Current Paediatrics (2006) **16**, 305–316

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/cupe

Advances in the understanding and management of autoimmune enteropathy

Simon Murch

Warwick Medical School, Clinical Sciences Research Institute, Clifford Bridge Road, Coventry CV2 2DX, UK

KEYWORDS
Autoimmune enteropathy;
IPEX syndrome;
Foxp3;
APS-1;
Aire;
Bone marrow transplantation

Summary
There have been real advances in understanding the pathogenesis of autoimmune enteropathy, including determination of specific autoantigens. The most important clinical association is with IPEX (X-linked immune polyendocrinopathy) syndrome, which is due to mutation in the *Foxp3* transcription factor, a molecule critical in generation of regulatory T cells. Association of non-IPEX autoimmune enteropathy with T cell activation defects further point to impairment of T cell tolerance mechanisms as the primary underlying cause of autoimmune enteropathy. This also explains the frequency of other autoimmune manifestations. The centrality of T cell responses in autoimmune enteropathy, rather than B cell autoantibody production, as previously thought, is further suggested by the finding of late-onset gut autoimmunity in APS-1 (autoimmune polyglandular syndrome-1), a condition where negative selection of T cells within the thymus is disrupted due to mutation in the *Aire* (autoimmune regulator) gene. However, this form of autoimmune enteropathy is milder because the immune target is within entero-endocrine cells rather than absorptive enterocytes. There have also been important changes in management, with introduction of more potent immunoregulatory therapy, and more recently the use of bone marrow transplantation, which may theoretically offer hope of a cure in what frequently used to be a fatal condition.

Practice points

- Autoimmune enteropathy is an important cause of childhood intestinal failure
- Careful clinical assessment—including stool analysis while nil by mouth and on carbohydrate challenge—is a cornerstone of diagnosis
- There are now recognized autoimmune enteropathy syndromes with identified gene mutations
- Scrupulous long-line care may be life-saving if parenteral nutrition is needed long-term
- Full immunological work-up including T cell activation is necessary
- Therapeutic options may now include bone marrow transplantation in selected cases

Introduction

Autoimmune enteropathy is a rare but important cause of severe persistent diarrhoea caused by an autoimmune

E-mail address: s.murch@warwick.ac.uk.

0957-5839/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cupe.2006.07.008

response to intestinal epithelium.[1–7] It presents classically in the first months of life with chronic diarrhoea, and is frequently thought to represent post-enteritis or food-sensitive enteropathy in the early stages. However, the diarrhoea fails to resolve despite all dietary exclusions, and weight loss is often so significant that parenteral nutrition is required. Diagnostic criteria are based on small intestinal villous atrophy, unresponsiveness to dietary restriction, circulating enterocyte antibodies and/or associated autoimmune conditions.[4]

The disease may be confined to the small intestine,[1] and sometimes also the colon,[8] or it may be part of a multisystem autoimmunity, most commonly in association with renal disease or polyendocrinopathy.[2–5] The lesion of autoimmune enteropathy, crypt hyperplastic villous atrophy, is induced by uncontrolled activation of T cells within the intestinal mucosa.[7] The main diagnostic test, detection of enterocyte autoantibodies, is, however, based on an associated B cell response. Several cases have been reported in which enterocyte autoantibodies have been undetectable or variable, and their titre often bears little relationship with clinical disease activity.[3,5,7–9] Thus the disease is now primarily viewed as T cell associated.[7,9]

The outcome of autoimmune enteropathy has often been poor, with many children dying from complications of long-term parenteral nutrition or because of involvement of other organs by the autoimmune process.[10–14] Recent trends have been towards more aggressive immunotherapy, or even bone marrow transplantation if an underlying constitutive immunodeficiency is identified.[9]

Presentation of autoimmune enteropathy

The classic presentation of autoimmune enteropathy is with chronic diarrhoea, beginning within the first months of life, and proceeding relentlessly despite all dietary manipulations.[6,11,12,15,16] The age at first onset is an important part of the history, as the primary epithelial enteropathies, the other major causes of intractable diarrhoea, usually present in the early neonatal period.[15,16] Perinatal onset may rarely occur in autoimmune enteropathy, but there is normally a history of good growth before the onset of symptoms. It is more common in males (often X-linked) and in consanguineous families. Affected children may develop autoimmunity in other organs, most commonly the pancreas and the kidney.[6] There is recent evidence that autoimmune enteropathy may occur in adults, sometimes enterocyte antibody negative[17,18] and potentially associated with thymoma.[19,20] Findings of gastric and colonic involvement in some cases have led to the introduction of the term generalized autoimmune gut disorder (GAGD).[8,18]

Autoimmune enteropathy may have either an aggressive or relatively insidious onset, and may not initially appear different to other diarrhoeal diseases. Primary immunodeficiency or autoimmune predisposition may be unmasked by enteric pathogens, and the disorders often considered initially are infective gastroenteritis and post-enteritis syndrome/cow's milk sensitive enteropathy.[6,21] Initial management includes milk exclusion with use of lactose-free oligoallergenic formulae, which may induce slight improvement in osmotic diarrhoea as secondary deficiency of lactase is common in enteropathy.[7] However, the loose stools and weight loss essentially continue.

Differential diagnosis of autoimmune enteropathy

There is a wide differential diagnosis for persistent diarrhoea in infants and young children, including common and rare diseases (Table 1).[21] The most common cause at this age is food-sensitive enteropathy, and appropriate exclusion diets should be performed for an adequate period. Chronic diarrhoea due to primary carbohydrate malabsorption syndromes (e.g. glucose–galactose malabsorption), will remit while nil by mouth, underlining the importance of this manoeuvre.[21]

Disorders of other transport systems induce malabsorption of lipids and fat-soluble vitamins (abetalipoproteinaemia, hypolipoproteinaemia, Anderson's disease, ileal bile-salt receptor deficiency), neutral amino acids (Hartnup disease), tryptophan, basic amino acids (cystinuria), electrolytes (chloridorrhoea, defective jejunal Na^+/H^+ exchange) and zinc (acrodermatitis enteropathica). Although vacuolated enterocytes and an abnormal blood film may be

Table 1 Causes of persistent diarrhoea in early life.

1. *Failure of specific absorption pathway* (e.g. Glucose-galactose malabsorption, primary alactasia, bile salt malabsorption).
2. *Food sensitive enteropathy or enterocolitis* (e.g. cow's milk sensitive enteropathy, coeliac disease, Food protein induced enterocolitis)
3. *Pancreatic malabsorption* (e.g. cystic fibrosis, Shwachman syndrome)
4. *Dysmotility with bacterial overgrowth* (potentially related to anatomical abnormality or pseudo-obstruction syndrome)
5. *Inflammatory bowel diseases* (classic inflammatory bowel disease is very rare in infancy—underlying systemic causes such as immunodeficiency should be sought)
6. *Immunodeficiency* (even severe immunodeficiencies may be clinically silent until unmasked by a pathogen—if a gut pathogen, this may prompt referral to the gastroenterologist rather than immunologist in the first instance)
7. *Systemic diseases* (e.g. mitochondrial cytopathy)
8. *Primary epithelial causes of intractable diarrhoea* (e.g. microvillous inclusion disease, tufting enteropathy, heparan sulphate deficiency)
9. *Cryptogenic enteropathies* (Syndromatic intractable diarrhoea)
10. *Autoimmune enteropathy*

seen in abetalipoproteinaemia, there may be no specific features of enteropathy in these disorders.[6,21]

