune function, malabsorption, growth faltering and, potentially, oral vaccine failure, all in a seemingly asymptomatic individual without overt diarrhea [2,3].

The most significant impact of EE may be on malnutrition, a well-recognized problem in the developing world where 26% of children under age 5 years are underweight. Even more alarming is the fact that 21% of deaths in children less than 5 years of age are attributable to sequelae

of malnutrition (http://www.un.org/millenniumgoals/pdf/MDG%20Report%202010%20En%20r15%20-low%20res%2020100615%20-.pdf). EE is also thought to contribute to the poor response to nutritional therapy. Various trials of complementary supervised feeding interventions have failed to show significant improvement in growth [4,5]. Even when breastfeeding is adequate, growth stunting and associated pathologic changes in the intestinal mucosa have been found in infants as young as 3 months. Models depicting the use of all known interventions – including vitamin A and zinc supplementation, balanced energy protein supplementation, complementary feeding, breastfeeding promotion, and micronutrient supplementation in pregnancy – showed that the use of these interventions in 99% of children worldwide would only decrease stunting by 33% [6]. Clearly, there is a large knowledge gap in our understanding of growth stunting and its associated mortality that cannot be explained by food insecurity. We must consider the impact of subclinical yet pathologic changes that are occurring in the absorptive and immune function of the gastrointestinal tract of infants and children in the developing world.

In addition to the failure of nutritional interventions, there has been the observation that oral vaccines, including those for polio and rotavirus, are less immunogenic in children of developing countries, suggesting that EE may cause altered mucosal immunity in these children [7–10]. EE causes fundamental changes in both small intestinal structure and function, as evidenced by biopsies and abnormal sugar absorption tests. The immunologic function of the small bowel may also be significantly altered. Because of constant exposure to enteric pathogens, in EE there is breach of the nonspecific host defenses and engagement of the innate and adaptive immune responses. If the mucosal immune system is chronically activated in children with EE, an oral vaccine might not elicit the same response in this population [6].

Here, we propose that the failure of health interventions in developing nations, specifically those targeting malnutrition and employing oral vaccines, can be directly attributed to EE, a subclinical, pathologic process occurring in the gastrointestinal tract of millions of people worldwide living in impoverished and unsanitary conditions (Figure 1).

Epidemiology

EE was originally dubbed 'tropical enteropathy' in the 1960s when there were several reports of abnormalities
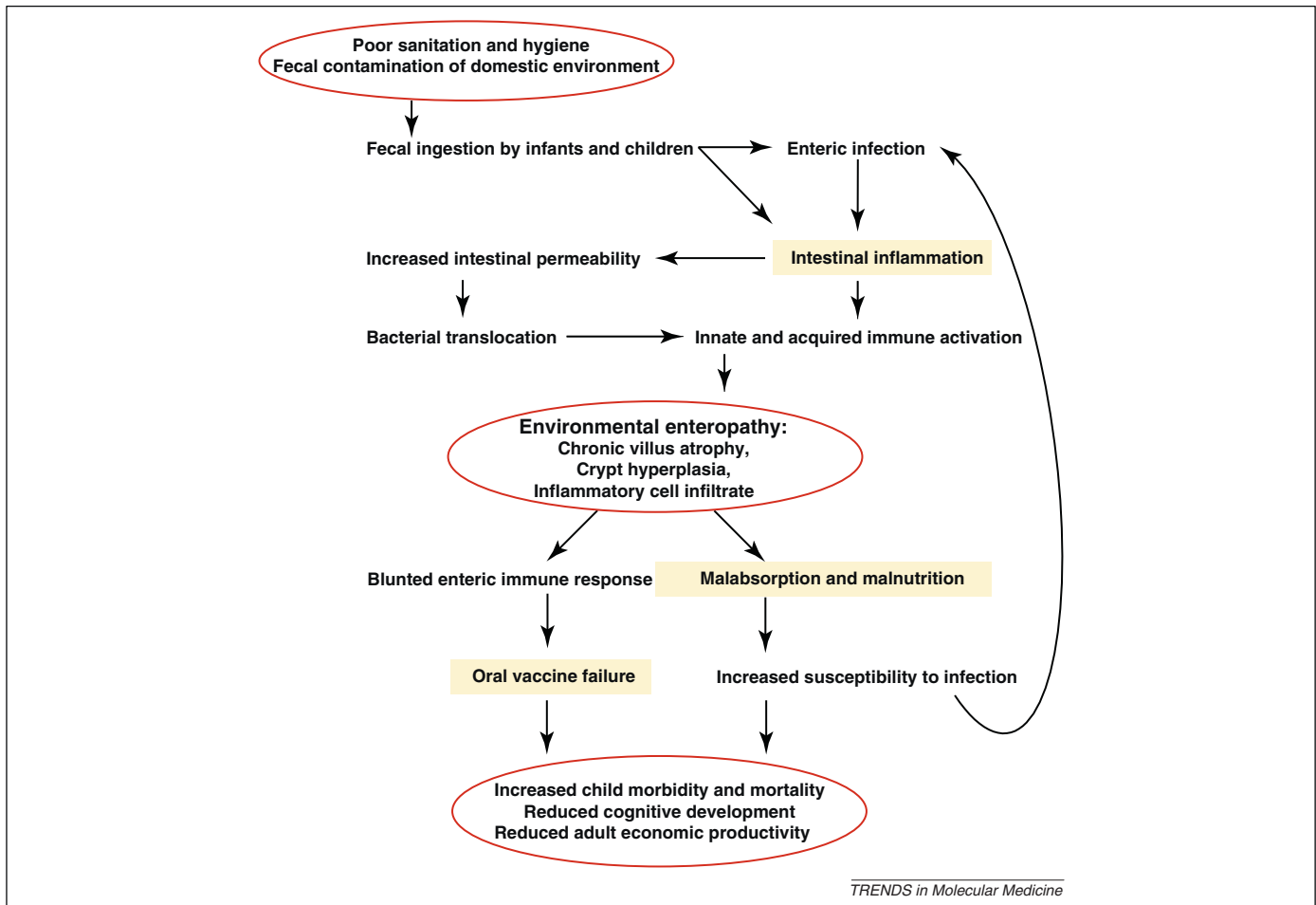
Figure 1. Model of the mechanism of development of environmental enteropathy. Adapted, with permission, from [82].

