Chronic Enteropathy: Molecular Basis

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Abstract

Major advances in the understanding of the pathophysiology of chronic and intractable diarrhea of infancy allow a new conceptual view of this heterogeneous group of disorders. Two major types of chronic ‘intractable’ enteropathies can be distinguished. (1) Congenital-constitutive forms are characterized by intrinsic enterocyte defects. To date three different types have been identified on a morphological-histological basis: microvillous inclusion disease, intestinal epithelial dysplasia and the so-called syndromatic diarrhea. These disorders are characterized by a high degree of consanguinity in the affected families. An autosomal recessive transmission was suggested, but the genes involved have not yet been identified. (2) Immunoinflammatory enteropathies starting within the first months of life, such as autoimmune enteropathies, can share the clinical picture of constitutive enteropathies; however, most often there are associated extraintestinal symptoms. A loss of function mutation in the \textit{FOXP3} gene located on Xp11.23-q13.3 causes a distinct X-linked form of severe autoimmune enteropathy. The functional consequences of \textit{FOXP3} mutations which point to a defect of regulatory T cells are currently under investigation. With the increasing understanding of the molecular basis of these distinct diarrheal disorders, new treatment strategies will emerge within the next years, giving new hope to these critically ill children and their families.

Introduction

Over the past years, major advances in the understanding of the pathophysiology of chronic and intractable diarrhea of infancy have been made allowing a new conceptual view of this heterogeneous group of gastrointestinal (GI) diseases. Today, according to the mode of onset, the clinical presentation, the presence of systemic symptoms or of immunoinflammatory reactions different classifications of chronic enteropathies can be proposed (fig. 1). In 1968, the descriptive term ‘intractable diarrhea of infancy’ was
introduced by Avery et al. [1] for children who within the first 3 months of life presented with severe and abundant noninfectious diarrhea, which persisted despite bowel rest. Diarrhea becomes most often life threatening, and patients are only stabilized by the use of total parenteral nutrition. With the rapid improvement of techniques of artificial nutrition in the late 1970s these critically ill babies and infants, formerly condemned to die within the first days or weeks after the onset of symptoms, survived for the first time. Subsequently, distinct clinical pictures emerged and new pathologies were discovered, such as microvillous atrophy (MVA)/microvillous inclusion disease (MVID) or epithelial dysplasia/tufting enteropathy (TE).

In the present review, we suggest a classification of this heterogeneous group of chronic enteropathies according to the age of onset (congenital or within the first weeks/months of life) as well as according to the presence or absence of inflammatory or immune reaction. An important differential diagnostic criterion will be the persistence of diarrhea on complete bowel rest. Besides the clinical picture and presentation, we will discuss in the following the molecular basis, as far as known, for the different constitutive and immunologic GI pathologies. Pathologies causing electrolyte secretory diarrhea, malabsorption or malassimilation will not be discussed in this review. Infectious and postinfectious syndrome and other self-limiting pathologies causing protracted but not persistent diarrhea are also not part of this review, which is

**Fig. 1.** Differential diagnostic approach for a child presenting with severe diarrhea with onset within the first days or weeks of life. CDG = Congenital disorders of glycosylation; dis. = disorder; IBD = inflammatory bowel disorder; Sd. = syndrome.
limited to chronic intestinal disorders defined by persistent or recurrent episodes of diarrhea over at least 3 weeks.

**Congenital- Constitutive Diarrheal Disorders**

*Clinical Considerations*

The typical clinical picture is abundant watery, sometimes mucous diarrhea starting within the first hours or days of life [2, 3]. Diarrhea can be so important that within a few hours a rapidly life-threatening situation develops due to massive dehydration and metabolic acidosis. The neonatal course (presence or absence of polyhydramnios) presents essential information contributing towards the differential diagnosis of a secretory (chloride or sodium) diarrheal disorder if polyhydramnios is present or a constitutive enterocyte disorder (absence of polyhydramnios). A major step in the differential diagnosis of congenital or early onset watery diarrhea is the response to complete bowel rest, as well as the determination of fecal and serum electrolytes. If diarrhea stops on total bowel rest it is most likely secondary to malabsorption or malassimilation (fig. 1), whereas, once defects in electrolyte transports are eliminated, massive and persistent diarrhea indicates a constitutive enterocyte disorder, such as MVA/MVID or intestinal epithelial dysplasia (IED)/TE or rarely syndromatric or phenotypic diarrhea.

