

## **CHAPTER 33**

### **CELIAC DISEASE**

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#### **DEFINITION**

Celiac disease (CD), also called gluten-sensitive enteropathy, is a permanent intestinal intolerance to dietary wheat gliadin and related proteins that produces mucosal lesions in genetically susceptible individuals.

#### **HISTORICAL BACKGROUND**

CD was first accurately described by Samuel Gee in 1888, but it was not until the early 1950s that Dicke in Holland established the role of wheat and rye flour in the pathogenesis of the disease and identified the protein known as gluten as the harmful factor in those cereals (1). A major contribution to the understanding of the disease came from the development of methods for peroral biopsy of the jejunal mucosa, which allowed definition of the mucosal lesion (2), and from the definition of diagnostic criteria published in 1969 by the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) (3). In recent years a substantial amount of data have been produced that have profoundly changed our understanding of epidemiology, clinical aspects and pathogenesis of CD, opening new perspectives for treatment.

## CEREAL PROTEINS AND OTHER ENVIRONMENTAL FACTORS

### Cereal Proteins

The cereals that are toxic for patients with CD are wheat, rye, and barley; rice and maize are non-toxic and are usually used as wheat substitutes in the diet of patients with CD. The toxicity of oats has been reassessed in recent years. It has in fact been shown that the use of oats as part of a gluten-free diet has no unfavourable effects on adult patients in remission and does not prevent mucosal healing in patients with newly diagnosed disease (4, 5). Nonetheless, a few celiacs seem not to tolerate oats, showing raised levels of intestinal interferon gamma mRNA after challenge (6); furthermore, the fear that small amounts of gliadin could contaminate oats, suggests caution before the inclusion of oats is advocated in the diet of celiac patients. Cereal grains belong to the grass family (Gramineae). Grains considered toxic for celiac patients (rye, barley, and, to a lesser extent, oats) bear a close taxonomic relationship to wheat, whereas nontoxic grains (rice and maize) are taxonomically dissimilar (Fig. 1). Wheat seed endosperm contains heterogeneous protein classes differentiated, according to their extractability and solubility in different solvents, into albumins, globulins, gliadins, and glutenins. Gliadins are monomers, whereas glutenins form large polymeric structures. Gliadins have been classified according to their N terminal amino acid sequences into alpha, gamma and omega types; glutenins are subdivided into high molecular weight glutenins and low molecular weight glutenins. The wheat toxicity results from the gliadin protein fraction, and the toxicity of cereals other than wheat is most likely associated with prolamin fractions equivalent to

gliadins in the grain of these other species; on the other hand, glutenin peptides have been shown to be immunogenic for mucosal T cells from celiac patients (7). The amino acid sequence(s) responsible for the disease have not been fully elucidated, also because different part of the gliadin molecules show different biological properties, all potentially involved in the pathogenesis of the disease; several HLA-DQ2 restricted T cell epitopes have been found clustering in regions of gliadin rich in proline residues (8), target of the tissue transglutaminase (TG2) deamidating activity (see pathogenesis); other sequences have been shown to activate innate immunity mechanisms (9), or interact with CD8+ cytotoxic T cells (10). Organ culture systems have validated the biological activity of some of these sequences, but there is no doubt that in vitro methodology must be paralleled by in vivo challenge studies. In fact, histologic changes have been shown to occur in the celiac intestinal mucosa after challenge with synthetic peptides encompassing the 31-49 (11), 31-43 and 44-55 (12), and, more recently, the 56-75 sequence of A-gliadin (13).

## **Other Environmental Factors**

The high concordance rate for monozygotic twins, and the similar risk shown by dizygotic twins and other siblings, suggest that a shared environment (gluten antigen aside) has little or no effect (14). On the other hand, the relevance of environmental factors other than gluten in CD is suggested by the significant changes in the incidence of the disease by time and place. Feeding practices seem to be relevant. Recently, Sweden has experienced an epidemic of symptomatic celiac disease in

children aged less than two years; the abrupt increase and decline in the incidence of the disease coincided with changes in the dietary pattern of infants (15). The risk of coeliac disease was found to be greater when gluten was introduced in the diet in large amounts (16); on the contrary, it was reduced if children were still breastfed when dietary gluten was introduced (16). This finding add to previous case referent studies showing that breast feeding is protective, while the age of introduction of gluten in infants diet has no effect on risk (17). An important question is whether favourable dietary habits simply postpone the onset of celiac disease, or reduce the overall lifetime risk of the disease; a challenging possibility that requires further studies is that celiac disease might be delayed, or even prevented, intervening in genetically susceptible individuals on dietary patterns of the first year of life. Among other environmental factors that could play a possible role in CD, infective factors have also been considered. The possible role played in pathogenesis by alpha interferon (18), and the epidemiological evidence of increased risk in relation to the month of birth (19), have suggested the possible involvement of a viral infection, but no evidence has so far supported this hypothesis.

