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Non-celiac wheat sensitivity: Differential diagnosis, triggers and implications



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A B S T R A C T

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Non allergy-non-celiac wheat sensitivity (NCWS) has become a common and often overrated diagnosis. Skepticism mainly relates to patients with prominent intestinal symptoms in the absence of general or intestinal signs of inflammation. There is consensus that the major wheat sensitivities, celiac disease and wheat allergy, have to be ruled out which may be difficult for wheat allergy. The non-inflammatory intolerances to carbohydrates, mainly lactose and FODMAPs (fermentable oligi-, di-, monosaccharides and polyols), which cause bloating or diarrhoea, can usually be excluded clinically or by simple tests. Recent studies and experimental data strongly indicate that NCWS exists in a substantial proportion of the population, that it is an innate immune reaction to wheat and that patients often present with extraintestinal symptoms, such as worsening of an underlying inflammatory disease in clear association with wheat consumption. Wheat amylase-trypsin inhibitors (ATIs) have been identified as the most likely triggers of NCWS. They are highly protease resistant and activate the toll-like receptor 4 (TLR4) complex in monocytes, macrophages and dendritic cells of the intestinal mucosa. Non-gluten containing cereals or staples display no or little TLR4 stimulating activity. Wheat ATIs are a family of up to 17 similar proteins of molecular weights around 15 kD and represent 2–4% of the wheat protein. With oral

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ingestion they costimulate antigen presenting cells and promote T cell activation in celiac disease, but also in other immune-mediated diseases within and outside the GI tract.
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Clinical definitions and differential diagnoses

Non-celiac gluten sensitivity (NCGS) has been described about 40 years ago, being defined as mainly abdominal symptoms related to ingestion of gluten containing cereals, after celiac disease and wheat allergy have been (largely) excluded. Since it is possible if not likely that non-gluten components of wheat, barley and rye induce the disease, we prefer the term non-celiac (non-allergy) wheat sensitivity (NCWS), at least in scientific discussions. An increasing number of subjects claim to suffer from NCWS, and physicians are challenged to better define the condition. In view of the “gluten free fad” we have to separate mere claims of a health benefit of gluten free diet (GFD) from real disease, and rule out numerous other differential diagnoses before diagnosing a patient with NCWS, a subject of recent consensus conferences [1–3]. Here, patients with a clear worsening of symptoms and signs shortly after consuming gluten containing food, with rapid improvement on a GFD best fulfil the criteria of having NCWS. For the experienced clinician extraintestinal symptoms which are frequent serve as the best indicator of the disease, and accordingly, are increasingly recognized as hallmarks of NCWS [4,5]. When it comes to mainly intestinal symptoms, there may be a significant overlap with the spectrum of irritable bowel syndrome (IBS) [6], wheat allergy which is frequently missed with conventional blood IgE and skin testing [7–9] and (less likely) intolerance to FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols [10]. Wheat allergy can be revealed with mucosal challenge followed by confocal endomicroscopy but this technology is not yet generally available [11]. However, clinically allergies and FODMAP intolerance are usually characterized by a quick onset after ingestion of the offending foods (minutes to a few hours), whereas NCWS has a slower onset, with symptoms appearing after hours to a few days [12]. This also sets these entities apart from celiac disease in which symptom onset can take days to weeks after onset of gluten challenge (Table 1). Notably, there appears to be a large number of patients with IBS whose complaints are at least partly attributable to food intolerances or food sensitivities, especially FODMAP intolerance and undetected allergies [10–13].

Toxic wheat components?

Much research has been invested in identifying bioactive components in wheat. Apart from allergens, gluten peptides that trigger T cell activation in celiac disease and wheat proteins that activate innate immunity (see below), research has failed to identify toxic constituents, rather described

Table 1
Guide to differential diagnosis of the three wheat sensitivities.

	Celiac disease	NCWS	Wheat allergy
Time from wheat (gluten) exposure to onset of symptoms	Days–weeks	Hours–days	Minutes–hours
Pathogenesis	Th1: autoimmunity/adaptive immunity	Innate immunity	Th2: Allergic immune response
HLA-DQ2/DQ8-restricted	Yes	No	No
Autoantibodies	Yes (anti-TG2)	No	No
Enteropathy	Yes	Minimal	Minimal
Symptoms	Intestinal and extra-intestinal	Intestinal and extra-intestinal	Intestinal and extra-intestinal
Complications	Co-morbidities, long term complications	Long term (extraintestinal) complications?	No co-morbidities, short-term complications (anaphylaxis)

