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Review Article

Celiac Disease Revisited: Prevention and Therapies

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Abstract

Celiac Disease (CD) is an autoimmune condition triggered by the ingestion of gluten, the protein fraction of wheat, barley, and rye. There is a strong linkage between celiac disease and HLA-DQ2 and HLA-DQ8 haplotypes. As in other autoimmune diseases, celiac disease results from of an immune response to self-antigens, leading to tissue destruction plus the production of auto antibodies and has a complex pattern of inheritance with influence from environmental as well as additive and non-additive genetic factors. In the past few years, therapies have advanced tremendously. Actually, these therapies are still in preclinical development. And they are not yet available for their use in the clinical practice, and recommendations are in progress. For instance, some therapies may be recommended for patients without complications but who remain symptomatic despite following a gluten-free diet. It is possible that some of these therapies can be applied preventively in subjects with genetic risk.

Keywords: Celiac disease; HLA; T cells; Gluten; Autoimmunity

Introduction

Celiac Disease (CD) is an autoimmune condition triggered by the ingestion of gluten, the protein fraction of wheat, barley and rye. There is a strong linkage between celiac disease and HLA-DQ2 and HLA-DQ8 haplotypes [1-3]. CD is not simply an intestinal disease; it is multifactorial, caused by many different genetic factors acting together with non-genetic causes [4]. Similar to other autoimmune diseases, CD is a polygenic disorder for which the Major Histocompatibility Complex (MHC) locus is the most important genetic factor [5]. Gluten protein is the pathogenic agent activated by the enzyme Tissue Transglutaminase (TTG), allowing its presentation to CD4+ T cells in the lamina propria of the small intestine [6]. These CD4+T cells recognize gluten peptides bound to HLA-DQ2.5 orHLA-DQ8 [7,8]. As in other autoimmune diseases, celiac disease is the result of an immune response to self-antigens leading to tissue destruction and the auto antibodies production [9].

It is important to consider that not all gluten T-cell epitopes are equally immunogenic and that many parts of gluten do not stimulate CD4+ T cells. In HLA-DQ 2.5-positive individuals, T-cells response directed against α- and ω-gliadins are clearly immune-dominant [10]. Why do some gluten peptides efficiently elicit inflammatory T-cell responses whereas others do not? It is influenced by at least three factors knowing: (a) resistance to proteolytic degradation, (b) substrate affinity to TG2 and (c) specificity to bind HLA molecules [11].

In the past few years, therapies have advanced significantly, and many of them are still in preclinical development. For instance, some therapies may be recommended for patients without complications but who remain symptomatic despite following a gluten-free diet. It is possible that some of these therapies can be applied preventively in subjects with genetic risk.

Suspected celiac disease

It is important to diagnose CD not only in patients with classic gastrointestinal symptoms, but also in patients with a less clear clinical manifestation, because the disease may have negative health consequences. CD may present with a wide variety of nonspecific signs and symptoms that suspected CD. So in children and adolescents with unexplained symptoms and signs of chronic or intermittent diarrhoea, failure to thrive, weight loss, stunted growth, delayed puberty, amenorrhoea, iron-deficiency anaemia, vomiting, chronic abdominal pain, cramping or distension, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetic form is–like rash, fracture with inadequate traumas/osteopenia/osteoporosis, and abnormal liver biochemistry between others should be considered as possible celiac disease [12,13].

In asymptomatic patients with an increased risk for CD such as type 1 diabetes mellitus, Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, selective IgA deficiency, autoimmune liver disease, and first-degree relatives with CD must be considered preferentially. The serological tests with high accuracy and other diagnostic tests available will allow making a diagnosis to differentiate between: i) symptomatic CD, ii) asymptomatic patients, iii) patients with the presence of extra intestinal symptoms or iv) at-risk groups with possible CD.

In general, first degree family members of CD patients have an increased risk for CD depending on the gender and HLA-haplotype [14]. The disease can be detected at a very early age, and about 50% of them already have CD by age three years [14,15]. Girls with familial risk for CD have a higher risk of develop the disease, and homozygous HLA-DQ2 children develop early CD more frequently than heterozygous ones [14]. Molecular genetic testing of first-degree relatives of individuals with celiac disease can identify those who are susceptible to develop celiac disease and who would benefit from serologic testing to screen for celiac disease or silent celiac disease [16]. Of note, first-degree relatives do not need to be symptomatic to consider molecular genetic testing. Early diagnosis of celiac disease and treatment with a gluten-free diet can prevent secondary complications [17].

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Table 1: Current non-dietary therapies for celiac disease.

