OPINION

New understanding of gluten sensitivity

Umberto Volta and Roberto De Giorgio

Abstract | Among gluten-related disorders, gluten sensitivity is an emerging entity that is characterized by a wide array of manifestations. In particular, patients complain of IBS-like symptoms and extraintestinal manifestations that occur shortly after the ingestion of gluten. Symptoms improve or disappear when gluten is withdrawn from the diet, and recur if gluten is reintroduced. Laboratory tests are usually unhelpful for diagnosis, although ~50% of patients are positive for IgG antigliadin antibodies. The natural history of gluten sensitivity is unknown; in particular, it is still to be clarified whether this disorder is permanent or transient and whether it is linked to autoimmunity. The pathogenesis of gluten sensitivity is unclear; data so far demonstrate a predominant activation of innate immune responses. Further research is necessary to establish the main clinicopathological features of gluten sensitivity, thus enabling physicians to improve their management of the increasing number of patients who are sensitive to dietary gluten.

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Introduction

Worldwide, the growing consumption of the Mediterranean diet, which includes a wide range of gluten-containing foods (such as bread, pasta and pizza), has contributed to an alarming increase in the incidence of gluten-related disorders.[1,2] Gluten is the main structural protein in wheat and other cereals (such as barley, rye and spelt) and its components—gliadins and glutenins—create a 3D network when flours are mixed with water, giving dough elasticity and viscosity.[3] New variants of wheat have arisen as a result of the mechanization of farming and the growing industrial use of pesticides and fertilizers that could have a leading role in the adverse immunologic reactions to gluten.[4] Moreover, the process of bread leavening has been progressively shortened, which has resulted in an increased concentration of toxic gluten peptides in bakery products.[5]

Gluten is one of the principal dietary components for most of the global population, particularly in Europe. For example, the mean daily gluten ingestion is 10–20 g in the Mediterranean area, and is even more in some populations.[6] Gliadins, a group of proteins that are rich in proline and glutamine, have been identified as the main toxic component of gluten. These proteins, which are resistant to digestion in the gastrointestinal tract, are known to cause a rearrangement of the cellular cytoskeleton through the zonulin pathway and cause the loss of tight junctions, which results in an increase in paracellular permeability of the small intestinal mucosa.[7,8] Moreover, gliadin has a toxic effect as it reduces the F-actin content of small intestinal mucosal cells, inhibits epithelial cell growth and induces apoptosis, thereby altering mucosal homeostasis.[9]

A wide array of disorders are related to gluten, ranging from well-known conditions such as celiac disease and wheat allergy to poorly defined illnesses (such as gluten ataxia and peripheral neuropathy) that are still a matter of clinical investigation.[1] Over the years, a thorough and dramatic change has occurred in the definition of celiac disease, that is, the disorder has gone from being considered an essentially malabsorptive condition to being recognized as a widely heterogeneous syndrome with digestive and extradigestive symptoms.[1] This improved definition of celiac disease and its clinical scenarios is largely attributable to improvements in diagnostic tests, such as the detection of autoantibodies and a superior appraisal of histopathology.[10] Growing evidence indicates that gluten sensitivity is emerging as a new entity.[11–13] Patients with gluten sensitivity do not have celiac disease, but they do experience symptoms when eating foods that contain gluten. The clinical features of gluten sensitivity include intestinal symptoms, commonly misdiagnosed as IBS,[14] and extraintestinal manifestations, which occur soon after gluten has been ingested and rapidly disappear once the patient is on a gluten-free diet.[12,13]

The concept of gluten sensitivity has challenged physicians and investigators over the years. Indeed, data published in 1980 and in 2000 suggested the existence of a syndrome caused by the ingestion of gluten in a subset of patients who did not have celiac disease or wheat allergy.[15,16] For many years, these patients continued to consume gluten-containing foods because gluten was not considered to be the cause of their symptoms. As a result, they were left in a no man's land, unrecognized by either allergists or gastroenterologists.[11] Much like patients who had IBS, patients with gluten sensitivity were commonly referred to psychiatrists because they were believed to have an underlying mental illness as a result of the poor awareness of the disease among doctors.[11]

This article discusses current knowledge of gluten sensitivity and defines its pathogenetic, clinical and diagnostic criteria. The aim is to provide a practical appraisal of gluten sensitivity that is useful for doctors and researchers in the diagnosis and appropriate management of patients with gluten sensitivity.