protective protein and non-protein components. Thus dietary plant lectins like wheat germ agglutinin and bean concanavalin A can induce intestinal epithelial shedding in rats, but this activity is unlikely to occur in the presence of the inhibitory monomeric or oligimeric sugars [14]. Wheat lectins and bean lectins (concanavalin A and phytohemagglutinins) rather protect from Helminth infection when added to chow [15]. Wheat arabinoxylans (belonging to FODMAPs) favour gut colonization with beneficial bifidobacteria and attenuate weight gain in obese mice [16]. Moreover, the wheat flavone tricin is protective in a rodent model of colorectal cancer [17]. Wheat and other grains and staples can be infested with fungi, especially fusarium and aspergillus, and thus may contain mycotoxins, such as ochratoxin, fusominin, aflatoxin and deoxynivalenol that are potent cellular toxins [18–20]. However, these toxins are not found in wheat that is appropriately harvested and stored. Much discussion is rotating around glyphosate (Roundup), a broadly used herbicide that inhibits the shikimic acid metabolic pathway unique to most plants and certain plant respiratory enzymes, with some activity also against human cytochromes. Some hypotheses correlate the increase in certain ailments, especially neurological inflammation, kidney disease, hepatotoxicity, mammary cancer, lymphoma and even celiac disease to glyphosate use and crop levels [21–23]. However, this herbicide is rapidly inactivated on the fields by hydrolysis, or in the body via N-acetylation, and there is no convincing study that provides a causal relationship with human disease, including the symptoms of patients with NCWS.

Immunogenicity of wheat

There is no doubt that wheat (and the related barley and rye) has immunogenic properties in a sizable number of human subjects. This also applies to ancient wheat variants like unicorn, emmer and spelt. Thus celiac disease in which defined gluten peptides induce a destructive intestinal T helper 1 (Th1) T cell activation in genetically predisposed individuals is discussed in other chapters of this issue. It affects between 0.5 and 1.5% of most populations worldwide causing significant morbidity [24–27]. In addition, wheat allergy may affect up to 2% of the population, usually increasing in prevalence with age, in contrast to many other common food allergies, e.g. to milk, egg and soy.

Allergies are characterized by T helper 2 (Th2) T cell response and either IgE or IgG antibody production. The allergic response to wheat shows a high individual variability, with a large spectrum of wheat proteins and peptide epitopes serving as potential allergens. Examples are alpha, gamma- and omega-gliadins, glutenins, lipid transfer protein, thioredoxin, peroxidase, and alpha-amylase-trypsin inhibitors (ATIs) [28–32]. Here, the family of ATIs, especially the variants 0.19, CM2, CM3 and CM16 have been implicated as prominent sensitizers in lung (baker's asthma), intestinal and skin allergy to wheat [33,34].

The role of innate immunity in NCWS

There is strong evidence that NCWS is caused by an innate immune response to wheat proteins (different from the adaptive, T cell mediated response to gluten in celiac disease, or to gluten and non-gluten proteins in wheat allergy) [1,3,12]. NCWS can usually be separated clinically from food intolerances, mainly lactase deficiency and FODMAP intolerance, conditions that lead to increased fermentation of carbohydrates in the intestine and that do not cause intestinal or general inflammation. Rather FODMAPs are considered sources of e.g. short chain fatty acids that have beneficial effects on the intestinal epithelium and mucosa. In contrast and as outlined above, celiac disease and food (wheat) allergy are Th1 and Th2 T cell mediated inflammatory diseases, caused by a type I and a type IV hypersensitivity to wheat proteins, respectively. In classical wheat allergy there is induction of IgE antibodies in Th2 T cells that trigger mast cell, basophil and eosinophil degranulation, while in celiac disease there is a destructive T cell inflammatory response. Importantly, a significant proportion of food allergies, including wheat allergy, does not induce IgE production, remains localized to the GI tract and rather resembles a type IV reaction which is not detectable using conventional tests (8,9), but may be diagnosed after direct mucosal provocation [11].

Innate immunity is a receptor mediated and immediate response to conserved and common bacterial and viral antigens, and to some danger molecules secreted by the body's own cells in response to

injury. Higher organisms are equipped with numerous innate, pathogen and danger signal recognizing receptors, such as the toll-like receptors that are prominently expressed on myeloid cells (monocytes, macrophages and dendritic cells) [35]. Earlier studies suggested that gluten peptides different from those that activate T cells in celiac disease could trigger innate immune responses in intestinal epithelia, monocytes, macrophages and especially in intestinal biopsy cultures of celiac patients *ex vivo*, but evidence for their role *in vivo* has been lacking [36–40]. Moreover, the receptor(s) remained elusive, except for implication of the (T cell) chemokine receptor CXCR3 on an intestinal epithelial cell line [39] or the toll like receptor pathway in monocytes [40].