In MVA/MVID, severe watery diarrhea typically starts within the first days of life [2–4]. This diarrhea becomes so abundant that within 24 h children can lose up to 30% of their body weight, resulting in profound metabolic acidosis and severe dehydration. MVID is most often severe and life-threatening. Accurate quantification of stool volumes reveals 150 to over 300 ml/kg/day with a high sodium content (over 100 mmol/l). Complete and prolonged bowel rest makes it possible to reduce stool volume moderately, but it nearly always remains above 150 ml/kg/day [4]. Typically, no additional clinical signs are associated with MVID; in particular, there are no malformations or involvement of other organs such as liver or kidney. However, a small number of children have massive pruritus secondary to elevated concentrations of biliary acids in the blood. Also, proximal renal tubular dysfunction was observed in some children with MVID/MVA. At clinical examination, no specific findings can be detected except enormous abdominal distension with fluid-filled intestinal and colonic loops. All children with congenital MVID urgently require total parental nutrition, which often causes rapidly evolving cholestasis and liver disease. A detailed multicenter analysis of 23 patients with MVID [4] distinguished two different forms and presentations of MVID on a clinical and morphological basis: the most frequent form, that is congenital early-onset MVID (starting within the first days of life), and some rare cases, that is late-onset MVID (with first symptoms appearing after 2 or 3 months of life). Intestinal failure is definitive in all children with early-onset
MVID and most with late-onset MVID. Some rare children with late-onset MVID were described who had a somewhat less severe course. In general, children with MVID are potential candidates for small bowel transplantation [5].

In IED/TE, massive watery diarrhea develops within the first days after birth in a way similar to MVA/MVID. Stool volumes are highly variable (100–200 ml/kg body weight/day) with stool electrolyte concentrations of sodium of 100–140 mmol/l [6, 7]. All children are highly dependent on parenteral nutrition. There is no past history of hydramnios or other antenatal or neonatal particularities. It is striking that in our experience most children with IED/TE have consanguineous parents and/or affected siblings and some of them died during the first months of life with severe diarrhea of unknown origin. Upon clinical examination, no malformations are observed; however, some children have somewhat rigid hair and a subgroup of patient shows clinical signs of photophobia. Distinct ophthalmologic examination reveals the presence of superficial keratitis in these children. The degree of severity of IED/TE is more variable compared to MVA/MVID with most children developing a severe course indicating lifelong and definitive intestinal failure. However, some patients have less severe disease and may acquire a certain degree of intestinal autonomy making it possible to reduce parenteral nutrition to three to four perfusions a week.

A distinct clinical group is the so-called phenotypic or syndromatic diarrhea. This disorder, first described by the group of Goulet [8] in Paris, is characterized by small for gestational age babies with severe persistent watery diarrhea associated with an abnormal phenotype, including facial dysmorphism, hypertelorism, and woolly, easily removable hair with trichorrhexis nodosa. In addition, immunological abnormalities were observed in all patients in the form of defective antibody responses despite normal serum immunoglobulin levels, and defective antigen-specific skin tests despite positive proliferative responses in vitro. The majority of patients followed in our center were the progeny of consanguineous marriages. The clinical course of these children is variable. Most of the children died in the past, but some of the survivors acquired a certain degree of intestinal autonomy.