## **GENETICS OF CELIAC DISEASE**

### **Family Studies**

Susceptibility to CD is determined to a significant extent by genetic factors. That is suggested by the occurrence of multiple cases in families, the prevalence of CD found among first-degree relatives being approximately 10% (20). Moreover, as

many as 75% of monozygotic twins have been found to be concordant with the disease (14). The concordance rate among HLA-identical siblings is about 30%, indicating that a significant part of the genetic susceptibility maps to the HLA region on chromosome 6.

## **Genetic Markers**

The strongest association of CD is with the HLA class II D region markers, class I and class II region gene associations being secondary to linkage disequilibrium. It has been suggested that the primary association of CD is with the DQ  $\alpha\beta$  heterodimer encoded by the DQA1 \*05 and the DQB1\*02 genes (21). Such a DQ molecule has been found to be present in 95% or more of celiac patients compared with 20% to 30% of controls. The data available on DQ2-negative celiac patients indicate that they almost invariably are HLA DQ8 positive (DQA1\*0301/DQB1\*0302) (22). A gene dosage effect has been suggested, and a molecular hypothesis for such phenomenon has been proposed based on the impact of the number and quality of the HLA DQ2 molecules on gluten peptide presentation to T cells (23). The most likely mechanism to explain the association with HLA class II genes is, in fact, that the DQ molecule binds a peptide fragment of an antigen involved in the pathogenesis of CD to present it to T cells.

Other non-HLA genes could confer susceptibility to CD. Considering the relevance of the immune response in the pathogenesis of the disease, candidate genes are those influencing the T cell response. Among those, several reports imply involvement of

the gene for the negative costimulatory molecule CTLA4 or a neighbouring gene (24). A series of whole genome screening studies have been performed in CD (25). The region that has most consistently been linked to CD is on the long arm of chromosome 5 (5q 31-33) (25). There is also evidence for susceptibility factors on the chromosomes 11q (26) and 19 (27).

## **EPIDEMIOLOGY**

The reported prevalence of symptomatic CD is 1 in 1000 live births, with a range from 1 in 250 (observed in Sweden) to 1 in 4000 (observed in Denmark) (28). The prevalence in women appears to be greater than in men. Population based screening studies have clearly shown that CD is underdiagnosed, clinical CD representing the top of the iceberg. A recent Finnish study has shown that the prevalence of biopsy proven CD is at least 1: 99 (29) children, indicating CD as one of the most common genetically based disease. Similar prevalence have been indicated in most European Countries, in North Africa, in South America and in USA (30).

Even when there is no policy for actually screening the entire population, the pick-up rate of coeliac patients within a health care system can be greatly increased by general awareness of the protean clinical manifestations of CD. By a policy of aggressive serological testing of family members and other at risk groups (Table

33.1), and by carrying out duodenal biopsy in every patient who has an upper GI endoscopy, a prevalence of CD similar to that emerged from mass screening studies has been revealed (31).

## **PATHOGENESIS**

Coeliac disease is a T cell mediated chronic inflammatory disorder with an autoimmune component. It is likely that the loss of tolerance is somewhat related to the peculiar physicochemical properties of gliadin. Altered processing by intraluminal enzymes, changes in intestinal permeability, activation of innate immunity mechanisms precede the activation of the adaptive immune response. Both epithelium and lamina propria are places where the immune response takes place (Fig 2).

### **Low digestibility of gliadin**

In the brush border of enterocytes are present enzymes able to hydrolyse peptide bonds containing proline (and glutamic acid); these enzymes show in treated celiac patients an activity comparable to that exhibited by controls (32). On the other hand, immunodominant epitopes from gliadin are highly resistant to intraluminal and mucosal digestion (33,34), although they are largely hydrolysed during the transport through the small intestinal mucosa (35); the hydrolysis is reduced only during the passage through the atrophic celiac mucosa (35). It is possible to hypothesize that incomplete degradation favour the immunostimulatory and toxic effects of these sequences.

