CELIAC SPRUE: AN UPDATE

Sami N. Arslanlar, MD

INTERNAL MEDICINE GRAND ROUNDS
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS

February 26, 2004

Sami N. Arslanlar, MD
William T. and Gay F. Solomon Division of General Internal Medicine
Internal Medicine Grand Rounds
February 26, 2004

Interests: GI/Hepatology and inflammatory bowel disease

INTRODUCTION

Celiac sprue, celiac disease, gluten-sensitive enteropathy – the number of names by which this disease has been known in the literature hints at the confusion that has been associated with it. For the purposes of this discussion, the term Celiac Sprue (CS) will be used, but the others are still used interchangeably in the literature. Celiac sprue (CS) is a fascinating disease with a long history. The insights that have emerged over the last century reveal that it is has long been recognized and recent growing understanding of the disease provides insight of this condition. It has become an example of a disease the product of in which unique interaction of genetic, environmental, and immunologic factors aspects meet to create a disease whose full aspects are increasingly coming to light. In this discussion, the term celiac sprue will be used preferentially but the other names are found extensively in the literature and should be kept in mind. Celiac sprue is characterized by (1) small intestinal malabsorption of nutrients after the ingestion of wheat gluten or related proteins from rye and barley; (2) a characteristic, though, not specific, villous atrophy of the small intestinal mucosa; (3) prompt clinical and histologic improvement after adherence to a strict gluten-free diet; and (4) clinical relapse when gluten is reintroduced.[1]

Classically, CS presents in the first and second years of childhood, when cereals are introduced to the diet for the first time. Signs and symptoms include steatorrhea with or without vomiting and occasional crampy abdominal pain. The child typically fails to thrive and is apathetic and irritable, and exhibits with muscle wasting, hypotonia, and abdominal distention. Increasingly this is also understood to be a disease diagnosed in adulthood, though the clinical presentation can vary greatly. In the past, celiac sprue was perceived to be a pediatric diagnosis but it is now often made in adult life. The clinical presentation of adults can vary greatly. Symptoms may be gastrointestinal, including diarrhea, steatorrhea, flatulence and weight loss or extraintestinal features, such as anemia and osteopenia.[1]

How and why these symptoms occur will be the topic of this discussion. These characteristics will be discussed in view of the current knowledge of the incidence of this disease, current understanding into the pathogenesis and pathophysiology of this disease, associated conditions, means of diagnosis and possible treatments.

HISTORICAL PERSPECTIVE

Celiac sprue was first described by Aretaeus of Cappadocia in the second century A.D. as an illness associated with the coelom or body cavity. [2] "If diarrhea does not proceed from a slight cause of only one or two days duration and if, in addition the patient's general system be debilitated by atrophy of the body, the celiac disease of a chronic nature is formed." "Sprue" was coined in the 18th century by Sir Patrick Manson in 1880[3] and is derived from the Dutch word "spruw," which means thrush, so named because of the high prevalence of aphthous mouth ulcers in these patients with chronic diarrhea and wasting.[1]

The first detailed clinical description of the "coeliac affection" is attributed to Samuel Gee.[4] Samuel Gee, a physician at St. Bartholomew's Hospital in London who in 1888 described the condition, and reported that the disorder affects all age groups. He described "a child who was fed upon a quart of the best Dutch mussels daily, throve wonderfully but relapsed when the season for mussels was over." He reported that "if the patient can be cured at all it must be by means of the diet. Next season he could not be prevailed upon to take them. This is an

experiment I have not yet been able to repeat, but if the patient can be cured at all, it must be by means of the diet." In 1910, Benecke provided the first description of the mucosal transformation characteristic of celiac disease.[4] In 1924, Sidney Haas described his treatment of celiac sprue with a banana-rich diet that he had used effectively in treating anorexia. He excluded bread, crackers, potatoes, and cereals. Bananas were gradually added to the diet usually from the fourth or eighth day. The treatment was continued indefinitely.[5] W. K. Dicke, a Dutch pediatrician during the early 1930s, became convinced that the consumption of bread and wheat flour had an adverse effect on patients with this condition. He noted that children in the Netherlands with CS did well during the war, when wheat flour was scarce, but relapsed when bread became available. His first report appeared in *Het Nederlands Tijdschriftvoor Geneeskunde* in 1941, and his observations were expanded in his doctoral thesis, submitted to the University of Utrecht in 1950. During World War II in the Netherlands, food, particularly cereals used to make bread, was scarce. During this time, the condition of children with celiac disease improved, only to relapse following supply, by the Swedish Air Force, of bread at the end of the war.[2] It was this serendipitous observation that led to the finding that wheat exacerbates celiac disease. Subsequent work in the second half of the 20th century clarified the biochemical, immunologic, and genetic factors that will be discussed later.

EPIDEMIOLOGY

The new availability of diagnostic tests for CS has resulted in revised estimates regarding prevalence of disease. It is suspected that only the tip of the "celiac iceberg" is being identified. With growing awareness of celiac sprue and the advent of serological means of diagnosis, data has emerged about the previously underestimated prevalence of celiac sprue. In 1997, Maki and Collin suggested the concept of the "celiac iceberg," the majority of cases go clinically undetected with either "silent (asymptomatic but with mucosal changes)" or "latent (normal small intestine on gluten-free diet but may develop architectural changes in the future)" disease.[6] The availability of serologic markers to screen patients and the increasing ease of biopsy has opened the possibility of celiac sprue as a diagnosis and the presentation has changed from previously defined "classic" cases. The proportion of the iceberg submerged is dependent on a variety of such variables: awareness of CS in the medical community and the public, access to diagnostic facilities, the age of introduction of dietary gluten in infants, the amount of gluten ingestion in community, the prevalence of GI infections, and probably other unknown genetic and environmental factors that influence clinical severity.[7]

The role of dietary gluten in the incidence and clinical presentation of CS is significant. Clinical features of CS have changed in young children during the last 10 years. The presentation has changed as well. Previously common complaints of diarrhea, suboptimal weight gain and suboptimal linear growth have become less common, with abdominal distension, thin extremities, irritability, and fatigue more common complaints associated with celiac sprue.[8] Iron deficiency anemia is most commonly seen in adult and children, followed by short stature in children and dermatitis herpetiformis in adults with diabetes and atopy most frequently associated conditions both in children and adults.[9] Incidence was three times higher ten years ago.[8] The reason for the change in incidence may be the change in timing of introduction of cereals into the diet.[9] The amount of gluten consumed and the time point of gluten exposure play a prominent role in disease modification. For example, data collected in Sweden show that there was a fourfold increase in the incidence of CS in children under the age of two when infant

formula was changed to include 40 times more gluten.[10] This phenomenon is in relation to the concomitant increase in the proportion of infants still breastfed at 6 months, decreased consumption of flour and the recommendation to introduce gluten in the infant's diet when the child is still breastfed.[10] This suggests that early exposure of the immature immune system to gliadin is a prominent cofactor for the manifestation of clinically overt CS. However, early patterns of gluten consumption or the duration of breast-feeding do not change the overall prevalence of CS in the population, when also the oligosymptomatic or asymptomatic cases of adolescent and adults are taken into account.[4]

The genetic and environmental factors contributing to the development of disease have been studied in relatives of CS patients and in relatively homogenous populations where it is common. Relatives of celiac patients have received particular interest. As they share common genetic and environmental characteristics, they are prime candidates to share the diagnosis of celiac sprue. Concordance for CS in first-degree relatives of affected persons ranges from 8% to 18% and reaches 70% in monozygotic twins.[1] Another study in at-risk groups, found the prevalence of CS was 1/22 in first degree relatives, 1/39 in second-degree relatives, and 1/56 in symptomatic patients. The overall prevalence of CS in not-at-risk groups was 1/133.[11] A study in Sweden found an 8.3% prevalence of CS in first degree relative of celiac patients. In 20 year follow up, 2/120 patients with initial biopsies showing minor mucosal changes developed celiac sprue by repeat biopsy.[12] Another study in families including two or more members with CS, a further 9.7% of healthy first-degree relatives showed positive IgA-EMA and at least 6.2% were shown to have CS with total percentage of CS patients in these families is as high as 40%. The prevalence of CS is particularly high in Scandinavia. In the Finnish population, depending on the screening test used, prevalence is between 1/67 and 1/130.

The high prevalence of celiac sprue in Europe, and in particular Scandinavia, has a long history of investigation. Screening of Finish population with EMA showed a frequency of 1/130.[13] Prevalence of celiac disease among Finnish schoolchildren is at least 1/99 using EMA and tTG. The prevalence of the combination of antibody positivity and an HLA haplotype associated with CS was 1/67.[14] Using IgA and IgG AGA, then EMA for positive results to screen healthy blood donors, prevalence of classic CS was 1/492 in southern Sweden which is comparable to that found in rest of Scandinavia , except Denmark where the historic prevalence was 1/10000.[15] Recent studies using EMA to screen healthy blood donors in the Dutch population showed a frequency of 1/333.[16] A study using IgA-EMA to screen 3482 inhabitants of Campogalliano, a region in Italy, found CS more frequent in younger age groups and estimated the prevalence in Italian general population is 1/204 up to 1/175 with inclusion of potential cases.[17] Using IgA and IgG AGA, then EMA to screen healthy blood donors in northern Spain found a frequency of 1/385.[18] A study in UK primary care population, found a seroprevalence of undetected CS in general adult population in Cambridge, UK, of 1.2%.[19] Studying a primary care population in the UK, the prevalence of CS was 3.3% in participants with IBS, 4.7% in participants with iron deficiency anemia, and 3.3% in participants with fatigue.[20]

Estimates of the prevalence of CS in North America vary. It may be as low as 1/3000 but this may represent significant underdiagnosis, however, as the prevalence of EMA in healthy blood donors in USA is 1/250[11] Another study in at-risk groups, found the prevalence of CS was 1/22 in first degree relatives, 1/39 in second-degree relatives, and 1/56 in symptomatic patients. The overall prevalence of CS in not-at-risk groups was 1/133.[21] In the US, females present younger and had longer duration of symptoms compared to males. The most common triggers

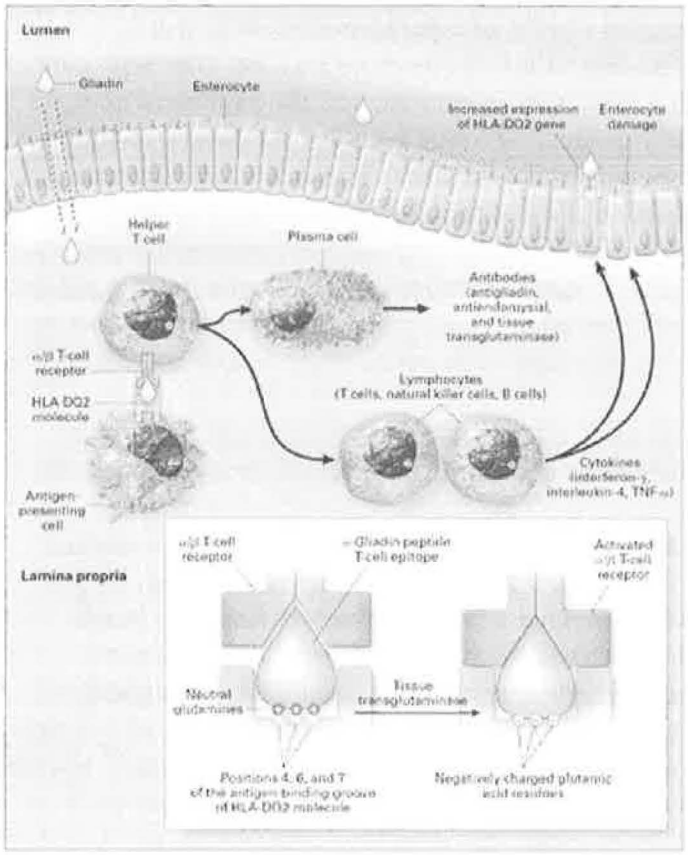
for diagnosis were GI symptoms. The mode of presentation were symptomatic (62%), anemia or reduced bone density (15%), screening of first-degree relatives (13%), and incidental diagnosis at endoscopy (8%). Lo compared those diagnosed before and after 1993, when serologic testing was first used, and noted a reduction in those presenting with diarrhea, 73% versus 43% and a reduction in the duration of symptoms from 9 +/- 1.1 years to 4.4 +/- 0.6 years.[22] A US survey of nationwide patient support groups with 1032 respondents revealed the median age of onset was 46 years and diagnosis of adult CS was often delayed, median 12 months with 21% delayed over 10 years. Only 32% adults were underweight, and only about 50% reported frequent diarrhea and weight loss. Another survey documented that common presenting symptoms were fatigue (82%), abdominal pain (77%), bloating or gas (73%), and anemia (63%). Initial physician diagnoses were often IBS (37%), psychological disorders (29%) and fibromyalgia (9%).[23] Another survey of 1612 celiac patients revealed 75% (1138) were biopsy proven with women predominating with a male:female ratio of 1:2.9. The majority of respondents were diagnosed in their fourth to sixth decades and symptoms were present a mean of 11 years before diagnosis. Diarrhea was present in 85%. Diagnosis was considered prompt by only 52% and 31% consulted two or more gastroenterologists.[24]

At one time, celiac sprue was thought to be rare in populations that were not of Northern European origins but it has been found worldwide. Furthermore, incidence is expected to increase worldwide in parallel with an increase in the consumption of wheat flour. For example, celiac sprue has been reported from the wheat-eating areas of Bengal and the Punjab, as well as in children who immigrate from India and Pakistan to England.[25] In India, the incidence of CS is expected to increase 203.08% over 10 yr and 409.6% over 20 yr.[26] Celiac sprue is found in Blacks, Arabs, Sudanese of Arab-Black stock, Cuban, Mexican, and Brazilians. It has been suggested that the incidence of celiac sprue in Asian children is as high as that in Caucasian children.[27] A recent study of Saharawi children from the Western Sahara found the prevalence of CS was 5.6%.[28] Huge prevalence of CS, 1/18 children, has been described in an Arab people living in the Sahara desert.[29] Screening Brazilian blood donors with AGA and EMA found a prevalence of undiagnosed CS of 1/681,[30] while another study estimated the prevalence of biopsy-proven CS in the Brazilian general population of 1/293, in adults 1/474 and in children 1/184.[31] Screening with AGA and EMA in prenuptial examination in La Plata, an urban area of Argentina, revealed a prevalence of 1/167.[32] Prevalence of CS in healthy, Iranian blood donors with AGA and EMA was 1/166.[33] Serologic screening in Israel estimated a prevalence of at least 1/157 in the general population.[34]

Special note needs to be made of the relationship of celiac sprue and immunoglobulin A deficiency because of its relevance in diagnosing celiac sprue that will be discussed later. In a study of 2098 celiac sprue patients, 54 (2.6%) had selective IgA deficiency. Patients with selective IgA deficiency had a higher incidence of silent forms (7/54, 13%), recurrent infection (16/54, 29.6%), and atopic disease (7/54, 13%).[35]

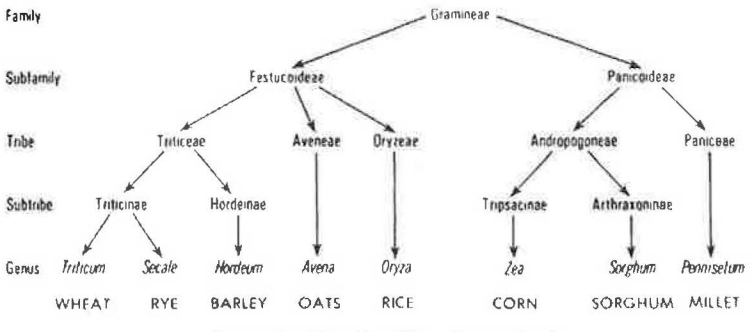
The increased risk of celiac sprue in Down syndrome is of particular interest. A study using EMA found the prevalence in US among children with Down syndrome is 1/250.[36] Another study of patients with Downs syndrome in east coastal USA found a similar result.[37] A survey of 1202 Down syndrome patients found a diagnosis of CS diagnosed in 55 patients. Diagnosis was made 3.8 years from initial presentation of symptoms with diarrhea, vomiting, failure to thrive, anorexia, constipation and abdominal distension more common. 69% had a classic presentation, 11% atypical and 20% silent. Iron deficiency anemia and hypocalcemia were also commonly seen. [38]

WHAT CAUSES CELIAC SPRUE?