Class of Treatment	*Therapeutic Agent*	*Mechanism of Action*	*Phase of development*
Tight Junction Modulators	Larazotide Acetate (AT-1001)	Modulation of intestinal epithelial tight junctions Reduce the paracellular transport caused by gluten Ameliorate the activation of the pathological immune cascade	Phase II
Endopeptidases	ALV003, AN-PEP	Enzymatic Degradation of Gluten, reduced the small intestinal mucosal injury PEP derive from Aspergillusniger	Phase II Phase I-II
Gluten binding polymer	BL-7010	non-absorbable, co-polymer with high affinity for gliadins	Phase I-II
Gluten Vaccine	Nexvax2	Desensitizing vaccine with three gluten peptides	Phase I
Immune modulation with parasitic infection	*Necatoramericanus*	Inhibits Th1 immune response Facilitate Th2-dependent immunological reaction	Phase II
Blocking of intestinal homing – CCR9 antagonist	CCX282B	Block the chemokine receptor CCR9 to prevent intestinal T-cell homing	Phase II
Inhibition of pro-inflammatory cytokines (IL-2/IL-15RBeta, CD20, Anti-TNF-a, integrin a4b7)	Infliximab Certolizumab Adalimumab Vedolizumab Hu-Mik- Beta-1	Monoclonal antibody	Approved

The diagnostic delay and eventually under-diagnosis of CD remains as an important issue. Diagnose delay referring to the time span from onset of first CD symptoms to consulting a physician and from this first physician visit until CD diagnosis. The magnitude of diagnostic delay appears to directly translate into clinical impairment in patients with undiagnosed CD, and not only reduces patients' quality of life but also may impact on associated important complications, as well as medical costs [18].

Prevention

A multicenter randomized and interventional trial has explored the relationship between age of gluten introduction and risk of CD, testing the hypothesis that gluten introduction should be delayed in infants who have a familiar risk [19-22]. These studies showed that neither the timing of gluten introduction nor breastfeeding had a significant impact on the risk to develop CD. The prevalence of autoimmunity and possible CD was significantly higher in children with high-risk HLA genotypes as compared with standard-risk HLA genotypes; so the major risk factor of early onset is the HLA-DQ2 homozygosis [21]. Early feeding practices and the amount of gluten introduced during weaning have been hypothesized to play a role in conferring risk of CD development. These studies suggest that high amount of gluten introduced during the first year of life may contribute to the risk of CD, although the small differences found in the final prevalence requires caution in the conclusions [22].

The prevention of celiac disease in at-risk people requires further investigations. The negative results of these recent randomized trials evaluating early low dose gluten exposure at four months after birth or delayed gluten exposure until 12 months have led to speculation why these interventions failed and whether celiac disease can be prevented at all. These trials provide evidence that the number of copies of the HLA DQ2 gene stratifies patients with regard to subsequent CD risk [20]. In these trials, more than 25% of homozygous children for HLA DQ2 developed the disease, indicating that this high risk group may be of particular interest when studying future interventions [20]. However, most of these CD patients do not have a family history and therefore are not high risk group.

Several studies have suggested that early life events related to maternity, pregnancy, delivery and neonatal life influence the risk of developing CD [23–25]. Both elective caesarean delivery and repeated antibiotic treatment for urinary tract infections may lead to dysbiosis, resulting in disturbed maturation of the immune system in the child [26]. The gut microbiota affects gut permeability, gut inflammatory activity (directly and *via.* the release of metabolites) and dysbiosis, all of which are suspected to play a role of increasing the risk of autoimmune disorders as CD [27].

It is interesting to identify novel disease biomarkers that could be used to identify people at-risk for CD. It would be valuable to obtain molecular data from samples in longitudinal population biobank cohort studies. The use of population biobank data might also enable the identification of other environmental factors (in addition to gluten) that affect CD outcome [28]. Such information could then be used to take preventive measures in stopping the disease from developing in people with a genetic predisposition to develop CD.

Another problem for prevention is the absence of sensitive and non-invasive biomarkers to monitor compliance to the GFD. The determination of serum tissue Transglutaminase enzyme (TG2A) or deamidated gliadin peptides (DPGA) are usually used during the follow-up of the patients as these markers improve with gluten elimination [29]. However, mucosal damage may still persist without TG2A or DPGA, and thus, antibody testing may be negative in patients with partial adherence to the GFD [30]. The validation of the novel method recently described to measure gluten immunogenic peptides in stools may represent a step forward in the assessment of dietary adherence [31].

In summary, prevention strategies could be implemented, particularly in families at-risk, based on the reduction of immunogenic gluten. In particular, the identification of wheat varieties with lower pro-inflammatory activity [32], or with less immunogenic sequences [33] could lead to initiatives to favor their diffusion among the general population with the result of an overall decline in the prevalence of CD.

Therapies

Currently, the only effective treatment for celiac disease is a strict lifelong gluten-free diet. However, it has been demonstrated that the mucosal recovery is not immediate and a large proportion of patients exhibit persistent low-grade inflammation despite a GFD [34].