Pathogenesis

The pathogenesis of gluten sensitivity is still largely unsettled, although current data, mainly derived from a single study by Sapone *et al.*,[17] suggest that the mechanisms involved are different from those underlying celiac disease (Figure 1). Two pathophysiological aspects have been tackled so far by investigators: the possible role of innate versus adaptive immunity and the epithelial barrier function of the intestinal mucosa.

Although both innate and adaptive immunity have a central role in the

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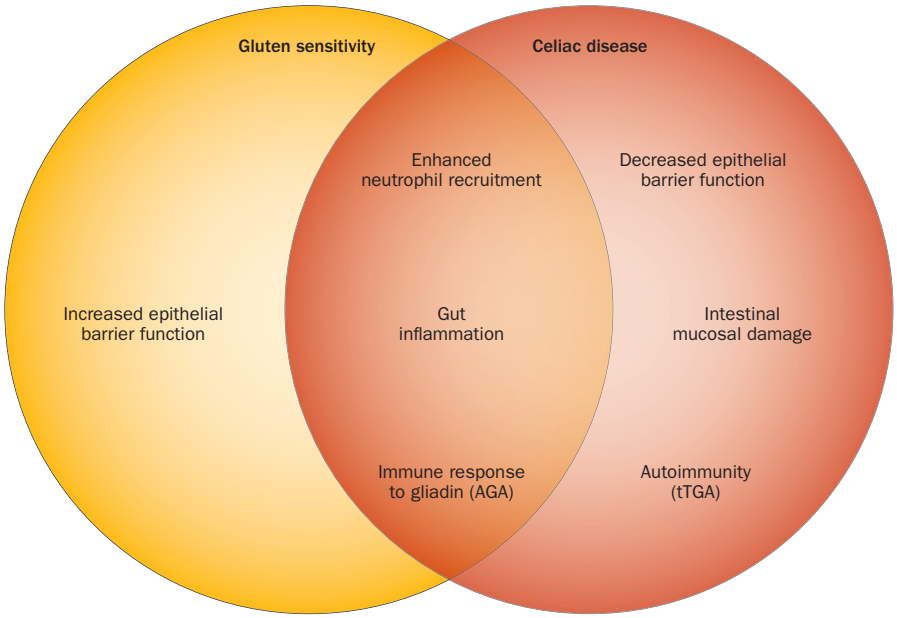

Figure 1 | Pathogenic mechanisms of gluten sensitivity and celiac disease. Enhanced neutrophil recruitment, gut inflammation (such as an increased number of intraepithelial lymphocytes) and an immune response to gliadin (AGAs) are features (orange field) common to gluten sensitivity and celiac disease. Decreased epithelial barrier function, intestinal mucosal damage and autoimmunity (that is, the presence of tTGA) are typical of celiac disease (red field), whereas enhanced epithelial barrier function has been demonstrated in gluten sensitivity (yellow field). Abbreviations: AGAs, antigliadin antibodies; tTGAs, tissue transglutaminase antibodies.

development of celiac disease, gluten sensitivity seems to be mainly associated with activation of the innate immune response.[1,17] The expression of the innate immunity marker Toll-like receptor (TLR)2 is considerably increased in the intestinal mucosa of patients with gluten sensitivity compared with patients who have celiac disease and control individuals.[17] Moreover, patients with gluten sensitivity had higher expression of *TLR1* and *TLR4* transcripts at the mucosal level than did patients with celiac disease or control individuals.[17] In addition, adaptive immune markers, including IL-6, T-helper-1 cytokine IFN-γ, IL-17 and IL-21, were expressed at increased levels in the small intestinal mucosa of patients with celiac disease, but not of those with gluten sensitivity.[17] Another interesting finding that differentiates gluten sensitivity from celiac disease concerned the mucosal expression of genes associated with T_{REG} cells. The expression of T_{REG} marker FOXP3 was found to be notably reduced in patients with gluten sensitivity compared with those who had celiac disease.[17] The expression of these messengers represents a quite controversial aspect of autoimmunity, as both downregulation and upregulation of FOXP3 and other T_{REG}-dependent molecules have been reported in patients with celiac disease and related conditions (such as type 1 diabetes mellitus).[18–20] Taken together, these preliminary data suggest that innate, rather than adaptive, immunity has a prominent pathogenetic role in gluten sensitivity.