The first theory of celiac sprue pathogenesis was the "enzyme" one in which an enzyme was thought to be missing, leading to an inability to digest gluten properly and thus creating an indigestible toxic fragment that causes the disease. Next the "lectin theory" entailed the presence in gluten of a toxic lectin, or vegetable protein that recognizes carbohydrate moieties of glycoproteins and glycolipids found on the brush border of the enterocyte and binds to them eliciting biological effects, such as cell agglutination, activation, and mitogenesis that would initiate a series of poorly defined toxic events. The current theory involves the interaction of peptides found in certain grains called gliadin, an enzyme called transglutaminase, and HLA class II molecules that have been associated with celiac sprue.

Humans started to cultivate grasses in Southwest Asia's Fertile Crescent at least 10,000 years ago. Primitive grasses were artificially selected and bred for their growing properties and nutritional value. Cereals represent a major carbohydrate source for most of the world's population, and the intensive cultivation has enabled civilizations to be fed. Wheat gluten is the cohesive mass that remains after washing dough. It consists of a complex mixture of many gliadin and glutenin polypeptides. Gliadins are monomers, whereas glutenins form large polymeric structures. On the basis of their amino-acid sequences, gliadins can be divided into α- γ-, ω-gliadins (250-300 residues), and glutenins can be subdivided into high molecular weight glutenins (650-800 residues) and low molecular weight glutenins (270-330 residues).[40] Many variants exist of each polypeptide type, with the greatest variation being in gliadins.



Taxonomic relationships of the major cereal grains

Generally, gluten proteins are rich in proline and glutamine residues, whereas some other amino acids, such as glutamic acid and aspartic acid are unusually rare.[41] Gliadin proteins appear late in the evolutionary process and seem to have evolved from the duplication of a gene encoding a sulfur-rich or cysteine-rich protein and repeated duplications of short DNA sequences encoding for glutamine and proline. The sulfur-rich proteins in wheat like γ-gliadin consist of an N-

terminal repetitive domain, based on repeats of the heptapeptide Pro-Gln-Gln-Pro-Phe-Pro-Gln, and a nonrepetitive C-terminal domain with three broadly based conserved cysteine-rich regions, designated A, B, and C. The sulfur-poor proteins consist of repetitive sequences alone, containing repeats, such as the octapeptide Pro-Gln-Gln-Pro-Phe-Pro-Gln-Gln. The high content of Pro and Gln, mainly found in these striking repetitive sequences, and the paucity of charged residues make the storage proteins particularly unique.[42] Proline residues are known to confer resistance against protein degradation by proteases of the GI tract. The lack of proline residues in avenin molecules thus results in higher susceptibility of the oats proteins for degradation by proteases in the GI tract.[43] Studies found α-gliadin from all wheat species investigated contained amino acid sequences potentially activating CS.[44] Minor variations in wheat gliadins are unlikely to explain the observed differences in disease expression across genetically similar populations.[45] For this reason, all cultivated wheat species are assumed to be celiac toxic cereals and should be avoided by celiac patients.[44]

Gliadin has been shown to have direct toxic effects. Using intestinal cell cultures to study the effects of gliadin revealed that gliadin was associated with significantly reduced cell viability. In particular, microscopic investigation of all the gliadin-treated cell cultures revealed cell damage similar to that mediated by lysosome enzyme release with signs of apoptosis and cellular cannibalism. These alterations are very similar to those observed in duodenal biopsy specimens from untreated celiac patients, which include a flattening reduction of the brush border, irregular microvillar surface, cytoplasmic vacuolization, prominent and ruptured lysosomes and increased enterocyte apoptosis. The reduction in the number of junctional complexes observed in the experiments may be important in determining the increased intestinal permeability described in patients with CS and in altering cell-to-cell communications.[46] Within just one hour of gliadin challenge, there was a marked upregulation of HLA-DR expression on both enterocytes and adjacent macrophages, most noticeable in villous enterocytes, on both the basal cytoplasm and the cell membrane.[47] Effects of gliadin on cell lines include changes in cell shape and size, the appearance of cytoplasmic vacuoli and inclusions, rearrangement of F-actin and tight junctions, reduction in growth and viability, directly induced apoptosis, decreased synthesis of nucleic acids and proteins, reduced content of glutathione and related enzymes, reduction in SH groups and increased lipid peroxidation. In conclusion, gliadin has a direct cytotoxic effect, which may be the first step in the pathogenic mechanism of CS by triggering and stimulating the immunological system.[48]

Data indicate that gliadin peptides, although poorly or not digested by intraluminal enzymes, can be fully digested by enterocytes in control and patients with treated CS. In patients with active CS, incomplete degradation gliadin lead to a 33-mer, residues 56-89, that may be responsible for the immunostimulatory and toxic effects of gliadin.[49] The 33-mer, residues 56-89 of α2-gliadin, was identified and was found to be stable toward breakdown by all gastric, pancreatic, and intestinal brush border membrane proteases.[50] The 33-mer carries multiple copies of three epitopes that are immunogenic in patients with CS. Similar peptide sequences are present in the hordeins and secalins.[51] It has been demonstrated that T cell cross-reactivity between gluten peptides and related peptide in the hordeins and secalins can be related to the toxicity of barley and rye for patients with CS. A general lack of proline residues and the presence of glutamic acid instead of glutamine residues in the core of the avenin peptides likely accounts for the lack of toxicity of oats in CS patients.[43] Peptide residues 56-75 of α–gliadin exacerbate CS in vivo.[52] Peptides corresponding to positions 57-68 and 62-75 of wheat α–gliadins stimulated all small intestinal gluten sensitive T cell clones from four adult

Norwegian celiac patients who carried HLA-DQ2.[53] Several Pro- and Gln- rich peptide sequences, most notably PQPQLPY, have been identified with potent immunogenic activity toward CD4+ T cells from small intestinal biopsies of CS patients. These peptide sequences have unusual properties: (1) they are relatively stable toward further proteolysis by gastric, pancreatic and intestinal enzymes, (2) they recognized and deamidated by human tTG with high selectivity, and (3) tTG catalyzed deamidation enhances their affinity for HLA-DQ2.[54] Selective deamidation of peptides residues 56-75 of α-gliadin increases circulating antibody recognition of gliadin peptides in CS patients.[55] Deamidation of glutamine residues at position 65 to glutamic acid in both molecules was essential for optimal HLA class II binding and subsequent T cell activation. T cell activation was obliterated by the substitution of a neutral alanine residue at that position. This is interesting because this residue is thought to point towards the T cell receptor when the peptide is positioned within the DQ2 binding cleft.[53]

It is interesting to note that gliadin peptides are transported inside enterocytes and processed by the intestinal mucosa. However, the mechanism of internalization of gliadin peptides is unknown. It might be suggested that the transport process of gliadin is probably mediated by the clathrin-mediated endocytic pathway and that an alteration of this process may be involved in the pathogenesis of CS enterocytes. An immune reaction was observed against the cytoskeleton in both children and adults with CS. In particular, anti-actin antibodies are shown to be more strongly associated with more severe degrees of villous atrophy. It is possible to hypothesize that gliadin peptides can trigger a cascade of events leading to the inappropriate presentation of tTG and cross-linked substrates to the immune system contributing to the immune aspect of CS. This mechanism could in part explain the increased prevalence of concomitant auto-immune disease, such as collagen diseases, type I diabetes, autoimmune alopecia, hypophysitis, and others in CS patients with prolonged gluten exposure.[56]

The first transglutaminase was identified by Heinrich Waelsch more than 40 years ago as a liver enzyme incorporating amines into proteins.[57] Transglutaminases are mediators of biological glues. Most common function is to catalyze the formation of isopeptide linkages between the carboxamide group of protein-bound glutamine residues and the ε-amino group of protein-bound lysine residues. One of the isoforms of tranglutaminase is factor XIIIa. Factor XIIIa is important for the clotting of blood by catalyzing the formation of ε(γ-glutamyl)lysine cross-links between fibrin monomers or fibrin and α2-plasmin inhibitor. It is activated by proteolytic cleavage of its proenzyme, factor XIII, and deficiencies cause protracted bleeding with a frequency of $1\text{-}5/10^6$ individuals in Japan.[58] Transglutaminase I cross links the proteins that comprise the cell envelope, a flexible insoluble barrier that lines the outer surface of fully differentiated cells of stratified squamous epithelia, which thereby protects against mechanical and chemical injury, or dehydration in the case of cornified epidermal cells. Mutations in transglutaminase 1 are responsible for a major form of autosomal recessive ichthyosis, lamellar ichthyosis, a disorder of keratinization that occurs spontaneously at a frequency between 1 and 3 x 10^5 individuals. Affected individuals are born embedded in hyperkeratotic skin, called collodion-baby syndrome, which later cracks and exposes the underlying tissue, leading to life-threatening dehydration and systemic infections. If the newborn survives the first few months of life, the disease eventually develops into large brown plate-like scales, which cover the entire body surface.[58]

Human transglutaminase 2 or tissue transglutaminase (tTG) is a 76-kD, consisting of 686 amino acids. Tissue transglutaminase is an inducible transamidating acyltransferase that catalyzes Ca^{2+}-dependent protein modifications. It acts as a G protein in transmembrane

signaling and as a cell surface adhesion mediator, which distinguishes it from other members of the transglutaminase family. Tissue transglutaminase is induced in cells undergoing apoptosis in vivo. Its overexpression primes cells for suicide and inhibition of its expression by antisense strategy results in decreased cell death. It has been reported recently that tTG sensitizes cells for apoptosis by interacting with mitochondria, shifting them to a higher polarized state and altered redox status. This might provoke activation of transglutaminase crosslinking activity. During the late stage of apoptosis, the massive increase of cytosolic Ca^{2+} determines the switch of tTG to its crosslinking configuration in all subcellular compartments leading to extensive polymerization of intracellular proteins, including actin and Rb, and formation of detergent-insoluble structures. These protein scaffolds stabilize the structure of the dying cell before its clearance by phagocytosis, limiting the release of harmful intracellular components and consequently inflammatory or autoimmune responses. Transglutaminase 2 deficient mice showed decreased adherence of primary fibroblasts and impaired would healing related to altered cytoskeletal dynamics of fibroblasts. The tTG participation in apoptosis could explain the findings that on increasing the frequency of cell death in the knock-out mice, clearance of apoptotic cells by phagocytosis is defective in the thymus and the liver and inflammatory as well as autoimmune reactions develop. They also show glucose intolerance and hyperglycemia because of reduced insulin secretion, similar to maturity-onset diabetes of the young.[57]

Anti-tTG antibodies found in type I diabetes mellitus belong to IgG and IgA immunoglobulin classes that suggests that these antibodies are formed by multiple mechanisms. Some diabetics with IgA anti-tTG antibodies also have CS, although the disease is asymptomatic. Celiac sprue with type I DM patients are usually homozygous for HLA-DQ2 which may drive the production of anti-tTG antibodies. Approximately 8% of these DM patients are positive for IgM anti-tTG antibodies and a striking 42% of patients have IgG anti-tTG antibodies. Patients with type 1 DM have been found to have increased intestinal permeability which may allow immunization against tTG by cells from the systemic and gastric circulation. Pancreatic beta cells may release tTG as a neoepitope. Successful management of diabetes, which attenuates beta cell destruction, results in decreased anti-tTG titers.[58]

The role of transglutaminases in inclusion body myositis is based on the putative involvement of these enzymes in the formation of the beta-amyloid aggregates in Alzheimer's disease. Transglutaminase 1 and 2 colocalize with amyloid deposits in vacuolated muscle fibers from the inclusion body myositis. Transglutaminase 2 is important in the development of extracellular matrix including fibrosis and atherosclerosis and may contribute to the formation of atherosclerotic plaques by catalyzing the incorporation of lipoprotein(a) into these structures. Transglutaminases colocalize with the plaques and tangles in the brains of Alzheimer's disease patients and likely contribute to the formation of extracellular neuritic senile plaques and intraneuronal neurofibrillary tangles. Presumed involvement of transglutaminases in Huntington's disease is based on demonstration of elevated transglutaminase activity in the affected regions of disease grains and studies showing huntingtin is a substrate for transglutaminase 2. The pathologic gain of function conferred by the elongated polyglutamine domain may be due to an increased propensity of such a domain to act as a transglutaminase substrate. In Parkinson's disease, a fragment of α-synuclein, known as the non-amyloid component (NAC), has been detected in the Lewy bodies of Parkinson's disease patients and the neuritic plaques of Alzheimer's disease patients. NAC acts as a substrate for transglutaminase and is neurotoxic to primary dopaminergic neurons as formation of NAC polymers is more toxic than monomeric forms. Progressive supranuclear palsy presents with unexpected falls, postural

instability, vertical gaze palsy, axial rigidity, dysarthria and dementia, with midbrain atrophy with dilation of the aqueduct of Sylvius and depigmentation of the substantia nigra and is associated with the loss of neurons in the substantia nigra, globus pallidus, subthalamic nucleus, basal ganglia, diencephalons and brain stem. The frequency of progressive palsy is approximately 1-2 per 100000. Transglutaminases may be responsible for the neurofibrillary tangles seen in progressive supranuclear palsy.[58]