Although its compliance is difficult to maintain due to that the gluten is ubiquitous and used extensively in the food industry, and food may be contaminated with traces or even moderate amount of gluten. Also, is present even in non-dietary sources. A significant number of non-food products contain ingredients derived from grains that can be problematic with celiac disease or gluten intolerance. Some cleanliness products turn to compounds derived from wheat or oats. Several make-ups contains ingredients like wheat or barley extracts and certain vitamins, supplements and even medications may contain gluten [35].

It should be remember that a small amount of gluten is immunogenic for a susceptible host. For this reason, dietary transgressions (either accidental or not) are common, representing up to 50 % of the CD patients where it prevents mucosal healing and therefore maintains mucosal atrophy [36]. Recurrent dietary transgressions and prolonged gluten consumption (e.g., in non-diagnosed individuals) contribute to refractory celiac disease where the patients are unable to respond to the GFD and maintain inflammation.

There is a need to develop alternative / adjuvant therapies for CD patients. (Table 1) summarizes the current non-dietary therapies for celiac disease. Strategies include developing reagents to modulate of antigen presentation and immune response: research with TG2 inhibitors, HLA blockers to prevent the binding of deamidated gluten peptides to the HLA binding pocket, thereby preventing antigen presentation and production of gluten-specific T cells. Cell-specific therapy in CD is being attempted in vaccine development, with the aim to induce oral tolerance to gluten [37]. Specific inhibition of pro-inflammatory cytokines as IL-15; protease inhibitors in the context of CD: elafin and microbiota-modulating therapy using probiotics has been suggested in CD [38].

An interesting area of research on non-dietary treatment of CD are the attempts to modulate the immunological response through infecting CD patients with parasites and facilitating the Th2-dependent immunological reaction, at the same time inhibiting the Th1-dependent reaction induced by gluten. This therapy promoted tolerance and stabilized indices in gluten toxicity by improving quality of life, reducing intestinal IFN-γ-expressing T cells and increasing Tregs [39].

Efforts have been geared toward the genetic manipulation of wheat cultivars to knockout immunogenic gluten sequences, which is complicated by the existence of numerous epitopes in each variant of actual hexaploid wheat [40]. Attempts to detoxify the gluten epitopes by a number of different methods are being taken. Genetic deletion of certain gliadin genes, notably the complete deletion of α-gliadin led to the decrease in the number of T cell-activating epitopes without affecting the baking quality of the wheat flour dough [41]. In fact, there are now efforts being made to decrease the immunogenic gliadin peptides in wheat using RNA interference technology [42].

A distinct strategy for attenuating the immunotoxicity of gliadin peptides employs orally administered polymeric resins, which sequester gliadin peptides in the small intestinal lumen before they can elicit their immunotoxic effects in the lamina propria. This polymer has been tested *in vitro* [43,44] avoiding induction of IELs, intestinal atrophy and decreased barrier function. This polymer is non-absorbable and is safe in animal use. Phase 1 clinical trials are currently underway

However, only two agents are in late Phase 2 clinical trials: ALV003 (2 recombinant, orally administered, gluten-specific proteases) reduced the small intestinal mucosal injury caused by 6 weeks of gluten challenge [45] and Larazotide acetate (AT1001), isanoctapeptide derived from a cholera toxin, ZOT and has been found to antagonize zonulin *via.* receptor blockade. Larazotide acetate is expected to reduce the paracellular transport caused by gluten and ameliorate the activation of the pathological immune cascade [46].

Future research

The interest to develop alternative therapies to GFD is supported by the increased prevalence of celiac disease and evidence of persistent disease activity. The successful therapies development has proven to be a challenge given the complexity of this autoimmune disease. Recent GWAS studies have increased our understanding of the genetic factors that increase CD susceptibility. Many of the identified genes have been implicated in most of other immune-modulated diseases in relation with T-cell maturation or immune response pathways. These genes may modulate gluten sensitivity, and future therapies can be derived from investigation into the role of these genes in the CD pathogenesis. Linking polymorphisms in these genes with autoimmune and inflammatory conditions are the target for therapeutic approach.

Conclusion

The common genetic background is the main factor determining the high prevalence of CD association with other autoimmune diseases. No conclusive findings clarify whether extrinsic gluten-related factors (age at the first introduction, concomitant breastfeeding, length of gluten exposure and gluten-free diet) may link CD to the autoimmune diseases. It is relevant to evaluate whether genetic background alone could explain the association between CD and autoimmune diseases or if gluten-related factors ought to be considered.

In summary, celiac disease is probably the best-understood immune mediated disease, but much is still unknown. There are still many issues to resolve as: i) Why do most genetically susceptible individuals not develop this disease? ii) Why does disease develop early in childhood in some people and others can tolerate gluten before symptoms appear? iii) Why are disease-associated symptoms so variable? iv) Why are patients intolerant to gluten and not to other food proteins?

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