## **Activation of innate immunity**

Gliadin has the ability to induce a complete activation of the immune system (adaptive and innate). In fact, in the treated celiac mucosa only the non-immunodominant peptide 31-43 (and not the peptide 56-68) induce rapid activation of the innate immune system, as revealed by rapid, IL-15, CD83 (a marker of mature dendritic cells), COX-2 (an enzyme rapidly induced during inflammation), and CD 25 expression by CD3- cells (non T-cells) of the innate immune system (9). A particular gliadin fragment, therefore, activates the innate immune system timing the in situ T cell recognition of dominant gliadin epitopes.

## **Lamina propria gliadin specific CD4+ activation**

The activation of lamina propria T cells by gliadin peptides in the contest of HLA-DQ2 or DQ8 molecules has been long recognised as one of the key events in pathogenesis of CD. A large number of T-cell stimulating peptides have been characterized in gluten proteins (7). Studies by Sollid and Koning have provided a model explaining the interplay between gliadin, DQ2 or DQ8 and TG2. In fact, these gliadin-specific T-cell responses have been found to be enhanced by the action of TG2 (36); the enzyme converts particular glutamine residues into glutamic acid, which results in higher affinity of these gliadin peptides for HLA-DQ2 or HLA-DQ8. The pattern of cytokines produced following gliadin activation is clearly dominated by  $\gamma$  IFN (Th1 skewed), but unexpectedly IL12 is not expressed. IL18 (37) and interferon alpha (18) are both candidate as driving factors for Th1 differentiation and gamma interferon production. The signalling pathway related to gamma interferon

has also been explored: persistent STAT1 activation was observed (38) as well as enhanced interferon regulations factor 1 (IRF1) expression (39). IL10 is also increased, but not enough to control the strong gamma IFN production (40). Downstream T cell activation, a complex remodelling of the mucosa takes place, involving increased levels of metalloproteinases (41) and growth factors (42) which leads to the classical flat mucosa.

### **Intraepithelial lymphocytes**

Intraepithelial lymphocytes are a hallmark of CD. Mechanisms other than lamina propria T cell activation are likely to be responsible for their recruitment. Alterations of intestinal epithelium occur in untreated CD; MIC and HLA-E are induced in response to stress and gamma interferon, or to gluten itself. MIC and HLA-E are recognised by NK receptors (NKG2D and CD94) present on intraepithelial lymphocytes and up-regulated by IL15 (43). More recently a direct recognition of gliadin peptides by CD8+ lymphocytes has also been demonstrated (10). Activation of intraepithelial lymphocytes is likely to contribute to epithelial cell apoptosis and villous atrophy via FAS/FAS ligand (44) and perforin-granzyme (45) pathways.

### **Coeliac disease and autoimmunity**

This Th1 skewed immunological picture is common to other conditions of organ autoimmunity; moreover, in recent years, the demonstration of autoimmune phenomena and the strict association with other autoimmune diseases have favoured the inclusion of CD itself in the number of autoimmune diseases. The most evident

expression of autoimmunity is the presence of serum antibodies to tissue transglutaminase (TG2) (46). Tests based on the measurement of IgA antibodies to the enzyme discriminates very efficiently coeliac patients (see diagnosis). As far as mechanisms of damage are concerned, antibodies to TG2 inhibit its activity in a dose dependent manner, both in vitro and in situ, although the inhibition is only partial (47). In in vitro models it has been shown that such antibodies interfere with differentiation of epithelial cells, probably disturbing TGF beta-mediated epithelial-fibroblast crosstalk (48). Several evidences suggest that TG2 autoantibodies are primarily produced in the gut mucosa of celiac patients (49), where they can be detected before they appear in the circulation. The finding of IgA deposits on extracellular TG2 in the liver, lymphnodes and muscles indicates that TG2 is accessible to the gut-derived autoantibodies (50). Several extraintestinal clinical manifestations of CD (e.g. liver, heart, nervous system) are possibly related to the presence of autoantibodies in situ. The mechanisms leading to autoimmunity are largely unknown. Upregulation of TG2 in inflamed sites may generate additional antigenic epitopes by crosslinking or deamidating external or endogenous proteins. Unmasking of cryptic epitopes has also been hypothesized in the context of an inflamed environment where antigen processing and presentation may be more efficient. Finally, help for the production of autoantibodies given by gliadin-specific T cells in the mucosa has been advocated to explain why these autoantibodies are dependent on the presence of gluten in the diet (51). TG2 are not the only autoantibodies present in CD: antibodies to actin (52) and to calreticulin (53) have

been detected in celiac sera. New autoantigens (enolase, ATP synthase beta chain) have recently been identified by mass fingerprinting approach (54).