Tissue transglutaminase can be considered a master regulator of CS.[59] Dieterich in 1997 described tTG as the target of EMA. Studies revealed that screening of anti-tTG is very specific and is linked to the humoral response and involved in generating gluten peptides that stimulate T cells present in the small intestine of CS patients. Glutamine residues in gluten- derived peptides are converted by tTG into glutamic acid. This introduction of negative charges results in peptides that bind with high affinity to the disease-associated HLA-DQ2 or DQ4 molecules and trigger inflammatory T-cell responses. Sollid showed that tTG can crosslink itself to gluten and these gluten-tTG complexes will be taken up by B-cells that express tTG-specific immunoglobulin on their membranes.[60] The gluten-tTG complex will be degraded intracellularly and gluten peptides will bind to HLA-DQ and be expressed on the cell surface. In CS patients, gluten-specific T cells will recognize this HLA-DQ-gluten peptide complex and result in T cell signaling for the production of tTG-specific antibodies by the B cells. Since only CS patients have measurable numbers of gluten-specific T cells, this explains why only CS patients make tTG-specific antibodies and the antibody titers drop after gluten withdrawal.[61] During disease development, the formation of tTG-gliadin complexes through tTG activity appears to be central for B-cell epitope spreading from gliadin to tTG. A cellular and humoral response against tTG can be induced in tTG null and wild type mice by subcutaneous immunization with human recombinant or guinea pig tTG in complete Freund's adjuvant. Immunized wild type, but not tTG null mice develop periductal lymphocytic infiltrates in lacrimal glands but no intestinal lesions are found. An example of epitope spreading is dermatitis herpetiformis. Transglutaminase 3 is the major autoantigen of dermatitis herpetiformis. While most celiac patients with or without dermatitis herpetiformis have IgA anti-tTG antibodies that cross react with transglutaminase 3, only patients with dermatitis herpetiformis have antibody populations that recognize transglutaminase 3 selectively and with high avidity.[62] T cell reactivity towards native gluten peptides in the intestine would result in tissue damage due to the release of inflammatory cytokines by these T cells. It is well established that tissue damage results in the release of cytoplasmic tTG, which can subsequently modify gluten peptides and generate a whole series of potent T cell stimulatory peptides. This would attract more gluten specific T cells into the small intestine, leading to more tissue damage, more tTG, and more T cell stimulatory gluten peptides in a vicious circle with oral tolerance broken as CS results.[63]

Molberg showed that tTG mediates it effect on T cell recognition of gliadin through an ordered and specific deamidation of gliadins that creates an epitope that binds efficiently to DQ2 and is recognized by gut-derived T cells.[60] Generation of epitopes by enzymatic modification is a new mechanism that may be relevant for breaking of tolerance and initiation of autoimmune diseases. This suggests that a tissue enzyme can modify exogenous antigen such that it is recognized more efficiently by CD4+ T cells. Tissue transglutaminase seems to be upregulated in inflammatory sites such as celiac mucosa, raising the possibility that intestinal infection or other local insults could predispose to the development of gluten sensitivity. tTG mRNA levels were one fold higher in CS patients than in controls. Immunohistochemistry and in situ demonstration of enzymatic activity in celiac mucosa clearly showed an increased expression of

active tTG in the extracellular matrix of the subepithelial region and in the enterocytes.[64] It remains unclear whether the tTG specific antibodies play a causal role in the intestinal pathology. As tTG play an important role in crosslinking components of the extracellular matrix, it seems possible that antibodies directed at the enzyme might interfere with the interactions between mesenchymal cells and the gut epithelium. tTG activity is frequently associated with tissue repair and remodeling, suggesting that its overexpression in CS may be secondary to ongoing inflammation and that resulting autoantibodies are epiphenomena.[65] The increased level of expression of transglutaminase 2, or tissue transglutaminase (tTG), in the mucosa of CS patients seems related to inflammation. Wounding markedly increases the expression of tTG and the tTG promoter contains response elements for the pro-inflammatory cytokines IL-6 and TNF. Inflammation, as induced by an infection, might, in addition, breach the epithelial barrier and lead to further influx of gluten peptides into the lamina propria. The increased concentration of gluten peptides would lead to over-consumption and depletion of lysine and polyamines, which, together with increased expression of tTG, would promote formation of deamidated peptides. So, it is probable that the generation of deamidated gluten peptides is linked closely with inflammation. Tolerogenic responses to oral antigens are difficult to evoke under inflammatory conditions, and if deamidated gluten epitopes are not formed under normal tolerogenic conditions, tolerance to deamidated gluten peptides would be hard to establish, once broken. Indeed, there is epidemiological evidence that infections are a risk factor for CS.[41] Environmental factors may play an important role in triggering overt disease in genetically predisposed individuals. Normal immune tolerance to dietary proteins appears to reflect the fact that such antigens do not stimulate the innate immune system and are presented to naïve CD4+ T cells by local antigen-presenting cells that lack the full complement of costimulatory molecules necessary for effective activation of T cells. Sensitisation to gluten could therefore be triggered by any environmental factor which disturbs this homeostatic balance, including the release of inflammatory mediators or other factors released during local infections, and can activate dendritic cells fully or alter other components of the local microenvironment. [66] It has been suggested that infection with adenovirus 12 might precipitate the clinical and histological appearance of CS. Kagnoff et al. reported that 89% of untreated CS patients had evidence of past adenovirus-12 infection.[27]

Role of autoantibodies in pathogenesis or as an epiphenomenon are unclear. The significant increase in the prevalence of CS among IgA-deficient individuals speaks against a role of the antibodies. However, most CS patients also have elevated levels of serum IgG EMA and tTG and little is known about the antibodies found locally in the mucosa of IgA-deficient patients. Interestingly, the EMA antibodies can, as suggested by Make, be involved in the disease development by blocking interactions between mesenchymal cells and epithelial cells during the migration of epithelial cells and fibroblasts from the crypts and epithelial cells of the villi. tTG is necessary for activation of TGF-β and indirect inhibition by tTG Ab can be envisaged to have broad effects as TGF-β is known to affect the differentiation of intestinal epithelium, to stimulate extracellular matrix formation, and to regulate the function of many immune competent cells within the gut microenvironment. tTG has been demonstrated to be involved in attachment of fibroblasts to the extracellular matrix, suggesting that the autoantibodies could also be involved in lesion formation by perturbing important contacts between fibroblasts and extracellular matrix components.[66]

In the current theory, the initial event in the pathogenesis of the celiac lesion is thought to be an abnormal permeability that allows the entry of gliadin peptides not entirely degraded by

the intraluminal and brush border-bound peptidases. It has been shown that increased permeability to macromolecules secondary to loosening of the intestinal tight junctions allows more meaningful entry of antigenic peptides. The upregulation of zonulin, an intestinal peptide involved in tight junctions regulation, seems to be involved in the increased gut permeability to gliadin peptides.[29] The early changes seen are highly suggestive of a direct toxic effect of gliadin on epithelial cells and macrophages or of a release of prestored cytokines. From a morphologic point of view, small bowel mucosal damage occurs as a result of gradual changes from normal mucosa to overt mucosal atrophy with crypt hyperplasia.[67] Direct toxicity of gliadin is not the only event in CS pathogenesis. CS is triggered by the binding of one or more gliadin peptides to CS-associated HLA-class II molecules. These putative CS peptides bind to oligosaccharide residues on HLA class II molecules distal to the peptide-binding groove invoking recognition and binding by specialized subsets of γδ T cell receptor bearing lymphocytes. The binding of these T cells serves as a signal for abrogation of oral tolerance to ingested protein setting in motion a series of immune responses directed against the small intestinal epithelium of CS patients. CS patients are victimized by this self-destructed immune response because of inheritance of certain combinations of HLA-DQ and DR haplotypes. Dimers encoded by HLA-DR may be the primary restriction elements for lectin-like, gliadin peptides while the degree or lack of immune suppression to ingested gliadins is governed by inherited HLA-DQ haplotypes. Molecular mimicry between one or more gliadin peptides and some, as yet unidentified, bacterial or viral superantigen plays a role in disease pathogenesis.[68] New multi-gene hypothesis for the initiation of CS in which deamidated free human peptides with T-cell epitope homology escape negative selection, and deamidation following peptide release due to injury triggers inflammation, thereafter repeatedly provoked by dietary gliadin immunodominant peptides concentrated in the proximal small intestine.[69]

Family studies reflect the importance of genetic factors in the pathogenesis of celiac sprue. Our understanding of the nature of this genetic predisposition began with the observation by Howell and colleagues that CS is associated with specific HLA class II DQ haplotypes. HLA-DQ2 is found in 95% of patients with CS, and HLA-DQ8 is found in most of the remaining patients.[1] An important link to genetic disposition was provided by the isolation of gliadin-specific HLA-DQ2-restricted T-cell clones from celiac sprue mucosa. Further evidence showed the primary mechanism underlying the association of CS with these HLA class II alleles is the preferential presentation of deamidated gluten peptide antigens by HLA-DQ2 and DQ8.[70] Up to 30% of persons of North European ancestry express HLA-DQ2 but CS develops in only a small proportion of these carriers. [71] A gliadin-derived epitope that is dominantly recognized by intestinal gluten-specific HLA-DQ8-restricted T cells has been identified.[72] It is now known that after gluten is absorbed, lamina propria antigen processing cells, probably dendritic cells that express HLA-DQ2 or DQ8, present gliadin peptides to sensitized T lymphocytes. These lymphocytes then activate B lymphocytes to generate immunoglobulin and stimulate other T lymphocytes to secrete cytokines, predominantly interferon-γ and to a lesser degree interleukin (IL)-4, IL-5, IL-6, IL-10, tumor necrosis factor (TNF)-α, and transforming growth factor-β. These cytokines not only damage enterocytes but also induce expression of aberrant HLA class II cell-surface antigens on the luminal surface of enterocytes, possibly facilitating additional direct antigen presentation by these cells to the sensitized lymphocytes.[1]

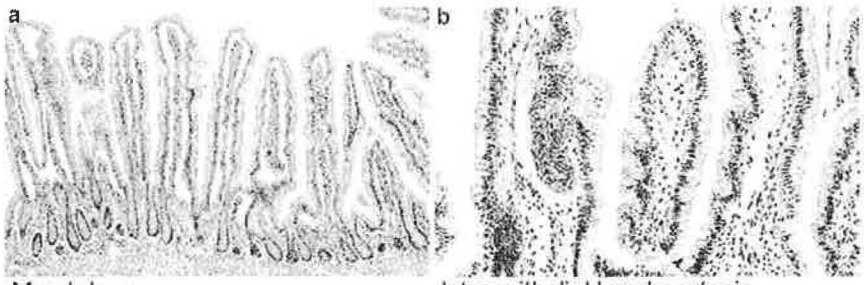
HLA-DQ2 is common in Europeans and is expressed in 25% to 30% of the normal population, yet celiac sprue develops in only a minority of them.[4] Thus another gene, or genes, at an HLA-unlinked locus must also participate in development of disease, and is likely to be a

stronger determinant of disease susceptibility than the HLA locus.[1] However, successive genome scans have not identified any single genetic risk factor. This suggests that there are a variety of different genetic mutations that strongly favor the development of CS. It is surprising that a disease with such great morbidity and mortality has not been eliminated by the process of evolution. One possible explanation is that gluten, a protein introduced in large quantities into the human diet only after the advent of agriculture activates mechanisms of innate immunity that are too important to the survival of the species to be eliminated.[73] A protective association between CS and hereditary hemochromatosis has also been suggested.[74]

An example of the interplay of environment, immunology and genetics is illustrated in an interesting case report. Carroccio et al report a case history of an infant who, at age of one year, developed diarrhea but was not investigated until he was 25 months old. At this time IgA AGA, EMA and tTG antibodies were detected in serum. Biopsy from proximal jejunum showed partial villous atrophy. Giardia lamblia was found in intestinal fluid aspirates. A diagnosis of CS with giardiasis made. He was treated with a course of metronidazole and gluten free diet was recommended. At two month follow up, it was noted that the diarrhea had resolved within seven days of receiving metronidazole, but gluten free diet had not been started. Repeat testing showed that IgA AGA and EMA were still present but in low titers. Normal diet continued, and at six month follow up, he was noted to be doing well with satisfactory growth. Serum antibodies had disappeared and repeat intestinal biopsy was structurally normal with only slight increase in intraepithelial lymphocytes. At age four, he had negative serologies for CS, with HLA typing characteristic for CS. Third jejunal biopsy showed normal villous architecture but slightly increased epithelial CD3+ lymphocyte and γδ lymphocyte count. Giardia was absent from intestinal fluid. At this time, when antibodies disappeared from the serum, IgA EMA was detected in the supernatant fluid from cultures of the intestinal mucosa incubated with gliadin. The child did well on normal diet with average intake of gluten of 11 g/day. The authors concluded that an active CS status with intestinal mucosa atrophy may regress to a latent CS status with normal intestinal mucosa histology after removal of the environmental factors that have presumably precipitated mucosa damage.[75]

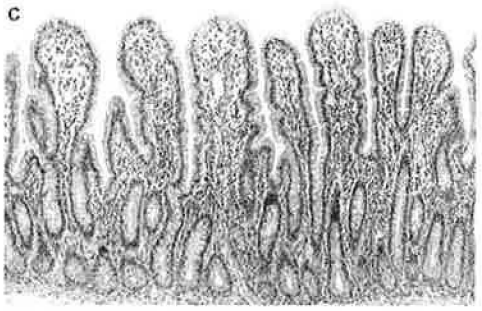
HISTOLOGY

The diagnosis of CS is based upon histological findings in duodenal or jejunal biopsies. In the past few years, it has turned out that the development of CS lesion in the small bowel is a dynamic process that may present in various histologic forms. At one end of the spectrum is a mucosa with normal architecture and an increase in intraepithelial lymphocytes (IEL), at the other end is the classical flat mucosa. An increase in the number of IEL is the first and most sensitive index of the effects of gluten on the mucosa and is therefore the single most important histologic feature in CS. Intraepithelial lymphocytes are also increased in number in the stomach and in the large bowel. The normal gut contains up to 40 IEL/100 EC, and so anything above indicates an ongoing immunologic process. In CS patients, the cell content of the lamina propria is increased in histologically