Molecular Considerations

There is strong evidence that all these congenital/early-onset structural enterocyte disorders have a distinct genetic basis [2]. The clinical features suggest an autosomal recessive transmission. Since the gene(s) involved are not yet identified for these congenital inherited autosomal recessive diseases, no genetic or prenatal diagnosis is possible. The majority of children with MVA/MVID are of Turkish origin; however, we follow up a couple of children with MVA/MVID (most with several affected siblings) of Caucasian origin. Patients with IED/TE are most often of Arab origin, that is Middle East including Turkey or North Africa. The prevalence in Malta Island, in the Mediterranean Sea, seems high but the phenotype might be milder. Once again, a small number of IED patients
followed up in our center are also of Caucasian origin without any evidence of consanguinity, indicating sporadic de novo mutations.

The precise molecular basis of MVA/MVID is still unknown. There is evidence of a major defect in membrane trafficking in intestinal epithelial cells (IEC), probably secondary to an altered structure of the cytoskeleton [9]. This disorder is morphologically characterized by the occurrence of so-called microvillous inclusions at the apical pole of enterocytes along with absent or atrophic microvilli on mature enterocytes easily visible on electron-microscopic analyses (fig. 2). The observation of morphologically normal microvilli on immature crypt cells in children with MVID indicates that the microvillous changes seen in differentiating and mature cells are of secondary nature or they are a consequence of yet unidentified events within the cell, such as membrane recycling or mechanisms controlling endo- or exocytosis [10, 11]. However, analysis of the membrane targeting of the disaccharidase sucrase-isomaltase revealed no abnormalities of the direct or indirect constitutive pathway. Another hypothesis suggesting a defect in the autophagocytosis pathway was proposed to explain the morphological and functional abnormalities in MVID. Very recent observations indicate a selective defect in glycoprotein exocytosis in patients with MVID [12]. These glycoproteins accumulate within the apical pole of enterocytes and form the characteristic secretory granules, easily observed on PAS staining on duodenal sections of patients with MVA/MVID (fig. 3).

In contrast to MVA/MVID which is most likely the consequence of an IEC membrane trafficking defect, IED/TE results from a regulatory defect of epithelial mesenchymal cell interactions, which are instrumental in intestinal development and differentiation. Alterations suggestive of abnormal cell-cell and cell-matrix interactions were seen in patients with IED without any evidence of abnormalities in epithelial cell polarization and proliferation [13].

Fig. 2. MVA/MVID: electron-microscopic picture showing typical atrophic microvilli on a mature IEC of the duodenum as well as microvillous inclusions (asterisk).
These alterations included abnormal distribution of the adhesion molecule $\alpha_2\beta_1$ integrin along the crypt-villous axis. The $\alpha_2\beta_1$ integrin is involved in the interaction of epithelial cells to various basement membrane components, such as laminin and collagen. To date, the pathophysiological mechanisms resulting in an increased immunohistochemical expression of desmoglein and the ultrastructural changes of desmosomes are unclear [13]. A major morphological feature of IED is the occurrence of epithelial tufts at the villi, along with crypt branching (fig. 4). Tufts correspond to rounded epithelial cells at the villous tips that are no longer in contact with the basement membrane. It can be speculated that a defect of normal enterocyte apoptosis at the end of their lifespan or an altered cell-cell contact is responsible for this effect. The primary or secondary nature of the formation of tufts remains to be determined. Mice with a disrupted gene encoding the transcription factor Elf3 display morphologic features resembling IED [14]. In this model, an abnormal morphogenesis of the villi was observed while progenitor crypt cells appear normal. IEC are characterized by a low transforming growth factor-$\beta$ type 2 receptor expression, which is implicated in the differentiation of immature IEC.

Fig. 3. MVA/MVID: high-power magnification of a duodenal section after periodic acid-Schiff (PAS) staining or anti-CD10 immunohistochemistry. As shown on both panels compared to normal controls, in MVA an enlarged intracytoplasmic band (arrow) at the apical pole of enterocytes can be seen along with an atrophic band instead of the normally well-defined small line representing the brush border (asterisk).
The molecular basis of phenotypic syndromatic diarrhea is completely unknown. The diagnosis of this syndrome is made by the association of different clinical symptoms. Characteristic or pathognomic histological alterations of the intestinal mucosa were not observed. Marked (subtotal to total) villous atrophy on duodenal biopsies along with a variable inflammatory infiltrate of the lamina propriety is seen in the majority of patients. In a recent study, we did not observe an increased expression of HLA molecules on IEC. To date, the genetic basis of this disorder remains unclear.

