Ages of celiac disease: From changing environment to improved diagnostics

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Abstract

From the time of Gee’s landmark writings, the recent history of celiac disease (CD) can be divided into many ages, each driven by a diagnostic advance and a deeper knowledge of disease pathogenesis. At the same time, these advances were paralleled by the identification of new clinical patterns associated with CD and by a continuous redefinition of the prevalence of the disease in population. In the beginning, CD was considered a chronic indigestion, even if the causative food was not known; later, the disease was proven to depend on an intolerance to wheat gliadin, leading to typical mucosal changes in the gut and to a malabsorption syndrome. This knowledge led to curing the disease with a gluten-free diet. After the identification of antibodies to gluten (AGA) in the serum of patients and the identification of gluten-specific lymphocytes in the mucosa, CD was described as an immune disorder, resembling a chronic “gluten infection”. The use of serological testing for AGA allowed identification of the higher prevalence of this disorder, revealing atypical patterns of presentation. More recently, the characterization of autoantibodies to endomysium and to transglutaminase shifted the attention to a complex autoimmune pathogenesis and to the increased risk of developing autoimmune disorders in untreated CD. New diagnostic assays, based on molecular technologies, will introduce new changes, with the promise of better defining the spectrum of gluten reactivity and the real burden of gluten related-disorders in the population. Herein, we describe the different periods of CD experience, and further developments for the next celiac age will be proposed.

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Key words: Antibodies; Autoimmunity; Celiac disease; Diagnostics; History; Intestinal mucosa

INTRODUCTION

The first descriptions of celiac disease (CD) refer to a disorder of the gut (koilakos in Greek), mainly characterized by fatty stools. While diarrhea was a symptom common to a number of diseases, fatty stools or steatorrhea was an uncommon symptom, characteristic of only a few dis-
cases, such as cystic fibrosis. The finding of steatorrhea in weaned children and in adults without cystic fibrosis was described as a single nosological entity by Samuel Gee, in a rapidly developing England at the end of the 19th century[3]. A similar disease was actually described by Aretaeus of Cappadocia, a physician active in Anatolia almost 2000 years earlier, during another period of rapid development, when agriculture had spread to the so-called region of the Fertile Crescent in the Middle-East.

We can refer to the Age from the first description by Aretaeus of Cappadocia to that of his English colleague 2000 years later as the “The Origins of the Celiac Age” (Figure 1 and Table 1). The cause of the disease was unknown, and the role of foods was conjecture. Gee described CD as a “chronic indigestion which is met with in persons of all ages, yet is especially apt to affect children between one and five years old. Signs of the disease are yielded by the feces; being loose, not formed, but not watery; more bulky than the food taken would seem to account for; pale in colour, as if devoid of bile; yeasty, frothy, an appearance probably due to fermentation; stinking, stench often very great, the food having undergone putrefaction rather than concoction”. Gee described, for the first time, that the only cure for the disease would be dietary, even if he failed to identify the foods causing the disorder. With his description, we can start the second age of CD: a disease of the gut, diagnosed on the basis of clinical features and curable with diet.

THE AGE OF STEATORRHEA: FAT IN STOOLS AND DIETARY ADJUSTMENT AS A TREATMENT

The first decades after the initial description by Samuel Gee were characterized by a clear medical description of the gastroenterological symptoms and signs of CD, and by an increasing recognition and identification of new cases, both in children and in adults. Fatty stools, together with abdominal bloating and failure to gain weight were the leading symptoms of the disease, which suggested a malabsorption of food nutrients. Since that time, different attempts were made to cure CD by employing different types of diets. Although the frequent onset of the disorder occurring in babies immediately after weaning suggested a role for cereals as the offending food, the first hypothesis focused on amides, and not on the protein content of flours. In fact, in 1921 the disease was still considered an intolerance to carbohydrates. In 1949, the success of a banana-based diet eliminated carbohydrates as the cause (Sydney Haas). However, scientific methods to identify the specific offending food were applied only around the mid 20th century, thanks to advances in chemical sciences.

THE AGE OF GLUTEN: STILL A VERY RARE DISORDER

In 1950, Dicke observed for the first time, that many children with celiac syndrome might be successfully treated with a diet free from wheat and rye flours. Two years later Anderson demonstrated that the gluten in wheat and rye was the harmful factor[2]. The ability to measure the fatty acids in stools permitted the systematic evaluation of the efficacy of different diets, and eventually confirmed that wheat, barley and rye were harmful to those with CD[3].