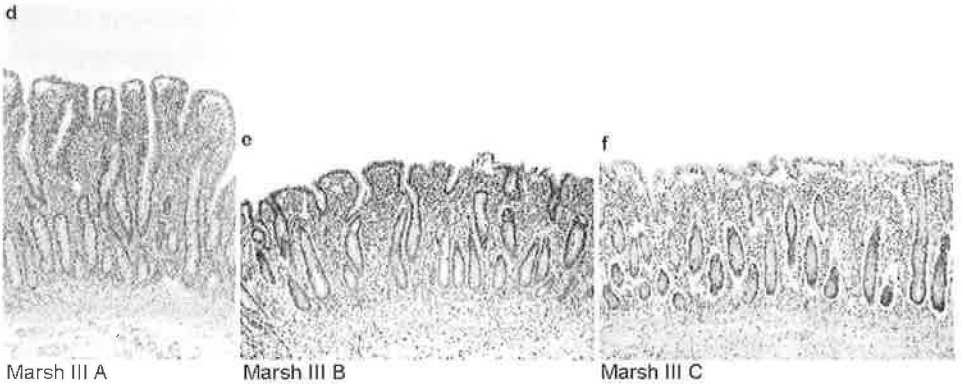
Marsh I Intraepithelial lymphocytosis

altered small bowel mucosa, in particular in its upper two-thirds. Plasma cells predominate with T cells increased to a lesser extent. In the dynamic process of the development of CS lesion, the first architectural change to be found is crypt hyperplasia. Initially, the elongated crypts are covered by normal appearing villi, and later on, when the lesion is more advanced, by shortened villi which may be absent in the most severe stage, the flat mucosa. Kinetic studies of the epithelium in CS show the rates of loss of surface enterocytes and the rate of crypt-cell proliferation are accelerated. Increased epithelial cell proliferation leads to crypt length increases. Enterocyte apoptosis is increased and correlates with proliferation. Local cytokine production can induce production of epithelial mitogens by stromal cells, which control epithelial renewal. Crypt hyperplasia is induced by growth factors, such as hepatocyte growth factor and keratinocyte growth factor, which are released by mesenchymal cells and possibly IELs. In more advanced stages of the CS lesion, matrix metalloproteinases, such as collagenase or stromelysine, are also active and seem to play a central role in the development of villous atrophy through degradation of the interstitial tissue.[76]



Marsh II

Marsh classified the distinctive histological changes seen in CS. He characterized the epithelial changes based on the presence of intraepithelial lymphocytes and villous structure in biopsies of small intestinal mucosa. Normal mucosa has than 40 intraepithelial lymphocytes (IEL)/ 100 enterocyte (EC) and is called type 0. Marsh type 1 is the infiltrative type characterized by a normal villous architecture, a normal height of the crypts and an increase in IEL numbers up to more than 40 IEL/100 EC. This stage is not diagnostic of CS, but is seen in patients with latent CS, dermatitis herpetiformis, and first degree relatives of patients with celiac sprue.[77] They usually do not have gastrointestinal symptoms and will have to be followed up for years to monitor for changes from type 1 to flat mucosa. Marsh type 2 is the hyperplastic type characterized by a normal villous architecture, an increase in IEL numbers up to more than 40 IEL/ 100 EC and crypt hyperplasia. This stage is only rarely encountered and has mainly been observed under experimental conditions or in patients with dermatitis herpetiformis.[77] Recent studies that both the symptoms and histologic findings are gluten-dependent, and the authors argue that the present diagnostic criteria for CS, currently requiring a definite villous atrophy, would thus appear to be in need of revision.[76] Marsh type 3 is the so-called "destructive" type of the CS lesion and is considered diagnostic. Marsh type 3 is divided into three different subgroups depending on the degree of villous atrophy, crypt hyperplasia, and increased volume of the lamina propria. All have increased IELs. Villous atrophy and crypt hypertrophy can also



Marsh III A Marsh III B Marsh III C

be seen in other food-sensitive enteropathies, severe giardiasis, nematode infection, mild Crohn's disease, graft-versus-host disease, tropical sprue and other rare inflammatory conditions.[77] Type 4 is a very rare hypoplastic lesion that is characterized by a flat mucosa with normal crypt heights and normal IEL counts.[76] It is an end stage lesion in a very small proportion of patients with severe gluten-induced lesions, who do not respond to gluten withdrawal and who may develop malignant complications.[77]

Histologic recovery in CS after starting gluten-free diet is different depending both on the age and on the histology at diagnosis. Up to 10% of adults with CS have villous atrophy after 5 years. A study of patients with Marsh IIIa, IIIb, or IIIc lesions found histologic remission was seen in 65% within 2 years, 85.3% within 5 years, and 89.9% in long term follow up. Eleven patients (7%) with persistent partial villous atrophy had symptoms and signs of malabsorption and were considered to have refractory CS; 5 of them developed an enteropathy-associated T-cell lymphoma. Children recovered up to 95% within 2 years and 100% in the long term.[78]

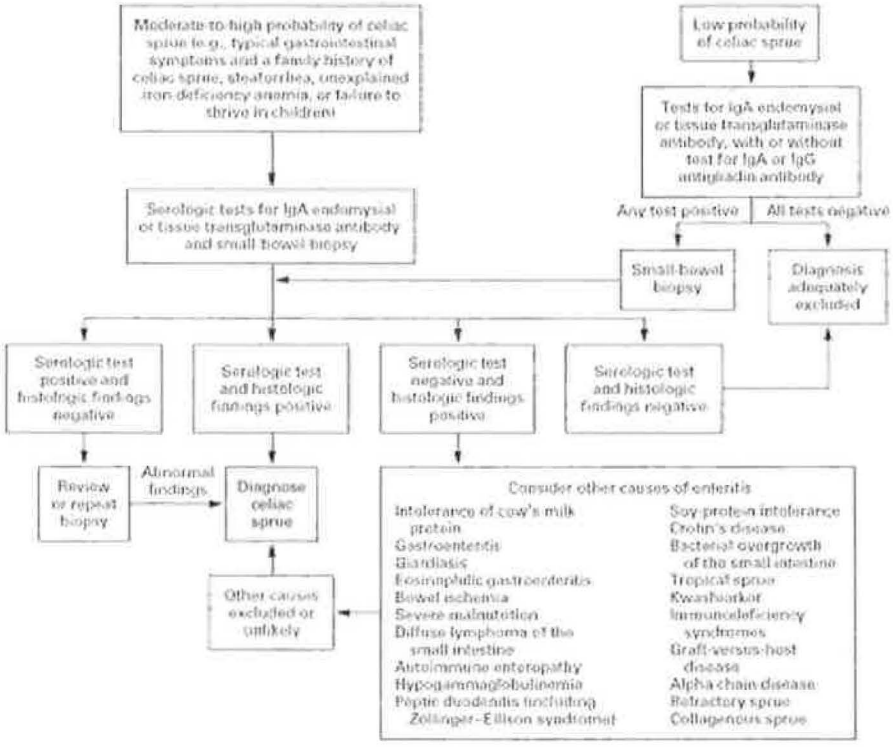
DIAGNOSIS

Small intestinal biopsy remains the gold standard for diagnosis of celiac sprue. However, serologic studies are useful in diagnosing celiac sprue. Endomysial antibodies (EMA) are felt to be the most sensitive and specific. Serum IgA EMA assay requires a tissue substrate, most commonly crytostat sections of the lower third of the monkey esophagus that has a high density of endomysium. Cost and ethical concerns led to the use of human umbilical cord as an alternate tissue source beginning in 1994. Like all indirect immunofluorescence assays, the IgA EMA test is a labour- and skill-intensive, with no prospect for automation, unlike ELISA tests.[80] The sensitivity of IgA EMA ranges from 57.9%[81] to 99.5%[82] and specificity from 87.1%[82] to 100%.[81]

Serologic testing became easier when it was elucidated that the epitope against which EMA is directed against is tissue transglutaminase (tTG). Enzyme-linked immunosorbent assay tests for IgA anti-tTG are now widely available, less costly, and easier to perform than the immunofluorescence assay used to detect IgA EMA. The sensitivity of IgA tTG range from 87%[83] to 100%[82] with specificity from 61%[84] to 96%.[85] Initially the test used guinea pig liver but more recently human recombinant sources have become available with increased specificity.[86] The utility of IgA anti-tTG testing in patients on gluten free diet or with minor mucosal injury is limited because the levels drop very quickly when gluten exposure ceases.[84] It is therefore not useful in monitoring compliance in patients.[87] The poor effectiveness of anti-tTG as an indicator of histologic recovery in celiac disease and its quick subsiding throughout the follow-up may also be explained by the Dieterich's hypothesis, that anti-tTG is generated in genetically predisposed individuals by complexes formed between anti-tTG and gluten. So, it is hypothesized that anti-tTG should disappear soon after gluten exposure ceases. Histologic recovery, however, is much slower as has been frequently recognized in clinical practice.[87]

Anti-gliadin antibodies (AGA) from CS patients react with structures on gliadin and potential autoantigens on enterocytes such as calreticulin.[88] Purified gliadin, a component of the wheat storage protein gluten, is readily available and is used as the antigen of enzyme-linked immunosorbent assays to detect serum anti-gliadin antibodies (AGA). The sensitivity of IgA AGA ranges from 55%[81] to 83%[89] with specificity ranging from 63.5% to 100%.[81] IgG AGA has similar sensitivities ranging from 68.4% to 95%[81] and specificities ranging from 76%[89] to 100%.[81] AGA varies closely with gluten intake and is thus useful in monitoring compliance.[90]

For initial diagnostic testing in an individual above the age of 2 years suspected of having CS, either IgA-EMA or IgA-tTG should be used. In one study, a one-step serologic testing (EMA) was found to be both more economical and more sensitive that the two step procedure with AGA or tTG screening followed by EMA for confirmatory testing.[18] Debate remains about recommendations to request serum IgA levels in conjunction with the IgA antibodies, in view of the increased prevalence of selective IgA deficiency in CS patients. In cases with proven IgA-deficiency, performing IgG-AGA, IgG-tTG or IgG-EMA provides useful diagnostic information.



Given the dynamic nature of this disease, and the fluctuating levels of serum markers, there is value in repeating serologic testing or even performing HLA-DQ typing in some clinical circumstances. If clinical suspicion is high, particularly in a member of a high - risk group, and initial screening is negative, discrepant or equivocal then retesting can help avoid more invasive small intestinal biopsy. When biopsies are equivocal or may have been performed during self-imposed gluten restriction, repeat serologic testing can be cost effective and offset the influence of sampling error.[80]

Celiac sprue has many of the features that make it suitable for mass screening: (1) the condition is common and often asymptomatic; (2) undiagnosed, there is significant morbidity and probably excess mortality; (3) a sensitive and specific screening test is available; (4) there is an effective treatment.[27] On the other hand there is no evidence that screening the general population will lead to reduction in morbidity or be cost effective. Patients identified by screening have shown poor compliance with a gluten-free diet and there is no data to suggest mortality from treatment in this group. Screening will only demonstrate lead-time bias.[92] Community-based case-control study found no association between recurrent abdominal pain and the prevalence of EMA. Data do not support screening for CS in the child with classic recurrent abdominal pain in the primary care setting.[93]

Despite the current data, there is still reason for active investigation of screening. The natural history of CS is unknown. Increased malignancy especially small bowel lymphoma, osteoporosis, infertility and pregnancy failure, growth retardation, and nonspecific low grade morbidity are the major rationales for diagnosis subclinical or asymptomatic CS. It is noteworthy

that malignancy occurs almost exclusively in undiagnosed or noncompliant CS patients, but it has not been demonstrated that gluten restriction in those with asymptomatic CS reduces risk of malignancy.[7]

Endoscopy should not be regarded as a screening method to judge which patients must be submitted to duodenal biopsy. Endoscopic signs must only be considered as an adjunctive tool that may improve the diagnostic yield.[94] One study showed that children with CS may have patchy villous atrophy of the duodenum. The bulb mucosa may be the only duodenal area involved, both at diagnosis and after gluten challenge. Therefore, multiple endoscopic biopsies should always be performed, not only in the distal duodenum, but also in the bulb.[95] Endoscopic markers of CS are scalloped folds, mosaic pattern nodular mucosa, and reduction in fold numbers. In studies, endoscopic markers had a sensitivity of 59-87.5% and specificity 92-100% with loss of folds most sensitive with mosaic pattern, nodularity and scalloping less sensitive.[96] Careful inspection of the duodenum during routine upper GI endoscopy allows accurate selection of patients for biopsy but may not detect patchy VA or milder enteropathy.[96] Videoendoscopy and vital dye staining have been shown to increase the sensitivity of these endoscopic markers.[97]

Tests of functional intestinal permeability can help diagnose CS. One study evaluated sucrosemia after an oral load as a noninvasive test for celiac sprue. All untreated celiacs but none of the treated celiacs had sucrose in their serum after the 8g oral challenge. None of the controls had sucrose in their serum after the 8 or 50g challenge. Brush border sucrase activity was low in untreated CS and may explain the findings.[98] Sorbitol H2-breath test and xylose-breath test have been shown to be very sensitive in detecting patients with slight histologic damage (Marsh I-IIIa).[99] A study assessed intestinal permeability to sucrose, lactulose, and mannitol to screen relatives of patients with celiac sprue. The addition of lactulose/mannitol permeability testing to the screening protocol allowed detection of all relatives who had evidence of gluten sensitivity.[100]

Small studies have looked at the usefulness of radiologic studies in diagnosing CS. Using ultrasound, celiac sprue was diagnosed in 12 patients. An increased gallbladder volume, the presence of free fluid in the abdominal cavity, and enlarged mesenteric lymph nodes showed a specificity of 96%, 96% and 97% respectively, whereas the presence of dilated small bowel loops with increased fluid content and increased peristalsis had a sensitivity of 92% an 83% respectively. 11/12 (92%) of patients with CS and 35/150 (23%) who did not have the disease had a least one ultrasound sign. All of the US signs were concomitantly present in 4 patients with CS (33%) and one patient without CS (0.6%).[101] Enteroclysis has been used to study the small intestines. Radiographic features indicative of adult CS were evaluated for diagnosis of CS. Reversal of jejunoileal fold pattern was the single best feature, with specificity 100% and sensitivity 59%. Accuracy was greatly improved if reversal of fold pattern and at least three of the following features, fold thickening, decrease of jejunal folds (colonization), increase of ileal folds (jejunization), dilatation, and flocculation, were also found.[102]

ASSOCIATED CONDITIONS

There is one large study of VA patients that provides the best information about associated diagnoses. The investigators looked at 458 patients with celiac sprue and reviewed records between 1986-1995. Diagnoses with significant association were dermatitis herpetiformis, lactase deficiency, lymphadenopathy, and lymphoma, pancreatic insufficiency, Crohn's disease, functional bowel symptoms, chronic nonalcoholic hepatitis, pulmonary

eosinophilia, nutritional marasmus, cachexia, weight loss, hypocalcemia, osteoporosis, vitamin B-complex deficiency, iron and vitamin-deficiency anemias[103]

Other lab abnormalities can be seen in celiac sprue. Celiac patients had a higher total plasma homocysteine level than the general population, indicative of poor vitamin status. The mean daily intake of folate and B12, but not vitamin B6, was significantly lower in celiac patients than in controls. This suggests that when following adults with CS, the vitamin status should be reviewed.[104] Another study of 159 of celiac patients with CS found 8% had low serum B12 at diagnosis with 6 additional patients who had been receiving B12 replacement therapy for 3-37 years before diagnosis. The overall prevalence of B12 deficiency in this group was 12% (19 patients). Only 2/19 patients had gastric corpus atrophy, one with intrinsic factor antibodies and the other with hypergastrinemia. Low B12 is common in CS and may be a presenting manifestation.[105] Red cell indices can also be used. Guidetti et al looked at RDW. Overall RDW increase was found in 80/149 (54.7%) patients with definite diagnosis of CS and in 14/49 (28.6%) patients in whom biopsy excluded the disease. A one year gluten withdrawal led to significant decrease in RDW, even in patients with obdurate mucosal involvement. They concluded that an elevated RDW despite normal hemoglobin might be a reliable predictor of the disease.[106]