**Immunological Inflammatory Diarrheal Disorders**

*Clinical Considerations*

The neonatal course is completely normal. Onset of intestinal symptoms is within the first months of life and it is rarely isolated, in contrast to congenital early onset enterocyte disorders. Diarrhea is often bloody and most often systemic inflammatory symptoms exist, i.e. fever, elevated inflammatory markers in blood and stools. Changes in the mode of alimentation, such as withdrawal of breastfeeding, the introduction of cow's milk proteins, but sometimes even a simple viral infection or a vaccination, may precede the onset of GI symptoms. A main characteristic of inflammatory or autoimmune
enteropathy (AIE) is a protein-losing enteropathy, seen in low serum albumin levels and a markedly enhanced α₁-antitrypsin clearance [15]. Complete bowel rest may improve diarrhea; however, in severe courses diarrhea often persists. This situation represents a diagnostic challenge, since the clinical pictures of children suffering from an early-onset enterocyte intrinsic defect and children with an immunological disorder overlap considerably. We recently evaluated the diagnostic value of measuring inflammatory markers in the stools to distinguish immunoinflammatory diarrheal GI disorders from constitutive enterocyte disorders in infants presenting with severe diarrhea within the first year of life [16]. The simple fecal test allowed with a specificity close to 100% to eliminate an inflammatory affection if fecal calprotectin levels were within the normal range. Contrary to autoimmune inflammatory enteropathies, allergic reactions always respond to and theoretically are healed on complete bowel rest. However, on re-alimentation, the symptoms reappear immediately if the causative antigen is re-introduced.

If a child presents with an AIE, the onset is often within the first 3 or 4 months in the form of severe diarrhea which can be bloody [15]. The majority of boys with AIE present in addition with severe atopic skin disease, hematological abnormalities along with endocrinopathy, such as insulin-dependent diabetes mellitus or thyroiditis. This association was described as IPEX (immune dysregulation, polyendocrinopathy, autoimmune enteropathy, X-linked) syndrome [15, 17, 18]. It is interesting to note that boys with IPEX also show severe immuneallergic symptoms with a strong Th2 response and hyper-IgE syndrome having some similarities with extremely severe food allergy. Isolated or oligosymptomatic forms of severe AIE exist in both, boys and girls. Prior to the onset of AIE/IPEX, these children develop completely normal, and no antenatal or neonatal particularities exist [19]. It is important to stress that the family history is most often positive for various autoimmune diseases. This indicates a particular genetic background, pointing to disease susceptibility genes. Another particularity is the exclusive occurrence of a subtype of AIE in boys, which indicates an X-linked mode of transmission. Inflammatory bowel disease (Crohn’s disease or ulcerative colitis) is extremely rare in this age group; however, it has to be considered as a differential diagnosis.

The onset of food allergy-induced enteropathy/colitis is less severe compared to AIE/IPEX. However, severe forms exist as – in extreme cases – food protein-induced enterocolitis syndrome. Besides the presentation as upper GI disease, the onset is in the form of diarrhea and rectal bleeding combined with failure to thrive [20]. Often atopic skin and pulmonary symptoms are associated with the GI symptoms. The family history for atopy is often positive, and several affected children within one family are not exceptional. Once more this points to a particular genetic background and potential susceptibility genes. Systemic inflammatory symptoms are rare; however, inflammatory signs at the intestinal mucosal level are typical for this disease entity, as is the

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occurrence of protein-losing enteropathy. Biological exams help to make the
diagnosis if IgE levels are enhanced with specific RASTs. However, food aller-
gies are frequently related to delayed type 4 hypersensitivity reactions and
show no biological or immunological abnormalities. Skin patch tests most
often are not helpful in the diagnosis. Food allergy can share the clinical pic-
ture of celiac disease, which has to be ruled out combining serological and if
necessary histological evaluations.