Anatomical abnormalities such as malrotation may present atypically and cause bacterial overgrowth with chronic diarrhoea and malabsorption. Severe disturbance of gut motility may also occur in pseudo-obstruction, inducing malabsorption through bacterial overgrowth. If chronic diarrhoea is accompanied by protein-losing enteropathy, lymphangiectasia should be considered, particularly if circulating lymphocytes are low.[13] Other disorders causing protein-losing enteropathy in infancy include congenital disorders of glycosylation and enterocyte heparan sulphate deficiency.[6,22] Protracted diarrhoea may also occur in metabolic diseases, notably mitochondrial cytopathy, and raised lactate should prompt further metabolic assessment.[23]

Immunodeficiency is always an important consideration in the infant presenting with chronic diarrhoea, and infants with severe immunodeficiency may present first with gastroenterological symptoms and/or have prolonged carriage of enteric pathogens. Primary immunodeficiency syndromes particularly recognized to cause chronic diarrhoea include severe combined immunodeficiency (SCID), thymic hypoplasia, class II major histocompatibility (MHC) deficiency, CD40 ligand deficiency,[24,25] and neutrophilic specific granule defect.[26] These conditions are often fatal in early childhood if untreated, and induce intractable diarrhoea because of chronic pathogen infection or their propensity to cause autoimmunity. The initial presentation may depend on patterns of pathogen exposure, and immunodeficiency may not be initially apparent. Acquired immune-deficiency syndrome (AIDS) may also present with enteropathy or persistent infection.[24]

Primary epithelial enteropathy is another important diagnostic consideration if diarrhoea is prolonged and continues while nil by mouth. In particular microvillous inclusion disease (microvillous atrophy) may induce intestinal failure with a combination of osmotic and secretory diarrhoea.[6,10,16] Similar, although initially less aggressive, presentation is seen with tufting enteropathy.[27,28] As mentioned above, an important difference between epithelial and autoimmune enteropathies is age at onset, as epithelial enteropathies classically present in the first days after birth while autoimmune enteropathy rarely begins during the first month. There is also a rare inherited form of infantile intractable diarrhoea, called syndromatic diarrhoea, which presents with recognizable dysmorphic features (hypertelorism with broad midface and nose), unpigmented and easily avulsed hair (trichorrhexis nodosa), subtle immunodeficiency and sometimes cirrhosis.[29,30]

Establishing the diagnosis

Diagnostic features of autoimmune enteropathy are listed in Table 2. Before establishing a specific diagnosis, there are important clinical characterizations to be performed, as with any unexplained chronic diarrhoeal disease.

Stool testing

Careful stool examination is very important, and should be repeated several times to exclude pathogens. Repeated analysis of reducing substances is also essential, and care should be taken to ensure that the liquid part of the stool is analysed to avoid false-negative results. If there is perianal excoriation, carbohydrate malabsorption is extremely likely (with the rare exception of congenital bile salt malabsorption[21]).

Nil by mouth

An essential clinical test is to determine whether diarrhoea continues while receiving nil by mouth over a period of 24 h.

Table 2 Diagnosis of autoimmune enteropathy.

1. *Stool electrolytes and reducing substances*. A mixed pattern of osmotic and secretory diarrhoea is seen. There may be improvement while nil by mouth, but stool losses remain increased due to underlying secretion. It is important to quantitate and characterise faecal losses.
2. *Therapeutic trial of food exclusions*. These are necessary but usually have little effect.
3. *Faecal inflammatory markers*. Faecal tumour necrosis factor (TNF)-α and calprotectin have been reported to be raised in autoimmune enteropathy.[108]
4. *Duodenal and colonic biopsies*. The classic duodenal findings are a coeliac-like crypt hyperplastic villous atrophy, but distinct from coeliac disease in that intraepithelial lymphocytes are not raised, and may even be reduced. Immunohistochemistry shows increase of T cells, often in a pericryptal distribution, and positive epithelial HLA-DR staining. Colonic changes are variable, but colitis is usually mild if present. Goblet cell depletion may sometimes be noted.
5. *Anti-enterocyte autoantibodies*. These are the hallmark of disease, but may be negative in some patients and vary with time (the primary response is T cell-mediated). AIE-75 (intestinal harmonin) is the major intestinal autoantigen).
6. *Anti-goblet cell autoantibodies* may be present, and should be sought if there is evidence of goblet cell depletion. Other autoantibodies should also be sought.
7. *In-vitro T cell activation studies* should be performed to identify underlying T cell activation defects. If impaired, flow cytometric assessment of CD3 staining intensity may identify potential CD3 mutations. It is important that all cases of autoimmune enteropathy should have full immunological review.
8. *Genotyping for mutations in Foxp3 gene* to diagnose IPEX syndrome.

Stool samples should be sent during this period to determine faecal electrolytes, and thus diagnose secretory diarrhoea (stool sodium >100 mmol/l). Complete resolution of diarrhoea on being placed nil orally would be unusual in autoimmune enteropathy and should raise the question of glucose–galactose malabsorption.

Glucose challenge

Although constitutive secretory diarrhoea is common in autoimmune enteropathy, this is exacerbated by osmotic diarrhoea following dietary intake. An important clinical test is to challenge orally with oral rehydration solution (ORS) after a period of nil by mouth; should this exacerbate underlying secretory diarrhoea by inducing osmotic diarrhoea with positive reducing substances, monosaccharide intolerance is confirmed and suggests severe enteropathy. Such infants will have major problems with tolerance of any enteral nutrition, and early introduction of parenteral nutrition is required.

Other dietary challenges

If the infant is able to tolerate ORS without purging, then the next phase of clinical testing is based on reintroduction of enteral feeds, while maintaining food exclusions that are sufficiently stringent to exclude severe food-sensitive enteropathy. Thus infants should be weaned onto a milk, soya and wheat-exclusion diet, usually by initial transfer to an amino acid formulae. If tolerated, then cautious graded weaning may continue. However, in most cases of genuine autoimmune enteropathy, the extent of successful weaning is limited, and the threshold for tolerance of enteral feeds is too low to maintain nutritional status without parenteral nutrition.

Immunological assessment

Assessment of immune status is important in any infant with unexplained persistent diarrhoea, and thus involvement of a paediatric immunologist is important. Immunodeficiency may present with chronic diarrhoea due to uncleared enteric pathogens, and is itself a risk factor for the development of autoimmunity. Autoimmune enteropathy was reported in cases of selective immunoglobulin deficiency and common variable immunodeficiency.[31–34] Potentially specific links have been established with conditions of defective T cell activation and low CD3 expression.[35–38] Thus assessment should include measurement of immunoglobulins, functional antibody testing (recall antigens), lymphocyte subset characterization by flow cytometry and in vitro T cell activation.

Autoantibodies

Enterocyte autoantibodies should be sought. Both brush-border and cytoplasmic staining patterns have been identified for IgG enterocyte autoantibodies, without clear clinical differences noted.[5,8] Should goblet cell depletion be prominent on histology, goblet cell autoantibodies should be looked for.[33,38,39] Additional tissue-specific autoantibodies should be sought when the diagnosis is established, and testing repeated as clinical circumstances dictate.

