Old Herborn University Seminar Monograph 27:
Persisting consequences of intestinal infection.
Editors: Peter J. Heidt, Dennis Lang, Mark Riddle, Richard Walker, and Volker Rusch.
Old Herborn University Foundation, Herborn-Dill, Germany: 71-86 (2014).

COELIAC DISEASE

JOSEPH A. MURRAY and ERIC V. MARIETTA

Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

SUMMARY

Coeliac disease, causes chronic inflammation of the proximal intestinal, is an increasingly common disorder impacting health and nutrition. It is also a model disease straddling gut epithelial responses and systemic autoimmunity. It is a disease about which we know much, but also presents mysteries. Why does it occur at any age and why is it increasing. Why do most individuals who carry the genetic predisposition and eat gluten but don't get the disease? It differs substantially from the more classic inflammatory bowel diseases of Crohn's disease and ulcerative colitis. Those latter diseases primarily affect the distal small intestine or colon and rarely the upper intestine. Furthermore, coeliac disease is a response to dietary glutens whereas the microbiota play the dominant role for triggering IBD. The colon and distal small intestine are filled with large numbers of bacteria and other microbiota, the proximal small intestine by contrast is almost sterile. It does contain some microbiota and is a target for pathogenic invasive organisms. The other difference is that the proximal small intestine is dealing primarily with ingesta, not resident microbiota. Indeed, there are further differences between the colon and the small intestine. The colon, which contains the largest resident biomass of our ecology, has built up effective barriers to the microbiota which when breached can result in devastating inflammation. By contrast, the small intestine is a far more permeable organ with its primary role being digestion and absorption. There is more cross talk between the luminal contents and the mucosal immune system. Hence, regulation of the inflammatory response within the small intestine is even more crucial to permit normal digestive functioning to occur than in the colon. The microbiota and coeliac disease may intersect in several ways. The microbiota may be inherently different in children/adults at risk for coeliac disease. Furthermore, pathogenic infections may perturb the intestinal milieu to an extent that individuals genetically prone to coeliac disease will lose tolerance to gluten. The gluten-free diet may change the microbiota that may affect the host. This review summarizes what is known about the aetiology, epidemiology, diagnosis, treatment, as well as the microbiology of coeliac disease.

INTRODUCTION

Coeliac disease is a chronic inflammatory condition predominately affecting the proximal small intestine (*Murray* et al., 2008). It occurs in people who have

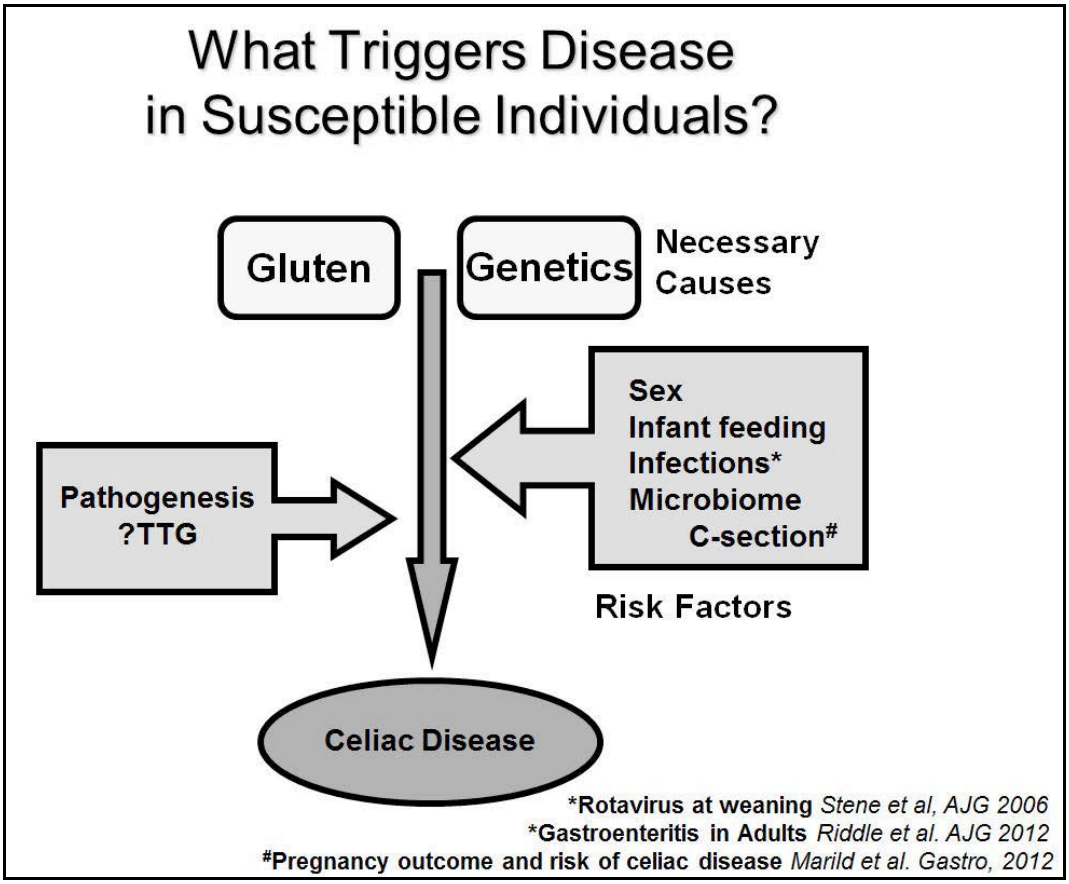

Figure 1: Coeliac disease has two required co-factors: 1) genetic predisposition, and 2) environmental factors, primarily the ingestion of gluten.

a specific genetic type, in particular the MHC class 2 gene pairs encoding the HLA molecules DQ2 or DQ8. Individuals must be exposed to dietary gluten for the disease to occur and, in most patients, the disease regresses and eventually heals when gluten is removed from the diet (*Murray* et al., 2004). The disease mostly affects Caucasians; however, this broadly affects many ethnic and racial groups across Europe, the Middle East, North Africa, India, and the Near East. It also affects Caucasians living in other areas, such as North America, South America, and Australia (*Rubio-Tapia* and *Murray,* 2007). The damage to the intestine produces inflammation as well as impacts the digestive and secretory function of the intestine. This leads to a wide variety of symptoms that can impact digestive function and also impact extra-intestinal sites (*Reilly* et al., 2012). While the rate of diagnosis of coeliac disease has increased dramatically (*Ludvigsson* et al., 2013), this rate of diagnosis probably still greatly underestimates the proportion of patients affected, with most patients remaining undiagnosed (*Rubio-Tapia* et al., 2012). The treatment of coeliac disease is based on avoidance of dietary gluten and, when diagnosed and treated in childhood, usually healing is prompt and complete. However, patients diagnosed as adults heal much less com-

monly and often take much longer to heal (*Lebwohl* et al., 2013). This review will focus on what is known about the triggering factors for coeliac disease, the impact of the microbiome on coeliac disease, as well as the potential impact of gluten on the microbiome and gut function.