This is not the end of this period. In fact, in the following years, the picture of CD continued to change every time improvements were made in diagnostics, which revealed new aspects of the disease. It will soon become evident that it is not just our knowledge changing over time, but CD itself, which may be due to the coincidence of several factors, including availability of large amounts of wheat with a high content of gluten, and changes in the epidemiology of gastro-enteric infections. In this Age, CD is still considered a rare disorder, affecting the gut directly as a consequence of a chronic indigestion of gluten. Late diagnosis, coincidence of malnutrition, and/or infections may account for the more severe form of the disease, the “celiac crisis”, which can lead to shock and death. Currently, this form is extremely rare, while other types of presentation are increasingly described in the literature. This age can be also remembered as the age of the Crosby-Kugler capsule[4]. This instrument assisted in the diagnosis of CD allowing a mini-invasive withdrawal of fragments of the small bowel mucosa for histological analysis. The description of the typical picture of flat mucosa and criptae hypertrophy constituted at the same time a confirmation of diagnosis, and a tool for investigating the pathogenesis of the intestinal damage in CD. Repeat biopsies could confirm the healing of mucosa after a period of being on a gluten-free diet and the relapse after a new challenge with wheat, suggesting that sensitivity to gluten is a permanent condition in CD.

On the evolutionary scenario, the observation that mucosal damage greatly diminished the available surface for nutrient absorption could suggest a different expression of the disease depending on the available food supply. Indeed, some changes in the clinical expression of CD in different countries or in different periods may be related to the amount of food available, as well as to different epidemiology of infectious diseases, which can synergize with gluten to induce gut damage.

THE AGE OF GLIADIN ANTIBODIES: CELIAC DISEASE IS AN IMMUNE DISORDER RECALLING THE IDEA OF A “CHRONIC INFECTION BY GLUTEN”

The identification of gluten antibodies (AGA) in those affected by CD revolutionized the view of the disease in 1964[5]. Similar to what was found in the first years of the 20th century by von Pirquet in allergic diseases, CD appeared to be due to the immune response to gluten rather than to a direct action of the protein. However, it
was not an allergy, as it involved different mechanisms, which resemble more strictly the response to gut infections. Thus, the analogy of a chronic “gluten infection” substituted the definition of gluten “indigestion” previously used. The finding of gluten antibodies in CD was even more revolutionary, as it became evident that the measurement of these antibodies could allow a more accurate diagnosis of the disease, and a convenient follow-up by dietary change.

As a further confirmation of the role of the immune...
system in the pathogenesis of CD, a close association between particular human leukocyte antigen (HLA) variants and the disease was observed. More importantly, the measurement of AGA, being a relatively non-invasive and low cost assay, allowed researchers to widen the search for CD in subjects with different clinical complaints, and to find that the disease could be associated with atypical, non-gastroenterological symptoms, such as anemia, short stature, or dermatitis herpetiformis.[6-8]. Intolerance to gluten was in fact more frequent than previously expected, and could even be diagnosed in people without any evident symptoms (silent CD), but presenting the typical jejunal lesion of the disease.

THE AGE OF ANTI-ENDOMYSIUM ANTIBODIES: CELIAC DISEASE IS CONNECTED TO AUTOIMMUNITY

As a result of the AGA assay, CD was found to be more common in individuals with type 1 diabetes, and other autoimmune disorders, than in the general population. It was thus not surprising to find that serum from a person diagnosed with CD could contain autoantibodies. Anti-reticulin antibodies were identified in CD in 1971[9]. The finding that these antibodies behaved in a gluten-dependent manner, similar to AGA, was of particular interest as it represented an autoimmune reaction induced by foods.[10]. Years before the relationship between gluten and reticulin was clarified, different assays for identifying CD-related autoantibodies entered clinical practice including reticulin antibodies in rat kidney; endomyosium antibodies in monkey esophagus; and, later human umbilical cord sections (AEA).[11]. AEA were soon considered a specific sign of CD permitting the definition of a new kind of gluten intolerance, in the absence of overt mucosal lesion (“latent celiac disease”)[12]. In these patients, the mucosal inflammation induced by gluten was only revealed by an infiltration of CD3-positive lymphocytes with an increase in the TCR-gamma/delta subset[13-15]. This is why we can also refer to the autoimmunity Age as the “AEA Age”.