Molecular Considerations

Genetic mapping studies of several families with boys suffering from AIE
made it possible to identify a disease causing mutation in a gene located on
the X chromosome Xp11.23-q13.3 [21, 22]. This gene was named FOXP3 and
encodes a 48-kD protein of the forkhead (FKH)/winged helix transcription
factor family, named scurfin. Scurfin is predominantly expressed in
CD4+/CD25+ T cells with regulatory functions. Experimental data suggest
that scurfin is implicated in the regulation and suppression of T cell activation
[23]. In a recent review of boys with IPEX [17], a loss of function mutations
was reported within the coding region of FOXP3, whereas in one family, a
mutation in the 3'-untranslated region of FOXP3 was observed. Two addi-
tional patients with typical clinical symptoms of IPEX did not show any muta-
tions within the coding regions of FOXP3, suggesting that regulatory or
conditional mutations may occur outside FOXP3, such as described by
Bennett et al. [24] in the polyadenylation signal following the final coding
exon of FOXP3. This may result in a decreased FOXP3 messenger ribonu-
cleic acid (RNA) expression, probably owing to nonspecific degradation of
aberrant RNA. Similar to loss of function mutations in FOXP3, this results in
a decreased or completely suppressed scurfin expression causing an altered
biological function. In addition, it is possible that other forms of IPEX or AIE
exist that are not related to mutations in the FOXP3 gene and that are trans-
mitted either with an X-chromosomal or an autosomic trait. Owen et al. [25]
reported on two families with several members presenting with clinical IPEX.
In one family a novel FOXP3 mutation was identified with a single base dele-
tion at codon 76 of exon 2, resulting in a frameshift mutation, whereas in the
second family the FOXP3 locus was excluded by recombination and muta-
tional analysis was negative. Since one girl was affected in this family, one can
speculate about an autosomal locus.

The structure of the FOXP3 protein, scurfin, suggests that it has deoxyri-
bonucleic acid (DNA) binding activity and may serve as nuclear transcription
factor. Schubert et al. [23] demonstrated that scurfin acts as a repressor of
transcription and regulator of T cell activation. Intact scurfin represses
transcription of a reporter containing a multimeric FKH-binding site. Such
FKH-binding sites are located adjacent to nuclear factor of activated T cells
(NFAT), regulatory sites in various cytokine promoters such as IL-2, or
granulocyte-macrophage colony-stimulating factor enhancer. Therefore, intact
scurfy seems to be capable of directly repressing NFAT-mediated transcription of the IL-2 gene in CD4+ T cells upon activation [23]. In addition, scurfin seems to play an important role in thymic maturation of regulatory T cells. Nonfunctional (due to maturation or structural defects) or absent regulatory T cells cause highly overactivated T cell reactions, since the natural inhibitor fails. A tremendous T cell-mediated inflammatory reaction within the GI tract causes severe epithelial cell and mucosal destruction without villous atrophy and erosions or ulcerations (fig. 5).

In general, allergic reactions can occur as acute type 1 IgE-mediated or as delayed type 4 IgE-independent and Th2-cell-mediated reactions. Food allergy is often IgE independent and we are only beginning to understand some of the molecular events of food allergy-induced enteropathy. Allergic reactions of the GI tract are particularly intriguing, since, normally, upon oral ingestion of a potential allergen an anti-inflammatory TGF-β-mediated response develops, a mechanism described as oral tolerance. In contrast, parenteral administration of the same antigen provokes a marked T cell-driven inflammatory response. Therefore, it was suggested that a defect in or a loss of anti-inflammatory control mechanisms might be a key step in the development of food allergy [20]. The number of TGF-β-producing T cells, presumably regulatory T cells, was reported to be reduced in the duodenal mucosa of children with food allergy [26]. That regulatory T cells play an important role

Fig. 5. AIE: complete villous atrophy and a major inflammatory infiltrate of mononuclear and some polynuclear cells within the lamina propria of the duodenum.
in food allergy is largely supported by the observation of allergic symptoms including food allergy in patients with the IPEX syndrome. The commensal intestinal flora was proposed as an environmental factor involved in the stimulation of the mucosal immune system. It was suggested that changes in the composition of the intestinal microflora early in life may result in an insufficient stimulation of innate immune response contributing to impaired development of regulatory T cells [27].