## PATHOLOGY

CD manifests itself pathologically as a disease of the small intestine. A distinct pattern of abnormalities has been observed; the features include 1) partial to total villous atrophy; 2) elongated crypts; 3) increased mitotic index in the crypts; 4) increased intraepithelial lymphocytes (IELs); 5) infiltrations of plasma cells and lymphocytes as well as mast cells, eosinophils, and basophils in the lamina propria; and 6) absence of identifiable brush border and abnormalities in the epithelial cells, which become flattened, cuboidal, and pseudostratified. However, these changes are not pathognomonic of CD, and most of them may be seen in other entities, such as cow's milk or soy protein hypersensitivity, intractable diarrhea of infancy, heavy infestation with Giardia lamblia, primary immunodeficiencies, tropical sprue, bacterial overgrowth, and intestinal lymphoma. Hence, it is crucial to establish the gluten dependence of the jejunal lesion.

It has now become clear that, from a pathological point of view, the small intestinal enteropathy in celiac disease may be of variable severity. A spectrum of pathological changes of increasing severity, ranging from a mild picture characterized by infiltration of the epithelium by lymphocytes, to crypt hyperplasia, to villous atrophy. In cases presenting with mild enteropathy immunohistochemical analysis has gained importance. Signs of activated mucosal cell mediated immunity (enhanced epithelial

expression of HLA -DR and -DP, increased density of CD25 positive cells) have been found in jejunal biopsies, for instance in first degree relatives of coeliac patients, considered for that to be “potential” coeliacs (55). More specific for coeliac disease, but both sensitivity and specificity far from being absolute, is the finding of increased density of intraepithelial lymphocytes with gamma/delta T cell receptor (56) (Fig 3). Nonetheless, these investigations are available only in specialised laboratories. In the presence of very mild signs of small intestinal enteropathy, such as the increased density of intraepithelial lymphocytes or the presence of immunohistochemical signs of T cell activation, the demonstration of the gluten dependence of such signs becomes central, in other words their disappearance after a period of gluten free diet; but this is not always easy to demonstrate (57).

## **CLINICAL PRESENTATION**

Clinical features of CD differ considerably. Intestinal symptoms are common in children diagnosed within the first two years of life; failure to thrive, chronic diarrhoea, vomiting, abdominal distension, muscle wasting, anorexia and irritability are present in most cases. With the shifting of the age at presentation of the disease later in childhood, and with the wider and more liberal use of serological screening tests, extra-intestinal manifestations, without any accompanying digestive symptom, have increasingly been recognized, affecting almost all organs.

Short stature has probably been the first isolated extraintestinal presentation of CD to be recognized; already in the early 80s, approximately 10% of patients with

isolated short stature undergoing jejunal biopsy were found to have a total villous atrophy (58). Nonetheless, both in children and in adults, the most frequent extraintestinal manifestation of CD is iron-deficiency anemia (59). The prevalence of CD in adult patients with microcytic anemia unresponsive to iron therapy is as high as 8.5% (60). As far as the locomotor apparatus is concerned, arthritis and arthralgia as presentation symptoms of CD were described by Maki (61). A screening study using determination of antigliadin antibodies in patients with idiopathic osteoporosis has shown an incidence of CD ten-fold higher than in healthy people (62). As a matter of fact the majority of adult coeliac patients suffer from metabolic osteopathy; gluten free diet normalizes bone mass only in a proportion of subjects. Patients whose CD was diagnosed in childhood and who have since then been receiving gluten free diet have a bone mineral density similar to that of healthy controls (63). The nervous system is also involved in CD. An Italian report has proposed an association between CD and epilepsy in patients with bilateral occipital calcifications (64) (Fig 4); in such patients gluten-free diet beneficially affects the course of epilepsy only when started soon after epilepsy onset. Moreover, gluten sensitivity is proposed to be common in patients with neurological diseases of unknown etiology. One example is gluten ataxia (65), a recently described condition, which seems to affect 60% of unclassified ataxia and which is identified on the basis of high serum titres of antigliadin antibody; up to 40% of them are coeliacs. Peripheral neuropathies of axonal and demyelinising types have also been reported and may respond to elimination of gluten from the diet. There is no doubt that the liver is a target of gluten toxicity in

CD. Isolated hypertransaminasemia has been recognized as a possible presentation of CD; it may be expression of chronic "cryptogenic" hepatitis resolving on a gluten-free diet (66). As 4% of patients with "cryptogenic" hepatitis are affected by otherwise silent CD, serological screening for CD is mandatory in such patients (67). Recently, patients with severe liver disease have been described in whom gluten-free diet prevented progression to hepatic failure, even in cases in which liver transplantation was considered (68).