AETIOLOGY

Coeliac disease affects individuals who have acquired genetic predisposition as well as ingesting gluten, the storage protein from wheat, barley and rye (Figure 1). If all that was required for coeliac disease to occur was the carriage of the appropriate risk factors as well as the ingestion of sufficient gluten on a daily basis, then fully 30% of the Caucasian population would develop the disease. However, between 1-2% of Caucasian population developed the disease, indicating that there must be other factors responsible for triggering the disease (*Walker* et al., 2010).

Genetic Basis of the Disease

Coeliac disease is associated primarily with the HLA genes initially thought to be Class 1 genes A1B8, but subsequently identified to be the HLA molecules DQ2 or DQ8. These are encoded by DQA1'05:DQB1'02XX and DQA1'03XXDQB1'0302, respectively. The carriage of one or other gene pairs is essential for the development of disease; however, this is not sufficient for the disease to occur. The known HLA genes contribute probably no more than 50% of the genetic familial risk for coeliac disease (*Bevan* et al., 1999). Siblings who carry the HLA type have an increased risk of coeliac disease compared to those who do not (*Murray* et al., 2007). Several genome-wide association studies (GWAS studies), now incorporating many thousands of patients and controls, have identified many other gene loci that are associated with risk of disease (*Garner* et al., 2009). These other loci are close to genes that regulate immune response and inflammation predominately (*Dubois* et al., 2010). Some of these genes may regulate immune responsiveness to microbial stimulation of the innate system. However, the attributable risk of coeliac disease to these other loci is relatively low, probably contributing no more than 10-15% of additional genetic risks. It is likely that coeliac disease is the result of the major HLA susceptibility genes combined with several other common genetic polymorphisms that increase immune responsiveness. Many of these genes associated with these loci are common to other inflammatory conditions, such as rheumatoid arthritis, Crohn's disease and type 1 diabetes, though there are some interesting genes that are negatively associated with particularly type 1 diabetes. The precise risk within family members varies depending on how close genetically the proband is to the patient. Monozygous twins have the highest concordance rate of 80%. Note, this is not 100% as there must be some environmental triggering differences between even identical twins. Siblings who share HLA risk factors have a greater risk than parents or children of patients with coeliac disease (*Book* et al., 2003). Second-degree relatives likely have a lower risk of coeliac disease (*Fasano* et al., 2003).

Dietary Gluten

The primary and required environmental factor for coeliac disease is dietary gluten. Gluten in this context represents the stored proteins from wheat, barley,

and rye, often given the term prolamines. These storage proteins provide the nitrogen store needed for seed germination. The original work identifying the protein fraction of these grains as being deleterious was performed in a series of challenge and withdrawal studies done in children in the Netherlands during and subsequent to the 2nd World War (*Dicke,* 1951). It was these seminal observations that laid the groundwork for the modern treatment of coeliac disease. Since that time, further work was done to identify the most immunogenic fragments of gluten, and these fragments are characterized by a large percentage of amino acids that glutamines and prolines. Particular motifs characterized by sequences of glutamines interspersed with prolines appear to be particularly immunogenic (*Jabri* and *Sollid,* 2006). In addition to their immunogenicity, is also their resistance of endopeptidase activity within the intestine. Within the human intestine, endopeptidases failed to break down some of the most immunogenic fragments of the gluten-derived proteins, particularly a gliadin. A specific 33-mer peptide or a gliadin is especially resistant to digestion. This same 33-mer peptide contains within it 3 very immunogenic peptides that, when each is complexed with the T cell, are presented to the T cells within the small intestine by the HLA molecule on the antigen-presenting cells, produce a very potent inflammatory response. The binding characteristics of these peptides can be greatly enhanced by deamidation of specific glutamine peptides, particularly glutamine amino acids (*Molbert* et al., 1998). Deamidation occurs in response to transglutaminase enzyme effects. Transglutaminases are enzymes present within the intestine and elsewhere. They are expressed constitutively but are especially expressed in the context of inflammation. There are also microbial transglutaminases present within the gut lumen and some have been used in food processing. The transglutaminases act to remove the amine side chain specific glutamines in the gliadin peptides. When these amine groups are removed, so-called deamidation, it renders the peptide far higher binding affinity to the class 2 HLA molecule binding site (*Shan* et al., 2002). By so binding, it then results in dramatically increased affinity to the T cells, which then produce both proliferate and trigger an inflammatory cascade within the intestine. Further complicating matters is the very rich genetic material of these grains. Wheat used for bread is hexaploid and has multiple repeat regions where the genes for these storage proteins. Hence, there are many (probably 50-100) epitopes within each wheat protein that can produce coeliac-responses from T cells derived from the small intestine. Children seem to respond more to native gliadin peptides than do adults and, in general, the deamidated peptides produce a much more potent effect than do the native, non-deamidated peptides. The peptides operant in DQ2 positive coeliacs differ from those in DQ8 positive coeliacs.

Triggers

It has also become clear from epidemiologic studies that coeliac disease can occur at any age (*Lohi* et al., 2007). Patients, in particular children, at genetic risk don't necessarily develop immune responses to gluten immediately. This may occur at discontinuous times over the 1st years of life. In addition, patients with type 1 diabetes who are often screened regularly for coeliac disease may develop coeliac disease later at the approximate age of 10 or 11, despite having been negative for some years previously despite being on a gluten-containing diet. Most intriguingly, el-

derly patients who were negative for coeliac disease may subsequently turn positively (*Norris* et al., 2005; *Vilppula* et al., 2009). The fact that patients, who apparently have tolerated gluten, then can lose tolerance to gluten and develop coeliac disease suggests that there may be environmental triggers. These factors may start at birth. Two studies have suggested that children born by Caesarean section are at increased risk of developing coeliac disease in childhood (*Marild* et al., 2012; *Decker* et al., 2010). Children born in the summer months who will likely wean in the winter are more prone to the disease (*Ivarsson* et al., 2003). While not studied in this largely epidemiologic study, it is well known that route of delivery affects the gut microbiota and even humoral immunity (*Huurre* et al., 2008).

In addition, coeliac disease may also be associated with intrauterine growth retardation and neonatal infections (*Sandberg-Bennich* et al., 2002). After birth, further exposures may be important for the occurrence of coeliac disease; in particular, many studies on breast-feeding have suggested that the failure of overlap between breast-feeding and the introduction of gluten might increase the risk of coeliac disease in childhood. Indeed, the now well-described Swedish epidemic of infant coeliac disease was ascribed to a combination of an abrupt termination of breast-feeding with a dramatic increased concentration of gluten in follow-on formula (*Ivarsson* et al., 2002). Recurrent rotavirus infection alone was initially thought to be an independent risk factor for coeliac disease occurring in infancy. However, subsequent work has demonstrated that perhaps it is recurrent rotavirus infection coinciding with cereal introduction that truly increases the risk for developing coeliac disease (*Stene* et al*.,* 2006).