Additional factors, potentially affecting the function of the intestinal barrier, also seem to play a key role in the onset of food allergy-induced enteropathy. Increased intestinal permeability and increased uptake of allergens were described in children with cow’s milk protein allergy [20]. The molecular basis of the pathological transport of allergens via intact enterocytes was recently revealed [28] with an IL-4-induced upregulation of CD23, a low-affinity IgE type II transmembrane glycoprotein, on IEC. This causes an exaggerated IgE-CD23-mediated endosomal uptake of allergens, which are subsequently released at the basolateral side of enterocytes. Eosinophils, basophils, monocytes, mast and T cells recruited to the lamina propria release upon interaction with this allergen large amounts of prostaglandins, leukotrienes, histamine, tryptase and various cytokines. In the resulting acute phase, inflammatory reactions provoke GI (vomiting, diarrhea, bleeding) and systemic symptoms (fever, anaphylaxis, etc.). Th2 cytokines, such as IL-4, IL-5 and IL-13, are responsible for an Ig switch to IgE, chemoattraction and accumulation of inflammatory cells such as eosinophils and basophils and T cells within the intestinal mucosa. On histological examination, villous atrophy along with an intense polymorph inflammatory infiltrate of the lamina propria and an enhanced number of intraepithelial T cells is seen in food-allergic enteropathy. Theoretically, food allergy is cured by an avoidance diet; however, this is not always so easy and a high degree of morbidity can be caused by this disorder.

**Conclusions**

The clinical stereotype presentation of abundant watery, sometimes mucous diarrhea within the first days or weeks of life can be related to a large variety of different diseases. According to the clinical characteristics, date of onset, persistence on complete bowel rest, fecal electrolyte concentration, presence of inflammatory elements or a protein-losing enteropathy, the diagnosis can be rapidly made. For early-onset constitutive enterocyte disorders a genetic background is suspected; however, no candidate genes have yet been identified. These constitutive enterocyte disorders can be related to intrinsic enterocyte defects or a defective anchoring mechanism of enterocytes. No causal or curative therapy is available; therefore, most of these children are on long-term parenteral nutrition or treated by intestinal transplantation.
Immunoallergic, inflammatory or autoimmune conditions include a second clearly distinct group of chronic enteropathies with onset early in life. Major advance were made in the role and understanding of the retrocontrol via reg-
ulatory T cells within the intestinal mucosa indicating new pathogenetic and therapeutic strategies for these disorders.

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Discussion

**Dr. Milla:** I would not be quite so pessimistic about bone marrow transplantation for autoimmune enteropathy. We have now transplanted something like 10 patients in the UK; the first 5 rather like yours were horrendous, and they took a long time to recover. With rather better conditioning regimes things got somewhat easier and the last 3 or 4 have come through relatively well. So I am quite clear in my mind this is the way to treat them because immunosuppression is extremely toxic if you want to control a severe disease like IPEX. I don’t believe we are any closer to understanding microvillous atrophy or microvillous inclusion disease, call it what you will. Whether there are really inclusions or whether it is just the apical surface thrown up into all sorts of convolutions so when you section it you appear to have an inclusion, I don’t know. But what I would like you to speculate on is why do the majority of these patients die from hepatic failure?

**Dr. Ruemmele:** I am aware of your good data on bone marrow transplantation in IPEX patients. After multiple discussions with our immunologists, we feel that bone marrow transplantation for IPEX is perhaps most difficult, but it is hopefully the future for these patients. To your question on microvillous atrophy or microvillous inclusion disease, call it what you will. Whether there are really inclusions or whether it is just the apical surface thrown up into all sorts of convolutions so when you section it you appear to have an inclusion, I don’t know. But what I would like you to speculate on is why do the majority of these patients die from hepatic failure?

