Celiac Disease

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Abstract and Introduction

Abstract

Purpose of Review: Recent advances in the clinical, epidemiological, genetic, and therapeutic aspects of celiac disease have made this condition a superb model of autoimmunity. This review will outline the most significant work that contributed to our current knowledge of the disease.
Recent Findings: Celiac disease is not confined to the Caucasian population as previously believed; rather its prevalence is approximately 1% worldwide. In addition to the HLA genes, many other genes involved in innate and adaptive immunity, intestinal barrier regulation, and autoimmunity have been identified as integral genetic components of the disease. Based on this information and on a better understanding of celiac disease pathogenesis, novel therapies alternative to the gluten-free diet are currently in advanced phase of development.
Summary: The outcome of these new findings will most likely have a significant impact in clinical practice, including diagnosis and management of the disease. Furthermore, celiac disease can be used as a unique model to gain more insights on the pathogenesis of autoimmune diseases.

Introduction

Celiac disease is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. Gluten is the major protein component of wheat, rye, and barley. The major predisposing genes are the histocompatibility leukocyte antigen (HLA)-DQ2 and DQ8 genotypes found in at least 95% of patients. Celiac disease is one of the most common lifelong disorders on a worldwide basis affecting 0.5-1% of the general population in the USA and other developed countries. The development of celiac enteropathy is paralleled by the appearance of serum antibodies, especially the IgA class antitissue transglutaminase (antitTG), antiendomysial antibody (EMA) and antigliadin antibodies (AGA), and eventually clinical manifestations. The clinical spectrum of celiac disease is wide including cases with either typical intestinal features (chronic diarrhea, weight loss, etc.) or 'atypical' extraintestinal features (anemia, osteoporosis, neurological disturbances, etc.), and silent forms that are occasionally discovered because of serological screening. Celiac disease prevalence is increased in at-risk conditions such as family history of celiac disease, autoimmune diseases, especially type 1 diabetes (T1D) and thyroiditis, IgA deficiency, and some genetic syndromes (Down, Turner, and William syndromes). Due to atypical features, many celiac disease cases currently escape diagnosis and are exposed to the risk of long-term complications, for example infertility and lymphoma. Celiac disease is a permanent condition that requires treatment by the complete exclusion of gluten-containing products from the diet. In Western countries, gluten-containing food makes a substantial contribution to daily energy intake and is enjoyable to eat. The changes needed to begin and maintain a gluten-free diet (GFD) are not trivial and have a major impact on

the quality of daily life.[1,2]

During these last years, there has been a growing interest on the different aspects of celiac disease. In this paper, we will briefly review some of the major advances on the epidemiology, genetics, and treatment of this common disorder.

Globalization of Celiac Disease

Until recently the geographical distribution of celiac disease was mostly restricted to Europe and other developed countries, such as the USA, Canada, and Australia. New epidemiological studies have provided evidence that this disorder is also common in other parts of the world including the Asian continent.

The major celiac disease predisposing genotypes (HLA-DQ2 and HLA-DQ8) are common throughout the Indian continent. In northern India, the prevalence of HLA-DQ2 and HLA-DQ8 in the general population has been estimated as being 9.9 and 15.6%, respectively, whereas in southern, Dravidian-speaking populations, the prevalence of *cis* DQ2 and DQ8 is 4.4-7.3 and 4.7-5.1%, respectively. Wheat consumption is higher in the so-called 'celiac belt', that is the northwestern group of states where this cereal is the staple diet. This finding explains why celiac disease has mostly been described in northern India.[3] Celiac disease cases reported from India were 130 between the years 1966 and 2000 versus 517 between the years 2001 and 2005. The major factor that resulted in increased reports of celiac disease from India was use of serologic testing, for example EMA and antitTG testing, to overcome diagnostic overlap with tropical sprue, tuberculosis, and small bowel bacterial overgrowth. By using a case-finding approach (antitTG serological testing on symptomatic individuals), Sood *et al.*[4] reported a prevalence of newly diagnosed celiac disease of one in 310 children on a sample of 4347 school-age children from Punjab, India. Clinical series from India usually described typical or 'hypertypical' cases, being chronic diarrhea, anemia, and stunting the commonest symptoms in children. However, more recently, atypical celiac disease cases presenting with short stature, anemia, abdominal distention, rickets, constipation, diabetes mellitus, and delayed puberty have been reported.[5] Children with atypical celiac disease are significantly older than classical cases.[5]