Work has been done showing that there are changes in the microbiota in coeliac disease. For example, there are changes in the population of microbiota on the duodenal mucosa. One study has suggested increased bacterial adherence to the duodenal mucosa of rod-like bacteria in coeliac disease (*Ou* et al*.,* 2009). It is not known if this phenomenon is primary or secondary to the damaged epithelium and loss of mucus that occurs in the context of coeliac disease. Reductions in the proportions of Lactobacillus and Bifidobacterium species have also been reported in the faecal samples from treated coeliac patients (*Nistal* et al., 2012). Another group has shown that there are differences in Bacteroides species and pathogenic features in coeliac patients as compared to healthy controls (*Sanchez* et al., 2012) based on faecal samples.

These results seem to be contradictory, but there are many explanations as to why they are not. The first explanation is that the majority of earlier identification analyses were based on culture screening methods; whereas today, the majority of studies are based on the sequencing of specific regions of the 16srRNA gene. The second explanation is that some studies evaluate duodenal biopsies, while others evaluate faecal samples, both of which would be sampling different niches that different bacteria can survive. Thus, all of these studies have provided valuable information as to how the composition of the intestinal microbiome of coeliac patients are different from healthy controls; the next step is to determine if any of these changes are causative for or consequential to intestinal damage.

Previous studies have suggested that two different types of situations could trigger intestinal inflammation in coeliac disease. The one type would be decreased levels of anti-inflammatory

bacteria such as Lactobacillus and Bifidobacterium, the other, an increase in inflammation promoting bacteria. In one study, *Bacteroides fragilis* was increased in both untreated and treated coeliac patients, and the *Bacteroides fragilis* associated virulence factors, bft and MPII , were also increased in the coeliac patients as compared to healthy controls (*Nistal* et al., 2012). All of these studies demonstrate then, that dysbiosis of the intestinal microbiota is present in coeliac patients and that this may indeed be causative as opposed to consequential. Potentially then, a combination of probiotics and targeted removal of specific intestinal bacterial species would greatly improve the health of coeliac patients.

There also appears to be differences in immunoglobulin binding of bacteria within the gut. A recent paper suggested that in patients with coeliac disease, both treated and untreated, there is reduction of immunoglobulin-bound bacteria within the intestine as compared to normal. It is interesting that patients with selective IgA deficiency, who do not secrete any IgA, are at greatly increased risk of coeliac disease, with about 10% of patients developing coeliac disease. Recent cases and work by Naval Medical Research in the Department of Defense has suggested that coeliac disease may be more likely to be diagnosed after an infectious gastroenteritis event (*Verdu* et al*.,* 2007; *Welander* et al., 2010; *Riddle* et al., 2012). It can be speculated that these infectious events may result in temporary inflammation activation of stress responses within the small intestinal mucosa, stimulate the innate immune system, which will then drive the adaptive response. Excess numbers of bacteria can also be seen in the duodenum of patients with unresponsive coeliac disease or even before treatment (*Tursi* et al., 2003). Also, co-occurrence of an inflammatory insult, such as rotavirus in childhood, along with the introduction of a novel peptide, such as gluten, especially one to which the child is genetically susceptible to react to, may result in activation of dendritic cells and presenting cells towards a pro-inflammatory response, thereby skewing naïve T cells to become effector T cells producing inflammation as opposed to T-reg cells. It is also possible that in patients who have already established tolerance to gluten that this tolerance could be broken in the context of severe on-going inflammation. Furthermore, wheat and like cereals contain other substances that can incite innate responses (*Junker* et al. 2012) and indeed do so in ways that simulate an LPS-like effect of bacteria (*Yamazaki* et al., 2008*)*.

Uptake of Gliadin Molecules

There are two primary pathways by which the peptides are thought to be taken up. One is an active pathway transcellularly stimulated by IFN-γ through the aberrant expression of IgA receptors on the surface (*Bethune* et al., 2009). In this circumstance, IgA-bound gliadin is taken up and transported through the enterocyte into the basolateral surface where the gliadin peptide is released and then processed by antigen-presenting cells and presented to the T cells responsive to gliadin. The other pathway is the paracellular pathway. It is has been shown the gluten acutely increases gut permeability (*Lammers* et al., 2008). It causes disruption of intercellular tight junctions and this is likely modulated through the release of a peptide called zonulin, also recently shown to be prehaptoglobin 2 (*Tripathi* et al., 2009). The zonulin appears to cause uncoupling of tight junctions that may allow the transit of some gluten peptides in a paracellular pathway, but also allow access of other antigens, particu-