General	GI	Extraintestinal	Associated Diseases
		Laboratory abnormalities	Definite association
Short stature	Diarrhea, steatorrhea, flatulence	Iron and folate deficiency anemia	IDDM
Weight loss	Abdominal distention, discomfort	Hypocalcemia, ↑ALP	Hyperthyroidism, hyperthyroidism
Failure to thrive	Anorexia, nausea, vomiting	Prolonged PT	IgA deficiency
Lassitude, lethargy	Recurrent aphthous stomatitis	Hypertransaminasemia	Sjrogren's syndrome
Clubbing, koilonychia	Angular cheilosis, glossitis	Skin	Microscopic colitis
Delayed puberty	Hepatic steatosis	Dermatitis herpetiformis	Primary biliary cirrhosis
Edema		Follicular keratosis	IgA mesangial nephropathy
		Pigmentation, bruising	Rheumatoid arthritis
		Hematological	Down syndrome
		Splenic atrophy, thrombocytosis	Epilepsy (±cerebral calcification)
		Musculoskeletal	Fibrosing alveolitis
		Osteopenia, osteoporosis, fractures	Recurrent pericarditis
		Bone pain, joint pain	Idiopathic pulmonary hemosiderosis
		Dental enamel defects	Possible association
		Arthritis, myopathy, cramps, tetany	Congenital heart defects
		Neurological	Inflammatory bowel disease
		Peripheral neuropathy, paraesthesia	Systemic lupus erythematosus
		Ataxia	Polymyositis, vasculitis
		Epilepsy (± cerebral calcification)	Myasthenia gravis
		Night blindness	Iridocyclitis, choroiditis
		Reproduction	Sarcoidosis
		Female and male infertility	Cystic fibrosis
		Recurrent abortion	Addison's disease
		Psychiatric/psychological	Autoimmune hemolytic anemia
		Anxiety, depression	Autoimmune thrombocytopenia
		Irritability, poor school performance	Schizophrenia

In adult patients with iron deficiency anemia, overall prevalence of CS was 5-6%. However, studies of adults with IDA refractory to oral iron have a much higher incidence of CS. In a small study of adults with iron deficiency anemia, immunological screening with AGA and EMA showed suspected CS in 5/85 cases (5.8%) with diagnosis confirmed on intestinal biopsy. Additional studies of patients with refractory IDA showed a higher prevalence of CS as high as 20%.[107] Another study looked at consecutive adult patients with IDA and response to GFD. Prevalence of CS in their study group was 13.7% with a strong female predominance. After 6 months of gluten free diet, 14/18 (77.8%) female patients recovered from anemia, but only 5/18 (27.8%) reversed from iron deficiency. At 12 months, all but one patient (94.4%) recovered from anemia and 9 (50%) patients from iron deficiency.[108]

Other investigators have looked at evidence of occult gastrointestinal blood loss in patients with histologic lesions of CS. One study found 2/8 patients with partial villous atrophy (25%) and 15/28 (54%) with total villous atrophy had positive Hemoccult tests. As occult GI bleeding can be detected in about half of patient with CS, the author felt that CS should be added to the list of factors that can contribute to IDA.[109] Logan et al investigated the utility of screening for CS in patients with positive FOBT. Five of 590 patients had had fecal occult blood tests at

time of diagnosis and four had positive tests during investigation of diarrhea and/or anemia. Twenty-two of 309 FOBT patients were positive for IgA AGA, but none had IgA EMA and two had positive anti-tTG assays. Logan et al concluded that CS does not need to be considered in the investigation of a positive FOBT, unless there are other indications.[110]

Celiac sprue has long been associated with progressive neurologic deficits, predominantly cerebellar ataxia and peripheral neuropathy. Frequent clinical association between CS and neuropsychiatric disorders has been reported. A study of CS patients in New York City found that approximately half of patients with CS had symptoms and signs of neuropsychiatric involvement, most, fortunately, are minor and reversible. Gobbi et al reported a consistent cohort of CS patients affected by drug-resistant epilepsy with cerebral calcifications and. In his study group up to half had improvement in seizure control with gluten-free diet.[111] Prevalent opinion is that nervous system involvement in CS is believed to be caused by the disease mechanism itself rather than by nutritional deficiencies related to malabsorption, such as vitamin E or B12 deficiencies. Recent studies suggested that idiopathic cerebellar ataxia might be the presenting manifestation of sensitivity to gluten with or without intestinal pathology. They found that the prevalence of gluten sensitivity is similar in patients with sporadic (27%) and hereditary (37%) ataxias, including patients with known ataxia genotypes. Another study found high AGA antibody titers in 23/52 (44%) patients with Huntington's disease, suggesting a previously unrecognized association between HD and gluten sensitivity. The role of AGA in these diseases is unclear.[112]

First report of liver involvement in celiac sprue appeared in 1977. Celiac hepatitis is characterized by the absence of hepatomegaly, splenomegaly, or any clinical features suggesting chronic liver disease such as hypergammaglobulininulemia. Serum autoantibodies other than anti-tTG-related autoantibodies are not found. Histologic changes of mild lobular and portal tract inflammation are reversible with GFD. In a group of 74 newly diagnosed adults with CS, Maggiore and Capri[113] found 40% had an elevation of serum aminotransferase activity that normalized in most cases during treatment with GFD. Liver biopsies in this group revealed signs of reactive hepatitis in five patients and histologic lesions of different types and severity ranging from steatosis with focal fibrosis to pronounced fibrosis and cirrhosis in another seven patients. Two retrospective studies in adults suggested that as many as 9% of patients with persistent and cryptogenic elevation of serum aminotransferases may be affected by asymptomatic CS.[114]

A high prevalence of CS (4.3%), four to ten times higher than expected, was recently found in Finland in a group of 185 adult patients who had undergone liver transplantation. Indication for liver transplantation varied: 4 patients had severe liver disease and CS, 1 had congenital liver fibrosis, 1 had massive hepatic steatosis, and 2 had idiopathic progressive hepatitis without apparent origin.[114]

All types of autoimmune liver disorder may be associated with CS, with autoimmune hepatitis having the closest association. Prevalence of CS in patients with autoimmune hepatitis is at least 2.7%.[115] Other authors have suggested CS occurs in up to 4.3% of type I AIH and up to 8% of type 2 AIH.[116] The correlation between primary biliary cirrhosis and celiac sprue is still unclear with numerous studies with conflicting results.[114] Prevalence of PBC has been reported to be as high as 3% in celiac patients, and about 6% of individuals with CS may be affected by PBC.[117] Clinical presentation can be strikingly different between PBC and CS. For example, dermatitis herpetiformis is not associated with PBC, thyroid disease and hyper-γ-globulinemia are seen in PBC while IDDM and selective IgA deficiency are associated with CS, and CS is

found in all ages while PBC is not diagnosed in children.[116] Recent studies refute the association demonstrating a high false positive rate of serologic tests for CS in patients with PBC.[118]

MALIGNANCY

Although serology-based estimation of the incidence of CS is high, only 10-20% of patients have an overt disease with apparent symptoms, and most have silent or asymptomatic disease. A major concern since the late 1980s has been the increased risk of malignancies, particularly of non-Hodgkin lymphoma, now identified as the main cause of increased mortality in patients with CS. Pioneering study by Holmes indicated CS patients had an overall two-fold relative risk of cancer with the risk increased 10-fold for certain GI carcinomas and 43-fold for NHL. Recent surveys, including a vast retrospective Swedish study, provided lower figures, with an overall risk of cancer only moderately increased and a three to six fold increased risk of NHL. However, the risk remained 10-fold increased for small intestinal carcinomas. In a recent European study, although prevalence of CS was increased only 2.6-fold in patients with NHL, the odds ratio increased significantly for small bowel lymphomas and enteropathy associated intestinal T lymphomas. Initial observations by Holmes provided evidence that a strict GFD had a protective effect; the risk of malignancy returning to that of the general population after 5 years. A study by Askling in Sweden showed the risk of cancer disappeared after a 10 year follow-up.[119] A study of 381 CS patients found 11% had a diagnosis of cancer, most before the diagnosis of CS.[120] Several case reports of small bowel adenocarcinoma are also reported, one of which was found adjacent to a jejunal villous adenoma.[121] However, a recent study did not find a significantly increased risk of duodenal adenoma in CS patients compared to non-celiac endoscoped population in New York City.[122] Patients with known CS who present with exacerbation of symptoms should be promptly investigated for occult GI malignancies, and considered for early surgical exploration.[123] In a recent study, dietary compliance was shown to reduce the risk of lymphoma and other malignancies and, if continued for more than five years, the risk becomes comparable to that of the general population. The risk of malignancies in asymptomatic cases the role of screening is unclear.

Tumorigenesis results from a loss of genome integrity that affects the function of genes controlling cell division, cell differentiation, and cell death. The chronic inflammation, crypt hyperplasia and increased presence of IELs in CS likely contribute to increased risk of malignancy.[119] A study of CS patients found the frequency of chromosome abnormalities in peripheral blood lymphocytes decreased significantly on a GFD and concluded that genomic instability is a secondary phenomenon, likely caused by chronic intestinal inflammation.[124] Another study in patients with CS looked at the effect of the gluten-induced mucosal injury on telomeres and found accelerated telomere shortening that would increase the process of end-to-end fusions resulting in chromosomal changes that play a role in the cancer predisposition observed in CS patients.[125]

AUTOIMMUNE DISORDERS

Sjorgen's syndrome is probably the most common rheumatic autoimmune condition occurring in connection with CS.[126] A study of Sjorgen's syndrome patients found CS in 15% with HLA DQ2 present in 56% patients, including all with CS.[127] The presence of false positive AGA in patients with SLE is common. Despite shared HLA loci there does not seem to be an

association between celiac disease and SLE.[128] Studies do not support an association with CS and seropositive RA or the screening of such patients for CS.[129] A study of EMA and tTG-positive relatives of celiac patients found autoimmune disease was present in 20%.[130] Serologic testing of CS patients found 25% were positive for at least one other autoantibody, with significant prevalence of anti-thyroid microsome, anti-nuclear, and anti-gastric parietal cells, while the relatives were positive 17.8%, with an increased prevalence of anti-nuclear and anti-thyroid microsome antibodies.[131] Another study of celiac patients had increased prevalence of serum anti-ssDNA (14%), anti-dsDNA (23%), anticardiolipin (14%), and anti-EMA (63%). The relevance of this finding on the extraintestinal manifestations of CS or the coexistence of autoimmune conditions and CS remains to be determined.[132] Relatives of CS patients have an increased prevalence of autoimmune disease compared to control groups, and relatives of CS patients with autoimmune diseases, have a risk as high as 25% of being silent celiacs and might need screening for CS.[133]

OSTEOPOROSIS

Osteomalacia and severe bone disease are relatively rare but low bone mineral density seems to follow dermatitis herpetiformis as the most common extraintestinal manifestation of CS.[134] In a study by Meyer et al., celiac patients (105 women and 23 men), with average age 56 yr old and on GFD a mean of 7.5 years, were found to have osteoporosis in 34% patients at lumbar spine, 27% at the femoral neck, 36% at the radius. Osteopenia was present in 38% at the lumbar spine, 44% at the femoral neck, and 32% at the radius. When matched to age-matched controls, men were more severely affected than women. BMD did not differ between those on GFD or who had not begun therapy and only 28% of participants had normal BMD.[135] Lindh et al found biopsy-proven CS in 3.2% of patients suffering from osteoporosis. Nuti et al found IgA tTG in 9.4% of patients with osteoporosis.[136] A study in Argentina showed that patients with CS seem to have an increased risk of fractures; 25% celiac patients had experienced one or more fractures, compared with 8% hospital controls.[136] Vazquez et al. found 25% of patients with CS have had one to five fractures in the peripheral skeleton versus 8% of controls. Most fractures were located in the wrist, radius, and ulna and occurred prior to diagnosis or while patients were noncompliant with GFD.[137] Fisher et al. found 347 consecutive older patients with hip fractures were AGA positive in 11-13% but none EMA positive. They concluded that CS does not appear be an important contributing pathogenic factor in older hip fracture population with osteoporosis and patients with CS do not represent a population at particularly high risk of osteoporotic fracture, and thus targeting them for osteoporosis screening and treatment is not justified.[138]

A strict GFD improves bone mineralization, even in one year.[139] Brera et al. found the rate of bone metabolism is altered in children with untreated CS and remarkable changes occur after the initiation of GFD.[140] A study of untreated CS patients found 34% had normal BMD, 40% had osteopenia, and 26% had osteoporosis. Between males and females, there were no statistical differences in bone metabolism or in most of the nutritional indices. In treated patients, whom showed a mucosal recovery in only 57%, GFD led to a significant improvement in BMD, bone metabolism and nutrition, even in postmenopausal women.[141]

The mechanisms of disturbances in bone metabolism in CS are poorly understood. In patients with symptomatic CS, low bone mass appears to be related to malabsorption, reduced calcium absorption due to intestinal villi atrophy, vitamin D deficiency and secondary hyperparathyroidism. Calcium malabsorption may be the result of vitamin D deficiency or the

reduction in the active absorptive surface area. Steatorrhea reduces calcium and vitamin D absorption by chelating them to intraluminal fat. Secondary hyperparathyroidism due to hypocalcemia accelerates bone turnover and bone loss. Sophisticated investigations discovered that vitamin D receptors are still normally present in the duodenal mucosa of celiac patients, even those with mucosal damage and villous atrophy. However, the vitamin D-regulated proteins that actively uptake calcium from the intestinal lumen are lost from the area of damaged small intestine mucosa. Calcium malabsorption is thus determined by the reduction in vitamin D-regulated proteins and enzymes involved in active calcium absorption, which are located in the functional zone of this area.[142] Impaired intestinal calcium malabsorption leads to secondary hyperparathyroidism, which is often present and may serve to aggravate the bone disease by increasing bone turnover. Consequently, markers of bone formation and bone resorption are often increased.[136] Results show that bone weakening in CS might result from metabolic disturbances of bone remodeling affecting trabecular and cortical bone masses, the mechanical quality of the bone material, and a reduction of muscle strength impairing the modeling-dependent optimization of bone structure. Dietary treatment seems to correct almost exclusively the metabolically induced disturbances, which were predominant in women. The induced secondary hyperparathyroidism would enhance the remodeling of trabecular, endosteal, and intracortical bone, resulting in trabecular thinning, cortical-subcortical bone mass loss, enhancement of the intracortical porosity, and impairment of the mechanical quality or intrinsic stiffness of the cortical bone. CS was shown to impair muscle mass and probably the mechanical usage of the skeleton. This could affect the natural distribution of the available bone mass and the achievement of an optimal bone architectural design.[143] There seems to be impairment of peripheral reduction of testosterone to the active dihydrotestosterone which may play a role as well.[144] Intestinal inflammation and cytokines may also play a role in reducing bone mass.