on jejunal biopsy of asymptomatic individuals from tropical countries [11–15]. Publications from Asia, Africa, and Latin America confirmed these findings in all age groups studied, from infants to adults. Changes seen on biopsy included decreased villous height, increased crypt depth, lymphocytic infiltration of the lamina propria, and increased intraepithelial lymphocytes (Figure 2). In addition, most of these individuals had abnormalities in the absorptive function of the small bowel.

Lindenbaum and colleagues set out to investigate whether this condition of subclinical malabsorption was acquired and, if so, whether it could be reversed. They studied Peace Corps volunteers living in Pakistan: many had mild diarrhea and a few had weight loss. On jejunal biopsy, the same abnormalities that were seen in native Pakistanis were observed, as well as abnormal carbohydrate absorption [16,17]. Two to three years after migrating back to the United States (US), the volunteers were found to have regained normal carbohydrate absorptive function. Five of these individuals also had repeat biopsies, all of which showed a return to normal jejunal histology. A second study, this time of a group of asymptomatic Indian and Pakistani immigrants living in New York City, demonstrated that almost half had malabsorption when studied within the first 2 years of migrating to the US, but absorption and changes on jejunal biopsies improved with increasing periods of residence in the US [18]. These

observed changes in histopathology in the same individuals in both the developed and developing country settings suggested there may be an environmental etiology to this condition.

A study from Vellore, India found that stillborn fetuses had normal finger-like intestinal villi, but abnormalities in architecture were seen in infants as early as 8 weeks of life, again making an environmental rather than genetic etiology plausible [19]. In Brazil, EE was documented in slum-dwelling infants living in poor sanitary conditions [20]. A study of adults in Zambia found that none of the 200 subjects had 'normal' jejunal biopsies. The subjects were noted to have more severe abnormalities in intestinal permeability than controls of higher socioeconomic status. As these subjects were studied serially over 3 years, it was also observed that there was seasonal variation in villous height and carbohydrate absorption [21].