In China and other Asian countries, the epidemiology of celiac disease is currently unknown. This region, however, shows a clear slowing of per capita consumption of rice and a parallel increased consumption of wheat-based products. Rising income and urbanization are driving forces in the increase in wheat consumption. Whereas wheat is considered an inferior food in Western societies, in the traditional rice-eating Asian countries, wheat is becoming a preferred staple. Because of these alimentary trends, an increasing incidence of celiac disease in Eastern countries can be expected in the near future.

The Genetics of Celiac Disease is Complex

Genetic predisposition plays a key role in celiac disease and considerable progress has been made recently in identifying genes that are responsible for celiac disease predisposition. It is well known that celiac disease is strongly associated with specific HLA class II genes known as HLA-DQ2 and HLA-DQ8 located on chromosome 6p21. Most celiac disease patients (around 95%) express HLA-DQ2 and the remaining patients are usually HLA-DQ8 positive. The HLA-DQ2 allele is common and is carried by approximately 30% of Caucasian individuals. Thus, HLA-DQ2 or HLA-DQ8 is necessary for disease development, but not sufficient, as its estimated risk effect is only 36-53%. Non-HLA genes contribute more than HLA to the celiac disease genetic background; however, this predisposition depends on a multitude of genes, each of them adding only a modest contribution to disease development. Due to small effect size and genetic heterogeneity between populations, the search for non-HLA celiac disease predisposing genes is like looking for a needle in a haystack. However, this process has been facilitated by the recent

application of genome-wide association studies (GWAS), a hypothesis-free approach that can test thousands of single nucleotide polymorphisms (SNPs) across the whole genome for association.[6••]

A provisional list of celiac disease predisposing loci includes *CELIAC1* on chromosome 6 (HLA-DQ2 and HLA-DQ8), *CELIAC2* on chromosome 5q31-33, *CELIAC3* on chromosome 2q33 (containing the T-lymphocyte regulatory genes *CD28, CTLA4*, and *ICOS*), and *CELIAC4* (the myosin IXB gene, *MYO9XB*) on chromosome 19p13.1. *MYO9B* encodes an unconventional myosin molecule that may have a role in actin remodeling of epithelial enterocytes. It has been hypothesized that this genetic variant might lead to an impaired intestinal barrier, which might allow the passage of immunogenic gluten peptides.[7] Although the MYO9B association has not been replicated in some European populations, it was a puzzling finding that MYO9B genetic variants predispose also to inflammatory bowel disease. These data imply shared causal mechanisms underlying intestinal inflammatory diseases.[8] Genetic variation in MYO9B was found to be associated also with systemic lupus erythematosus and rheumatoid arthritis, suggesting that MYO9B is a general risk factor for autoimmunity.[9] Furthermore, associations with tight junction genes *PARD3* and *MAGI2* has been reported in Dutch patients affected with either celiac disease or ulcerative colitis, again suggesting a common defect of the intestinal barrier in these two conditions.[10•]

The first GWAS in a large cohort of UK celiac disease patients and controls identified risk variants in the 4q27 region harboring *IL2* and *IL21* genes.[11] IL-2, secreted in an autocrine fashion by antigen-stimulated T cells, is a key cytokine for T-cell activation and proliferation. Another T-cell-derived cytokine, IL-21, enhances B-cell, T-cell, and natural killer cell proliferation and IFN-_ production. Both cytokines are implicated in the mechanism of other autoimmune conditions, namely T1D and rheumatoid arthritis, suggesting that the 4q27 region might represent a general autoimmune disease risk locus.[12] A further GWAS on follow-up samples from three independent European celiac disease collections identified seven previously unknown regions contributing significantly toward disease risk.[13••] These seven newly identified regions, together with *IL2-IL21*, explained 3-4% of the heritability of celiac disease. Six out of seven regions harbored genes controlling immune responses, for example leukocyte signaling in response to IL-18 and IFN-_ production. Together with other recent GWAS reports,[14] these findings suggested possible common mechanisms between celiac disease and T1D at the *SH2B3* region and the 3p21 *CCR* gene region, and between celiac disease and Crohn's disease at the *IL18RAP* region.