**Dr. Imanzadeh:** What are the definition and scientific criteria for intestinal failure?

**Dr. Ruemmele:** Intestinal failure can be defined as the critical reduction of functional gut mass below the minimal amount necessary for adequate digestion and absorption to satisfy body nutrient and fluid requirements, resulting in dependency on
parenteral nutrition [1]. I am not sure if there is a real validated definition. In our hands it is a dependency on parenteral nutrition.

**Dr. Davidson:** You mentioned that the incidence of microvillous inclusion disease seems to be restricted to certain population groups, but in my experience that may not be the case. The first families that I saw with this condition were in Melbourne, Australia and they were of European Caucasian origin with two children dying in each family. Similarly in Toronto there were several Caucasian families [2]. I think this condition is not restricted to particular population groups even though it may be more common in certain groups such as the Navajo American Indian population.

**Dr. Ruemmele:** I completely agree with you; most of the patients we saw are of Turkish or Arab origin, but we have also French or Italian families. There are different phenotypes of the disease and, perhaps I have to add this to the question of Dr. Milla, some children have very itchy pruritus indicating perhaps a hepatic involvement for some of them. We never saw a particular genetic background.

**Dr. Davidson:** With regard to hepatic failure, many of these children are on long-term parenteral nutrition and this may be a contributing factor.

**Dr. Milla:** We are very skilled at parenteral nutrition; we know how to stop patients getting hepatic failure. But it is almost as if they are following a time table once you have kept them alive long enough, and now we have quite a large group of these patients. They almost inevitably develop hepatic failure, especially if they are those who present in the first week or so of life. It is something about their disease; I just don't believe it is parenteral nutrition, it is an acquired thing.

**Dr. Saavedra:** Our experience at John Hopkins is rather limited; most people have anecdotal cases more than series and hopefully we can put a large series together. We have had children with microvillous inclusion disease, a total of 3 for more than a year, who have been on parenteral nutrition without any problem, and in one case up to 3 years without liver disease. I don't know if it may or may not be geographic. The one child with liver disease had on biopsy the features of what we would consider parenteral nutrition-associated cholestasis. Intestinal failure, or what we prefer calling intestinal dysfunction, which is persistent to the point that the patient requires transplantation, we typically call failure, so there are varying degrees of dysfunction as we would call them. The dysfunction persists to the point of getting liver disease or occasionally running of central line sites, which is the other excuse for transplantation, at the point that we would typically call it failure, otherwise it is a prolonged intestinal dysfunction. You did discuss the digestive potentials of chronic illnesses that present early in life, certainly the absorptive group. But there is this large group of children with motility problems and digestive absorptive motility and barrier. Would you comment briefly on where we are with myopathies and neuropathies at the molecular level if you had to compare them to where we are with microvillous inclusion.

**Dr. Ruemmele:** When I presented my data I started with diarrhea and I did it this way because enteropathy is a large topic and it is very difficult to cover all aspects. If you start with the general problem and definition of intestinal failure this topic would be completely different, you have to talk about short bowel syndrome which is partially caused by intestinal failure, motility disorders and constitutive enterocyte disorders that I presented today. On intestinal failure the pathologies must be grouped differently but this is not really the point I wanted to make. A lot is known about motility disorders, such as Hirschsprung disease, there are a lot of hypotheses. Regarding mitochondrial disorders or neurological-muscular disorders, only very few data exist. I am not really able to answer all aspects of your question.

**Dr. Lack:** Your speculation that this might be a good model for food allergy is fascinating. One of the main differences that I see though is that this is a generic regulatory T cell defect, so it presumably would be evidence of multiple food allergies,
whereas in food allergy there are 1 or 2 or 3 food allergens. First is the polyconal IgE elevated; do you actually find antigen-specific IgE on RAST testing, and do these children have positive skin prick tests?