ATIs as triggers of innate immunity in wheat

In our own studies we could not confirm published gliadin peptides, including alpha-gliadin peptide p31–49, as an activator of innate immunity in intestinal epithelia of myeloid cells, whereas a water soluble fraction and a peptic-tryptic digest of commercial gliadin strongly activated mouse and human myeloid cells *in vitro*, most potently dendritic cells > macrophages > monocytes. This activity was not due to e.g. lipopolysaccharide (LPS) contamination and not associated with alpha or gamma-gliadins, or synthetic gliadin peptides, leading to the identification of the family of amylase-trypsin inhibitors (ATIs) as triggers of innate immunity in wheat [41]. Notably, ATIs belong to the family of water soluble globulins and represent between 2 and 4% of total wheat protein (as compared to ~80–90% for gluten) [42].

We also demonstrated that ATIs engage the toll like receptor 4 (TLR4)-MD2-CD14 complex, lead to an upregulation of dendritic cell maturation markers and the release of proinflammatory cytokines in myeloid cells. Accordingly, mice deficient in TLR4 or TLR4-signalling are protected from the intestinal and systemic innate immune activation after oral challenge with ATIs [41]. Moreover, reactivity of cells of healthy controls is comparable to that of celiac patients, in line with innate immune activation.

ATIs are present in many plants where they may be involved in grain maturation and carbohydrate storage [43], but they also inhibit enzymes of common parasites such as mealworms and mealbugs [44]. When added to *ex vivo* celiac biopsy cultures ATIs serve as immune adjuvants for the gluten specific T-cell response [41]. *In vivo*, mice that are fed ATIs show an increased intestinal and systemic release of cytokines and chemokines like TNF α , IL-8 and MCP-1 (CCL-2) within 1–12 h. Therefore, ATIs qualify as the prominent triggers of innate immunity in CD and NCGS. ATIs are molecules of ~120–150 amino acids length that display a compact secondary structure with five intramolecular disulfide bonds which endows them with relative resistance to proteolytic digestion by the gastrointestinal proteases trypsin and pepsin [41,45]. They therefore maintain their biological activity of TLR4 activation during their intestinal passage after oral ingestion. To date, it is not clear how and to what extent ATIs are sensed by the intestinal myeloid cells. However, it is likely that either dendritic cells that have been shown to extend their bodies into the intestinal lumen of mice to sense luminal antigens [46] and/or active transport of (intact) ATIs through the intestinal epithelial layer which occurs with gliadin peptides [47] may be operative. Both scenarios would imply that ATI sensing is increased in the inflamed gut.

The modern wheat genome encodes up to 17 different ATI species [48]. They demonstrate variability in primary sequence but share key amino acids and a similar secondary structure, even when wheat ATIs are compared to ATIs of the related barley and rye [41,49]. TLR4-activating activity is dependent on the secondary structure and destroyed after reduction of disulfide bonds [41]. Wheat ATIs form mainly noncovalently linked homodimers and –tetramers, and the dimeric 0.19 and the tetrameric CM3 are the prevalent ATIs in modern wheat. 0.19 and CM3, either when isolated from wheat or expressed as recombinant proteins in eukaryotic cells, display comparable TLR4 activating activity [41].

Importantly, TLR4 stimulating activity is mainly confined to ATIs of gluten containing grains. Thus plants other than wheat, rye, barley or their related ancestors show only minimal or absent TLR4-activating activity, although they also contain (structurally different) inhibitors of amylase and trypsin-like activities (Zevallos VF et al., unpublished data). Therefore, a gluten-free diet is also essentially ATI-free. This is important, since ATI effects are dose-dependent, other than the often minor amounts of gluten that are needed to trigger allergies or celiac disease. Modern hexaploid wheat

contains higher ATI activity than some older wheat variants, but even among modern wheat there appears to be a great variability dependent on the wheat strain (Zevallos VF et al., unpublished). Importantly, orally ingested (luminal) bacterial lipopolysaccharide which is the most potent TLR4 agonist, is completely inactivated by gastric acid and intestinal epithelial alkaline phosphatase, as shown after oral gavage [41], which makes ATIs the only relevant luminal TLR4 activator in the gastrointestinal tract.