Constant exposure to fecal–oral contamination, and sustained episodes of self-limited infectious gastroenteritis, may lead to a perpetual state of small bowel injury. This injury could cause hyperstimulation of the mucosal immune system and result in the small bowel pathology that has been described. These structural and immune changes may be the adaptive response of the body to a particularly hostile environment. If there is constant pathogenic infiltration into the mucosa, it could be advantageous to remain in an inflammatory, hyperimmune state.
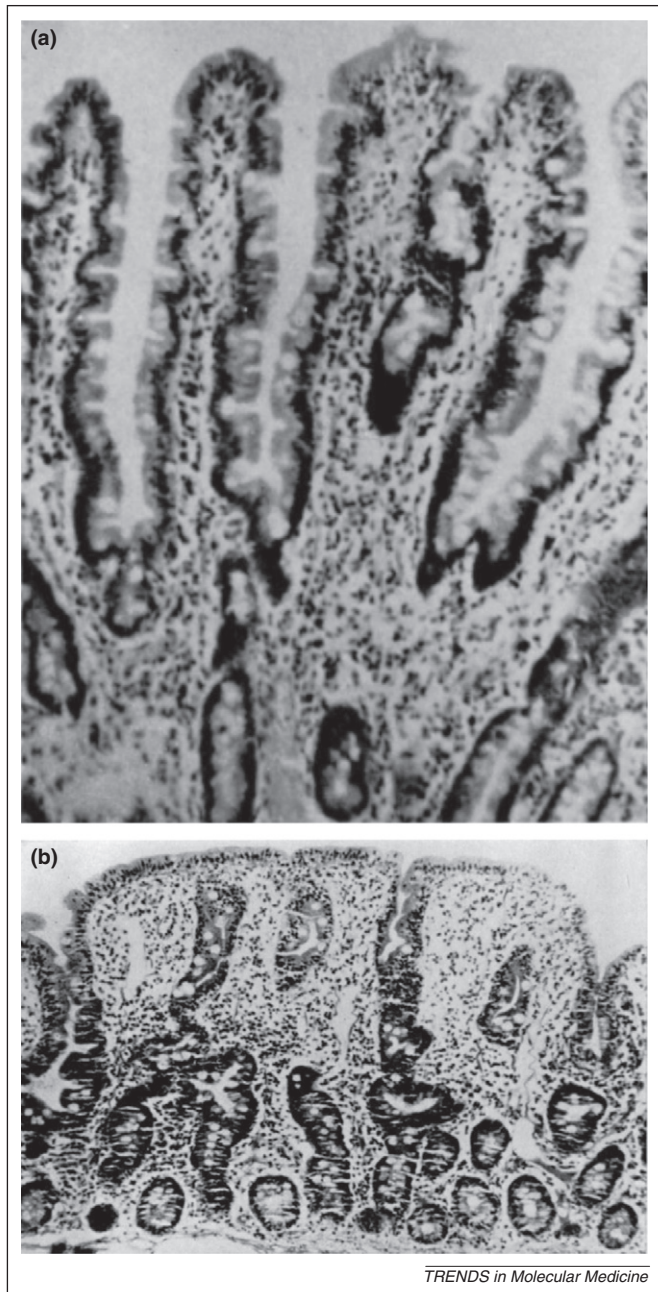
Figure 2. Jejunal biopsy of **(a)** a healthy individual and **(b)** an individual with environmental enteropathy (EE). **(a)** Healthy jejunal tissue has finger-like villi and short and narrow crypts. **(b)** This biopsy was taken from a malnourished Mexican adult, who may have had concurrent diarrhea. Note the severely flattened villi and inflammatory infiltration in the lamina propria and epithelium that are characteristic of EE. Reproduced, with permission, from [15].

Although EE was widely reported in the 1970s and 1980s as an interesting phenomenon, there were few attempts to elucidate the mechanism of disease, and the condition appeared to be almost forgotten over the next 20 years. More recently, there has been increasing interest in the condition as the scientific community struggles to understand disparate responses to health interventions between the developing and developed worlds [22].

EE as a distinct entity

Even though EE is marked by increased intestinal permeability, impaired gut immune function, malabsorption, and growth faltering, individuals with this condition are seemingly asymptomatic, rarely having overt gastrointestinal symptoms [2,3,23]. The differential for conditions causing malabsorption in developing countries is fairly long, and includes tropical sprue, a distinct entity from EE (Table 1). Tropical sprue is a condition that affects both residents and travelers to countries in Asia, Africa, South and Central America, and the Caribbean [24,25]. Patients with tropical sprue have chronic diarrhea, steatorrhea, weight loss, and manifestations of nutritional deficiencies. Although a specific pathogen has not been identified, the etiology of tropical sprue is thought to be infectious as these patients do respond to treatment with broad-spectrum antibiotics [26].

There are reports that small bowel bacterial overgrowth (SBO) may play a role in EE. Khin-Maung *et al.* found an association between abnormal breath hydrogen test (a measure of SBO) and growth faltering in Burmese children [27]. Another study from Brazil reported that slum-dwelling children were more likely to have an abnormal breath hydrogen test when compared with a control group with access to running water and adequate sewage [28]. This study, however, did not find an increased rate of growth faltering among slum-dwelling children with SBO. Although both studies may suggest that children with SBO have malabsorption, they did not investigate measures of intestinal permeability. Trehan *et al.* treated Malawian children with rifaximin for 7 days and found no improvement in markers of intestinal permeability, suggesting that although SBO may play a role in malabsorption, it may not contribute to leaky intestinal barrier [29].

T cell mediated enteropathy

The majority of studies that have investigated EE have reported the same findings: blunting of the villi, crypt hyperplasia, and lymphocytic infiltration of the lamina propria [2,3]. Neonates born in developing countries had normal intestinal villi at birth, but then developed these pathologic changes within 2–12 weeks of age. Slum-dwelling infants in Brazil had abnormal villous/crypt ratio and inflammatory cell infiltration in the lamina propria. These infants were also found to have abnormal D-xylose absorption when compared with infants from middle-class families with access to potable water and sewage facilities [30].