ENDOCRINE DISORDERS

An estimated 1-1.5% of diabetic children suffer from celiac sprue. Patients with diabetes and celiac sprue usually present with classical symptoms poor glycemic control with frequent hypoglycemia. Diarrhea is often misinterpreted as due to autonomic diabetic neuropathy or exocrine pancreatic insufficiency, and therefore the diagnosis of CS delayed. Studies report the frequency of autoimmune IDDM in celiac patients ranges from 3.5% to 7.4%. There is no evidence that the risk of CS in type 2 DM is increased compared to population at large. The diabetic susceptibility is also associated with HLA DR3-DQ2 and DR4-DQ8. The association between the two diseases could thus be partially explained by the sharing of a common genetic factor in HLA region. Not et al. showed that the prevalence of other autoimmune diseases was significantly higher in first-degree relatives of autoimmune IDDM patients found to have silent CS than in relatives without CS antibodies. Saukkonen et al observed that the prevalence of CS in siblings of patients with autoimmune IDDM did not differ from that reported from population-based screening studies.[136] Subclinical hypothyroidism is an especially frequent finding in CS. In patients with autoimmune thyroid disease, a celiac prevalence of approximately 2-4% has been reported.[136] The prevalence of autoimmune diseases was significantly higher in patients with both CS and autoimmune thyroiditis than in patients with autoimmune thyroiditis alone.[145] In a recent screening survey, five of 41(12.5%) patients with Addison's disease of autoimmune origin were found to be suffering from CS. Alopecia areata has been found in approximately 2% of CS patients and GFD may initiate hair growth in some patients.

It has been hypothesized that increased intestinal permeability in untreated CS predisposes to other autoimmune disorders by facilitating further external antigens such as food proteins, bacterial products, and endotoxins to enter the intestinal lamina propria, thus leading to the activation of autoimmune phenomena. Ventura et al examined the relationship between prevalence of autoimmune diseases and the duration of exposure to gluten. They observed that the development of autoimmune conditions was related to the age at diagnosis of CS which related to the time of gluten exposure. The number of autoimmune conditions in those celiac patients in whom the GFD was adopted in childhood was not significantly different from that of controls. By contrast, in celiac patients who had started GFD at the age of 10 years or more, the frequency of autoimmune conditions was significantly increased. Age at diagnosis of CS was the only independent predictor for the development of autoimmune conditions. The authors suggested that an early diagnosis and treatment of CS might protect against the development of autoimmune diseases.[136]

GASTROINTESTINAL DISORDERS

In a study of 600 first-degree relatives of patients with celiac sprue, 10 cases of inflammatory bowel disease, seven cases of ulcerative colitis and three cases of Crohn's disease, were identified among first-degree relatives, whereas only one case of IBD was identified among the 1,196 first-degree relatives of control patients. Only the prevalence of UC was statistically significant.[146] Sanders et al reported a prevalence of CS of 5% among population of 300 patients with newly diagnosed IBS. O'Leary et al found a prevalence of 20% of celiac patients screened for IBS symptoms by Rome I criteria versus 5% of controls. Small intestinal bacterial overgrowth is most common among those with predisposing motility or structural disorders, with authors proposing that disturbed intestinal motility secondary to CS may account in part for the unexpectedly high proportion of CS with small intestinal bacterial overgrowth. Although prolonged orocecal transit is a recognized phenomenon in untreated celiac patients with active mucosal lesions, it reverts to normal within 6-8 months of GFD.[147]A case control study of 300 consecutive new patients who fulfilled Rome II criteria for IBS found 66 with positive antibodies of whom 14 had CS. There was significant association of CS with IBS compared with controls. Thirteen of the fourteen patients described an improvement in IBS-type symptoms since commencing GFD.[148] Of 150 celiac patients reviewed, 30/150 (20%) fulfilled ROME I criteria. Celiac patients with IBS-type symptoms had a markedly lower quality of life than those without and GFD improved quality of life.[149] These findings have been found in other recent studies.[150]

REFRACTORY CELIAC SPRUE

Refractory celiac sprue is a rare syndrome with a poor prognosis, defined by malabsorption due to gluten-related enteropathy after initial or subsequent failure of a strict GFD and after exclusion of any disorder mimicking CS. Refractory celiac sprue type (RCS) I has normal IELs by phenotyping, while type II has phenotypically immature IELs defined by characteristic T-cell markers. Recent evidence suggests that RCS comprises a heterogenous group of patients with diverse underlying causes with a small proportion of these patients seem to have an adult form of autoimmune enteropathy, characterized by the presence of antienterocyte antibody; however, a larger group of patients seem to have a cryptic intestinal T-cell lymphoma, characterized by the presence of phenotypically abnormal, monoclonal

intraepithelial lymphocytes, despite benign cytology. Recent estimated prevalence of true RCS is 7-8%. The clinical response to gluten withdrawal, with resolution of diarrhea and weight gain, tends to occur in the first weeks of a GFD, although histologic improvement may continue for up to 2 years so a one-year gluten-free period is a reasonable arbitrary period to await histologic resolution before making a diagnosis of RCS.[151] In a study of reported nonresponsive CS, 50% were found to have gluten contamination while 18% were due to RCS.[152] Refractory celiac sprue is a diagnosis of exclusion. Other conditions that may resemble RS are intestinal lymphoma, ulcerative jejunitis, autoimmune enteropathy, collagenous sprue, intolerance to nongluten dietary proteins, such as milk, egg and soy-induced enteropathies, giardiasis, bacterial overgrowth, Whipple's disease, pancreatic insufficiency, irritable bowel syndrome, lymphocytic colitis, collagenous colitis, T-cell lymphoma, pancreatic cancer, fructose intolerance, protein losing enteropathy, cavitating lymphadenopathy syndrome and tropical sprue.[152] Topical steroids have been used in patients with uncomplicated celiac disease on a normal diet, but their efficacy has not been evaluated in RCS. It has been suggested that steroids should be used in three situations in CS: (1) inability or refusal to tolerate a GFD despite deteriorating health (2) complicating ulcerative jejunitis and (3) deteriorating health despite adherence to a GFD and milk-free diet, such as RCS. There is limited experience with azathioprine and cyclosporine in treating RCS.[153] A study confirmed earlier anecdotal reports of efficacy of azathioprine in RCS with clear clinical and histological improvement shown in most patients. However, monoclonality persisted after treatment.[154] In another study, 8 of 13 adult RCS treated with cyclosporine in therapeutic doses had histological improvement (61%) in five of whom (38%) normalization of villi was demonstrated.[155] A case of patient with RCS in whom remarkable clinical and histological improvement was achieved on elemental diet was reported.[156] Other attempts at immunomodulation are reported.[157] IL-10 and blocking of IL-15 have been proposed as treatments.[158]

Ulcerative jejunoileitis is a rare condition characterized by chronic idiopathic ulcerations affecting the small bowel, unrelated to drugs, ischemia, infections, or other known causes. Although UJI is a well known complication of CS, its relationship to CS may not be obvious in patients failing to respond to a GFD. Mortality rate is high due to obstruction, bleeding and perforation. GFD may be effective in some patients but may require steroids and often surgery.[159] Enteropathy associated T cell lymphoma (EATCL) of the small intestine is a well documented complication of CS. The 5 year survival of patients with CS associated lymphoma of the small intestine is about 10%, which is much lower than those with primary GI lymphomas[160]

TREATMENT

The importance of gluten withdrawal has been known since the 1950s and remains the essential treatment for celiac sprue. This involves the complete removal of gluten-containing cereal grains, such as wheat, barley and rye, from the diet. One gram of native gliadin is adequate to induce small bowel reactions after 4-6 hours.[52] There is no conclusive data on the threshold of gluten sensitivity of celiac patients. Every effort should be made to keep the diet of celiac patients as gluten-free as possible.[161] This is often very difficult to initiate and maintain. The role of patient education is essential and the assistance of a dietitian can not be underestimated. National celiac associations and support groups are available with helpful recipes and information readily available over the internet. Gluten-free products are available

but access and cost can put undue pressure on patients. The possibilities of contamination as well as inadvertent ingestion are issues that must be addressed in the follow-up of patients with celiac sprue. There is a tendency for higher levels of contamination with increased processing. The level of contamination was in most cases low. The Codex Alimentarius Commission is the international body responsible for the execution of the Joint FAO/WHO Food Standards Program. A standard for gluten-free has been suggested by the committee that allows a maximal content of 20 mg/kg gluten (on a dry matter basis) in products naturally free from gluten, and 200 mg/kg (on a dry matter basis) in products based on wheat starch rendered "gluten-free"[162]

Avoid all foods containing wheat, rye, and barley gluten.
Avoid all foods containing oats (at least initially).
Avoid foods containing lactose initially.
Use only rice, corn, maize, buckwheat, potato, soybean, or tapioca flours, meals, or starches.
Look for foods that have the gluten-free symbol.
Try foods containing wheat starch from which gluten has been removed after the diagnosis of celiac sprue is established.
Read all labels and study the ingredients of processed foods.
Beware of gluten in medications, food additives, emulsifiers, and stabilizers.
Avoid all beers, lagers, ales, and stouts.
Wine, liqueurs, most ciders, and other spirits, including whiskey and brandy, are allowed.
Give essential medications parenterally initially if malabsorption is severe.

In the vast majority of cases, CS is a benign disorder. Its current treatment, although constraining, is safe and efficient, and the costs and benefits of any other treatment will require thorough appraisal. Furthermore, one unresolved key issue is to simply define who deserves treatment. The wide clinical spectrum of the disease might reflect a wide level of individual sensitivity, some of which could be compatible with a normal diet. Epidemiologic studies providing a precise appraisal of the risk of complications are therefore needed to substantiate the need for a treatment in individuals with silent or pauci-symptomatic disease determined by serologic studies.[163]

The impact of a diagnosis of celiac sprue and its concomitant change in lifestyle should not be underestimated. Celiac women adhering to the treatment regimen for several years perceive the disease burden to be worse than men do. In light of similar differences in their quality of life, inquiry is warranted into the way celiac men and women are coping with the disorder. Women were less satisfied with the outcome at ten years than men were, and expressed more concern about the impact on socializing with friends and having to abstain from important things in life.[164] After ten years on a GFD, adult celiac patients fail to attain the same degree of subjective health as the general population. This is particularly true for female patients and suggests that factors beyond normalization of the intestinal mucosa are of importance for the perceived health status of celiacs diagnosed in adult life.[165] Celiac patients showed good knowledge of the disease, directly correlated to their socioeconomic level. At diagnosis, relief was most intense feeling. Anger was inversely correlated with actual compliance to diet.[166] Quality of life was poorer in both celiac and diabetic patients than in healthy controls and significantly correlated with anxiety. In CS, affective disorders should be ascribed to difficulties in adjusting to the chronic nature of the disease rather than directly to the disease itself.[167]A recent survey of members of the Canadian Celiac Association found that 45% of respondents reported that following GFD was very or moderately difficult. Quality of life of individuals with CS was comparable to the mean quality of life of Canadians.[168] Improved quality of life after diagnosis was reported by 77%.[24] Interestingly, the prevalence of surgery in CS patients is greater than the general population, specifically appendectomy, tonsillectomy, hernia repair and cosmetic surgery.[169]

The toxicity and tolerance of oats has been examined in several studies. In a recent study, nineteen celiac sprue patients on gluten free diet were challenged with 50g oats/day for 12 weeks. Purity of the oats was verified by ELISA, western blot, and mass spectrometry, with

contamination with barley found in one sample. Oats generally well tolerated but several patients reported initial abdominal discomfort and bloating. One patient developed partial villous atrophy and rash during first oat challenge. She improved on oats free diet but developed subtotal villous atrophy and dramatic dermatitis during second challenge. This study concluded that most celiac sprue patients can tolerate oats but contamination of commercial oats by other cereals is a problem and celiacs should select oats from manufacturers that are devoted to producing oats without such contamination.[170] In another randomized, controlled study, adults had a mean oat intake in oat group of 49.9 +/- 14.7 g/day at 6 months for patients in remission and 46.6+/-13.3 g/day at 12 months for patients with newly diagnosed CS. The oat and control groups did not differ significantly in nutritional status, symptoms or laboratory measures. Patients in remission, regardless of diet, did not have worsening architecture of the duodenal villi or increase in mononuclear cell infiltration. This study concluded that moderate amounts of oats can be included in a gluten-free diet for most adult patients with CS without adverse effects but severe CS patients were excluded.[171] This same group examined the effect of oats on serologic markers. In this study, the rate of antigliadin and antireticulin antibodies did not differ between diet groups in patients with newly diagnosed CS. They found oats had no effect on antibody levels in patients with remission and no increase in number of intraepithelial lymphocytes in oats group. These results were felt to strengthen the view that adult patients with CS can consume moderate amounts of oats without adverse immunological effects.[172] This group also reported no difference in controls versus oats consuming patients after five years of follow up.[173]

CONCLUSION

Celiac sprue is a gluten-sensitive disorder with small intestinal malabsorption that is the result of genetic, environmental, and immunologic factors that have recently been better understood. It is more common than previously thought and an awareness of the varied presentations and associated conditions can help raise clinical suspicion. Diagnosis can be readily made with serologic testing and small intestinal biopsy. Treatment with gluten free diet is effective and readily available, and continued research into celiac sprue will further explain its pathogenesis and lead to other effective treatments.