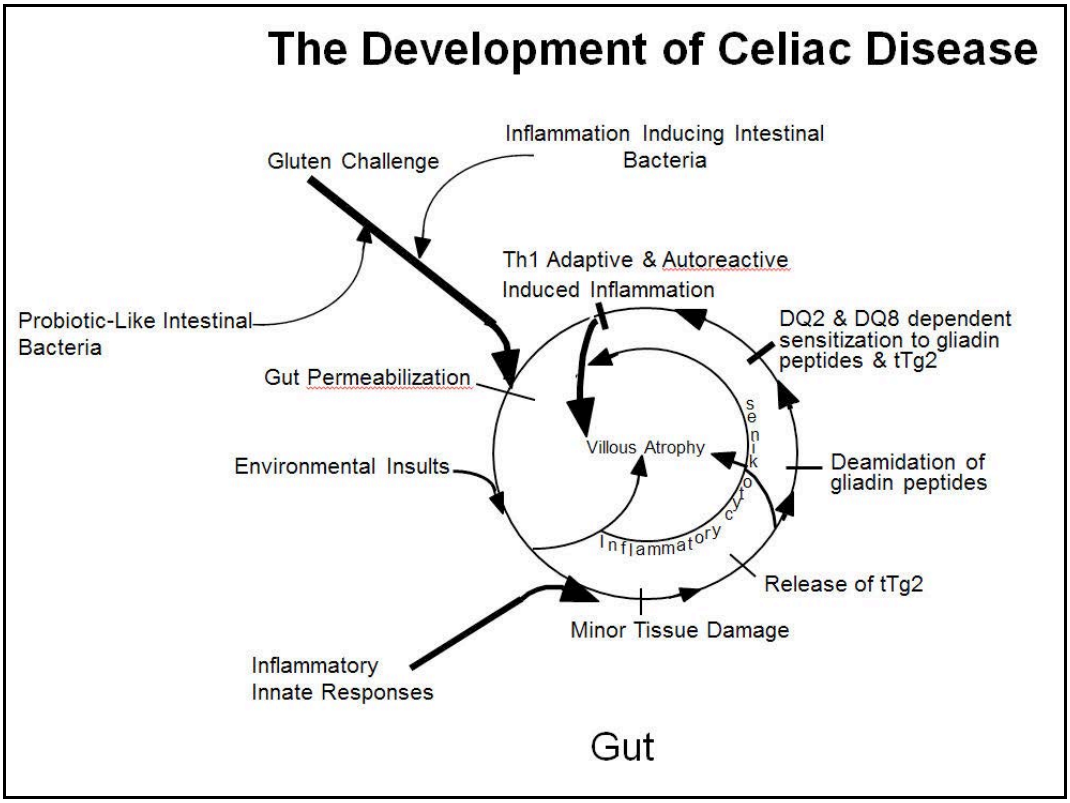

Figure 2: The maelstrom of inflammation that leads to established coeliac disease requires many conspirators affecting both the innate and adaptive parts of the immune system. Reprinted with permission from: Nehra, V., Marietta, E., and Murray, J.A.: Coeliac disease. In: Encyclopedia of human nutrition (Caballero, B., Allen, L., Prentice, A., Eds.). Elsevier, Oxford, 1, 407-418 (2006).

larly bacterial antigens that may excite an innate and amplify an adaptive response to gluten (*Jabri* et al., 2005). Recent clinical trials have shown potential benefit of larazotide acetate, a modulatory inhibitory of gluten's effects on permeability (*Kelly* et al., 2012). This permeability can be exacerbated by the use of an agent that increases inflammation and blocked by larazotide acetate (*Natividad* et al., 2009).

IMMUNOPATHOGENESIS

The immunopathogenesis of coeliac disease involves several cell types within the intestine (*Jabri* et al., 2009). The enterocyte or epithelial cells lining the intestine become distressed and express aberrant class 1 molecules on their surface. These also can express IL15. Intraepithelial lymphocytes are predominately CD8+ T cells, and these cells, while generally thought to have a regulatory effect, in the context of coeliac disease can become cytotoxic and can express NK receptors on their surface (*Meresse* et al., 2004). These NK receptors interacting with the class 1 molecules on the enterocyte can cause enterocyte injury. These intraepithelial enterocytes also respond to IL15, either

produced from the lamina propria or by enterocytes on the surface in response to stress. Key essential ingredients to coeliac disease pathogenesis are gluten-responsive CD4 cells in the lamina propria. These CD4 cells respond to specific gluten peptides presented in the context of class 2 molecules and produce a cascade of cytokines characterized by INFγ and to a lesser extent IL2 and TNF-α. These cytokines drive a cascade of responses that produce both cellular as well as humoral responses (Figure 2). The cellular responses further cascade, drawing in macrophages and activating other cells, including a complement system, neutrophils, eosinophils and mast cells. These combination cells like lead to the destruction of the architecture of the small intestine, resulting in increased thickening or crypt hyperplasia, villous atrophy, disruption of enterocyte function and absorption, increased secretion, and consequent inflammation and malabsorption. Metalloproteases are elaborated, which further alter the architecture. The inflammatory response also includes a potent humoral response and antibodies directed against the deamidated gliadin peptides as well as against tissue transglutaminase are also elaborated within the intestinal mucosa they are secreted, and also increased in circulation. The IgA isotype antibodies are more commonly expressed, particularly directed against the autoantigen tissue transglutaminase. However, for gliadin or deamidated gliadin antibodies, the IgG is equally expressed, suggesting that the primary target for the humoral response is the exogenous antigen and not the autoantigen. Making coeliac disease further different from many autoimmune diseases, with which it shares genetic predisposition, is the observation that the antibodies diminish in quantity once the exogenous antigen is removed. Indeed typically patients who have been on a gluten-free diet for >1 year will become IgA negative. Other antibody responses are often seen. These are antibodies directed at microbial antigens present within the intestine. For example, false positive ASCA antibodies are seen in the context of coeliac disease and usually these diminish or disappear with treatment of the patient with a gluten-free diet, suggesting that these are seen because of injury.

DIAGNOSIS

Coeliac disease is typically detected first by serologic tests. The primary serologic test and most accurate serologic test is the tissue transglutaminase IgA antibody with sensitivities of approximately 95% and specificities of 95% (*Rostom* et al., 2005; *Hadithi* et al., 2007). The performance of this test has been further refined in that patients with extremely positive tests >10 times the upper limit of normal are essentially almost guaranteed to have coeliac disease (*Klapp* et al., 2013). The test which immediately preceded tissue transglutaminase was the endomysial Ig antibody test done by indirect immunofluorescence. This test has a very high specificity, virtually 100% in most laboratories, but a sensitivity that is quite variable, likely due to variability in laboratory methods and interpretations (*Li* et al., 2009). Accompaniment of the tissue transglutaminase IgA test is a measurement of total IgA. This is performed in order to detect those patients with selective IgA deficiency that not only are at greatly increased risk of coeliac disease but for whom the stand-

ard IgA-based tests will be negative even in the setting of coeliac disease (*Rubio-Tapia* et al., 2013). For patients who have known or suspected selective IgA deficiency, deamidated gliadin IgG or tissue transglutaminase IgG tests can be performed, though their sensitivity is not perfect for coeliac disease (*Rashtak* et al., 2008a). Tissue transglutaminase IgG is not useful in the context of normal IgA levels (*Rashtak* et al., 2008a). Antibodies directed against native gliadin antibodies are not of any additional benefit and indeed are fraught with greatly reduced specificity and can negatively impact the accuracy of diagnostic testing when included in diagnostic panels for coeliac disease (*Rubio-Tapia* et al., 2013). All of the antibodies have reduced sensitivity when the patient reduces gluten intake (*Rashtak* et al., 2008b).

Intestinal biopsies showing the classic features of villous atrophy, crypt hyperplasia, and increased intraepithelial lymphocytes are still regarded as the mainstay of diagnosis. Adequate numbers of samples should be taken (*Lebwohl* et al., 2012). Recent guidelines promulgated by the European Society for Pediatric Gastroenterology and Hepatology and Nutrition have suggested that in patients meeting certain very strict criteria, a biopsy may be unnecessary to confirm the diagnosis (*Husby* et al., 2012, 2013; *Rubio-Tapia* et al., 2013).

TREATMENT

Once the disease is detected and confirmed by biopsy, then treatment is usually instituted. Treatment includes a strict, gluten-free diet. While theoretically simple, there are many challenges to be able to adhere to a gluten-free diet long-term. Patients are usually evaluated for any vitamin deficiencies that may require repletion. Most common is iron deficiency, calcium, vitamin D and other fat-soluble vitamins. B12 deficiency can affect over 20% of patients with coeliac disease. It is unclear if this mandates parenteral B12 for life as is often necessitated in patients who have B12 deficiency due to ileal resection or pernicious anaemia (*Murray* and *Ross*, 2004).