Another intriguing factor that is probably involved in the pathogenesis of gluten sensitivity concerns changes to the epithelial barrier of the small intestine mucosa. Loss of intestinal barrier function, which has been clearly established in celiac disease, represents a key mechanism for the development of autoimmunity through the continuous aberrant passage of antigens across the intestinal epithelium.[21] However, in contrast to celiac disease, the study by Sapone *et al.*[17] showed that patients with gluten sensitivity did not have changes in intestinal mucosal permeability as assessed by the lactulose–mannitol test. In particular, an increased lactulose to mannitol urinary ratio, which is indicative of enhanced permeability of the small intestine, was detected in patients with celiac disease, but not in those with gluten sensitivity.[17] Nonetheless, it should be noted that the lactulose–mannitol test might not be reliable enough to identify subtle abnormalities of the intestinal barrier function in patients with gluten sensitivity. Indeed, Biesiekierski *et al.*, using the dual sugar absorption test, did not find any significant difference in the intestinal barrier function of two randomly treated groups of patients who had gluten sensitivity (one challenged by gluten, the other by placebo).[22] In addition, PCR analysis of tight junction components, that is claudins (CLDN), tight junction protein 1 and occludin, showed a notably higher expression of *CLDN4* mRNA in duodenal biopsy samples from patients with gluten sensitivity than those with celiac disease. As increased *CLDN4* expression is indicative of reduced intestinal permeability, this finding suggests that patients with gluten sensitivity might have a less permeable mucosa than those with celiac disease.[17] Clearly, further studies are necessary to confirm this result and to establish whether mucosal epithelial barrier function is actually increased in patients with gluten sensitivity.

Finally, experimental models of gluten sensitivity are an exciting investigational research area. Findings have indicated that gastrointestinal neuromuscular abnormalities observed in animal models can also contribute to the generation of symptoms in patients with this syndrome. HLA-DQ8 transgenic mice sensitized and gavaged with gliadin displayed an increased release of acetylcholine from the myenteric plexus, leading to muscle hypercontractility and an epithelial prosecretory state. Both abnormalities improved upon gluten withdrawal.[23] Moreover, luminal antigens, such as components of the intestinal microbiota, might contribute to enhanced inflammatory responses to dietary antigens such as gluten.[24] Taken together, these results from animal models provide a proof of concept that neuromuscular abnormalities and gut microbiota can trigger gliadin-induced symptoms. This experimental knowledge is now expected to be translated into clinically relevant data.

Clinical picture

Although the epidemiology of gluten sensitivity is far from being established, its presumed prevalence is higher than that of celiac disease.[12,13] Although it can occur at any age, gluten sensitivity seems to be more frequent in adults than in children, with a median age of onset of 40 years (range 17–63 years), and as with functional bowel disorders (including IBS), gluten sensitivity is more prevalent in females than in males (male to female ratio 1:2.5).[25]