Patients having fertility problems may have subclinical CD: unexplained infertility may be the only sign of CD (69). Similarly, unfavourable outcome of pregnancy such as recurrent abortions, or premature delivery, or low weight at birth, are more often observed in undiagnosed or untreated CD patients (70). Different degrees of dental abnormalities have been described in children with CD; severe enamel hypoplasia is present in up to 30 percent of untreated CD children (71). Alopecia areata has been reported to be the only clinical manifestation of CD (72).

A special place in this list is taken by dermatitis herpetiformis (DH), a gluten dependent condition characterized by a symmetric pruritic skin rash with subepidermal blisters and granular subepidermal deposits of IgA in remote uninvolved skin. Most patients with DH have abnormal small intestinal biopsy pathology, histologically indistinguishable from that of CD, although usually less severe. Approximately 60% of children with DH have been reported to have subtotal villous atrophy, and 30% have partial villous atrophy on jejunal biopsy (73). The histologic changes return to normal after dietary exclusion of gluten. Therapy with

dapsone usually leads to prompt clinical improvement; a strict gluten-free diet permits a reduction or discontinuation of dapsone over a period of months. Improvement of skin lesions on a gluten-free diet seems to occur also in patients with no evident mucosal abnormality; in the same patients the rash recurs with a gluten rechallenged.

Different may be the mechanisms operating in these different situations. Such extradigestive manifestations may more likely result from the intestinal damage and consequent nutritional deficiencies (e.g. anemia, osteopenia) and/or due to the deranged (auto?)immune response (e.g. skin, liver, joints, CNS involvement).

## **ASSOCIATED DISEASES**

Some diseases, many with an autoimmune pathogenesis, are found with a higher than normal frequency in celiac patients; among these are thyroid diseases (74), Addison's disease (75), pernicious anemia (76), autoimmune thrombocytopenia (77), sarcoidosis (78), insulin-dependent diabetes mellitus (79), alopecia (80) and cardiomyopathies (81). Such associations have been interpreted as a consequence of the sharing of identical HLA haplotypes (e.g., B8, DR3). Nevertheless, the relation between CD and autoimmunity is more complex. In CD patients there is evidence that the risk of developing autoimmune diseases seems to be directly correlated to the duration of gluten exposure (82).

An increased incidence of CD has been found in Down syndrome patients compared with the general population (83). Similarly in Turner's syndrome and Williams

syndrome higher number of CD cases was also observed (84,85). Selective IgA deficiency is also a condition associated with celiac disease (86). Screening test alternatives to those based on the measurement of IgA isotype antibodies must be adopted in such patients.

## **LABORATORY FINDINGS**

### **Tests for Malabsorption and permeability**

Tests for malabsorption may be of help in approaching the diagnosis of CD. Determination of hemoglobin, serum iron, calcium, phosphorus, alkaline phosphatase, magnesium, and protein levels may be indicative of malabsorption. In particular, red blood cell folate levels have been found to be a sensitive index (20) Prothrombin levels should be checked in any case before intestinal biopsy is performed.

Over the last few years, tests based on intestinal permeability to sugars have been found of value as a noninvasive screening tool. Most of them are based on the differential intestinal absorption of two nonmetabolized sugars. In untreated CD the absorption of the smaller probe (mannitol, rhamnose) is reduced owing to the loss of intestinal surface area, and that of the larger one (lactulose, cellobiose) is reported as increased, because paracellular pathways are "leakier" and/or increased in number. Expression of the results as a ratio of disaccharide: monosaccharide recovery gives clear separation between normal cases and patients with CD (87,88). Although this test has a sufficient sensitivity for abnormalities of jejunal mucosa, it is also

characterized by a low specificity for CD and false-positive results occurring mainly in patients with mucosal abnormalities due to other causes (e.g., Crohn's disease, atopic eczema, food allergy, and damage induced by nonsteroidal anti-inflammatory agents).