In adult patients diagnosed with coeliac disease, bone density is typically performed as diminished bone density, either due to osteomalacia or osteoporosis, is very common. Fracture risk is increased in patients with coeliac disease prior to diagnosis, and the rate does not apparently drop in follow-up (*Jafri* et al., 2008).

PROGNOSIS

Most patients diagnosed as children will promptly respond to a gluten-free diet and will resume normal growth and development. As long as they remain on a gluten-free diet, they will remain healed and well. Adult patients diagnosed with coeliac disease heal much more slowly and are at increased risk of complications and increased mortality, especially within the 1st year of diagnosis (*Rubio-Tapia* et al., 2010; *Lanzini* et al., 2009). This seems to be a relatively modest increase in overall mortality in diagnosed coeliac disease. Patients with undiagnosed coeliac disease, however, may be at greatly in-

creased risk of mortality with an almost 4-fold increase in mortality over 45 years (*Rubio-Tapia* et al., 2009). The increase in mortality associated with coeliac disease is not universally found and variances such as the rate of detection of coeliac disease, in particular geography, may impact the likely long-term mortality. Early detection couple with vigorous treatment with a gluten-free diet is most likely to result in a good outcome.

FUTURE TREATMENTS

Several new treatments are in development for coeliac disease given that we know much about the processes involved. Treatments directed at altering the nature of wheat itself, detoxifying the wheat within the gastrointestinal tract or even before ingestion, binding the peptides within the intestine to prevent presentation to the immune system (*Liang* et al., 2010). The modulation of the permeability or prevention of increased permeability induced by gluten, as well as the degradation of the immune peptides within the gut by potent endopeptidases all are in pre-clinical or even clinical trials (*Gass* et al., 2007; *Cerf-Bensussan* et al., 2007; *van den Broeck* et al., 2009). Other approaches, such as an immunotherapy approach to try to reduce tolerance to gluten, are also under study (*Senger* et al., 2003; *Vickery* et al., 2009; *Keech* et al., 2009). Other targets, such as blocking the deamidation by tissue transglutaminase and blocking the binding of the peptides to DQ2 or DQ8, are also areas that have sparked development of agents in preclinical study (*Xia* et al., 2007; *Klock* et al., 2011). Also, use of worms to skew the immune response away from a coeliac-like response has been used in humans (*Daveson* et al., 2011).

PREVENTION

Coeliac disease has dramatically increased in prevalence over the last 50 years (*Rubio-Tapia* et al., 2009). This is almost certainly due to environmental factors. Identifying and abrogating these environmental factors is crucial if we are to stem the tide of coeliac disease. Whilst it affects still somewhat just less than 1% of the population of the U.S., it now affects over 2% of other Caucasian populations such as Sweden and Finland, rates that appear to be increasing (*Dube* et al., 2005). It is vitally important to identify the factors that have led to this increase so that this increase can be mitigated. Several approaches to the prevention of coeliac disease are under study. The Prevent CD trial primarily in Europe and North America addresses the issue of timing of introduction and quantity of gluten in the infant diet. Other studies looking at using systems biology approach to address the role of the microbial community on tolerance to gluten is also under study. It is interesting to note that the phenomena of oral tolerance require the presence of microbiota within the gut. Studies looking at the co-administration of beneficial bacterial with gliadin molecules have also been studied and may prevent or reverse gluten sensitization (*Huibregtse* et al., 2009).

SUMMARY

In summary, coeliac disease is an increasingly common chronic disease affecting primarily the upper small intestine associated with significant morbidity and mortality. It is often overlooked, but presents both opportunities and challenges for understanding of the interaction between the environment and immune system, and may present an example of a disorder that can be the result of triggering of loss of homeostasis due to food or microbial influences within the intestine.

LITERATURE

Book, L., Zone, J.J., and Neuhausen, S.L.: Prevalence of celiac disease among relatives of sib pairs with celiac disease in U.S. families. Am. J. Gastroenterol. 98, 377-381 (2003).

Bethune, M.T., Siegel, M., Howles-Banerji,S., and Khosla, C.: Interferon-gamma released by gluten-stimulate celiac disease-specific intestinal T cells enhances the transepithelial flux of gluten peptides. J. Pharmacol. Exp. Ther. 329, 657-668 (2009).

Bevan, S., Popat, S., Braegger, C.P., Busch, A., O'Donoghue, D., Faith-Magnusson, K., Ferguson, A., Godkin, A., Hogberg, L., Holmes, G., Hosie, K.B., Howdle, P.D., Jenkins, H., Jewell, D., Johnston, S., Kennedy, N.P., Kerr, G., Kumar, P., Logan, R.F.A., Love, A.H.G., Marsh, M., Mulder, C.J.J., Sjoberg, K., Stenhammer, L., Walker-Smith, J., Marossy, A.M., and Houlston, R.S.: Contribution of the MHC region to the familial risk of coeliac disease. J. Med. Genet. 36, 687-690 (1999).

Cerf-Bensussan, N., Matysiak-Budnik, T., Cellier, C., and Heyman, M.: Oral proteases: a new approach to managing coeliac disease. Gut 56, 157-160 (2007).

Daveson, A.J., Jones, D.M., Gaze, S., McSorley, H., Clouston, A., Pascoe, A., Cooke, S., Speare, R., MacDonald, G.A., Anderson, R., McCarthy, J.S., Loukas, A., and Croese, J.: Effect of hookworm infection on wheat challenge in celiac disease--a randomised double-blind placebo controlled trial. PLoS One 6, e17366 (2011).

Decker, E., Engelmann, G., Findeisen, A., Gerner, P., Laass, M., Ney, D., Posovszky, C., Hoy, L., and Hornef, M.W.: Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics 125, e1433-e1440 (2010).

Dicke, W.K.: Treatment of celiac disease. Ned. Tijdschr. Geneeskd. 95, 124-130 (1951).

Dube, C., Rostom, A., Sy, R., Cranney, A., Saloojee, N., Garritty, C., Sampson, M., Zhang, L., Yazdi, F., Mamaladze, F., Pan, I., Macneil, J., Mack, D., Patel, D., and Moher, D.: The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review. Gastroenterology 128, S56-S67 (2005).