A low-titre antibody recognizing a 75 kDa autoantigen (AIE-75), localized to intestinal epithelium and kidney, was identified in Japanese children patients with autoimmune enteropathy and associated nephropathy.[40,41] This is not found in every case, and was negative in patients without renal involvement.[41] AIE-75 has been characterized as the intestinal isoform of harmonin, a protein thought to function as a molecular anchor within the cytoskeleton.[42] The major expression of harmonin is within the inner ear, and its germline mutation causes Usher syndrome type 1c, which causes congenital deafness, vestibular dysfunction and retinitis pigmentosa but not enteropathy.[43] More extensive harmonin gene deletion in one family (probably including its intestinal isoform) induced inflammatory enteropathy,[44] suggesting that autoimmune disruption of intestinal harmonin function may promote inflammation. Intestinal harmonin interacts with the tumour-suppressor gene MCC2 (mutated in colonic cancer 2), on chromosome 19p13,[42] and may regulate intestinal proliferation.[45]

Histology

Endoscopic examination of small bowel and colon, with multiple biopsies, is a cornerstone of diagnosis. While involvement of the stomach, ileum and colon may occur,[8,18] there is more usually severe small intestinal enteropathy, with a coeliac-like crypt hyperplastic villous atrophy (Fig. 1). This varies from mild villous blunting to total villous atrophy, and is variable from time to time within the same patient. One characteristic distinguishing autoimmune enteropathy (Fig. 2) from coeliac disease is the relative paucity of intraepithelial lymphocytes (IELs).[7,16] The cellular infiltrate may be intense within the lamina propria, and is composed of T cells (Fig. 3), plasma cells and variable numbers of eosinophils and neutrophils.[13,15,16] In some cases, there is a mucosal lesion more akin to graft-versus-host disease, with abnormal surface epithelium and evidence of apoptosis.[46] In others, there may be reduced or absent goblet cells, associated with goblet cell autoantibodies.[33,38,39]

The basis of autoimmune enteropathy

There have been important advances in determining the molecular basis of autoimmune enteropathy,[6,9] which may have several potential causes (Table 3). Underpinning its known causes are the mechanisms involved in T cell tolerance.[9,47,48] Autoimmune enteropathy is associated with constitutive defects in both central and peripheral T cell tolerance. Although extensive discussion of these mechanisms is beyond the scope of this review, a basic outline aids understanding these disorders (Table 4).

Central immune tolerance is established within the thymus, where most developing T cells actually fail to survive two stringent purging mechanisms.[47] First, developing T cells have to be 'positively selected' at the early CD4+CD8+ stage for their ability to recognize MHC molecules presented by epithelial cells in the thymic cortex—95% of

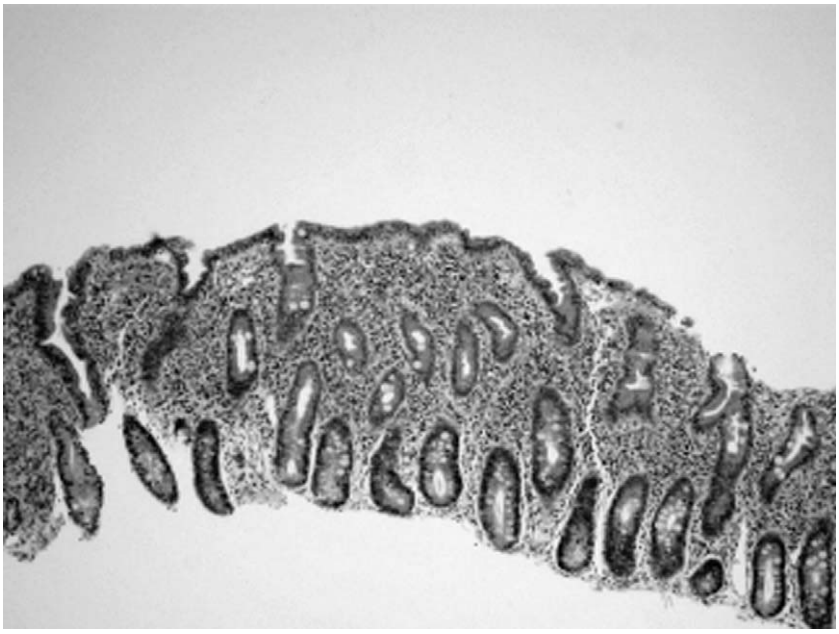

Figure 1 Subtotal villous atrophy in a case of autoimmune enteropathy due to IPEX syndrome. There is crypt hyperplastic villous atrophy with minimal increase of intraepithelial lymphocytes. Goblet cells are present but reduced in number.

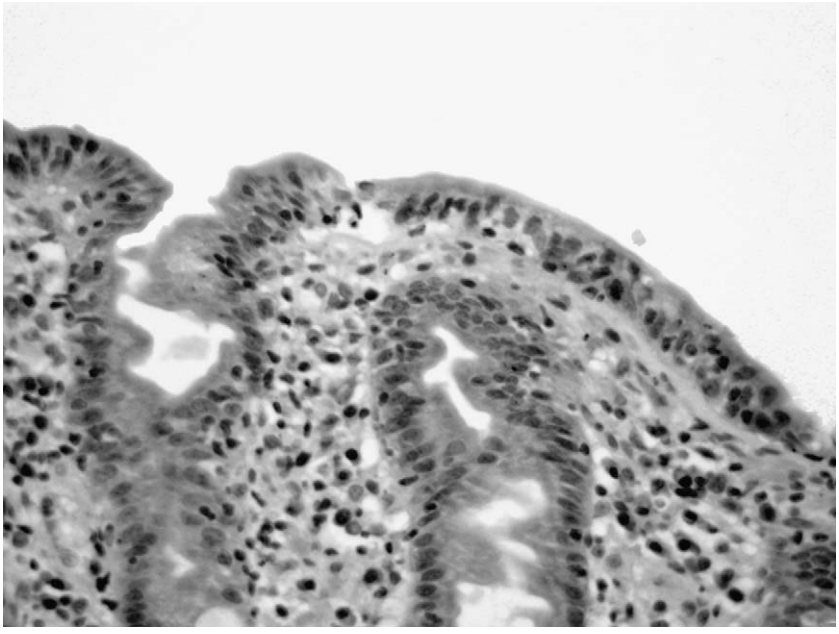

Figure 2 A higher-power view of the same biopsy, showing the presence of intraepithelial lymphocytes, but with only minor increase above normal. This is quite distinct from typical findings in coeliac disease, where their numbers are usually very high.

cells fail this challenge and are eliminated. Second, the surviving cells are challenged in the thymic medulla by self-antigens presented by antigen-presenting cells (dendritic cells and macrophages). T cells that bind self-peptides with too great an affinity are then eliminated by induced apoptosis ('negative selection').

Thus the surviving emigrants from the thymus will include cells with lesser degrees of self-reactivity, which must then be controlled by peripheral tolerance mechanisms. Key amongst these are the regulatory lymphocyte (T_{REG}) populations,[9,48] including subsets of CD4 cells (CD4+CD25+), CD8 cells (CD8+CD28-), TGF-β1 producing T_H3 cells and interleukin (IL)-10 producing TR1 cells). If thymic negative selection is impaired, either because self-antigens are not presented or because T cells of high affinity for the self-antigens do not activate normally, highly self-reactive T cells will escape into the periphery and may induce autoimmune damage even if peripheral regulatory mechanisms are intact. Conversely, if the mechanisms of peripheral tolerance fail, particularly if adequate T_{REG} cells are not

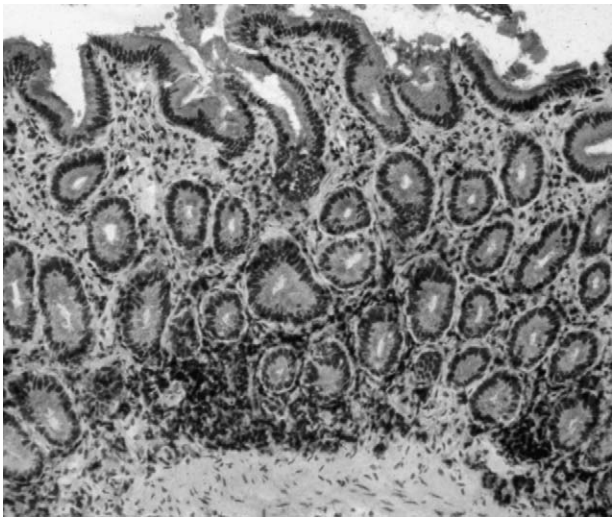

Figure 3 Immunostaining for T cells in a case of autoimmune enteropathy associated with polyglandular autoimmunity in a female child. The T cells (dark staining) cluster round the deep crypt epithelium. Circulating autoantibodies were variably present in this case, and did not correlate with enteropathy. Goblet cells are markedly depleted.