Dr. Ruemmele: These children have the highest IgE levels you ever saw. In our institution, we normally see IgE levels lower than 40 kU during the first year of life. However IPEX boys often have several thousands, up to 70,000 kU, and if you perform RAST testing they are all positive. We did not go further to test if these IgE levels are really functional. It is not very surprising when you have 10,000 times the normal level, you find cross-reaction.

Dr. Lack: Do they have immediate hypersensitivity when you give them foods?

Dr. Ruemmele: They show both. They have immediate and also delayed symptoms. They never have pulmonary symptoms. In our institution we followed 12 patients and none of them developed pulmonary symptoms. I am not sure if this is part of this entity. IPEX boys never wheeze; they only have skin and GI symptoms, both immediate and delayed type.

Dr. S. Koletzko: Did you or anybody in the audience also see mild cases of IPEX syndrome or are they always severe? If we intervene with bone marrow transplantation it should be done early before the toxic effect of the therapy comes on. Or is there any mild form?

Dr. Ruemmele: There are very few data in the literature. Powell et al. [3] described a family with 18 male members who have a very mild form. They have survived until adult age and have minimal immunosuppression. The genetic defect in this family does not affect the FOXP3 gene but it does affect the polyacids just before the gene. It is incomplete regulation of the gene. With Michael Lentze we had a patient in Bonn who presented with the first symptoms at the age of 7 years. He had been treated for severe food allergy, which was not as severe as IPEX. His disease worsened and at the age of 15 years we made the diagnosis on a genetic basis revealing a substitution of one single amino acid in the FOXP3 gene. So yes, mild forms or atypical forms exist, and it is worth testing all patients we suspect to have a form of autoimmune enteropathy on FOXP3 mutations.

Dr. Milla: I guess it also depends on what you mean by autoimmune enteropathy because IPEX is not the only form of autoimmune enteropathy. There are at least 4 different syndromes and if you call autoimmune enteropathy the ability of an enterocyte to produce an autoantibody or the ability of the immunocytes to produce an autoantibody against enterocyte components, then there are probably a lot of different syndromes. If you look at the early literature that describes autoimmune enteropathy there are quite a few patients who do not fit into the IPEX phenotype. Many of them are girls and they often have rather mild disease, and respond just to food elimination. The last part of Dr. Ruemmele's talk about barrier function is very interesting because of the consequences of the loss of barrier function and the way in which the gut-associated lymphoid tissue responds to that. Surely one possibility is that it responds by producing autoantibodies and maybe not in very severe IPEX-like phenotype. At least that is what is in the literature.

Dr. Ruemmele: I completely agree, I emphasized IPEX because we know the molecular basis; however other forms of autoimmune enteropathy exist. Girls do not develop IPEX, at least it has never been described, but we know some little girls with autoimmune GI diseases. For me, the definition of autoimmune enteropathy is the production of autoimmune antibodies directed against the gut combined with the clinical picture of a protein-losing enteropathy. IPEX is a particular form, very severe, in a subgroup of patients.

Dr. Sorensen: Do girls really never develop it? In many immune deficiency diseases, in situations of extreme ionization, female carriers can express a form of the disease.
Dr. Ruemmele: You are right and in fact there are some patients who you think have IPEX but it cannot be proven on a molecular basis. All the publications, except one, a very curious publication, checked the girls and the family members. I showed you over 4 generations with 22 female carriers who do not have a single symptom; they are not atopic, they have nothing. So it is very unlikely but I have never heard of this kind of pathology.

Dr. Lentze: I talked to one of my Dutch colleagues today because children in the Netherlands start to eat peanut butter very early and he told me they don’t have peanut allergies in the Netherlands. So perhaps Dr. Kneepkens can say something. Is that true?

Dr. Kneepkens: Of course we have peanut allergy in the Netherlands but not to the extent that you see it in England or in the US. Maybe that is the point, maybe we are more like Israel.

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