In the AEA Age, attention was focused on the particular relationship between CD and autoimmunity, which was initially thought to reflect the clustering of different autoimmune disorders due to the sharing of the same HLA variants. More recently a multicenter study from the SIGEP suggested that in genetically predisposed subjects, the longer the exposure to gluten the higher the risk of developing autoimmune disorders[16]. In this picture, the risk of developing autoimmunity in CD could be higher in cases without the typical gastroenterological symptoms of the disease in patients who were more likely to be diagnosed later, and likely to remain exposed to gluten longer. It is noteworthy that postponing gluten intake in the first year of life could make the gastrointestinal symptoms less intense, thus delaying the diagnosis, and possibly increasing the risk of developing autoimmunity.[17].

A milder gastroenterological presentation, because of a different environmental setting, could also be the cause of underestimating the prevalence of CD in the United States. However, the existence of AEA allowed for new screening and testing, which eventually demonstrated a similar prevalence of the disease in the United States, compared to many other countries, in the range of about 1%.[18] A good outcome of large screenings has been the increase in awareness of the disease in the population, making easier the clinical diagnosis and diet-based treatment.

The AEA Age ended with the idea that much still remained to be understood regarding CD, with the simile of the “celiac iceberg”: while the tip is represented by cases with typical symptoms, the majority of individuals with gluten intolerance are under the water, and are difficult to identify because of atypical or even absent symptoms and/or due to apparently normal mucosa.[19]. The iceberg idea was intriguing, as it suggested that a percentage of normal people exist, who can respond to gluten with different pathological reactions and that different diagnostic tools could unravel the disease. As a matter of fact, the AEA Age marked a major change in the knowledge of CD, from a rare gut disorder due to gluten and expressed with gastrointestinal complaints (just the tip of the iceberg), to a common autoimmune disorder triggered by gluten in the gut but expressed with a wide variety of clinical symptoms involving different systems. It is noteworthy that this submerged part of the iceberg is much bigger compared to the tip and, in the same way, clinical symptoms other than gastrointestinal are much more common than typical symptoms, where the disease itself is much more common than what was previously considered. Indeed, CD could be suspected in patients with a variety of autoimmune disorders such as diabetes, thyroiditis, dermatitis herpetiformis, autoimmune ataxia, alopecia, as well as symptoms directly due to malabsorption (Table 2).

THE AGE OF TRANSGLUTAMINASE: FROM TARGET TO DIAGNOSTIC TOOL

HLA variants DQ2 and DQ8 were the genetic factors most closely associated with CD. The isolation in duodenal biopsies of T cell clones recognizing gluten peptides in association with these HLA molecules further confirmed the pathogenic role of these genetic variants[20]. Furthermore, anti-gluten CD4 T cells produced large amounts of interferon gamma, which seemed to account for the typical mucosal damage seen in CD-affected mucosa. However, even this knowledge failed to explain why a HLA DQ2/DQ8 patient can present CD, yet another, not. In this scenario, the identification of the single antigen targeted in the Endomysial staining reaction was expected to permit a better knowledge of CD pathogenesis, and a better understanding of the origins of CD[21].

Thus, the search for the “endomysial antigen” represented an amazing adventure for most researchers involved in CD in the 1990s. In 1997, Dieterich and col-
leagues found that the endomyal antigen involved in the autoimmune response in CD was the enzyme tissue transglutaminase or Type 2 transglutaminase (tTG or TG2)[22]. Indeed, tTG is present in the endomysial net, where it stabilizes the connective tissue by catalyzing the link between glutamine and lysine of different structural proteins. This activity is very important in tissue repair processes and an increased activity of the enzyme can be evidenced in damaged tissues, including the mucosa in CD. Furthermore, tTG plays another important role, in the packaging of debris after cell apoptosis, which allows for the correct removal of apoptotic bodies containing inflammatory response materials.

Ludwig Sollid was the first to publicly hypothesize a model linking gluten to tTG and to anti-tTG autoantibodies. Briefly, when large amounts of gluten enter the mucosa because of increased epithelial permeability (may be favored by other factors, such as infections), the anti-gluten response causes mucosal damage, causing the release and activation of tTG. Gluten itself, due to its high content in glutamine, can be a target of tTG and can be cross-linked with other proteins, including tTG. As a consequence, macro-molecular complexes containing both gluten peptides and tTG can be recognized by AGA-producing B cells, as well as by AEA-producing B cells. According to the “Sollid hypothesis”, B cells recognizing these macro-complexes, regardless of their antibody specificity will present gluten peptides to gluten-recognizing these macro-complexes, regardless of their antigen spreading.