From a clinical stand-point, gluten sensitivity is characterized by symptoms that usually occur soon after gluten ingestion,

disappearing or improving (within hours or a few days) with gluten withdrawal and relapsing following gluten challenge. The clinical presentation of gluten sensitivity is a combination of IBS-like symptoms, including abdominal pain, bloating, bowel habit abnormalities (either diarrhea or constipation), and systemic manifestations such as 'foggy mind', headache, fatigue, joint and muscle pain, leg or arm numbness, dermatitis (eczema or skin rash), depression and anemia (Figure 2).[25] In a double-blind, randomized, placebo-controlled trial by Biesiekierski *et al.*,[22] IBS-like symptoms and tiredness reoccurred more frequently in the gluten rechallenged group than in patients on placebo (68% and 40%, respectively), thus suggesting a link between gluten and symptom generation. Although the frequency of intestinal IBS-like symptoms is higher than that of extraintestinal manifestations, all patients usually display two or more extraintestinal symptoms, the most common being fatigue (36%) and 'foggy mind' (42%), the latter defined as a sensation of lethargy that occurs after eating gluten-containing foods.[25] However, Biesiekierski *et al.* reported that only one extraintestinal manifestation (that is, tiredness) was associated with IBS-like symptoms.[22] Thus, other data are needed to establish the actual prevalence and type of extraintestinal symptoms in patients with gluten sensitivity.

Despite having a normal small intestinal mucosa, relatives of patients with celiac disease often display evidence of immune responsiveness to gluten.[11] This observation has been confirmed by the demonstration that 10 of 78 (12.8%) patients with gluten sensitivity were first-degree relatives of patients with celiac disease.[25] Local instillation of gluten in the rectum can be a useful test for identifying mucosal evidence of gluten sensitivity at an early stage in asymptomatic first-degree relatives of patients with celiac disease.[26,27] At variance with celiac disease, patients with gluten sensitivity do not seem to show major autoimmune comorbidities.[17] Indeed, in the study of 78 patients with gluten sensitivity none had type 1 diabetes mellitus and only one (1.3%) had autoimmune thyroiditis, compared with 5% and 19%, respectively, of 80 patients with celiac disease.[25] Natural history data on gluten sensitivity are still lacking and it is therefore difficult to draw firm conclusions on the outcome of this condition. Whether patients with gluten sensitivity are at risk of complications, such as intestinal lymphoma or other gastrointestinal neoplasms, is yet to be determined.[25]

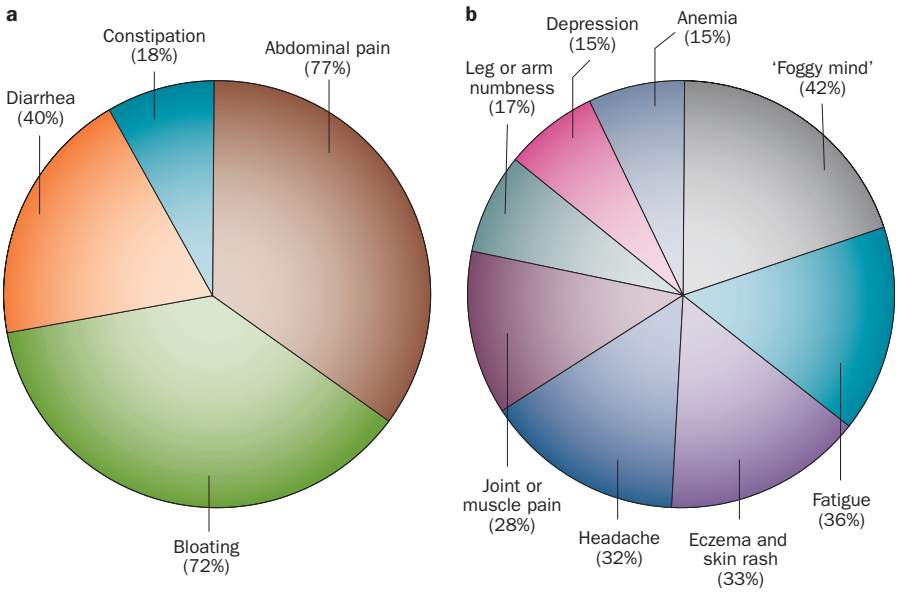

Figure 2 | Symptoms reported by 78 patients with gluten sensitivity; most patients complained of two or more symptoms.[25] **a** | Gastrointestinal symptoms. **b** | Extraintestinal symptoms.