Jejunal biopsies performed on children in the Gambia revealed a range of pathology, from normal architecture to flattened villi, with most specimens having crypt hypertrophy and elevated concentrations of intraepithelial CD8+ lymphocytes [31]. Further studies on children from the same region found histological evidence of cell-mediated enteropathy, independent of nutritional status, with an increased ratio of inflammatory cytokines (interferon-γ, IFN-γ) over regulatory cytokines (transforming growth factor β, TGF-β) [32]. The authors hypothesized that the mucosal changes seen in these children were histologically and immunologically suggestive of a proinflammatory state and indicated a T cell mediated response to an environmental antigen, in many ways analogous to celiac disease.

Celiac sprue is a T cell mediated gluten insensitivity that occurs in genetically predisposed individuals. Histologically, celiac sprue and EE are similar, with villous

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Table 1. Differential for noninfectious tropical malabsorption

	Environmental enteropathy	Tropical sprue	Celiac sprue	Crohn's disease
Characteristic signs and symptoms	Subclinical; can have mild malabsorption and be associated with growth faltering	Chronic diarrhea, weight loss, manifestations of nutritional deficiencies	A minority of patients have the classic diarrhea-predominant form; more patients have 'silent' disease, characterized by lack of symptoms or extraintestinal symptoms, such as arthritis, infertility, anemia, and osteoporosis	Perianal abscesses, intestinal fistulas, and strictures; individuals are at increased risk for Primary Sclerosing Cholangitis (PSC), ankylosing spondylitis, and psoriasis
Histopathology	Blunting of intestinal villi, increased crypt lengthening, intraepithelial lymphocytes and lymphocytic infiltration of the lamina propria	Blunting of intestinal villi, increased crypt lengthening, increased mononuclear inflammatory cells and intraepithelial lymphocytes	Indistinguishable from EE	Active and chronic inflammation, variable distortion to villous architecture, metaplastic epithelial changes, possible granulomas
Serologic diagnosis	No established biochemical parameters for diagnosis	No established biochemical parameters for diagnosis	Antitissue transglutaminase antibody; antiendomysial antibody; antigliadin Ab (nonspecific)	No established biochemical parameters for diagnosis
Etiology	Recurrent environmental exposure to fecal contamination	Infectious	Genetic predisposition with presence of HLA-DQ2 or HLA-DQ8 initiated by exposure to gluten proteins; pathogenesis due to both T cell and antibody-mediated response	Unknown; genetic predisposition to certain forms of the disease, involving NOD2 and autophagy gene (ATG161L)
Available treatment	None	May respond to tetracycline therapy	Avoidance of gluten in diet	Corticosteroids and immunomodulating drugs
Refs	[2,66]	[2,62]	[33,67]	[63]