Dubois, P.C.A., Trynka, G., Franke, L., Hunt, K.A., Romanose, J., Curtotti, A., Zhernakova, A., Heap, G.A.R., Adany, R., Aromaa, A., Bardella, M.T., van den Berg, L.H., Bockett, N.A., de la Concha, E.G., Dema, B., Fehrmann, R.S.N., Fernandez-Arquero, M., Fiatal, S., Grandone, E., Green, P.M., Groen, H.J.M., Gwiliam, R., Houwen, R.H.J., Hunt, S.E., Kaudinen, K., Kelleher, D., Korponay-Szabo, I., Kurppa, K., MacMathuna, P., Maki, M., Mazzilli, M.C., McCan, O.T., Mearin, M.L., Mein, C.A., Mirza, M.M., Mistry, V., Mora, B., Morley, K.I., Mulder, C.J., Murray, J.A.,

Nunez, C., Oosterom, E., Ophoff, R.A., Polanco, I., Peltonen, L., Plattel, M., Rybak, A., Salomaa, V., Schweizer, J.J., Sperandeo, M.P., Tack, G.J., Turner, G., Veldink, J.H., Verbeek, W.H., Weersma, R.K., Rolters, V.M., Urcelay, E., Cukrowska, B., Greco, L., Neuhausen, S.L., McManus, R., Barisani, D., Deloukas, P., Barrett, J.C., Saavalainen, P., Wijmegna, C., and van Heel, D.A.: Multiple common variants for celiac disease influencing immune gene expression. Nat. Genet. 42, 295-302 (2010).

Falth-Magnusson, K., Franzen, L., Jansson, G., Laurin, P., and Stenhammar, L.: Infant feeding history shows distinct differences between Swedish celiac and reference children. Pediatr. Allergy Immunol. 7, 1-5 (1996).

Fasano, A., Berti, I., Gerarduzzi, T., Not, T., Colletti, R.B., Drago, S., Elitsur, Y., Green, P.H., Guandalini, S., Hill, I.D., Pietzak, M., Ventura, A., Thorpe, M., Kryszak, D., Fornaroli, F., Wasserman, S.S., Murray, J.A., and Horvath, K.: Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch. Intern. Med. 163, 286-292 (2003).

Garner, C.P., Murray, .JA., Ding, Y.C., Tien, Z., van Heel, D.A., and Neuhausen, S.L.: Replication of celiac disease UK genome-wide association study results in a US population. Hum. Mol. Genet. 18, 4219-4225 (2009).

Gass, J., Bethune, M.T., Siegel, M., Spencer, A., and Khosla, C.: Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. Gastroenterology 133, 472-480 (2007).

Hadithi, M., von Blomberg, B.M., Crusius, J.B., Bloemena, E., Kostense, P.J., Meijer, J.W., Mulder, C.J., Stehouwer, C.D., and Pena, A.S.: Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. Ann. Intern. Med. 147, 294-302 (2007).

Huibregtse, I.L., Marietta, E.V., Rashtak, S., Koning, F., Rottiers, P., David, C.S., van Deventer, S.J., and Murray, J.A.: Induction of antigen-specific tolerance by oral administration of Lactococcus lactis delivered immunodominant DQ8-restricted gliadin peptide in sensitized nonobese diabetic Abo Dq8 transgenic mice. J. Immunol. 183, 2390-2396 (2009).

Husby, S., Koletzko, S., Korponay-Szabo, I.R., Mearin, M.L., Phillips, A., Shamir, R., Troncone, R., Giersiepen, K., Branski, D., Catassi, C., Leigeman, M., Maki, M., Ribes-Koninckx, C., Ventura, A., Zimmer, K.P., ESPGHAN Working Group on Coeliac Disease Diagnosis, ESPGHAN Gastroenterology Committee, and European Society for Pediatric Gastro-enterology, Hepatology and Nutrition.: European Society for Pediatric Gastro-enterology, Hepatology, and Nutrition Guidelines for the diagnosis of coeliac disease. J. Pediatr. Gastroenterol. Nutr. 54, 136-60 (2012).

Husby, S. and Murray, J.A.: New aspects of the diagnosis of celiac disease in children, adolescents, and adults. Mayo Clin. Proc. 88, 540-543 (2013).

Huurre, A., Kalliomaki, M., Rautava, S., Rinne, M., Salminen, S., and Isolauri, E.: Mode of delivery - effects on gut microbiota and humoral immunity. Neonatology 93, 236-240 (2008).

Ivarsson, A., Hernell, O., Stenlund, H., and Persson, L.A.: Breast-feeding protects against celiac disease. Am. J. Clin. Nutr. 75, 914-921 (2002).

Ivarsson, A., Hernell, O., Nystrom, L., and Persson, L.A.: Children born in the summer have increased risk for coeliac disease. J. Epidemiol. Community Health 57, 36-39 (2003).

Jabri, B., Kasarda, D.D., and Green, P.H.: Innate and adaptive immunity: the yin and yang of celiac disease. Immunol. Rev. 206, 219-231 (2005).

Jabri, B. and Sollid, L.M.: Mechanisms of disease: immunopathogeneis of celiac disease. Nat. Clin. Pract. Gastroenterol. Hepatol. 3, 516-525 (2006).

Jabri, B. and Sollid, L.M.. Tissue-mediated control of immunpathology in coeliac disease. Nat. Rev. Immunol. 9, :858-870 (2009).

Jafri, M.R., Nordstrom, C.W., Murray, J.A., Van Dyke, C.T., Dierkhising, R.A., Zinsmeister, A.R., and Melton, L.J. 3rd: Long-term fracture risk in patients with celiac disease: a population-based study in Olmsted County, Minnesota. Dig. Dis. Sci. 53, 964-971 (2008).

Junker, Y., Zeissig, S., Kim, S.J., Barisani, D., Wieser, H., Leffler, D.A., Zevallos, V., Libermann, T.A., Dillon, S., Freitag, T.L., Kelly, C.P., and Schuppan, D.: Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J. Exp. Med. 209, 2395-2408 (2012).

Keech, C.L., Dromey, J., Chen, Z.J., Anderson, R.P., and McCluskey, J.: 355 Immune tolerance induced by peptide immunotherapy in an HLA DQ2-dependent mouse model of gluten immunity. Gastroenterology 136, Suppl. 1, A-57 (2009).

Kelly, C.P., Green, P.H., Murray, J.A., Dimarino, A., Colatrella, A., Leffler, D.A., Alexander, T., Arsenescu, R., Leon, F., Jiag, J.G., Arterburn, L.A., Paterson, B.M., Fedorak,R.N., and Larazotide Acetate Celiac Disease Study Group: Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomized placebo-controlled study. Aliment. Pharmacol. Ther. 37, 252-262 (2013).

Klapp, G., Masip, E., Bolonio, M., Donat, E., Polo, B., Ramos, D., and Ribes-Koninckx, C.: Celiac disease: the new proposed ESPGHAN diagnostic criteria do work well in a selected population. J. Pediatr. Gastroenterol. Nutr. 56, 251-256 (2013).