BIBLIOGRAPHY

1. Farrell, RJ and Kelly, CP. Celiac sprue and refractory sprue, in Feldman, M, Friedman, LS, and Sleisenger, MH (eds): Sleisenger & Fordtrans's Gastrointestinal and Liver Disease: pathophysiology, diagnosis, management. Philadelphia; W. B. Saunders, 7th edition. 2002, 1817-1841.
2. Ciclitira PJ and Ellis HJ. Celiac disease. In: Textbook of gastroenterology. Yamada T, et al., eds, Chapter 76. Philadelphia: Lippincott-Raven, 2003.
3. Westergaard H. The sprue syndromes. UTSW Internal Medicine Grand Rounds, June 13, 1985.
4. Schuppan D. Current concepts of celiac disease pathogenesis. Gastroenterology 2000;119:234-242.
5. Ciclitira PJ. AGA technical review on celiac sprue. Gastroenterology 2001;120:1526-1540.
6. American Gastroenterological Association medical position statement: celiac sprue. Gastroenterology 2001;120:1522-1525.
7. Cronin CC and Shanahan F. Exploring the Iceberg – the spectrum of celiac disease. Am J Gastroenterol 2003;98:518-520.
8. Ludvigsson JF, Ansved P, Fälth-Magnusson K, et al. Symptoms and signs have changed in Swedish children with coeliac disease. J Pediatr Gastroenterol Nutr 2004;38-181-186.
9. López-Rodríguez MJ, Canal Macías ML, Lavado Garcia JM, et al. Epidemiological changes in diagnosed coeliac disease in a population of Spanish children. Acta Pædiatr 2003;92:165-169.
10. Troncone R, Greco L, and Auricchio S. The controversial epidemiology of coeliac disease. Acta Pædiatr 2000;89:140-141.
11. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States. Arch Intern Med 2003;163:286-292.
12. Högberg L, Fälth-Magnusson K, Grodzinsky E, and Stenhammar L. Familial prevalence of coeliac disease: a twenty-year follow-up study. Scand J Gastroenterol 2003;38:61-65.
13. Kolho K-L, Färkkilä MA, and Savilahti E. Undiagnosed coeliac disease is common in Finnish adults. Scand J Gastroenterol 1998;33:1280-1283.
14. Mäki M, Mustalahti K, Kokkonen J, et al. Prevalence of celiac disease among children in Finland. N Engl J Med 2003;348:2517-2524.
15. Sjöberg K and Eriksson S. Regional differences in coeliac disease prevalence in Scandinavia? Scand J Gastroenterol 1999;34:41-45.
16. Rostami K, Mulder CJJ, Werre JM, et al. High prevalence of celiac disease in apparently healthy blood donors suggests a high prevalence of undiagnosed celiac disease in the Dutch population. Scand J Gastroenterol 1999;34:276-279.
17. Volta U, Bellentani S, Bianchi FB, et al. High prevalence of celiac disease in Italian general population. Dig Dis Sci 2001;46:1500-1505.
18. Riestra S, Fernández E, Rodrigo L, Garcia S, and Ocio G. Prevalence of coeliac disease in the general population of Northern Spain. Scand J Gastroenterol 2000;35:398-402.
19. West J, Logan RFA, Hill PG, et al. Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. Gut 2003;52:960-965.
20. Sanders DS, Patel D, Stephenson TJ, et al. A primary care cross-sectional study of undiagnosed adult coeliac disease. Eur J Gastroenterol Hepatol 2003;15:407-413.
21. Not T, Horvath K, Hill ID, et al. Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. Scand J Gastroenterol 1998;33:494-498.
22. 105 Lo W, Sano K, Lebwohl B, Diamond B, and Green PHR. Changing presentation of adult celiac disease. Dig Dis Sci 2003;48:395-398.
23. Zipser RD, Patel S, Yahya KZ, Baisch DW, and Monarch E. Presentations of adult celiac disease in a nationwide patient support group. Dig Dis Sci 2003;48:761-764.
24. Green PHR, Stavropoulos SN, Panagi SG, et al. Characteristics of adult celiac disease in the USA: results of a national survey. Am J Gastroenterol 2001;96:126-131.
25. Poddar U, Thapa BR, Nain CK, Prasad A, and Singh K. Celiac disease in India: are they true cases of celiac disease? J Pediatr Gastroenterol Nutr 2002;35:508-512.
26. Sood A, Midha V, Sood N, Kaushal V, and Puri H. Increasing incidence of celiac disease in India. Am J Gastroenterol 2001;96:2804-2805.
27. Lebenthal E and Branski D. Celiac disease: an emerging global problem. J Pediatr Gastroenterol Nutr 2002;35:472-474.
28. Mandal A and Mayberry J. How common is celiac disease in South America? Am J Gastroenterol 2000;95:579-580.
29. Catassi C, Fornaroli F, and Fasano A. Celiac disease: from basic immunology to bedside practice. Clin Applied Immunol Rev 2002;3:61-71.
30. Gandolfi L, Pratesi R, Cordoba JCM, Tauil PL, Gasparin M, and Catassi C. Prevalence of celiac disease among blood donors in Brazil. Am J Gastroenterol 2000;95:689-692.
31. Pratesi R, Gandolfi L, Garcia SG, et al. Prevalence of coeliac disease: unexplained age-related variation in the same population. Scand J Gastroenterol 2003;7:747-750.
32. Gomez JC, Selvaggio GS, Viola M, et al. Prevalence of celiac disease in Argentina: screening of an adult population in the La Plata area. Am J Gastroenterol 2001;96:2700-2704.
33. Shahbazkhani B, Malekzadeh R, Sotoudeh M, et al. High prevalence of coeliac disease in apparently healthy Iranian blood donors. Eur J Gastroenterol Hepatol 2003;15:475-478.
34. Shamir R, Lerner A, Shinar E, et al. The use of a single serological marker underestimates the prevalence of celiac disease in Israel: a study of blood donors. Am J Gastroenterol 2002;97:2589-2594.
35. Cataldo F, Marino V, Ventura A, et al. Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. Gut 1998;42:362-365.
36. Book L, Hart A, Black J, Feolo M, Zone JJ, and Neuhausen SL. Prevalence and clinical characteristics of celiac disease in downs syndrome in a U.S. study. Am J Med Gen 2001;98:70-74.
37. Pueschel SM, Ramano C, Failla P, et al. A prevalence study of celiac disease in persons with down syndrome residing in the United States of America. Acta Pædiatr 1999;88:953-956.
38. Bonamico M, Mariani P, Danesi HM, et al. Prevalence and clinical picture of celiac disease in Italian down syndrome patients: a multicenter study. J Pediatr Gastroenterol Nutr 2001;33:139-143.
39. Farrell RJ and Kelly CP. Celiac Sprue. N Engl J Med 2002;346:180-188.
40. McAdam SN and Sollid LM. Getting to grips with gluten. Gut 2000;47:743-745.
41. Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. Immunology 2002;2:647-655.
42. Godkin A and Jewell D. The pathogenesis of celiac disease. Gastroenterology 1998;115:206-210.
43. Vader LW, Stepniak DT, Bunnik EM, et al. Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. Gastroenterology 2003;125:1105-1113.
44. Wieser, H. Comparative investigations of gluten proteins from different wheat species. III. N-terminal amino acid sequences of α-gliadins potentially toxic for coeliac patients. Eur Food Res Technol 2001;213:183-186.
45. Kcaveny AP, Offner GD, Bootle E, and Nunes DP. No significant difference in antigenicity or tissue transglutaminase substrate specificity of Irish and US wheat gliadins. Dig Dis Sci 2000;45:755-762.
46. Dolfini E, Elli L, Ferrero S, et al. Bread wheat gliadin cytotoxicity: a new three-dimensional cell model. Scand J Clin Lab Invest 2003;63:135-142.
47. Elli L, Dolfini E, and Bardella MT. Gliadin cytotoxicity and in vitro cell cultures. Toxicology Letters 2003;146:1-8.
48. Matysiak-Budnik T, Candalh C, Dugave C, et al. Alterations of the intestinal transport and processing of gliadin peptides in celiac disease. Gastroenterology 2003;125:696-707.
49. Shan L, Molberg Ø, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. Science 2002;297:2275-2279.
50. McManus R and Kelleher D. Celiac disease – the villain unmasked? N Eng J Med 2003;348:2573-2574.
51. Fraser JS, Engel W, Ellis HJ, et al. Coeliac disease: in vivo toxicity of the putative immunodominant epitope. Gut 2003;52:1698-1702.
52. Ellis HJ, Pollock EL, Engel W, et al. Investigation of the putative immunodominant T cell epitopes in coeliac disease. Gut 2003;52:212-217.
53. Parrot I, Huang PC, and Khosla C. Circular dichroism and nuclear magnetic resonance spectroscopic analysis of immunogenic gluten peptides and their analogs. J Biol Chem 2002;277:45572-45578.
54. Alcanzi M, Demonte AM, Esper C, Garcilazo S, and Waggener M. Celiac disease: antibody recognition against native and selectively deamidated gliadin peptides. Clinical Chemistry 2001;47:2023-2028.
55. Orrù S, Caputo I, D'Amato A, Ruoppolo M, and Esposito C. Proteomics identification of acyl-acceptor and acyl-donor substrates for transglutaminase in a human intestinal epithelial cell line. J Biol Chem 2003;278:31766-31773.
56. Fesus L and Piacentini M. Transglutaminase 2: an enigmatic enzyme with diverse functions. TRENDS in Biochemical Sciences 2002;27:534-539.
57. Kim S-Y, Jeitner TM, and Steinert PM. Transglutaminases in disease. Neurochemistry International 2002;40:85-103.
58. Reif S and Lerner A. Tissue transglutaminase – the key player in celiac disease: a review. Autoimmunity Reviews 2003.

59. Mcarin ML and Koning F. Tissue transglutaminase: master regulator of celiac disease? J Pediatr Gastroenterol Nutr 2003;36:9-11.
60. Molberg Ø, McAdam S, Lundin KEA, et al. T cells from celiac disease lesions recognize gliadin epitopes deamidated *in situ* by endogenous tissue transglutaminase. Eur J Immunol 2001;31:1317-1323.
61. Freitag T, Schulze-Koops H, Nicdobitek G, Melino G, and Schuppan D. The role of the immune response against tissue transglutaminase in the pathogenesis of coeliac disease. Autoimmunity Reviews 2003.
62. Koning F. The molecular basis of celiac disease. J Mol Recognit 2003;16:333-336.
63. Esposito C, Paparo F, Caputo I, et al. Expression and enzymatic activity of small intestinal tissue transglutaminase in celiac disease. Am J Gastroenterol 2003;98:1813-1820.
64. Mowat AM. Dietary modifications: food dependent autoimmunity in coeliac disease. Gut 1998;43:599-600.
65. Sollid LM. Molecular basis of celiac disease. Annu Rev Immunol 2000;18:53-81.
66. Mowat AM. Coeliac disease – a meeting point for genetics, immunology, and protein chemistry. Lancet 2003;361:1290-1292.
67. Guandalini S and Gokhale R. Update on immunologic basis of celiac disease. Curr Opin Gastroenterol 2002;18:95-100.
68. Barbeau WE, Novascone MA, and Elgert KD. Is celiac disease due to molecular mimicry between gliadin peptide-HLA class II molecule-T cell interactions and those of some unidentified superantigen? Molecular Immunology 1997;34:535-541.
69. Kumar R, Eastwood AL, Brown ML, and Laurie GW. Human genome search in celiac disease: mutated gliadin T-cell-like epitope in two human proteins promotes T-cell activation. J. Mol Biol 2002;319:593-602.
70. De Wal YV, Kooy Y, Veelen PV, Vader W, Koning F, and Peña S. Coeliac disease: it takes three to tango! Gut 2000;46:734-737.
71. Clot F, Gianfrani C, Babron M-C, et al. HLA-DR53 molecules are associated with susceptibility to celiac disease and selectively bind gliadin-derived peptides. Immunogenetics 1999;49:800-807.
72. Van De Wal Y, Kooy YMC, van Veelen PA, et al. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. Proc Natl Acad Sci 1998;95:10050-10054.
73. Fasano A. Celiac disease – how to handle a clinical chameleon. N Eng J Med 2003;348:2568-2570.
74. Singhal, A et al. Coeliac disease and hereditary haemochromatosis: association and implications. Eur J Gastroenterol Hepatol 2004; 16: 235-237.
75. Carroccio A, Cavataio F, Montalto G, Paparo F, Troncone R, and Iacono G. Treatment of giardiasis reverses 'active' coeliac disease to 'latent' coeliac disease. Eur J Gastroenterol Hapatol 2001;13:1101-1105.
76. Kaukinen K, Mäki M, Partanen J, Sievänen H, and Collin P. Celiac disease without villous atrophy. Revision of criteria called for. Dig Dis Sci 2001;46:879-887.
77. Lionetti P. The enteropathy of celiac disease. J Pediatr Gastroenterol Nutr 2002;34:S18-S21.
78. Oberhuber G. Histopathology of celiac disease. Biomed & Pharmacother 2000;54:368-372.
79. Wahab PJ, Meijer JWR, Goerres MS, and Mulder CJJ. Coeliac disease: changing views on gluten-sensitive enteropathy. Scand J Gastroenterol 2002;37 Suppl 236:60-65.
80. Wong RCW, Steele RH, Reeves GEM, Wilson RJ, Pink A, and Adelstein S. Antibody and genetic testing in coeliac disease. Pathology 2003;35:285-304.
81. Murray J, Herlein J, Mitros F, and Goeken J. Serologic testing for celiac disease in the United States: results of a multilaboratory comparison study. Clinical and Diagnostic Lab Immunology 2000;7:584-587.
82. Scoglio R, Di Pasquale G, Pagano G, Lucanto MC, Magazzù G, and Sferlazzas C. Is intestinal biopsy always needed for diagnosis of celiac disease? Am J Gastroenterol 2003;98:1325-1331.
83. Amin M, Eckhardt T, Kapitza S, et al. Correlation between tissue transglutaminase antibodies and endomysium antibodies as diagnostic markers of coeliac disease. Clinica Chimica Acta 1999;282:219-225.
84. Reeves GEM, Burns C, Hall ST, Gleeson M, Lemmert K, and Clancy RL. The measurement of IgA and IgG transglutaminase antibodies in celiac disease: a comparison with current diagnostic methods. Pathology 2000;32:181-185.
85. Schuppan D and Hahn EG. IgA anti-tissue transglutaminase: setting the stage for coeliac disease screening. Eur J of Gastroenterol Hepatol 2001;13:635-637.
86. Blackwell PJ, Hill PG, and Holmes GKT. Autoantibodies to human tissue transglutaminase: superior predictors of coeliac disease. Scand J Gastroenterol 2002;37:1282-1285.
87. López-Rodríguez MJ, Canal Macías ML, Lavado Garcia JM, et al. Epidemiological changes in diagnosed coeliac disease in a population of Spanish children. Acta Pædiatr 2003;92:165-169.
88. Krupičková S, Tučková L, Flegelová Z, et al. Identification of common epitopes on gliadin, enterocytes, and calreticulin recognised by antigliadin antibodies of patients with coeliac disease. Gut 1999;44:168-173.
89. Stern M for the Working Group on Serologic Screening for Celiac Disease. Comparative evaluation of serologic tests for celiac disease: a European initiative toward standardization. J Pediatr Gastroenterol Nutr 2000;31:513-519.
90. Tursi A, Brandimarte G, Giorgetti G, Gigliobianco A, Lombardi D, and Gasbarrini G. Low prevalence of antigliadin and anti-endomysium antibodies in subclinical/silent celiac disease. Am J Gastroenterol 2001;96:1507-1510.
91. Parnell NDJ and Ciclitira PJ. Review article: coeliac disease and its management. Aliment Pharmacol Ther 1999;13:1-13.
92. Kumar PJ. European and North American populations should be screened for coeliac disease. Gut 2003;52:170-171.
93. Fitzpatrick KP, Sherman PM, Ipp M, Saunders N, and Macarthur C. Screening for celiac disease in children with recurrent abdominal pain. J Pediatr Gastroenterol Nutr 2001 33:250-252.
94. Brocchi E, Tomassetti P, Biasco G, Corazza G, Gasbarrini G, and Corinaldesi R. Celiac disease: improving the diagnosis. Am J Gastroenterol 2002;97:1269-1270.
95. Bonamico M, Mariani P, Thanasi E, et al. Patchy villous atrophy of the duodenum in childhood celiac disease. J Pediatr Gastroentrol Nutr 2004;38:204-207.
96. Oxentenko AS, Grisolano SW, Murray JA, Burgart LJ, Dierkhising RA, and Alexander JA. The insensitivity of endoscopic markers in celiac disease. Am J Gastroenterol 2002;97:933-938.
97. Dickey W and Hughes D. Prevalence of celiac disease and its endoscopic markers among patients having routine upper gastrointestinal endoscopy. Am J Gastroenterol 1999;94:2182-2186.
98. Cox MA, Lewis KO, and Cooper BT. Sucrosemia in untreated celiac disease. A potential screening test. Dig Dis Sci 1998;43:1096-1101.
99. Casellas F, Sardi J, de Torres I, and Malagelada J-R. Hydrogen breath test with D-Xylose for celiac disease screening is as useful in the elderly as in other age groups. Dig Dis Sci 2001;46:2201-2205.
100. Smecuol E, Vazquez H, Sugai E, et al. Sugar tests detect celiac disease among first-degree relatives. Am J Gastroenterol 1999;94:3547-3552.
101. Fraquelli M, Colli A, Colucci A, et al. Accuracy of ultrasonography in predicting celiac disease. Arch Intern Med 2004;164:169-174.
102. Lomoschitz F, Schima W, Schober E, Turetschek K, Kaider A, and Vogelsang H. Enteroclysis in adult celiac disease: diagnostic value of specific radiographic features. Eur Radiol 2003;13:890-896.
103. Delcò F, El-Serag HB, and Sonnenberg A. Celiac sprue among US military veterans. Associated disorders and clinical manifestations. Dig Dis Sci 1999;44:966-972.
104. Hallert C, Grant C, Grehn S, et al. Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. Aliment Pharmacol Ther 2002;16:1333-1339.
105. Dickey W. Low serum vitamin B12 is common in coeliac disease and is not due to autoimmune gastritis. Eur J Gastroenterol Hepatol 14:425-427.
106. Guidetti CS, Scaglione N, and Martini S. Red cell distribution width as a marker of coeliac disease: a prospective study. Eur J Gastroenterol Hepatol 2002;14:177-181.
107. Carroccio A, Iannitto E, Cavataio F, et al. Sideropenic anemia and celiac disease: one study, two points of view. Dig Dis Sci 1998;43:673-678.
108. Annibale B, Severi C, Chistolini A, et al. Efficacy of gluten-free diet alone on recovery from iron deficiency anemia in adult celiac patients. Am J Gastroenterol 2001;96:132-137.
109. Fine KD. The prevalence of occult gastrointestinal bleeding in celiac sprue. N Engl J Med 1996;334:1163-1167.
110. Logan RFA, Howarth GF, West J, Shepherd K, Robinson MHE, and Hardcastle JD. How often is a positive faecal occult blood test the result of coeliac disease? Eur J Gastroenterol Hepatol 2003;15:1097-1100.
111. Gentile V, Violante V, D'Amico B, Illiano M, and Luongo A. Tissue transglutaminase and coeliac disease pathogenesis: potential molecular mechanisms for other human diseases. Neurochemistry International 2002;40:79-83.
112. Bushara KO, Nance M, and Gomez CM. Antigliadin antibodies in Huntington's disease. Neurology 2004;62:132-133.
113. Maggiore G and Caprai S. The liver in celiac disease. J Pediatr Gastroenterol Nutr 2003;37:117-119.
114. Kaukinen K, Halme L, Collin P, et al. Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. Gastroenterology 2002;122:881-888.