atrophy and increased lymphocytic infiltration. Clinically, both can result in malabsorption, malnutrition, and their sequelae. Etiologies of both EE and celiac sprue are believed to be due to continued exposure to an environmental insult, therefore it may be helpful to draw upon the celiac sprue literature to gain better understanding into the pathology of EE.
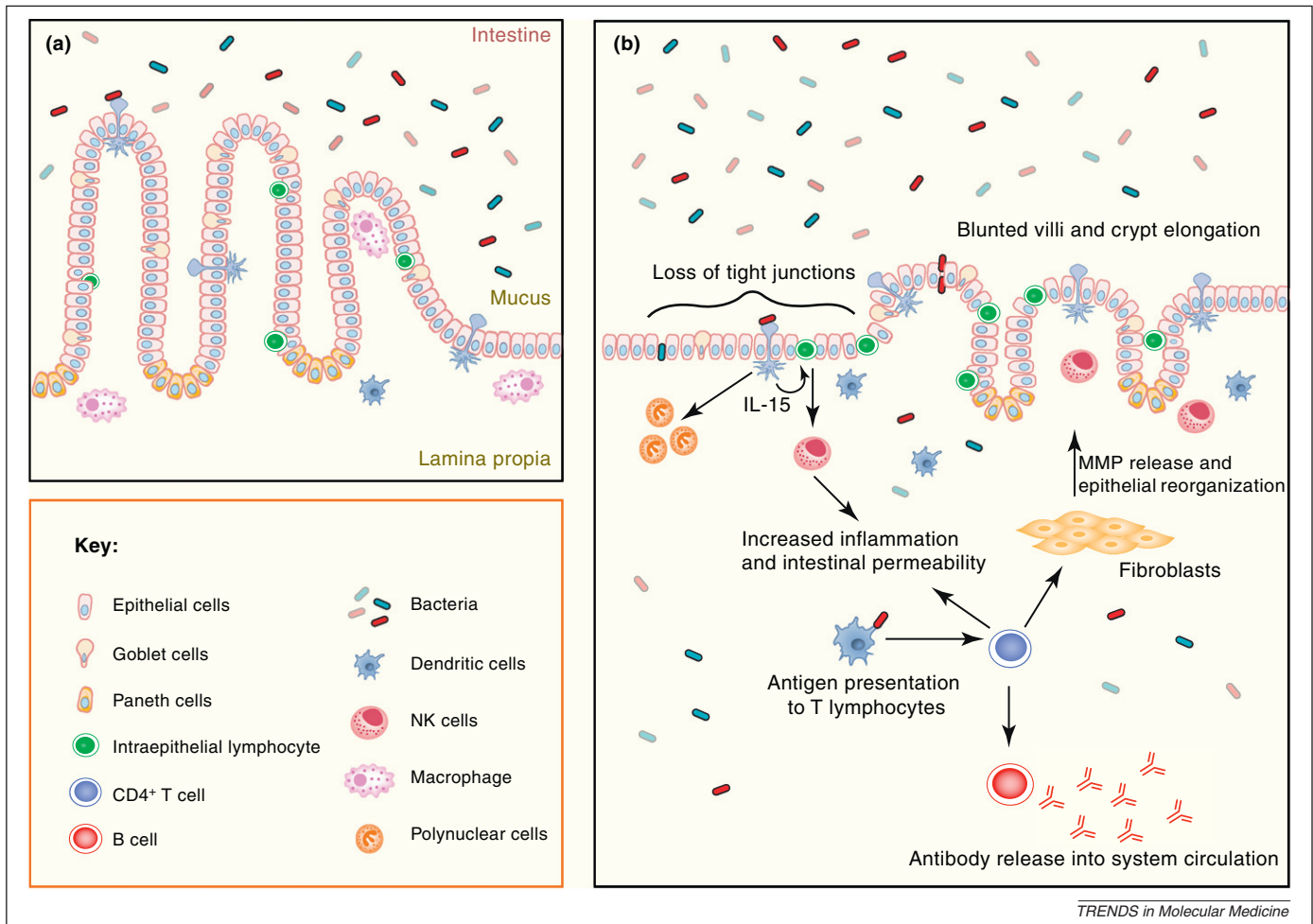
Celiac sprue is triggered by gluten exposure in genetically susceptible individuals. Almost all patients with the disease have HLA class II genes HLA-DQ2 (DQA1*05 and DQB1*02) or HLA-DQ8 (DQA1*03 and DQB1*0302), which encode molecules that present gluten peptides and are expressed on antigen presenting cells (APCs). The gluten-presenting APCs activate CD4$^+$ T cells, which subsequently release IFN-γ. This stimulates myofibroblast secretion of matrix metalloproteinases (MMPs) which induce matrix remodeling and villus atrophy. Additionally, release of mucosal interleukin-15 (IL-15) release in the small bowel activates intraepithelial lymphocytes to act as natural killer cells and results in epithelial damage and increased mucosal permeability. The increased permeability allows gluten peptides to be picked up by mucosal APCs and causes further T cell activation [33].

Adults in Zambia with EE have evidence of increased T cell activation as measured by CD69 and HLA-DR expression [34]. It is plausible that in EE there is constant T cell stimulation due to luminal exposure of enteric pathogens, ultimately resulting in pathogenic changes in the small bowel (Figures 2 and 3). Thus, EE may simply be the body's response to an overstimulating environment. A state of chronic inflammation could be a protective mechanism against constant infiltration by foreign pathogens.

Chronic immune stimulation

In theory, persistent damage to the mucosal barrier could permit translocation of foreign macromolecules into the circulation that may cause activation of a systemic immune response [35]. Lipopolysaccharide (LPS) is a component of the Gram-negative bacterial cell wall and the receptor for endotoxin, Toll-like receptor 4 (TLR4), is present on monocytes, macrophages, and dendritic cells. Measurement of serum LPS is used to assess bacterial translocation across the gut wall. Presence of bacterial ribosomal 16S RNA is also indicative of microbial translocation and has been shown to correlate with elevated LPS [36]. Naturally occurring antibodies to the LPS core oligosaccharide (EndoCab) are produced by B cells in a T cell-dependent manner after LPS exposure. In conditions involving acute bacterial translocation, such as with gastrointestinal surgery or sepsis, peripheral EndoCab levels are decreased because these antibodies are actively involved in clearing LPS from the circulation [37].