generated, the moderately self-reactive T cells that escape normal thymic purging may induce tissue damage. In this latter scenario, the fragile existing tolerance may be broken by encounter with pathogens expressing similar antigenic determinants to self-tissue (molecular mimicry).[49]

If peripheral immune regulation is adequate, such nascent autoimmune reactions may be brought under control. There are indeed reports of spontaneous resolution of even severe autoimmune enteropathy.[50] Transiently positive enterocyte antibodies and chronic diarrhoea are sometimes seen in children with cow's milk and other food allergic enteropathies,[51] which are associated with delayed immune maturation and defective mucosal generation of regulatory TGF-β producing T cells.[52] As children outgrow milk allergy when they generate T_{REG} cell populations,[53] it is possible that transient low-grade autoimmune enteropathy remits with such immune maturation. Conversely, enterocyte autoantibodies may develop later when immune regulation is disturbed, as demonstrated by their high incidence in AIDS-associated diarrhoea.[54] Thus transient low-grade autoimmune enteropathy may not be a rare event, and the ability to generate a peripheral regulatory immune response may determine whether full-blown disease occurs. Although abnormalities of selection processes in extrathymically maturing intestinal lymphocytes would presumably have a

Table 3 Recognised types of autoimmune enteropathy.

1. *IPEX-syndrome* (due to mutation in the Foxp3 gene). X-linked disorder, so male infants predominate. Often associated with polyendocrinopathy.
2. *IPEX-like syndrome* (without IPEX mutation). Candidate molecules from known actions would include the CTLA-4/CD28 co-stimulatory pathway, the adhesion molecule CD18 and the regulatory cytokines interleukin (IL)-10 and TGF-β1.
3. *Immunodeficiency-associated autoimmune enteropathy* (a particular link has been established with T cell activation deficiencies).
4. *Autoimmune polyglandular syndrome-1 (APS-1)*—also known as autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED) syndrome (due to mutation in the AIRE) gene. Only some cases develop enteropathy, and it is generally less severe than IPEX syndrome.
5. *Autoimmune enteropathy with colitis—generalised autoimmune gut disorder (GAGD)*. Also reported in adults, associated with infiltration of unusual population of CD4 αE/$\beta7^-$ intraepithelial lymphocytes.

Table 4 Management of autoimmune enteropathy.

1. *Optimise enteral nutrition*. This may need to include modular feeds to allow titration of individual fats and carbohydrates as tolerated.
2. *Parenteral nutrition (PN)* is almost always required, even if transiently. This will allow appropriate assessment of enteral absorption while maintaining adequate nutrition. Long-term total PN is frequently needed. Anticoagulation may be necessary if there is an underlying pro-coagulant state or phospholipids antibodies develop. Scrupulous line care is vital, and many children have died because line access is exhausted.
3. *Involvement of a multi-disciplinary nutrition team* is necessary to ensure adequate macro- and micro-nutrient delivery.
4. *Immunomodulatory therapy* is almost always needed. Corticosteroids are frequently used initially, but most infants require more potent immunomodulation. Agents used include azathioprine, cyclosporine, tacrolimus and sirolimus. Intravenous immunoglobulin therapy has also been used in some cases.
5. *Bone marrow transplantation* may be required in cases with underlying immunodeficicncy, particularly in IPEX syndrome and T cell activation deficiencies.

high chance of inducing gut-specific autoimmunity,[7,54,55] there is little yet known about this process in humans.

Specific autoimmune enteropathy syndromes

X-linked immune polyendocrinopathy (IPEX) syndrome

The most important cause of autoimmune enteropathy currently recognized is IPEX syndrome. This was initially reported as a syndrome of unknown cause affecting 17 male infants from a Mormon family, characterized variably by diabetes mellitus, haemolytic anaemia or eczema, with about half also suffering from intractable diarrhoea.[2] X-linked inheritance was demonstrated, and the gene correctly postulated to have a function preventing overactivity of the immune system.[2] The responsible gene was eventually mapped within the X chromosome near to the locus for Wiskott–Aldrich syndrome.[56] The clinical features were sufficiently distinct from Wiskott–Aldrich syndrome to suggest another gene would be identified. Importantly, this turned out to be a region of the X chromosome in which the human homologue of the mouse *scurfin* gene turned out to be located. Scurfy mice, first reported in the 1940s, develop a spontaneous multifocal inflammatory condition characterized by tissue infiltration of activated T cells.[57] Focused gene mapping studies finally identified the *scurfin* gene.[58] Because of the overlapping phenotype with human IPEX syndrome and the suggestive chromosomal location, genetic analysis by two groups identified that the human homologue of *scurfin*, *Foxp3* (a winged helix/forkhead transcription factor) was indeed mutated within the DNA-binding region in IPEX syndrome.[59,60] A third group meanwhile located the same molecule by gene mapping in affected families.[61]

The clinical features of IPEX syndrome are variable, although eczematous skin disease is very common. Extraintestinal features include most commonly type 1 diabetes mellitus, which may predate the onset of enteropathy, and variably high IgE, hypothyroidism, liver abnormalities and anaemia.[62,63] Despite the X-linkage, female infants have been described with a similar presentation, and males with clinical IPEX but without *Foxp3* mutation.[64]

The *Foxp3* gene has subsequently attracted enormous interest, as it turned out to be critical for the generation of T_{REG} cells.[65,66] While *Foxp3* plays a, so far, poorly characterized but apparently essential role in normal thymic development,[67] it is better studied as a critical regulator of peripheral immune tolerance, as an essential checkpoint in the generation of T_{REG} cells, which act to inhibit activation of proinflammatory cells in autoimmune or immunopathological lesions.[68] The development of autoimmune enteropathy in IPEX syndrome is thus due to the inability of affected children to generate T_{REG} populations (eg CD4+CD25+ cells). Follow-up from birth of a child with IPEX syndrome identified abnormal T cell activation by 2 weeks age, before the onset of recognizable disease.[69]

It is notable that scurfy mice have a very similar phenotype to mice deficient in the regulatory cytokine TGF-β1 and the activation inhibitor CTLA-4.[6,55] CTLA-4 acts by blocking the CD28 co-stimulation pathway, whose potency in T cell activation was shown by the recent TGN1412 drug trial.[70] It is very likely that other immunodeficiencies predisposing to abnormal thymic selection or impaired peripheral regulatory responses will be identified in subsequent cases of autoimmune enteropathy.

Autoimmune polyglandular syndrome type 1 (APS1).