## Serological Tests

Serological tests have also acquired a strong importance, in particular the search for antiendomysial antibodies; more recently, after the demonstration that tissue transglutaminase is the main autoantigen recognised by endomysial antibodies (46), antitissue transglutaminase antibodies have shown a great sensitivity and specificity for the diagnosis of celiac disease (89) (Table 33.2). The specificity is almost absolute also considering that subjects with positive serum endomysial antibodies and normal histology have a high chance to develop enteropathy in the following years (90). On the other hand, a note of caution comes from studies in adult patients indicating a lower sensitivity, particularly in subjects with milder form of enteropathy (91). In the last years after first generation test based on the use of guinea pig antigen, the most recent assays, based on the use of recombinant human enzyme as coating antigen in ELISA, have further improved the diagnostic efficacy. We can expect further improvement by the definition of the epitopes of tissue transglutaminase recognised by coeliac sera (49). However, a series of technical problems common to other ELISA tests still are present, for example the correct definition of cut-off values. Furthermore, while it has now been clearly shown that the site of production

of endomysial and tissue transglutaminase antibodies is the gut mucosa (92), and that their presence in the serum is the result of their spill-over, is more than a working hypothesis the possibility that there are “seronegative” subjects with presence of such antibodies only in their intestinal secretions (93).

## **HLA**

As already mentioned, coeliac disease is strongly associated with some HLA allele specificities, namely those serologically recognised as HLA DQ2 (90-95% of cases) and HLA DQ8 (approximately 5% of cases); less than 2% of coeliac patients lack both HLA specificities; at the same time approximately one third of our “normal” population has one or the other marker; that means that the demonstration of being DQ2 and/or DQ8 positive has a strong negative predictive value, but a very weak positive predictive value for the diagnosis of coeliac disease. With these limitation the test may prove useful to exclude coeliac disease in subjects on a gluten-free diet, or in subjects belonging to at risk group (eg first degree relatives, insulin-dependent diabetics, patients with Down’s syndrome) to avoid long term follow-up. Simplified methods based on molecular typing of only the alleles associated to coeliac disease have been set up (94).

## **DIAGNOSIS**

The two requirements mandatory for the diagnosis of CD remain: 1) the finding of villous atrophy with hyperplasia of the crypts and abnormal surface epithelium, while

the patient is eating adequate amounts of gluten; and 2) a full clinical remission after withdrawal of gluten from the diet. The finding of circulating IgA antibodies to gliadin, reticulin, and endomysium at the time of diagnosis, and their disappearance on a gluten-free diet, adds weight to the diagnosis. A control biopsy to verify the consequences on the mucosal architecture of the gluten-free diet is considered mandatory only in patients with equivocal clinical response to the diet and in patients asymptomatic at first presentation (as is often the case in patients diagnosed during screening programs, e.g., first-degree relatives of celiac patients) (95).

Gluten challenge is not considered mandatory, except under unusual circumstances. These include situations where there is doubt about the initial diagnosis, for example when no initial biopsy was done, or when the biopsy specimen was inadequate or not typical of CD. Gluten challenge should be discouraged before the age of 7 years and during the pubertal growth spurt. Once decided, gluten challenge should always be performed under strict medical supervision. It should be preceded by an assessment of mucosal histology and performed with a standard dose of at least 10 g of gluten per day without disrupting established dietary habits. A further biopsy is taken when there is a noticeable clinical relapse or, in any event, after 3 to 6 months. Serologic tests (IgA gliadin, reticulin and endomysium antibodies, absorptive and permeability tests), more than clinical symptoms, can be of help in assessing the timing of the biopsy to shorten the duration of the challenge (96).