115. Clot F and Babron M-C. Genetics of celiac disease. Molecular Genetics and Metabolism 2000;71:76-80.
116. Volta U, de Franceschi L, Molinaro N, et al. Frequency and significance of anti-gliadin and anti-endomysial antibodies in autoimmune hepatitis. Dig Dis Sci 1998;43:2190-2195.
117. Neuberger J. PBC and the gut: the villi atrophy, the plot thickens. Gut 1999;44:594-595.
118. Habior A, Lewartowska A, Orłowska J, et al. Association of coeliac disease with primary biliary cirrhosis in Poland. Eur J Gastroenterol Hepatol 2003;15:159-164.
119. Verkarre V, Romana S-P, and Cerf-Bensussan N. Gluten-free diet, chromosomal abnormalities, and cancer risk in coeliac disease. J Pediatr Gastroenterol Nutr 2004;38:140-142.
120. Green PHR, Fleischauer AT, Bhagat G, Goyal R, Jabri B, and Neugut AI. Risk of malignancy in patients with celiac disease. Am J Med 2003;115:191-195.
121. Rampertab SD, Forde KA, and Green PHR. Small bowel neoplasia in coeliac disease. Gut 2003;52:1211-1214.
122. Rampertab SD, Fleischauer A, Neugut AI and Green PHR. Risk of duodenal adenoma in celiac disease. Scand J Gastroenterol 2003;38:831-833.
123. Pricolo VE, Mangi AA, Aswad B, and Bland KI. Gastrointestinal malignancies in patients with celiac sprue. Am J Surg 1998;176:344-347.
124. Kolaček S, Jadrešin O, Petković I, Mišak Z, Sonicki Z, and Booth IW. Gluten-free diet has a beneficial effect on chromosome instability in lymphocytes of children with coeliac disease. J Pediatr Gastroenterol Nutr 2004;38:177-180.
125. Cottliar A, Palumbo M, La Motta G, et al. Telomere length study in celiac disease. Am J Gastroenterol 2003;98:2728-2731.
126. Bizzaro N, Villalta D, Tonutti E, Tampoia M, Bassetti D, and Tozzoli R. Association of celiac disease with connective tissue diseases and autoimmune diseases of the digestive tract. Autoimmunity Reviews 2003;2:358-363.
127. Iltanen S, Collin P, Korpela M, et al. Celiac disease and markers of celiac disease latency in patients with primary Sjögren's syndrome. Am J Gastroenterol 1999;94:1042-1046.
128. Rensch MJ, Szyjkowski R, Shaffer RT, et al. The prevalence of celiac disease autoantibodies in patients with systemic lupus erythematosus. Am J Gastroenterol 2001;96:1114-1115.
129. Francis J, Carty JE, and Scott BB. The prevalence of coeliac disease in rheumatoid arthritis. Eur J Gastroenterol Hepatol 2002;14:1355-1356.
130. Cataldo F and Marino V. Increased prevalence of autoimmune diseases in first-degree relatives of patients with celiac disease. J Pediatr Gastroenterol Nutr 2003;36:470-473.
131. Utiyama SRDR, Kotze LMDS, Nisihara RM, et al. Spectrum of autoantibodies in celiac patients and relatives. Dig Dis Sci 2001;46:2624-2630.
132. Lerner A, Blank M, and Shoenfeld Y. Increased prevalence of autoantibodies in celiac disease. Dig Dis Sci 1998;43:723-726.
133. Petaros P, Martelossi S, Tommasini A, Torre G, Caradonna M, and Ventura A. Prevalence of autoimmune disorders in relatives of patients with celiac disease. Dig Dis Sci 2002;47:1427-1431.
134. Collin P, Kaukinen K, Välimäki M, and Salmi J. Endocrinological disorders and celiac disease. Endocrine Reviews 2002;23:464-483.
135. Vazquez H, Mazure R, Gonzalez D, et al. Risk of fractures in celiac disease patients: a cross-sectional, case-control study. Am J Gastroenterol 2000;95:183-189.
136. Meyer D, Stavropolous S, Diamond B, Shane E, and Green PHR. Osteoporosis in a North American adult population with celiac disease. Am J Gastroenterol 2001;96:112-119.
137. Thomason K, West J, Logan RFA, Coupland C, and Holmes GKT. Fracture experience of patients with coeliac disease: a population based survey. Gut 2003;52:518-522.
138. Barera G, Beccio S, Proverbio MC, and Mora S. Longitudinal changes in bone metabolism and bone mineral content in children with celiac disease during consumption of gluten-free diet. Am J Clin Nutr 2004;79:148-154.
139. Sategna-Guidetti C, Grosso SB, Grosso S, et al. The effects of 1-year gluten withdrawal on bone mass, bone metabolism and nutritional status in newly-diagnosed adult coeliac disease patients. Aliment Pharmacol Ther 2000;14:35-43.
140. Pedrera JD, López MJ, Canal ML, et al. Quantitative phalangeal bone ultrasound is normal after long-term gluten-free diet in young coeliac patients. Eur J Gastroenterol Hepatol 2001;13:1169-1173.
141. Vogelsang H, Suk EK, Janisiw M, Stain C, Mayr WR, and Panzer S. Calcaneal ultrasound attenuation and vitamin-D-receptors genotypes in celiac disease. Scand J Gastroenterol 2000;35:172-176.
142. Ferretti J, Mazure R, Tanoue P, et al. Analysis of the structure and strength of bones in celiac disease patients. Am J Gastroenterol 2003;98:382-390.
143. Scott EM, Gaywood I, and Scott BB for the British Society of Gastroenterology. Guidelines for osteoporosis in coeliac disease and inflammatory bowel disease. Gut 2000;46(Suppl I):i1-i8.
144. Bianchi ML and Bardella MT. Bone and celiac disease. Calcif Tissue Int 2002;71:465-471.
145. Badenhoop K, Dieterich W, Segni M, et al. HLA DQ2 and/or DQ8 is associated with celiac disease-specific autoantibodies to tissue transglutaminase in families with thyroid autoimmunity. Am J Gastroenterol 2001;96:1648-1649.
146. Chiarioni G, Bassotti G, Germani U, et al. Gluten-free diet normalizes mouth-to-cecum transit of a caloric meal in adult patients with celiac disease. Dig Dis Sci 1997;42:2100-2105.
147. O'Leary C and Quigley EMM. Small bowel bacterial overgrowth, celiac disease, and IBS: what are the real associations? Am J Gastroenterol 2003;98:720-722.
148. O'Leary C, Wieneke P, Buckley S, et al. Celiac disease and irritable bowel-type symptoms. Am J Gastroenterol 2002;97:1463-1467.
149. Shahbazkhani B, Forootan M, Merat S, et al. Coeliac disease presenting with symptoms of irritable bowel syndrome. Aliment Pharmacol Ther 2003;18:231-235.
150. Ryan BM and Kelleher D. Refractory celiac disease. Gastroenterology 2000;119:243-251.
151. Abdulkarim AS, Burgart LJ, See J, RDLD, and Murray JA. Etiology of nonresponsive celiac disease: results of a systematic approach. Am J Gastroenterol 2002;97:2016-2021.
152. Mauriño E, Niveloni S, Cherñavsky A, et al. Azathioprine in refractory sprue: results from a prospective, open-label study. Am J Gastroenterol 2002;97:2595-2602.
153. Wahab PJ, Crusius JBA, Meijer JWR, Uil JJ, and Mulder CJJ. Cyclosporin in the treatment of adults with refractory coeliac disease – an open pilot study. Aliment Pharmacol Ther 2000;14:767-774.
154. Mandal A and Mayberry J. Elemental diet in the treatment of refractory coeliac disease. Eur J Gastroenterol Hepatol 2001;13:79-80.
155. Mulder CJJ, Wahab PJ, Meijer JWR, and Metselaar E. A pilot study of recombinant human interleukin-10 in adults with refractory coeliac disease. Eur J Gasterenterol Hepatol 2001;13:1183-1188.
156. Mention J-J, Ahmed MB, Bègue B, et al. Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology 2003;125:730-745.
157. Biagi F, Lorenzini P, and Corazza GR. Literature review on the clinical relationship between ulcerative jejunoileitis, coeliac disease, and enteropathy-associated T-cell lymphoma. Scand J Gastroenterol 2000;8:785-790.
158. Varadarajulu S and Lewin D. Enteropathy-associated T-cell lymphoma involving the colon and extraintestinal B-cell lymphoma in celiac disease. Dig Dis Sci 2003;48:1298-1302.
159. Stern M, Ciclitira PJ, van Eckert R, et al. Analysis and clinical effects of gluten in coeliac disease. Eur J Gastroenterol Hepatol 2001;13:741-747.
160. Størsrud S, Yman IM, and Lenner RA. Gluten contamination in oat products and products naturally free from gluten. Eur Food Res Technol 2003;217:481-485.
161. Benahmed M, Mention J-J, Matysiak-Budnik T, and Cerf-Bensussan N. Celiac disease: a future without gluten-free diet?? Gastroenterology 2003;125:1264-1267.
162. Hallert C, Grännö C, Hultén S, et al. Living with coeliac disease: controlled study of the burden of illness. Scand J Gastroenterol 2002;37:39-42.
163. Hallert C, Grännö C, Grant C, et al. Quality of life of adult coeliac patients treated for 10 years. Scand J Gastroenterol 1998;33:933-938.
164. Ciacci C, Iavarone A, Siniscalchi M, Romano R, and De Rosa A. Psychological dimensions of celiac disease: toward an integrated approach. Dig Dis Sci 2002;47:2082-2087.
165. Fera T, Cascio B, Angelini G, Martini S, and Guidetti CS. Affective disorders and quality of life in adult coeliac disease patients on a gluten-free diet. Eur J Gastroenterol Hepatol 2003;15:1287-1292.
166. Cranney A, Zarkadas M, Graham ID, and Switzer C. The Canadian celiac health survey – the Ottawa chapter pilot. BMC Gastroenterol 2003;3:1-6.
167. Ciacci C, Cavallaro R, Romano R, et al. Increased risk of surgery in undiagnosed celiac disease. Dig Dis Sci 2001;46:2206-2208.
168. Lundin KEA, Nilsen EM, Scott HG, et al. Oats induced villous atrophy in coeliac disease. Gut 2003;52:1649-1652.
169. Janatuinen EK, Pikkarainen PH, Kemppainen TA, et al. A comparison of diets with and without oats in adults with celiac disease. N Engl J Med 1995;333:1033-1037.
170. Janatuinen EK, Kemppainen TA, Pikkarainen PH, et al. Lack of cellular and humoral immunological responses to oats in adults with coeliac disease. Gut 2000;46:327-331.
171. Janatuinen EK, Kemppainen TA, Julkunen RJK, et al. No harm from five year ingestion of oats in coeliac disease. Gut 2002;50:332-335.
172. De Sousa JS. Enzymatic treatment of celiac disease. J Pediatr Gastroenterol Nutr 2004;38:229.
173. Cerf-Bensussan N and Heyman M. A bacterial enzyme to treat celiac disease? J Pediatr Gastroenterol Nutr 2003;36:585-586.