Box 1 | Diagnostic criteria for gluten sensitivity*

- Gluten ingestion elicits the rapid occurrence of intestinal and extraintestinal symptoms
- Symptoms disappear rapidly after gluten withdrawal
- Reintroduction of gluten causes symptoms
- Specific IgE to gluten and wheat and skin prick tests results are negative
- Celiac disease serology (IgA endomysial antibodies, IgA tissue transglutaminase antibodies, IgG deamidated gliadin antibodies) results are negative
- Antigliadin antibodies (mainly of IgG class) are positive in about 50% of patients
- Normal mucosa or mild increase in the number of intraepithelial lymphocytes at histopathology
- HLA-DQ2 and/or HLA-DQ8 possibly positive in ~40% of patients

*Criteria proposed by the authors.

Similar to patients with celiac disease, patients with clinical features that are compatible with gluten sensitivity should change their dietary habits and consume foods with minimal gluten content. Cereals such as buckwheat, rice, corn and millet and vegetables such as quinoa, amaranth and soybean are recommended as substitutes for gluten-containing products. In the absence of controlled trials, commercially available gluten-free products that are used by patients with celiac disease can be proposed to patients with gluten sensitivity to achieve a thoroughly gluten-free regimen. As no clues exist as to whether gluten sensitivity is a permanent or a transient condition, the reintroduction of gluten after 1–2 years on a gluten-free diet might be advised.[13,25]

Diagnostic criteria

In the absence of a specific biomarker, the diagnosis of gluten sensitivity relies on the accurate assessment of clinical features, along with the exclusion of wheat allergy and celiac disease (Box 1). Gluten withdrawal is associated with a dramatic improvement or even the disappearance of IBS-like and extraintestinal symptoms, and reintroducing gluten causes symptom recurrence.[11–13,25] Symptom cessation or reoccurrence attributable to the absence or presence of dietary gluten should be considered a test indicative of gluten sensitivity. However, as a placebo effect produced by gluten withdrawal cannot be excluded, double-blind, placebo-controlled challenge studies are awaited to confirm the diagnosis of gluten sensitivity. Wheat allergy should be ruled out by testing patients for serum IgE antibodies that are specific to gluten and wheat fractions as well as by skin prick tests.[13] Another diagnostic prerequisite is the lack of highly specific markers of celiac disease, that is, IgA tissue transglutaminase antibodies (tTGAs), IgA endomysial antibodies (EmA) and IgG deamidated gliadin (DGP) antibodies.[17,25]

Box 2 | Unresolved issues and future research in gluten sensitivity

- Is gluten sensitivity a permanent or transient condition?
- Is there a risk that complications (such as lymphoma or small bowel carcinoma) will develop?
- Are there any biomarkers that would help the diagnosis of gluten sensitivity?
- A double-blind, placebo-controlled gluten challenge test is needed in each suspected patient to confirm the diagnosis and exclude a placebo effect induced by gluten exclusion

Serological analyses of patients with gluten sensitivity have found a high prevalence (40–50%) of first-generation antigliadin antibodies (AGAs).[12,13,25] AGA positivity is almost always confined to the IgG class, while only occasionally occurring in the IgA class. Antibody titers of IgG AGAs in patients with gluten sensitivity are usually as high as those found in patients with celiac disease.[25] Although lower than in patients with celiac disease (80–90%), the prevalence of IgG AGAs in patients with gluten sensitivity is much higher than in those with a variety of other conditions, for example, IBS (20%)[28] or nongastrointestinal diseases (connective tissue disorders and autoimmune liver disease, 9% and 21.5%, respectively),[29] and in the general population and healthy blood donors (ranging from 2% to 8%).[30,31]

In the presence of clinical symptoms suggestive of gluten sensitivity, AGA positivity, together with a lack of EmA, tTGAs and DGP antibodies, supports the diagnosis of gluten sensitivity. Another interesting serological finding in patients with gluten sensitivity is the very low ELISA activity of IgA tTGAs (usually <1 AU in over 30% of cases).[25] In contrast to celiac disease, where IgG AGAs remain positive in half the patients after gluten withdrawal, IgG AGAs disappear in most patients with gluten sensitivity within 6 months of them beginning a gluten-free diet.[32] It is tempting to speculate that an ongoing immunological memory might be selectively operational in celiac disease but not in gluten sensitivity.[32]