Bacterial translocation may play an important role in chronic immune activation and disease progression in patients with HIV/AIDS [38–40]. North American patients infected with HIV have higher levels of LPS in serum than HIV-negative controls, indicating gut barrier breakdown and immune dysfunction. Acutely infected HIV patients have low levels of LPS and low levels of EndoCab, whereas chronically infected HIV patients have higher levels of LPS but low levels of EndoCab, suggesting that in chronic disease circulating EndoCab levels are inadequate to clear serum LPS, and thus unable to prevent systemic immune activation that occurs in response to LPS [35].

Figure 3. Proposed pathogenesis associated with environmental enteropathy (EE). **(a)** In normal intestines, goblet cells secrete a layer of mucus that protects epithelial cells from exposure to bacteria. Phagocytosis by macrophages protects against bacteria entering the lamina propria while minimizing tissue injury. Tight junctions are undisrupted. Villi are long and finger-like and crypts are shallow in comparison, with a villus:crypt ratio of 3:1. Adapted, with permission, from [68]. **(b)** Proposed pathogenesis associated with EE. (i) Recurrent gastrointestinal infection results in disruption of the mucus layer and loss of barrier function. Luminal bacteria bind to Toll-like receptor (TLR) (not shown) on dendritic cells and intraepithelial lymphocytes (IELs) on the basolateral surface of epithelial cells. (ii) Pattern-recognition receptors and intracellular sensors (NOD2) in innate immune cells (NK cells, macrophages, and dendritic cells) induce recruitment of inflammatory cells, whereas dendritic cell sampling results in activation of T cells. (iii) There is loss of tight junctions and increased paracellular leakage as a result of IEL activation. (iv) This increased intestinal permeability leads to increased pathogenic infiltration into the lamina propria, resulting in increased production of proinflammatory cytokines and increased recruitment of immune cells. (v) This cycle of inflammation ultimately leads to remodeling of the intestinal architecture with increased IELs and lymphocytic infiltration in the lamina propria, as well as villus atrophy and crypt hyperplasia with a decreased villus:crypt ratio. Adapted, with permission, from [33].

What are the implications of bacterial translocation? In HIV patients, Brenchly *et al.* studied monocytes that were chronically stimulated by LPS *in vivo* and found these cells were hyporesponsive to further stimulation by LPS *in vitro*. Other studies have shown that elevated serum LPS levels correlate with decreased expression of IL-12 and IFN-α after stimulation with TLR agonists, suggesting that chronic immune stimulation by LPS results in a blunted innate immune response [39].

Among HIV-negative patients, although serum LPS levels do not differ significantly between inhabitants of North America versus those of East Africa, EndoCab levels are significantly higher among Africans, implicating chronic bacterial translocation in this population [41]. Presumably, sustained exposure to intestinal pathogens via ingestion leads to subsequent blunting of intestinal villi, increased intestinal permeability, microbial translocation, and chronic systemic exposure to LPS. Thus, among residents of the developing world, this exposure causes chronic immune stimulation and, ultimately, a dampened mucosal immune response. This downregulated mucosal immune response could be responsible for the poor immunogenicity of oral vaccines in the developing world.

Under normal conditions, the mucosal immune system can discriminate between commensal and pathogenic bacteria in the gut. Exposure to commensal bacteria causes downregulation of TLR expression and induces regulatory T cell differentiation. By contrast, dendritic cells in the lamina propria (on the basolateral side) recognize pathogenic bacteria and induce nuclear factor-κB (NF-κB) activation. Recently, it has been found in mice that segmented filamentous bacteria induce the appearance of Th17 cells in the lamina propria. Thus, the presence of commensal organisms may enhance mucosal immunity to pathogens [42]. Conversely, recent data show that commensal organisms may facilitate infection and reproduction of enteric viruses. *Kane et al.* demonstrated that mouse mammary tumor virus, a retrovirus, binds to bacterial LPS in the gut [43]. Once bound to LPS, the virus can interact with TLR4 (a natural LPS receptor) on leukocytes, causing release of

IL-10, an anti-inflammatory cytokine that suppresses the antiviral immune response. Kuss *et al.* reported that antibiotic depletion of gut microbiota in mice resulted in reduced susceptibility to enteric viruses, including polio virus [44]. They found that polio virus binds LPS and that exposure to commensal bacteria enhances infection of host cells, suggesting that viruses may exploit commensal flora. The interplay between commensal bacteria and enteric viruses could have important implications for the efficacy of oral vaccines against polio and rotavirus, both enteric viruses. We do not know how the microbiome varies in children with EE; presumably, constant exposure to fecal contamination could cause marked alterations in commensal flora with resulting variation in mucosal immunity and response to oral vaccines.

The presence of helminth infection may also have a large influence on the modulation of mucosal immunity. Colonization by intestinal worms has been shown to induce certain populations of T cells, influence production of regulatory cytokines, and suppress intestinal inflammation in animal models [45,46]. The eradication of helminth infection from higher income countries is thought to contribute to the increased incidence of atopic and inflammatory disorders to the extent that there are ongoing clinical trials investigating the benefits of 'helminth therapy' on a variety of inflammatory conditions, including Crohn's disease and ulcerative colitis [47]. The impact of helminth colonization on EE has not been well studied, but considering that prevalence of intestinal worm infection is as high as 50% in children in certain parts of the world, this is a crucial issue that needs further attention [48].