In a situation where jejunal histology has lost specificity, and with the growing

contribution by serology, and to a less extent by HLA, many propose to move to a new diagnostic approach mainly based on antibodies and genetics. Nonetheless, the contribution of the analysis of jejunal biopsies may still be very important; in particular the study of the intestinal mucosa could prove decisive in the definition of the disease state. In fact, considering that more than 30% of the population has the HLA alleles implicated in celiac disease and more than 1% a positive serology, with an increasing number of subjects showing signs of minor enteropathy, the same definition of disease and the consequent need of a gluten free diet still awaits a definitive response. It is quite clear that subjects with severe gluten dependent enteropathy face a series of health risks, mainly nutritional; they probably have also higher risk of developing autoimmunity, and, although less than previously thought, of presenting neoplastic complications. On the contrary, little is known of those with minor enteropathy, maybe silent from a clinical point of view, for instance subjects belonging to groups such as first degree relatives or insulin-dependant diabetics. A recent report (57) showing nutritional deficiencies also in patients with minor enteropathy, positive serology and “right” genetics, resolving on a gluten free diet, seems to indicate in all these subjects the need for a gluten free diet. Prospective studies are necessary. Until serological methods are improved, the genetic make up of celiac patients is better defined, it seems wise for a diagnosis of coeliac disease still rely on a combined approach based of clinical criteria, histology, serology and genetics.

## **THERAPY**

### **Gluten-Free Diet**

Since the identification of gluten as etiologic factor in CD, a strict gluten-free diet has become the cornerstone of the management of such patients. Their diet should exclude wheat, rye, and barley; the inclusion of oats is still debated, because the toxicity has not been definitively disproved and because of the fear that small amounts of gliadin could contaminate oats; rice and maize are nontoxic and are usually used as wheat substitutes.

The clinical response to withdrawal of gluten is often dramatic, but it must be stressed that the gluten-free diet is recommended for both symptomatic and asymptomatic patients with CD. Normalization of the jejunal histology occurs after about 6 months. The most likely cause of lack of response is failure to adhere strictly to the diet, but the possibility of sensitivity to other dietary proteins, lymphoma, and immunodeficiency should also be considered.

All the present evidence strongly supports the view that restriction of gliadin and related prolamines should be complete and for life for all patients. A gluten-free diet is thought to be protective against the development of maligned disease. Patients with celiac disease have a risk of small bowel adenocarcinoma that is about 80-fold greater than that of the general population (97). The predominant celiac-associated lymphoma is the enteropathy-associated T-cell lymphoma, which doses not respond well to chemotherapy and is rapidly fatal (98). Malignancies are not the only risk that celiac patients not compliant with gluten-free diet are exposed to; nutritional

deficiencies (99), osteoporosis, and more recent evidence that the risk of developing autoimmune diseases is related to the duration of exposure to gluten are additional concerns (82).

## **Other Therapeutic Measures**

Specific vitamin, mineral, and trace element deficiencies should be corrected. Replacement therapy can generally be discontinued after clinical and histologic recovery on gluten-free diet has been documented.

Other dietary measures are rarely needed. The disaccharidase activity is greatly depressed in atrophic celiac mucosa, but it is advisable to remove milk and lactose-containing products only if intolerance is clinically manifest. Secondary lactase deficiency resolves rapidly after institution of a gluten-free diet, and milk can usually be tolerated after 2 to 4 weeks of the diet.

When patients present in celiac crisis, rapid correction of volume depletion and fluid and electrolyte abnormalities is crucial; steroid therapy is helpful. Short-term administration of steroids (2 mg/kg per 24 hours of prednisone for 1-2 weeks) may also be used in severely ill infants in whom anorexia and malabsorption do not rapidly respond to the gluten-free diet.

## **Therapeutic strategies for the future.**

Significant progress has been made in recent years in the understanding of the cellular and molecular basis of CD and in the consequent identification of possible targets for therapy. Recently it has been shown that, because of the high

proline content, gliadin peptides are highly resistant to digestive processing by pancreatic and brush border proteases. Enzyme supplement therapy using bacterial endopeptidases has been proposed to promote complete digestion of cereal proteins and thus destroy T cell multipotent epitopes. The identification of gliadin peptide sequences having biological effects, either through non immune-mediated mechanisms, or by activation of T cells, is important. Breeding programs and/or transgenic technology may lead to production of wheat that is devoid of biologically active peptide sequences. The identification of specific epitopes may also provide a target for immunomodulation of antigenic peptides. Engineered peptides may potentially bind to HLA molecules but not T cell receptors (TCR), or bind TCR but switch a proinflammatory Th1 to a Th2 or protective Th3 response. Other promising areas include inhibition of the innate immune response activated by gliadin peptides, preventing gliadin presentation to T cells by blocking HLA binding sites, use of TG2 inhibitors, and assessing IL-10 as a tool to promote tolerance. An immunomodulatory approach will need to have a safety profile equivalent to that of the gluten-free diet, but with the advantage of increased compliance.

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