Klock, C., Jin, X., Choi, K., Khosla, C., Madrid, P.B., Spencer, A., Raimundo, B.C., Boardman, P., Lanza, G., and Griffin, J.H.: Acylideneoxoindoles: a new class of reversible inhibitors of human transglutaminase 2. Bioorg. Med. Chem. Lett. 21, 2692-2696 (2011).

Lammers, K.M., Lu, R., Brownley, J., Lu, B., Gerard, C., Thomas, K., Rallabhandi, P., Shea-Donohue, T., Tamiz, A., Alkan, S., Netzel-Arnett, S., Antalis, T., Vogel, S.N., and Fasano, A.: Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology 135, 194-204 (2008).

Lanzini,A., Lanzarotto, F., Villanacci, V., Mora, A., Bertolazzi, S., Turini, D., Carella, G., Malagoli, A., Ferrante, G., Cesana, B.M., and Ricci, C.: Complete recovery of intestinal mucosa occurs very rarely in adult coeliac patients despite adherence to gluten-free diet. Aliment. Pharmacol. Ther. 15, 1299-1308 (2009).

Lawler, M., Humphries, P., O'Farrelly, C., Hoey, H., Sheils, O., Jeffers, M., O'Briain, D.S., and Kelleher, D.: Adenovirus 12 E1A gene detection by polymerase chain reaction in both the normal and coeliac duodenum. Gut 35, 1226-1232 (1994).

Lebwohl, B., Rubio-Tapia, A., Assiri, A., Newland, C., and Guandalini, S.: Diagnosis of celiac disease. Gastrointest. Endosc. Clin. N. Am. 22, 661-677 (2012).

Lebwohl, B., Granath, F., Ekbom, A., Montgomery, S.M., Murray, J.A., Rubio-Tapia, A., Green, P.H., and Ludvigsson, J.F.: Mucosal healing and mortality in coeliac disease. Aliment. Pharmacol. Ther. 37, 332-339 (2013).

Li, M., Yu, L., Tiberti, C., Bonamico, M., Taki, I., Miao, D., Murray, J.A., Rewers, M.J., Hoffenberg, E.J., Agardh, D., Mueller, P., Stern, M., Bonifacio, E., and Liu, E.: A report on the Internatonal Translutaminase Autoantibody Workshop for Celiac Disease. Am. J. Gastroenterol. 104, 154-163 (2009).

Liang, L., Piier, M., Leroux, J.C., and Subirade, M.: Interaction of alpha-gliadin with polyanions: design considerations for sequestrants used in supportive treatment of celiac disease. Biopolymers 93, 418-428 (2010).

Lohi, S., Mustalahti, K., Kaukinen, K., Laurila,

K., Collin, P., Rissanen, H., Lohi, O., Bravi, E., Gasparin, M., Reunanen, A., and Maki, M.: Increasing prevalence of coeliac disease over time. Aliment. Pharmacol. Ther. 26, 1217-1225 (2007).

Ludvigsson, J.F., Rubio-Tapia, A., van Dyke, C.T., Melton, L.J., 3rd, Zinsmeister, A.R., Lahr, B.D., and Murray, J.A.: Increasing incidence of celiac disease in a North American population. Am. J. Gastroenterol. 108, 818-824 (2013).

Marild, K., Stephansson, O., Montgomery, S., Murray, J.A., and Ludvigsson, J.F.: Pregnancy outcome and risk of celiac disease in offspring: a nationwide case-control study. Gastroenterology 142, 39-45 (2012).

Meresse, B., Chen, Z., Ciszewski, C., Tretiakova, M., Bhagat, G., Krausz, T.N., Raulet, D.H., Lanier, L.L., Groh, V., Spies, T., Ebert, E.C., Green, P.H., and Jabri, B.: Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity 21, 357-366 (2004).

Molberg, O., Mcadam, S.N., Körner, R., Quarsten, H., Kristiansen, C., Madsen, L., Fugger, L., Scott, H., Norén, O., Roepstorff, P., Lundin, K.E., Sjöström, H., Sollid, L.M.: Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat. Med. 4, 713-717 (1998).

Murray, J.A., Watson, T., Clearman, B., and Mitros, F.: Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease. Am. J. Clin. Nutr. 79, 669-673 (2004).

Murray, J.A. and Ross, M.A.: Malabsorption and neurological disease. In: Neurogastro-enterology (Quigley, E.M.M., Pfeiffer, R.F., Eds.). Butterworth-Heinemann, Phil-adelpha, 203-53 (2004).

Murray, J.A., Moore, S.B., Van Dyke, C.T., Lahr, B.D., Dierkhising, R.A., Zinsmeister, A.R., Melton, L.J., 3rd, Kroning, C.M., El-Yousseff, M., and Czaja, A.J.: HLA DQ gene dosage and risk and severity of celiac disease. Clin. Gastroenterol. Hepatol. 5, 1406-1412 (2007).

Murray, J.A., Rubio-Tapia, A., Van Dyke, C.T., Brogan, D.L., Knipschield, M.A., Lahr, B., Rumalla, A., Zinsmeister, A.R., and Gostout, C.J.: Mucosal atrophy in celiac disease: extent of involvement, correlation with clinical presentation, and response to treatment. Clin. Gastroenterol. Hepatol. 6, 186-193 (2008).

Natividad, J.M., Huang, X., Slack, E., Jury, J., Sanz, Y., David, C., Denou, E., Yang, P., Murray, J., McCoy, K.D., and Verdu, E.F.: Host responses to intestinal microbial antigens in gluten-sensitive mice. PLoS One 4, e6472 (2009).

Nistal, E., Caminero, A., Vivas, S., Ruiz de Morales, J.M., Sáenz de Miera, L.E., Rodriguez-Aparicio, L.B., and Casqueiro, J. Differences in faecal bacteria populations and faecal bacteria metabolism in healthy adults and celiac disease patients. Biochemie 94, 1724-1729 (2012).

Norris, J.M., Barriga, K., Hoffenberg, E.J., Taki, I., Miao, D., Haas, J.E., Emery, L.M., Sokol, R.J., Erlich, H.A., Eisenbarth, G.S., and Rewers, M.: Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. JAMA 293, 2343-2351 (2005).

Ou, G., Hedberg, M., Hörstedt, P., Baranov, V., Forsberg, G., Drobni, M., Sandsröm, O., Wai, S.N., Johansson, I., Hammarström, M.-L., Hernell, O., and Hammarström, S.: Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease. Am. J. Gastroenterol. 104, 3058-3067 (2009).

Pinier, M., Verdu,E.F., Nasser-Eddine, M., David, C.S., Vezina, A., Rivard, N., and Leroux, J.C.: Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium. Gastroenterology 136, 288-298 (2009).

Rashtak, S., Ettore, M.W., Homburger, H.A., and Murray, J.A.: Comparative usefulness

of deamidated gliadin antibodies in the diagnosis of celiac disease. Clin. Gastroenterol. Hepatol. 6, 426-432, quiz 370 (2008a).