To summarize, it appears that the genetic predisposition to celiac disease depends on one gene with a large effect (HLA-DQ2/DQ8) on the adaptive immune response to gluten peptides and many other genes influencing different aspects of innate and adaptive immune reactions, intestinal permeability, and general predisposition to autoimmunity.

The Treatment: Gluten-free Diet

The cornerstone of treatment of celiac disease is a lifelong adherence to a strict GFD devoid of proteins from wheat, rye, barley, and related cereals. Gluten is, however, a common (and in many countries unlabeled) ingredient in the human diet, presenting a big challenge for celiac disease patients. Gluten-free products are not widely available and are more expensive than their gluten-containing counterparts. Dietary compliance is therefore suboptimal in a large proportion of patients. Furthermore, even when compliance is not an issue, a high percentage of celiac disease individuals on a GFD that are symptom-free and test negative to celiac disease serology show persistence of severe intestinal damage.[15,16] This persistence damage may be in part due to gluten cross-contaminations and lack of information on safe gluten thresholds.

How Much of Gluten is Too Much?

It is almost impossible to maintain a diet with zero gluten content because gluten contamination is very common in food. 'Hidden' gluten (used as a protein filler) may be found in commercially available products, such as sausages, soups, soy sauces, and ice cream. Even products specifically targeted to dietary treatment of celiac disease may contain trace amounts of gluten proteins, either because of the cross-contamination of originally gluten-free cereals during their milling, storage, and manipulation or because of the presence of wheat starch as a major ingredient. Until recently, the potential toxicity of trace amounts of gluten was not clear. This is a hot topic that has not only clinical but also trading and regulatory implications. In northern European countries, up to 100 ppm of gluten is permitted in special food for celiac disease patients as an ingredient. Conversely, a more prudent threshold of 20 ppm has been adopted in North American and southern European countries.

New data are now available on the issue of the gluten threshold. By using a prospective, double-blind and placebo-controlled study design and the quantitative morphometry on small intestinal biopsies as the biomarker of gluten-induced damage, Catassi *et al.*[15] showed that 50 mg of daily gluten, if introduced for 3 months, was sufficient to cause a significant decrease in the villus height/crypt depth ratio in the small bowel mucosa of treated celiac disease patients. Neither the clinical nor the serological (IgA antitTG and AGA) findings showed a correlation with the minimal mucosal changes induced by these gluten traces. Because of the limited number of patients studied, no firm conclusions could be reached about the potential toxicity of 10 mg gluten/day, which remained a 'grey' area. A recent systematic review of the literature suggested that a daily gluten intake of less than 10 mg is unlikely to cause significant histological abnormalities.[17] These results should be interpreted in light of recent data regarding the consumption of wheat substitutes by celiac disease patients. In a large sample of European celiac disease patients, the median intake of wheat substitutes was 173-268 g/day, whereas 10% of patients consumed 400-531 g/day of these products.[18]

It can be concluded that the previously used 200 ppm value is not a safe threshold because the harmful gluten intake of 50 mg/day could be ingested even by patients consuming a moderate amount (250 g/day) of nominally gluten-free products. Even a 100-ppm threshold is not suitable for generalized use, especially when consumption of wheat substitutes is occasionally as high as 500 g/day. The threshold of 20 ppm keeps the intake of gluten from 'special celiac food' well below the amount of 50 mg/day, which allows a safety margin for the variable gluten sensitivity and dietary habits of patients.

Potential New Therapies: A Future Without Gluten-free Diet?

The problems related to gluten thresholds outlined above and the major progress achieved during the last decade in the understanding of the cellular and molecular basis of celiac disease led to the identification of potential targets for novel therapies alternative to the GFD.

Enzyme Therapy

It has been shown that because of the high proline content, gliadin peptides are highly resistant to digestive processing by pancreatic and brush border proteases.[19] Enzyme supplement therapy with the use of bacterial prolyl endopeptidases has been proposed to promote complete digestion of cereal proteins and thus destroy T-cell multipotent epitopes.[20,21•] It remains to be assessed to what extent such intraluminal digestion may detoxify peptides particularly active in the most proximal part of the small intestine. An alternative approach to reduce gluten toxicity is based on a pretreatment of whole gluten or gluten-containing food with bacterial-derived peptidase.