The second rare disorder linking polyendocrinopathy and autoimmune enteropathy, APS1 (also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome or APECED), is extremely rare apart from within formerly isolated communities in Iran, Sardinia and Finland.[71] Affected individuals develop multifocal autoimmune disorders, but later than in IPEX syndrome (usually at several years age or as teenagers), and usually with less severe intestinal involvement.[72–74] Surprisingly, in view of its molecular basis in thymic selection,[71,75] chronic candidiasis is often the first presenting feature, followed more predictably by autoimmune hypoparathyroidism and/or Addison's disease. Clinical diagnosis is based on presentation with at least two of these features,[73] and a variety of autoantibodies have been reported.[76] Intestinal malabsorption has been reported in around 9% of cases at presentation, and around a quarter of patients suffer episodic gastrointestinal symptoms including dysmotility and malabsorption.[74] The autoantibodies specific for the intestine usually recognize enteroendocrine cells, and absent endocrine cells with cholecystokinin deficiency was reported in one patient, in whom variable symptoms were related to the presence or absence of cholecystokinin-producing cells.[77] Specific intestinal autoantigens include trypophan hydroxylase and histidine decarboxylase.[78–80]

The locus for APS1 was mapped to chromosme 21p22.3 and the gene identified to encode the autoimmune regulator (*Aire*) protein.[71,81,82] Several mouse models of *Aire* mutation confirm that the principal effect of *Aire* deficiency is to engender defects in negative selection of autoreactive T cells within the thymus.[71,75] *Aire* plays a critical role in central immune tolerance by its expression within medullary epithelial cells of the thymus,[75,83] under regulation by lymphotoxin.[84] Within the thymus it assists presentation of self-antigens to developing thymocyte at the time of negative selection, thus promoting deletion of potentially autoreactive T cells.[47,71,82,83]

There is also evidence of peripheral *Aire* expression within monocytes and dendritic cells.[85,86] Peripheral dendritic cells from both humans and mice with *Aire* mutations show excess expression of vascular cell adhesion molecule-1 (VCM-1) and activate naïve T cells unusually strongly.[87] This suggests a role for *Aire* protein in peripheral, as well as central, immune tolerance. Although *Aire* gene expression is seen within most epithelia, at much lower levels than within the thymus, intestinal expression appears confined to goblet cells.[88] There is thus so far no evidence pointing to a role of *Aire* in the extrathymic maturation of intestinal lymphocytes. The relatively less severe autoimmune enteropathy in APS1 than in IPEX is therefore intriguing. However, this may simply relate to the localization of the autoantigen within enteroendocrine cells, rather than within absorptive enterocytes.

T cell activation defects

It is probably no accident that the group of constitutive immunodeficiencies most linked with autoimmune enteropathy are those with impaired T cell activation. Although usually less severe than the SCID syndromes, the potential for T cell-mediated autoimmunity is higher because of the requirement for activation of self-reactive lymphocytes within the thymus to induce their negative selection.[47] Thus activation-deficient autoreactive T cells may not be deleted because they mimic normal self-tolerant cells, and may then promote autoimmunity in the periphery by providing help to potentially autoreactive B cells.[38] Autoimmune enteropathies have been reported in children with mutations in the T cell receptor/CD3 complex,[35–37] in the IL-2 receptor α chain (CD25)[89] and with impaired T cell activation of unknown molecular basis.[38] In CD25 mutation there was abnormal thymic expression of CD1 and bcl-2, molecules involved in regulating thymic selection, and evidence of oligoclonal expansion of autoreactive clones within the intestine.[89] These cases further underline the important role of T cell tolerance in autoimmune enteropathy.

The mucosal lesion in autoimmune enteropathy

The T cell infiltrate within the lamina propria may be dominated by CD4+ T cells, or may show increase of both CD4 and CD8 cells.[9,13,16] In contrast to coeliac disease, neither $\gamma\delta$ T cells nor intraepithelial lymphocytes (IELs) are increased in autoimmune enteropathy,[16] although there is usually strong expression of HLA-DR on the epithelium, suggesting local production of T_H1 cytokines such as interferon-γ.[90] The cause of the reduced IEL numbers by contrast to coeliac disease is unknown, but may theoretically be due to impaired expression of trophic factors or critical adherence ligands by the epithelium (e-cadherin normally binds $\alpha E\beta 7$ integrin on CD8 and $\gamma\delta$ IELs).[7,91] There is one report in an adult patient of an expanded population of CD4+$\alpha E\beta 7$-T cells in the epithelium, a phenotype expected in lamina propria lymphocytes.[18]

B cell and plasma cell populations are increased within the mucosa in autoimmune enteropathy but have not yet been characterized in detail.[9,16] It is thus unclear whether autoreactivity amongst B cells may play a role in the development of autoimmune enteropathy, as suggested by links with immunoglobulin deficiencies,[31,34,42] or whether B cell responses are secondary to autoreactive T cells. Experimental studies support the clinical links of autoimmune enteropathy with T cell abnormalities.

Experimental models of autoimmune enteropathy

In a model of gut autoimmunity, in which influenza haemagglutinin (HA) expression was engineered in intestinal epithelium of mice carrying HA-specific T cells, there was increased infiltration into the intestine of these T cells (which had escaped deletion within the thymus) with evidence of their local activation.[92] Potential disease was minimized by a regulatory T cell response, mediated by the IPEX gene foxp3.[92] This confirms that a peripheral regulatory response may hold in check a propensity for autoimmune enteropathy, even if deletion of autoreactive cells has not occurred within the thymus.

Another model, based on similar engineering of epithelial gene expression (ovalbumin), demonstrated that 'self'-antigen derived from the epithelium could induce a tolerant response from CD8 cells within the mucosal immune system, but that tolerance could be broken by presentation of the autoantigen during listeria infection.[93] This suggests that innate predisposition to gut autoimmunity may be unmasked by chance encounter with pathogens (and may explain why many cases present after infection).

Prognosis of autoimmune enteropathy

The Italian Society of Paediatric Gastroenterology (SIGEP) study[11] estimated an incidence of severe protracted diarrhoea of 0.64–0.92 per 10^5 infants per year, of which autoimmune enteropathy and primary enterocyte disorders were the most common causes. Classic autoimmune enteropathy has previously had a poor prognosis, with around half affected infants dependant on parenteral nutrition in the long-term or dying from complications.[10–12,14] Recent reports suggest a more hopeful prognosis.

Management of autoimmune enteropathy

Parenteral nutrition is almost always required and may have to be very prolonged. This mandates scrupulous care of Hickman lines, as line infections may cause life-threatening sepsis and also lead to line removal. Repeated line removals are eventually fatal, as venous access becomes impossible. The frequency of line infections is reduced in units with extensive experience in managing prolonged parenteral nutrition, and this may be an important limiting factor in the long term.[14] A multi-disciplinary approach, involving skilled nursing staff, dieticians, psychologists, pharmacists, paediatric surgeons, immunologists and gastroenterologists may be needed.[10–14]

One important aim is to maximize enteral input, which may require frequent determinations of the threshold for absorption as medical treatment changes. It is often necessary to use modular feeding, as intake of standard formulae is limited by purging of malabsorbed carbohydrates. An individualized feed may be developed in close liaison with experienced paediatric dieticians, and carbohydrate intake may be optimized by using a combination of glucose (absorbed by the glucose–galactose transporter SGLT-1), fructose (absorbed separately by the glut-5 transporter) and glucose polymer.[7,13] It may be better to use continuous enteral feeds rather than intermittent bolus feeding. The threshold for absorption will hopefully be improved by successful use of immunosuppression, and attempts can then be made to increase enteral intake.