Noninvasive measures of EE

Because this condition is incompletely understood, a clear gold standard for diagnosis of EE is not yet defined. The most widely accepted surrogate marker for epithelial integrity is the lactulose:mannitol test, in which the subject is given an oral solution containing lactulose and mannitol. Mannitol is a monosaccharide and is completely absorbed by the small intestine; lactulose is a disaccharide and is only partially absorbed. Two hours after ingestion, urine is collected from the subject and chromatography is used to assess the levels of the sugars. Urine collected at this time point will reflect permeability and absorption of both the small intestine and colon, whereas urine collected later than 2 h may exclusively reflect colonic function. In patients with normal gut barrier, there should be a low level of lactulose compared with mannitol in the urine. In patients with leaky gut mucosa, the amount of absorbed lactulose, and thus lactulose in the urine, will be higher and result in a higher lactulose:mannitol ratio. This test has been validated as a screening test for celiac disease [49,50].

Studies from the Gambia have examined the association between increased intestinal permeability and growth faltering [51,52]. Lunn *et al.* reported that 43% of growth faltering in the first 15 months of life was associated with mucosal enteropathy by biopsy [23]. These same children had an abnormal lactulose:mannitol test when compared with British children of the same age. Interestingly, the abnormal lactulose:mannitol ratio was marked by both elevated lactulose and decreased mannitol, suggesting increased gut permeability as well as malabsorption.

Several serum and fecal biomarkers have been studied as indicators of intestinal inflammation (Table 2). Most of these markers have been developed for evaluation in patients with inflammatory bowel disease (IBD), and a few have been studied in populations at risk for EE. Fecal calprotectin, a cytoplasmic protein within neutrophils, has been correlated with active intestinal inflammation in patients with IBD [53,54]. As mentioned previously, Endo-Cab is a measure of exposure to LPS core antigen. Endo-Cab correlates with endotoxemia and increased intestinal permeability in patients with acute pancreatitis [55]. Among Gambian infants, elevated serum EndoCab is

Table 2. Biomarkers that may be informative in the study of environmental enteropathy[a]

Biomarker	Description	Refs
Zonulin	Human homolog of zonula occludens toxin from *V. cholerae* that modulates tight junctions. In celiac sprue, gliadin induces zonulin release by binding to the CXCR3 receptor causing cytoskeletal rearrangement and ZO-1 reorganization, leading to increased intestinal permeability.	[64,65,69]
Fecal calprotectin	A complex of S100A8/S100A9, this protein accounts for 60% of neutrophilic cytosol. It has been used to predict relapse in IBD patients.	[53,54,70,71]
Fecal S100A12	Marker of neutrophil activation, elevated in IBD and bacterial enteritis, but not viral enteritis.	[71–73]
EndoCab[a]	Antibody to bacterial cell wall LPS core oligosaccharide. EndoCab has is the only biomarker that has been studied in EE.	[52,69]
Granulocyte macrophage colony stimulating factor autoantibody	Reported to be associated with increased intestinal permeability as measured by the lactulose:mannitol test in patients with Crohn's disease.	[74]
Neopterin	Protein produced by monocytes and macrophages after stimulation with IFN-γ. Marker of TH1 cell activation.	[75]
Myeloperoxidase	A lysosomal protein in neutrophilic granulocytes. Found to be elevated in mouse model of IBD.	[76]
Citrulline	Amino acid produced by enterocytes. Proposed as measure of gross enterocyte mass.	[56,57]
Reg 1A, Reg 1B	Gene family involved in tissue regeneration. Protein found in colonic crypt epithelial cells with antiapoptotic and pro-proliferative properties. Upregulated during amebiasis and may play a protective role against parasite-induced apoptosis. Also being studied for diagnosis and monitoring of celiac disease.	[77,78]
Lactoferrin	Iron-binding glycoprotein found in secondary granules of leukocytes. Used as a surrogate for fecal leukocytes and a measure of intestinal inflammation.	[79–81]

[a]EndoCab is the only biomarker that has been evaluated in EE, the rest have been studied in other inflammatory bowel conditions and may be potentially useful in understanding EE.