An intestinal biopsy sample should always be obtained from patients with suspected gluten sensitivity when they are on a gluten-containing diet to exclude the presence of villous atrophy, the hallmark of celiac disease histopathology. About 60% of patients with gluten sensitivity have a normal intestinal mucosa that is composed of <25% intraepithelial lymphocytes (IELs)[13,17,25] ('grade 0' according to the Marsh–Oberhüber modified classification).[33] The remaining 40% of patients have a mild increase in the number of IELs of up to 40% ('grade 1'), which is less than the percentage of IELs usually found in patients with celiac disease.[12,25] Nonetheless, grade 1 lesions are known to occur not only in gluten-related conditions, but also in a wide array of diseases, that is, autoimmune disorders (such as Hashimoto thyroiditis and type 1 diabetes mellitus), intestinal infections (bacterial, viral and parasitic), *Helicobacter pylori* infection, lactose intolerance, food allergies and common variable immunodeficiency.[34] When a grade 1 lesion is demonstrated, T-cell receptor γδ IELs should be assessed, as their levels are considerably increased in patients with celiac disease but not in those with gluten sensitivity.[12] In the context of a grade 1 lesion, the detection of IgA tTGAs in the intestinal mucosa would suggest a diagnosis of potential celiac disease rather than gluten sensitivity.[35,36]

Positivity for HLA-DQ2 and/or HLA-DQ8 has been reported in ~40% of patients with gluten sensitivity.[13,17,25] This figure is much lower than that found in patients with celiac disease (99%) and is comparable to the general population (~30%).[17,25] Thus, data acquired so far indicate that gluten sensitivity is unrelated to the genetic pattern that is found in the vast majority of patients with celiac disease. Nonetheless, the possibility that patients who are positive for HLA-DQ2 or HLA-DQ8 but do not have a manifest gluten-related disorder might eventually develop either celiac disease or gluten sensitivity cannot be ruled out.

Conclusions

In the array of gluten-related disorders, patients sensitive to dietary gluten are increasingly recognized in daily practice. As a result of the broad spectrum of symptoms, gluten sensitivity might be regarded as a syndrome rather than a single entity. Indeed, IBS-like and extraintestinal, mainly neurological, symptoms improve or disappear upon gluten withdrawal and recur when gluten-containing foods are reintroduced into a patient's diet. Altogether, these features provide a conceptual link between gluten and the generation of symptoms. Nonetheless, other proteins contained in wheat and bread have the potential to cause the symptoms that are experienced by patients with this syndrome. In this context, a protein that is highly resistant to intestinal proteases and elicits innate immunity—wheat amylase-trypsin inhibitor—might contribute to gluten sensitivity.

The diagnosis of gluten sensitivity should be corroborated by the exclusion of celiac disease and wheat allergy along with positivity for IgG AGAs. However, current facilities for the diagnosis of gluten sensitivity are comparable to those in the early 1970s, when no serological marker was available to identify celiac disease objectively. Thus, future efforts should be directed to the identification of reliable biomarkers for the diagnosis of gluten sensitivity (Box 2). Another relevant issue concerns the definition of the histological hallmarks underlying gluten sensitivity. In particular, the characterization of IELs and their distribution along the villus axis in the gut mucosa is expected to improve the diagnostic yield. Furthermore, genomic and proteomic assessments will contribute to expand our knowledge of gluten sensitivity.

In conclusion, further research is necessary to shed light on gluten sensitivity, a condition that requires an accurate evaluation by gastroenterologists and general practitioners alike. For the time being, limiting the amount of gluten in the diet is required to obtain symptom improvement. Advances are expected in the next few years that will help to provide a reliable diagnosis and improved management for an increasingly high number of patients who are sensitive to dietary gluten.

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Author contributions

Both authors contributed equally to all aspects of this article.