Immunosuppressive therapy

A variety of immunosuppressive therapies have been used in autoimmune enteropathy, with varying success. In milder cases, spontaneous or treatment-induced permanent

remission can happen.[50,94] Some cases have responded in the short-term to corticosteroids, but most need more potent therapies, including azathioprine and cyclophosphamide.[10,95] Although cyclosporine and tacrolimus have been effective in some cases,[94,96,97] it is notable that T cells from scurfy mice require very high doses of cyclosporine to suppress in vitro activation.[57] A recent report suggests that sirolimus, which suppresses T cell activation by a calcineurin-independent pathway may give therapeutic efficacy in cyclosporine-resistant disease in IPEX syndrome,[98] and similar benefit has been seen with mycophenolate movetil.[99] There are promising recent reports suggesting that the anti-TNF-α monoclonal antibody, infliximab, may induce disease remission in otherwise resistant disease.[20,100,101]

Bone marrow transplantation

In view of the profound immune dysregulation induced by foxp3 mutation in IPEX syndrome, bone marrow transplantation has been attempted in several cases. In the first case, there was substantial improvement in intractable diarrhoea and endocrinopathy but unexplained late death from haemophagocytosis.[102] More cases have been transplanted subsequently than currently reported in the literature,[103] and outcome reportedly mixed, but successful transplantation has led to complete remission of autoimmune enteropathy. As experience is gained, it will be possible to determine whether partial bone marrow transplantation may be as successful, as seen in foxp3-deficient mice, where the transplanted cells clonally expand to provide adequate T cell regulation.[104]

Bone marrow transplantation is unlikely to be effective in APS-1, as the underlying thymic lesion would be unaffected. For the T cell activation deficits, much would depend on the severity of the associated condition. One severely affected infant with unclassified T cell activation defect unfortunately died just before the planned bone marrow transplant.[38]

Genetic engineering

A potential future approach may be to genetically engineer lymphocytes to express the missing protein, as has been used for other immunodeficiencies.[105] For IPEX syndrome, the potential benefit would be to induce appropriate regulatory populations,[106] but the hazard would be of overexpressing *Foxp3* sufficient to inhibit T cell responses to pathogens. Indeed overexpression of *Foxp3* led to impaired T helper and humoral responses in mice.[107] This will require much greater research before it moves into the clinical arena.

References

1. Walker-Smith JA, Unsworth DJ, Hutchins P, et al. Autoantibodies against gut epithelium in a child with small intestinal enteropathy. *Lancet* 1982;**i**:566.
2. Powell BR, Buist NRM, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J Pediatr* 1982;**100**:731–7.
3. Unsworth DJ, Hutchins P, Mitchell J, Phillips A, Hindocha P, Holborow J, et al. Flat intestinal mucosa and autoantibodies against the gut epithelium. *J Pediatr Gastroenterol Nutr* 1982;**1**:503–13.
4. Unsworth DJ, Walker-Smith JA. Autoimmunity in diarrhoeal disease. *J Pediatr Gastroenterol Nutr* 1985;**4**:375–80.
5. Mirakian R, Richardson A, Milla PJ, Walker-Smith JA, Unsworth J, Savage MO, et al. Protracted diarrhoea of infancy: evidence of an autoimmune variant. *Br Med J* 1986;**293**:1132–6.
6. Murch SH. Protracted diarrhea. In: Wyllie R, Hyams JS, editors. *Pediatric gastrointestinal and liver disease*. 3rd ed. Amsterdam: Elsevier; 2006 (in press).
7. Murch SH. The molecular basis of intractable diarrhoea of infancy. *Baillière's Clin Gastroenterol* 1997;**11**:413–40.
8. Hill SM, Milla PJ, Bottazzo GF, Mirakian R. Autoimmune enteropathy and colitis: is there a generalised autoimmune gut disorder? *Gut* 1991;**32**:36–42.
9. Ruemmele FM, Brousse N, Goulet O. Autoimmune enteropathy: molecular concepts. *Curr Opin Gastroenterol* 2004;**20**:587–91.
10. Goulet OJ, Brousse N, Canioni D, Walker-Smith JA, Schmitz J, Phillips AD. Syndrome of intractable diarrhoea with persistent villous atrophy in early childhood: a clinicopathological survey of 47 cases. *J Pediatr Gastroenterol Nutr* 1998;**26**:151–61.
11. Guarino A, Spagnuolo MI, Russo S, Albano F, Guandalini S, Capano G, et al. Aetiology and risk factors of severe and protracted diarrhoea. *J Pediatr Gastroenterol Nutr* 1995;**20**:173–8.
12. 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.
13. Walker-Smith JA, Murch SH. Intractable diarrhoea. In: Diseases of the small intestine in childhood. 4th ed. Oxford: Isis Medical Media; 1999. p. 279–98.
14. Gambarara M, Bracci F, Diamanti A, Ambrosini MI, Pietrobattista A, Knafelz D, et al. Long-term parenteral nutrition in pediatric autoimmune enteropathies. *Transplant Proc* 2005;**37**:2270–1.
15. Sherman PM, Mitchell DJ, Cutz E. Neonatal enteropathies: defining the causes of protracted diarrhea of infancy. *J Pediatr Gastroenterol Nutr* 2004;**38**:16–26.
16. Cuenod B, Brousse N, Goulet O, de Potter S, Mogenot J- F, Ricour C, et al. Classification of intractable diarrhea in infancy using clinical and immunohistochemical criteria. *Gastroenterology* 1990;**99**:1037–43.
17. Corazza GR, Biagi F, Volta U, Andreani ML, De Franceschi L, Gasbarrini G. Autoimmune enteropathy and villous atrophy in adults. *Lancet* 1997;**350**:106–9.
18. Leon F, Olivencia P, Rodriguez-Pena R, Sanchez L, Redondo C, Alvarez I, et al. Clinical and immunological features of adult-onset generalized autoimmune gut disorder. *Am J Gastroenterol* 2004;**99**:1563–71.
19. Mais DD, Mulhall BP, Adolphson KR, Yamamoto K. Thymoma-associated autoimmune enteropathy. A report of two cases. *Am J Clin Pathol* 1999;**112**:810–5.
20. Elwing JE, Clouse RE. Adult-onset autoimmune enteropathy in the setting of thymoma successfully treated with infliximab. *Dig Dis Sci* 2005;**50**:928–32.
21. Murch SH. Unusual enteropathies. *Gastrointest Endosc Clin North Am* 2001;**11**:741–66.
22. Murch SH. Protein-losing enteropathy. In: Weinstein WM, Hawkey CJ, Bosch J, editors. *Clinical gastroenterology and hepatology*. Philadelphia: Elsevier Mosby; 2005. p. 309–14.
23. Cormier-Daire V, Bonnefont JP, Rustin P, Maurage C, Ogler H, Schmitz J, et al. Mitochondrial DNA rearrangements with onset as chronic diarrhea with villous atrophy. *J Pediatr* 1994;**124**:63–70.