Engineered Grains and Inhibitory Gliadin Peptides

Either breeding programs or transgenic technology or both may lead to production of wheat that is devoid of biologically active peptide sequences. Site-directed mutagenesis of wheat, which would not affect the baking properties, has also been proposed, although the number and the repetition of such sequences in wheat render this approach difficult. The identification of specific epitopes may also provide a target for immunomodulation of antigenic peptides by engineering peptide analogues of gliadin epitope(s) with antagonistic effects of native peptide(s).

Immunomodulatory Strategies

The autoantigenic tTG is mainly expressed in the lamina propria and its expression is upregulated by various stimuli, such as mechanical stress or bacterial/viral infection, during active celiac disease. Although the precise molecular details of this interaction *in vivo* remain unclear, selective inhibition of tTG in the small intestine might represent a therapeutically useful strategy for countering the immunotoxic response to dietary gluten in celiac disease. Other immunomodulatory targets, including IL-10, are possible alternative tools for promoting tolerance. However, evidence that gluten toxicity is not dependent only on T-cell recognition is growing. Activation of innate immunity has been demonstrated, and antibodies to IL-15 have been proposed, particularly in the treatment of refractory sprue because of the intraepithelial-lymphocyte-activating role of IL-15.[22] Nevertheless, one should realize that treated celiac disease is a benign condition and dietary treatment is safe, although strenuous. Therefore, any immunomodulatory approach must have a safety profile equivalent to that of the GFD, but with the advantage of increased compliance.

Correction of the Intestinal Barrier Defect

The ability of the intestinal mucosa to regulate the trafficking of macromolecules between the environment and the host is an extremely important function of the intestine. Together with the gut-associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular tight junctions, controls the equilibrium between tolerance and immunity to nonself antigens. When the finely tuned trafficking of macromolecules is dysregulated in genetically susceptible individuals, both intestinal and extraintestinal autoimmune disorders can occur.[23] This new paradigm subverts traditional theories underlying the development of autoimmunity, which are based on either molecular mimicry or the bystander effect or both, and suggests that the autoimmune process can be arrested if the interplay between genes and environmental triggers is prevented by re-establishing the intestinal barrier function. Indeed, in many cases, increased intestinal permeability seems to precede disease and causes an abnormality in antigen delivery that triggers the multiorgan process leading to the autoimmune response.[23] Therefore, correction of the intestinal barrier defects may represent an innovative therapeutic alternative to the treatment of autoimmune diseases, including celiac disease.

The inhibition of zonulin, a modulator of intestinal permeability, has been already successfully explored in an animal model of autoimmunity.[24] More recently, the zonulin inhibitor AT-1001 has been tested in an inpatient, double-blind, randomized placebo-controlled human clinical trial to determine its safety, tolerability, and preliminary efficacy.[25••] Following acute gluten exposure, a 70% increase in intestinal permeability was detected in the placebo group, whereas no changes were seen in the AT-1001 group.[25••] Gastrointestinal symptoms were significantly more frequent among patients of the placebo group as compared with the AT-1001 group.[25••] Combined, these data suggest that AT-1001 is well tolerated and appears to reduce gluten-induced intestinal barrier dysfunction, proinflammatory cytokine production, and gastrointestinal symptoms in celiac patients.

Conclusion

Celiac disease is a unique model of autoimmunity in which some of the genes involved, the target

autoantigen, and, most importantly, the environmental trigger, are all known. Therefore, celiac disease represents a superb model to study the genetic, immunological, epidemiological, and clinical aspects of multifactorial diseases. Given the undisputable role of gluten in inducing the autoimmune intestinal insult typical of celiac disease, the GFD is considered the only effective treatment for individuals with celiac disease. However, the implementation of a GFD is challenging and most of the time suboptimal. A better understanding of the complexity of the genetic/environmental interaction responsible for celiac disease development opens the way to explore alternative therapeutic strategies. It is possible that reducing the 'strength' or the access of the environmental component will prevent disease recurrence, particularly in those patients with a lower genetic load of predisposing genes.

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