Box 1. Zonulin

Zonula occludens toxin (Zot), produced by *Vibrio cholerae*, is a recently identified molecule that opens tight junctions in the intestine. Fasana *et al.* identified a human analog of Zot, which they named Zonulin. Zonulin and Zot act on the same intestinal epithelial receptor. Fasana *et al.* propose that just as Zot can reversibly open intestinal tight junctions, zonulin (activated by intestinal flora imbalance or other immunogenic stimulation) causes an intracellular signaling cascade that results in actin filament rearrangement and opening of tight junctions. Increased intestinal permeability and disruption of intestinal tight junctions is an important aspect of the pathogenesis of EE. As zonulin is responsible for paracellular leakage, it may play a significant role in development of EE. Once tight junctions are opened, antigens can pass into the lamina propria and stimulate an immune response. Intestinal expression of zonulin and serum zonulin IgA are elevated in celiac patients with active disease, further supporting the hypothesis that zonulin modulates tight junction permeability. It has been proposed that by mediating immune exposure to luminal antigens, zonulin may be involved in the development of autoimmune diseases, such as diabetes and celiac sprue [64,65].

Box 2. Outstanding questions

- How prevalent is EE?
- What causes EE? Does it have an infectious or toxic etiology?
- What indicators (biomarkers) can we use to identify children at risk for development of EE?
- Are there nutritional interventions that can facilitate healing of the gut?
- How is the immune system different in children with EE?
- How can we design vaccines that will elicit a response in children with EE?
- What is the impact of EE in adults?
- Is there a genetic predisposition?
- Does EE in a mother result in malnutrition in her child?
- How do helminth infections influence the development of EE?
- What is the role of commensal versus pathogenic microbes in development of EE?
- Can EE be transferred from one person to the next by fecal contamination?
- Can one develop immunity to EE?

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associated with poor height and weight growth and increased intestinal permeability, as measured by the lactulose:mannitol test [52].

Other studies have focused on metabolic byproducts of enterocytes or regulators of tight junctions (Box 1, Table 2). Citrulline is an amino acid produced by intestinal epithelial cells, and levels of citrulline were lower in patients with villous atrophy from IBD when compared with controls. Additionally, citrulline levels correlated with length of remnant small bowel in patients with short gut syndrome. Thus, citrulline is being proposed as a marker of gross enterocyte mass and may be useful for following patients with villous atrophy [56]. Citrulline levels did not correlate with the D-xylose absorption test in patients with HIV-related enteropathy [57].

Concluding remarks and future perspectives

Children and adults who inhabit a fecally contaminated environment are at risk for the development of EE, which is marked by alterations in intestinal architecture, increased intestinal inflammation and permeability, deranged gut immune response, and growth faltering. These changes have important implications for dealing with maladies that affect the developing world, including malnutrition and control of infectious diseases.

Some studies have investigated potential treatments to ameliorate the detrimental effects of EE on nutritional status. Lima *et al.* studied dietary supplementation with glutamine in underweight children aged 2 to 60 months [58]. Although there was no difference in weight gain among the placebo and treatment groups, the treatment group did show a significant improvement in lactulose:mannitol ratio, indicating improved intestinal barrier function and suggesting that glutamine supplementation may promote enterocyte proliferation and intestinal repair. Sullivan *et al.* found significant histological improvement in jejunal biopsies of children who underwent a 4-week feeding program, suggesting that improved nutrition can foster mucosal repair independent of effect on nutritional status [59]. Perhaps both of these studies demonstrate that although enterocyte repair may occur acutely with specific nutritional interventions, the 'catch up' in growth and increase in overall body mass may take longer to appreciate and, therefore, long-term follow-up of these children is necessary.

A few studies have shown a correlation between enteropathy and exposure to specific pathogens, including hookworm and *Citrobacter rodentium* [12,21,60]. In Malawi, treatment of children with the antibiotic rifaximin failed to improve gut barrier function [29]. However, if a clear relationship between specific pathogens and EE can be established, then antimicrobials or vaccines could play a role in preventing EE development.

Promotion of basic sanitation as a means to reduce fecal–oral contamination is necessary to reduce the impact of EE in the developing world [61,62] (http://www.unicef.org/wash/index_43084.html). Ensuring universal access to potable water and plumbing would render the sequelae of EE obsolete. However, until the political will, stability, and resources come to fruition to make this feasible, it is imperative that we better elucidate the complexities of EE.

This subclinical condition may have a larger impact on worldwide disability adjusted life years (DALYs) than does any specific pathogen, particularly because EE increases susceptibility to other morbidities. Before treatments can be devised, better methods of diagnosing EE are needed (Box 2). Noninvasive diagnostic parameters are especially important as endoscopy is impractical at the public health scale of intervention that is likely to be required for an ailment affecting up to 40% of the world's children. Finally, treatment and prevention will require filling the 'knowledge gap' of pathogenesis, for which *in vivo* animal models will play an important role [63]. It is this type of progressive approach that will be required to help us better understand and treat EE, and ultimately address undernutrition, growth faltering, and vaccine efficacy to promote a more healthy and productive global population.

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