Rashtak, S., Ettore, M.W., Homberger, H.A., and Murray, J.A.: Combination testing for antibodies in the diagnosis of coeliac disease: coparison of multiplex immunoassay and ELISA methods. Aliment. Pharmacol. Ther. 28, 805-813 (2008b).

Reilly, N.R., Fasano, A., and Green, P.H.: Presentation of celiac disease. Gastrointest. Endosc. Clin. N. Am. 22, 613-221 (2012).

Riddle, M.S., Murray, J.A., and Porter, C.K.: The incidence and risk of celiac disease in a healthy U.S. adult population. Am. J. Gastroenterol. 107, 1248-1255 (2012).

Rostom, A., Dube, C., Cranney, A., Saloojee, N., Sy, R., Garritty, C., Sampson, M., Zhang, L., Yazdi, F., Mamaladze, V., Pan, I., MacNeil, J., Mack, D., Patel, D., and Moher, D.: The diagnostic accuracy of serologic tests for celiac disease: a systematic review. Gastroenterology 128, S38-S46 (2005).

Rubio-Tapia, A. and Murray, J.A.: Celiac disease. In: GI Epidemiology (Talley, N.J., Locke, G.R. III, and Saito, Y.A., Eds.). Blackwell Publishing, Malden, 157-163 (2007).

Rubio-Tapia, A., Kyle, R.A., Kaplan, E.L., Johnson, D.R., Page, W., Erdtmann, F., Brantner, T.L., Kim, W.R., Phelps, T.K., Lahr, B.D., Zinsmeister, A.R., Melton, L.J., 3rd, and Murray, J.A.: Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology 137, 88-93 (2009).

Rubio-Tapia, A., Rahim, M.W., See, J.A., Lahr, B.D., Wu,T.-T., and Murray, J.A.: Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. Am. J. Gastroenterol. 105, 1412-1420 (2010).

Rubio-Tapia, A., Ludvigsson, J.F., Brantner, T.L., Murray, J.A., and Everhart, J.E.: The prevalence of celiac disease in the United States. Am. J. Gastroenterol. 107, 1538-1544; quiz 1537, 1545 (2012).

Rubio-Tapia, A., Hill, I.D., Kelly, C.P., Calderwood, A.H., and Murray, J.A.: ACG clinical guidelines: diagnosis and management of celiac disease. Am. J. Gastroenterol. 108, 656-676; quiz 77 (2013).

Sanchez, E., Laparra, J.M., and Sanz, Y.: Discerning the role of Bacteriodes fragilis in celiac disease pathogenesis. Appl. Environ. Micobiol. 78, 6507-6515 (2012).

Sandberg-Bennich, S., Dahlquist, G., and Kallen, B.: Coeliac disease is associated with intrauterine growth and neonatal infections. Acta Paediatr. 91, 30-33 (2002).

Senger, S., Luongo, D., Maurano,F., Mazzeo, M.F., Sicilliano, R.A., Gianfrani, C., David, C., Troncone, R., Auricchio, S., and Rossi, M.: Intranasal administration of a recombinant alpha-gliadin down-regulates the immune response to wheat gliadin in DQ8 transgenic mice. Immunol. Lett. 88, 127-134 (2003).

Shan, L., Molberg, O., Parrot, I., Hausch, F., Filiz, F., Gray, G.M., Sollid, L.M., and Khosla, C.: Structural basis for gluten intolerance in celiac sprue. Science 297, 227-2279 (2002).

Stene, L.C., Honeyman, M.C., Hoffenberg, E.J., Haas, J.E., Sokol, R.J., Emery, L., Taki, I., Norris, J.M., Erlich, H.A., Eisenbarth, G.S., and Rewers, M.: Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am. J. Gastroneterol. 101, 2333-2340 (2006).

Tripathi, A., Lammers, K.M., Goldblum, S., Shea-Donohue, T., Netzel-Arnett, S., Buzza, M.S., Antalis, T.M., Vogel, S.N., Zhao, A., Yang, S., Arrietta, M.C., Meddings, J.B., and Fasano, A.: Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. Proc. Natl. Acad. Sci. USA 106, 16799-16804 (2009).

Tursi, A., Brandimarte, G., and Giogetti, G.: High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms

after gluten withdrawal. Am. J. Gastroenterol. 98, 839-843 (2003).

van den Broeck, H.C., van Herpen, T.W., Schuit, C., Salentijn, E.M., Dekking, L., Bosch, D., Hamer, R.J., Smulders, M.J., Gilissen, L.J., and van der Meer, I.M.: Removing celiac disease-related gluten proteins from bread wheat while retaining technological properties: a study from Chinese Spring deletion lines. BMC Plant Biol. 9, 41 (2009).

Verdu, E.F., Mauro, M., Bourgeois, J., and Armstrong, D.: Clinical onset of celiac disease after an episode of Campylobacter jejuni enteritis. Can. J. Gastroenterol. 21, 453-455 (2007).

Vickery, B.P. and Burks, A.W.: Immunotherapy in the tretment of food allergy: focus on oral tolerance. Curr. Opin. Allergy Clin. Immunol. 9, 364-370 (2009).

Vilppula, A., Kaukinen, K., Luostarinen, L., Krekela, I., Patrikainen, H., Valve, R., Maki, M., and Collin, P.: Increasing prevalance and high incidence of celiac disease in elderly people: a population-based study. BMC Gastoenterol 9, 49 (2009).

Vilppula, A., Kaukinen, K., Luostarinen, L., Krekela, I., Patrikainen, H., Valve, R., Luostarinen, M., Laurila, K., Maki, M., and Collin, P.: Clinical benefit of gluten-free diet in screen-detected older celiac disese patietns. BMC Gastoenterol 11, 135 (2011).

Walker, M.M., Murray, J.A., Ronkainen, J., Aro, P., Storskrubb, T., D'Amato, M., Lahr, B., Talley, N.J., and Agreus, L.: Detection of celiac disease and lymphocytic enteropathy by parallel serology and histopathology in a population-based study. Gastroenterology 139, 112-119 (2010).

Welander A., Tjernberg, A.R., Montgomery, S.M., Ludvigsson, J., and Ludvigsson, J.F.: Infectious disease and risk of later celiac disease in childhood. Pediatrics 125, e530-e536 (2010).

Xia, J., Bergseng, E., Fleckenstein, B., Siegel, M., Kim, C.Y., Khosla, C., and Sollid, L.M.: Cyclic and dimeric gluten peptide analogues inhibiting DQ2-mediated antigen presentation in celiac disease. Bioorg. Med. Chem. 15, 6565-6573 (2007).

Yamazaki, K., Murray, J.A., and Kita, H.: Innate immunomodulatory effects of cereal grains through induction of IL-10. J. Allergy Clin. Immunol. 121, 172-178 (2008).