Another finding connecting tTG and gluten relies on the capacity of the enzyme to deaminate gluten-derived peptides increasing their affinity to the DQ2 and DQ8 HLA, thus worsening the consequences of anti-gluten immunity.[23,24] Recently, measurement of the immune response to deaminated gliadin peptides (DGP)[25] has been utilized to increase the performances of the AGA assay. This model could partly explain the role of environment, with gastrointestinal infections, in precipitating the pathogenic mechanisms of CD with a vicious circle of tissue damage, activation of tTG, entry and deamination of gluten, anti-gluten response and the spreading of autoantibodies. Hyper-production of IL-15 is associated with these mucosal changes, which could affect the production of the immunoregulatory cytokine TGF-beta.[27,28]. Even if this model does not illuminate the specific relationship between CD and other autoimmune disorders, it describes a dysregulated mucosal immunity, which is likely to interfere with the normal mechanisms of immune tolerance.

Apart from contributing to pathogenic knowledge, the identification of the main CD autoantigen allowed for a further improvement of diagnostics for CD by using ELISA assays based on human recombinant tTG (hTGG). Using hTGG, population screening has been performed starting from finger puncture producing as little as a few drops of blood in children from primary schools[27], and more recently rapid tests have been produced for the consumer market. Due to the reliability of hTGG assays, CD diagnosis can now be confirmed with just one jejunal biopsy without any need for repeating biopict examinations after the start of the diet. In some cases, it is even thought that a confirmation by means of jejunal biopsy may not be necessary. In fact, considering that a strong correlation has been demonstrated between high levels of tTG antibodies and a higher grade of mucosal damage (Marsh score)[29,30], the ESPGHAN is currently evaluating the possibility of making the diagnosis without a confirmatory jejunal biopsy in patients who have symptoms that can be referred to CD, if IgA-tTG antibodies are > 10x the upper normal limit, AEA and HLA DQ2 and/or DQ8 are positive.

**THE FUTURE AGE: WILL NEW TOOLS IDENTIFY NEW DISEASES?**

Cut-off values generated for a quantitative tTG assay assume a semi-Gaussian distribution of the values in the healthy population, with a tail of high values representing true celiac patients. This means that positive results represent a statistical correlate of the disorder and are not to be confused with the disease itself. Even if these assays are very useful and reliable in assisting the diagnosis of CD, they just represent our best for today, not the confidence of identifying all individuals in whom a gluten-free diet could give measurable advantages. While it is almost certain that very high tTG antibody titers indicate the presence of the disease[29,30], it is less easy to give significance to low titers and border-line results. In fact, there are several lines of evidence that gluten-dependent pathology can develop even in some patients with negative tTG antibodies, albeit rarely. On the other hand, even some patients with positive tTG might not develop symptoms on a gluten-containing diet, the gluten-free diets should still be prescribed, as we are not able to predict the risk of developing pathology in individuals.

Indeed, CD is a multifactorial disorder. It just might be that, in considering the picture of the “celiac ice-
berg”, there are different levels of intolerance to gluten, and exposure to gluten could have different consequences in each patient. In other words, we still know just a part of what made the iceberg. The forthcoming Age will clarify if we will be able to identify a single definite disorder by advances in molecular diagnostics, or if we will be faced with different forms of gluten intolerance (see Figure 1). Recently, it has been argued that intra-mucosal production of anti-tTG antibodies may precede their appearance in serum and could represent a specific indicator of gluten intolerance as well. An immunofluorescence technique on jejunal biopsies allows the detection of IgA deposits that co-localize with tTG in the villus connective tissue, which are considered bona fide tTG antibodies. These antibodies could be detected in patients with latent CD, regardless of their presence in serum, and have been shown to predict the development of villous atrophy and to disappear during a gluten-free diet[13]. Analysis of phage display antibody libraries confirmed that anti-tTG antibodies are indeed produced by mucosal lymphocytes and provided a further tool to identify latent CD, where tTG antibodies were produced in mucosa before that they can be found increased in serum[12]. These techniques have a role not only in research. In clinical practice, patients with a potential risk of developing gluten-related diseases, such as relatives of those diagnosed with CD, or with autoimmune disorders, may be positive for DQ2 HLA, but have normal levels of serum tTG antibodies. It was of particular interest to find that some of these patients did have mucosal tTG antibodies that behaved as gluten-dependent[13]. The characterization of such individuals, affected by intermediate or latent forms of CD is one of the goals of modern diagnostics and, a new key to better unravel knowledge on the disease. Further studies will address how gluten may interact with other environmental and genetic factors to condition the risk of developing different types of associated diseases.

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