24. Klein N, Jack D. Immunodeficiency and the gut: clues to the role of the immune system in gastrointestinal disease. *Ital J Gastroenterol Hepatol* 1999;**31**:802–6.
25. Lin SC, Shyur SD, Ma YC, Huang LH, Lee WI. Hyper-IgM1 syndrome with interstitial pneumonia and diarrhea caused by coxsackievirus B4 in a 3-month-old infant. *Ann Allergy Asthma Immunol* 2005;**95**:93–7.
26. Wynn RF, Sood M, Theilgaard-Monch K, Jones CJ, Gombart AF, Gharib M, et al. Intractable diarrhoea of infancy caused by neutrophil specific granule deficiency and cured by stem cell transplantation. *Gut* 2006;**55**:292–3.
27. Goulet O, Kedinger M, Brousse N, Cuenod B, Colomb V, Patey N, et al. Intractable diarrhoea of infancy with epithelial and basement membrane abnormalities. *J Pediatr* 1995;**127**: 212–9.
28. Reifen RM, Cutz E, Griffiths AM, Ngan BY, Sherman PM. Tufting enteropathy: a newly recognized clinicopathological entity associated with refractory diarrhea in infants. *J Pediatr Gastroenterol Nutr* 1994;**18**:379–85.
29. Giraut D, Goulet O, Ledeist F, Brousse N, Colomb V, Cesarinin JP, et al. Intractable diarrhea syndrome associated with phenotypic abnormalities and immune deficiency. *J Pediatr* 1994;**25**:36–42.
30. Landers MC, Schroeder TL. Intractable diarrhea of infancy with facial dysmorphism, trichorrhexis nodosa, and cirrhosis. *Pediatr Dermatol* 2003;**20**:432–5.
31. McCarthy DM, Katz SI, Gazze L, Waldmann TA, Nelson DL, Strober W. Selective IgA deficiency associated with total villous atrophy of the small intestine and an organ-specific anti-epithelial cell antibody. *J Immunol* 1978;**120**:932–8.
32. Van Thiel DH, Smith Jr. WI, Rabin BS, Fisher SE, Lester R. A syndrome of immunoglobulin A deficiency, diabetes mellitus, malabsorption, a common HLA haplotype. Immunologic and genetic studies of forty-three family members. *Ann Intern Med* 1977;**86**:10–9.
33. Moore L, Xu X, Davidson G, et al. Autoimmune enteropathy with anti-goblet cell antibodies. *Hum Pathol* 1994;**26**: 1162–8.
34. Catassi C, Mirakian R, Natalini G, Sbarbati A, Cinti S, Coppa GV, 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**:608–13.
35. Alarcón B, Regueiro JR, Arnaiz-Villena A, Terhorst C. Familial defect in the surface expression of the T-cell receptor-CD3 complex. *New Engl J Med* 1988;**319**:1203–8.
36. Arnaiz-Villena A, Timon M, Rodriguez-Gallego C, et al. T lymphocyte signalling defects and immunodeficiency due to the lack of CD3 γ. *Immunodeficiency* 1993;**4**:121–9.
37. Martín-Villa JM, Regueiro JR, de Juan D, et al. T-lymphocyte dysfunctions together with apical gut epithelial cell autoantibodies. *Gastroenterology* 1991;**101**:390–7.
38. Murch SH, Fertleman CR, Meadows N, Morgan G, Phillips AD, Walker-Smith JA. Autoimmune enteropathy with unique mucosal features in T cell activation deficiency: the contribution of T cells to the mucosal lesion. *J Pediatr Gastroenterol Nutr* 1999;**28**:393–9.
39. Hori K, Fukuda Y, Tomita T, Kosaka T, Tamura K, et al. Intestinal goblet cell autoantibody associated enteropathy. *J Clin Pathol* 2003;**56**:629–30.
40. Kobayashi I, Imamura K, Yamada M, Okano M, Yara A, Ikema S, et al. A 75-kD autoantigen recognized by sera from patients with X-linked autoimmune enteropathy associated with nephropathy. *Clin Exp Immunol* 1998;**111**:527–31.
41. Kobayashi I, Imamura K, Kubota M, Ishikawa S, Yamada M, Tonoki H, et al. Identification of an autoimmune enteropathy-related 75-kilodalton antigen. *Gastroenterology* 1999;**117**: 823–30.
42. Ishikawa S, Kobayashi I, Hamada J, Tada M, Hirai A, Furuuchi K, et al. Interaction of MCC2, a novel homologue of MCC tumor suppressor, with PDZ-domain Protein AIE-75. *Gene* 2001;**267**: 101–10.
43. Verpy E, Leibovici M, Zwaenepoel I, Liu X- Z, Gal A, Salem N, et al. A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nat Genet* 2000;**26**:51–5.
44. Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, et al. A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. *Nat Genet* 2000;**26**: 56–60.
45. Hirai A, Tada M, Furuuchi K, Ishikawa S, Makiyama K, Hamada J, et al. Expression of AIE-75 PDZ-domain protein induces G2/M cell cycle arrest in human colorectal adenocarcinoma SW480 cells. *Cancer Lett* 2004;**211**:209–18.
46. Ciccocioppo R, D'Alo S, Di Sabatino A, Parroni R, Rossi M, Doglioni C, et al. Mechanisms of villous atrophy in autoimmune enteropathy and coeliac disease. *Clin Exp Immunol* 2002; **128**:88–93.
47. Kyewski B, Derbinski J. Self-representation in the thymus: an extended view. *Nat Rev Immunol* 2004;**4**:688–98.
48. Buckner JH, Ziegler SF. Regulating the immune system: the induction of regulatory T cells in the periphery. *Arthritis Res Ther* 2004;**6**:215–22.
49. Fujinami RS, von Herrath MG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin Microbiol Rev* 2006;**19**:80–94.
50. Savage MO, Mirakian R, Wozniak ER, Jenkins HR, Malone M, Phillips AD, et al. Specific autoantibodies to gut epithelium in two infants with severe protracted diarrhoea. *J Pediatr Gastroenterol Nutr* 1985;**4**:187–95.
51. Latcham F, Merino F, Winter C, Lang A, Garvey J, Thomson MA, et al. A consistent pattern of minor immunodeficiency and subtle enteropathy in children with multiple food allergy. *J Pediatr* 2003;**143**:39–47.
52. Pérez-Machado MA, Ashwood P, Thomson MA, Latcham F, Sim R, Walker-Smith JA, et al. Reduced transforming growth factor-β1-producing T cells in the duodenal mucosa of children with food allergy. *Eur J Immunol* 2003;**33**:2307–15.
53. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004;**199**:1679–88.
54. Martín-Villa JM, Camblor S, Costa R, Arnaiz-Villena A. Gut epithelial cell autoantibodies in AIDS pathogenesis. *Lancet* 1993;**342**:380.
55. Murch SH. Towards a molecular understanding of complex childhood enteropathies. *J Pediatr Gastroenterol Nutr* 2002; **34**:S4–S10.
56. Bennett CL, Yoshioka R, Kiyosawa H, Barker DF, Fain PR, Shigeoka AO, et al. X-linked syndrome of polyendocrinopathy, immune dysfunction, and diarrhea maps to Xp11.23–Xq13.3. *Am J Hum Genet* 2000;**66**:461–8.
57. Clark LB, Appleby MW, Brunkow ME, Wilkinson JE, Ziegler SF, Ramsdell F. Cellular and molecular characterization of the scurfy mouse mutant. *J Immunol* 1999;**162**:2546–54.
58. Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001;**27**:68–73.
59. Wildin RS, Ramsdell F, Peake J, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001; **27**:18–20.
60. Bennett CL, Christie J, Ramsdell F, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked

syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001;**27**:20–1.
61. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J Clin Invest* 2000;**106**:R75–81.
62. Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet* 2002;**39**:537–45.
63. Myers AK, Perroni L, Costigan C, Reardon W. Clinical and molecular findings in IPEX syndrome. *Arch Dis Child* 2006; **91**:63–4.
64. Owen CJ, Jennings CE, Imrie H, et al. Mutational analysis of the FOXP3 gene and evidence for genetic heterogeneity in the immunodysregulation, polyendocrinopathy, enteropathy syndrome. *J Clin Endocrinol Metab* 2003;**88**:6034–9.
65. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;**299**:1057–61.
66. Ochs HD, Ziegler ZF, Torgerson TR. FOXP3 acts as a rheostat of the immune response. *Immunol Rev* 2005;**203**:156–64.
67. Chang X, Gao JX, Jiang Q, Wen J, Seifers N, Su L, et al. The Scurfy mutation of FoxP3 in the thymus stroma leads to defective thymopoiesis. *J Exp Med* 2005;**202**:1141–51.
68. Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003;**15**:430–5.
69. Bakke AC, Purtzer MZ, Wildin RS. Prospective immunological profiling in a case of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX). *Clin Exp Immunol* 2004;**137**:373–8.
70. Goodyear M. Learning from the TGN1412 trial. *Br Med J* 2006;**332**:677–8.
71. Ulmanen I, Halonen M, Ilmarinen T, Peltonen L. Monogenic autoimmune diseases—lessons of self-tolerance. *Curr Opin Immunol* 2005;**17**:609–15.
72. Padeh S, Theodor R, Jonas A, Passwell JH. Severe malabsorption in autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy syndrome successfully treated with immunosuppression. *Arch Dis Child* 1997;**76**:532–4.
73. Perheentupa J. APS-I/APECED: the clinical disease and therapy. *Endocrinol Metab Clin North Am* 2002;**31**: 295–320.
74. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med* 1990;**322**:1829–36.
75. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002;**298**:1395–401.
76. Soderbergh A, Myhre AG, Ekwall O, Gebre-Medhin G, Hedstrand H, Landgren E, et al. Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 2004; **89**:557–62.
77. Hogenauer C, Meyer RL, Netto GJ, Bell D, Little KH, Ferries L, et al. Malabsorption due to cholecystokinin deficiency in a patient with autoimmune polyglandular syndrome type I. *N Engl J Med* 2001;**344**:270–4.
78. Ekwall O, Hedstrand H, Grimelius L, Haavik J, Perheentupa J, Gustafsson J, et al. Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* 1998;**352**:279–83.
79. Gianani R, Eisenbarth GS. Autoimmunity to gastrointestinal endocrine cells in Autoimmune Polyendocrine Syndrome Type I. *J Clin Endocrinol Metab* 2003;**88**:1442–4.
80. Skoldberg F, Portela-Gomes GM, Grimelius L, Nilsson G, Perheentupa J, Betterle C, et al. Histidine decarboxylase, a pyridoxal phosphate-dependent enzyme, is an autoantigen of gastric enterochromaffin-like cells. *J Clin Endocrinol Metab* 2003;**88**:1445–52.
81. Zuklys S, Balciunaite G, Agarwal A, Fasler-Kan E, Palmer E, Holländer GA. Normal thymic architecture and negative selection are associated with aire expression, the gene defective in the Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED). *J Immunol* 2000;**165**:1976–83.
82. Villasenor J, Benoist C, Mathis D. AIRE and APECED: molecular insights into an autoimmune disease. *Immunol Rev* 2005;**204**:156–64.
83. Rizzi M, Ferrera F, Filaci G, Indiveri F. Disruption of immunological tolerance: role of AIRE gene in autoimmunity. *Autoimmun Rev* 2006;**5**:145–7.
84. Chin RK, Lo JC, Kim O, Blink SE, Christiansen PA, Peterson P, et al. Lymphotoxin pathway directs thymic Aire expression. *Nat Immunol* 2003;**4**:1121–7.
85. Kogawa K, Nagafuchi S, Katsuta H, Kudoh J, Tamiya S, Sakai Y, et al. Harada M: Expression of AIRE gene in peripheral onocyte/dendritic cell lineage. *Immunol Lett* 2002;**80**: 195–8.
86. Sillanpää N, Magureanu CG, Murumagi A, Reinikainen A, West A, Manninen A, et al. Autoimmune regulator induced changes in the gene expression profile of human monocyte-dendritic cell-lineage. *Mol Immunol* 2004;**41**:1185–98.
87. Ramsey C, Hassler S, Marits P, Kampe O, Surh CD, Peltonen L, et al. Increased antigen presenting cell-mediated T cell activation in mice and patients without the autoimmune regulator. *Eur J Immunol* 2006;**36**:305–17.
88. Adamson KA, Pearce SH, Lamb JR, Seckl JR, Howie SE. A comparative study of mRNA and protein expression of the autoimmune regulator gene (Aire) in embryonic and adult murine tissues. *J Pathol* 2004;**202**:180–7.
89. Roifman CM. Human IL-2 receptor alpha chain deficiency. *Pediatr Res* 2000;**48**:6–11.
90. Mirakian R, Hill S, Richardson A, Milla PJ, Walker-Smith JA, Bottazzo GF. HLA product expression and lymphocyte subpopulations in jejunum biopsies of children with idiopathic protracted diarrhoea and enterocyte autoantibodies. *J Autoimmun* 1988;**1**:263–77.
91. Cepek KL, Shaw SK, Parker CM, et al. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the αEβ7 integrin. *Nature* 1994;**372**:190–3.
92. Westendorf AM, Templin M, Geffers R, Deppenmeier S, Gruber AD, Probst-Kepper M, et al. CD4+ T cell mediated intestinal immunity: chronic inflammation versus immune regulation. *Gut* 2005;**54**:60–9.
93. Liu Z, Lefrancois L. Intestinal epithelial antigen induces mucosal CD8 T cell tolerance, activation, and inflammatory response. *J Immunol* 2004;**173**:4324–30.
94. Seidman EG, Lacaille F, Russo P, et al. Successful treatment of autoimmune enteropathy with cyclosporine. *J Pediatr* 1990; **117**:929–32.
95. Oliva-Hemker MM, Loeb DM, Abraham SC, Lederman HM. Remission of severe autoimmune enteropathy after treatment with high-dose cyclophosphamide. *J Pediatr Gastroenterol Nutr* 2003;**36**:639–43.
96. Sanderson IR, Phillips AD, Spencer J, et al. Response to autoimmune enteropathy to cyclosporin A therapy. *Gut* 1991;**32**:1421–5.
97. Bousvaros A, Leichtner AM, Book L, et al. Treatment of pediatric autoimmune enteropathy with tacrolimus (FK506). *Gastroenterology* 1996;**111**:237–43.
98. Bindl L, Torgerson T, Perroni L, Youssef N, Ochs HD, Goulet O, et al. Successful use of the new immune-suppressor sirolimus

in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). *J Pediatr* 2005;**147**:256–9.
99. Quiros-Tejeira R, Ament ME, Vargas JH. Induction of remission in a child with autoimmune enteropathy using mycophenolate mofetil. *J Pediatr Gastroenterol Nutr* 2003;**36**:482–5.
100. Vanderhoof JA, Young RJ. Autoimmune enteropathy in a child: response to infliximab therapy. *J Pediatr Gastroenterol Nutr* 2002;**34**:312–6.
101. von Hahn T, Stopik D, Koch M, Wiedenmann B, Dignass A. Management of severe refractory adult autoimmune enteropathy with infliximab and tacrolimus. *Digestion* 2005;**71**:14114.
102. Baud O, Goulet O, Canioni D, Le Deist F, Radford I, Rieu D, et al. Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. *N Engl J Med* 2001;**344**:1758–62.
103. Mazzolari E, Forino C, Fontana M, D'Ippolito C, Lanfranchi A, Gambineri E, et al. A new case of IPEX receiving bone marrow transplantation. *Bone Marrow Transplant* 2005;**35**:1033–4.
104. Smyk-Pearson SK, Bakke AC, Held PK, Wildin RS. Rescue of the autoimmune scurfy mouse by partial bone marrow transplantation or by injection with T-enriched splenocytes. *Clin Exp Immunol* 2003;**133**:193–9.
105. Cavazzana-Calvo M, Hacein-Bey S, Yates F, et al. Gene therapy of severe combined immunodeficiencies. *J Gene Med* 2001;**3**:201–6.
106. June CH, Blazar BR. Clinical application of expanded CD4+25+ cells. *Sem Immunol* 2006;**18**:78–88.
107. Kasprowicz DJ, Smallwood PS, Tyznik AJ, Ziegler SF. Scurfin (FoxP3) controls T-dependent immune responses in vivo through regulation of CD41 T cell effector function. *J Immunol* 2003;**171**:1216–23.
108. Kapel N, Roman C, Caldari D, Sieprath F, Canioni D, Khalfoun Y, et al. Faecal tumor necrosis factor-alpha and calprotectin as differential diagnostic markers for severe diarrhea of small infants. *J Pediatr Gastroenterol Nutr* 2005;**41**: 396–400.