An adult person in wheat consuming societies usually ingests 150–250 g of wheat flour per day and will therefore be exposed to 0.5–1 g of ATIs. As predicted by the mechanism of action that indicates a mild to moderate activation of innate immunity in the gut, the majority of (healthy) people ingesting ATI-containing grains should not develop symptoms. Morphological effects of ATIs in the gut are discrete and may rather reflect dendritic cell or macrophage activation than mirror an increase in inflammatory cell numbers, which is well in line with histology previously described in patients with NCGS. From this it can also be deduced that the modest to moderate immune activation in the gut of healthy individuals does not cause disease.

However, this may change in subjects with pre-existing inflammatory diseases, where both sensing (uptake) of ATIs may increase and where even a modest innate immune activation can exacerbate pre-existing chronic diseases. Thus our unpublished data indicate that in mouse models of chronic inflammation/autoimmunity, such as inflammatory bowel disease, systemic lupus erythematosus, autoimmune encephalitis, fatty liver disease or inhalative allergy the feeding of ATIs at doses equivalent to average human consumption exacerbates disease manifestation (Zevallos VF, Pickert G, Ashfaq-Khan M et al., unpublished data). This occurs via promotion of a stronger antigen-specific adaptive immune response compared to their disease controls fed a defined ATI- or gluten/ATI-free diet. This can be explained by the adjuvant effect of TLR4 stimulation of antigen presenting cells, once they already maintain a T cell mediated Th1 or Th2 reaction. Notably, this is not limited to the gut, but can extend to nearby or more remote lymph nodes or lymphatic organs where T cell priming to

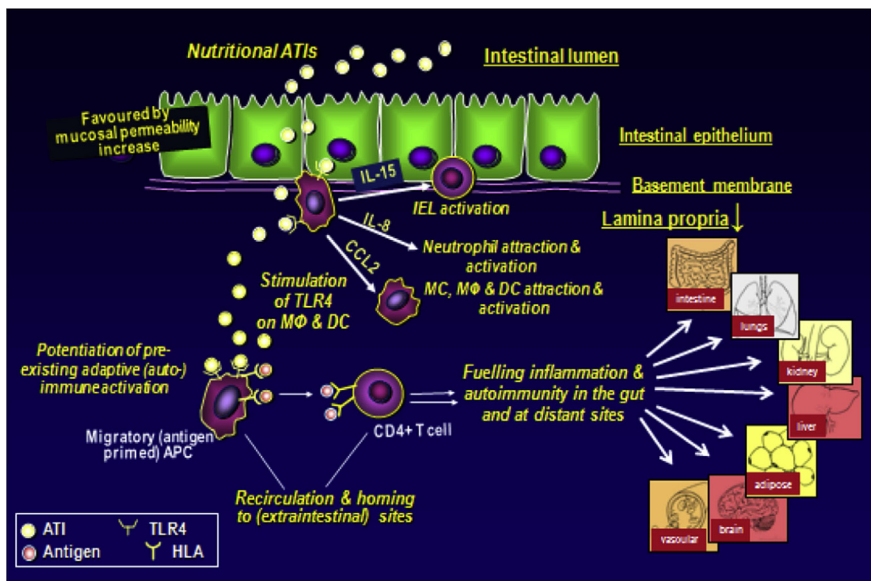


Fig. 1. Suspected role of nutritional ATIs in non-celiac wheat sensitivity. ATIs resist intestinal proteolysis and reach the small intestine and the colon, where they are sensed by mucosal innate immune cells, dendritic cells (DC), macrophages (MΦ) and monocytes (MC), inducing the release of proinflammatory chemokines and cytokines via activation of toll-like receptor 4 (TLR4). Moreover, ATIs serve as immune adjuvant, potentiating inflammatory antigen presentation on antigen presenting cells (APCs), thus driving an ongoing adaptive T cell response, as occurs in chronic inflammatory, allergic and autoimmune diseases. Much of the encounter between ATI-activated APCs and specific T cells may occur at extraintestinal sites, such as mesenteric lymph nodes, IEL, intraepithelial lymphocyte; HLA, human lymphocyte antigen.

specific antigen occurs (Fig. 1). This effect is dose-dependent, and a significant reduction of daily ATI-intake by a >90% avoidance of gluten containing foods could be sufficient to decrease their costimulatory effect to an irrelevant level. This should have relevant consequences for dietary recommendations, since in contrast to celiac disease or wheat allergy where a strictly gluten free diet has to be maintained, patients with proven NCWS could adapt a liberal gluten free diet.

Implications and future avenues

Apart from the hygiene hypothesis [49], the innate stimulatory activity of wheat (rye, barley) via ATIs could also explain the increasing prevalence of allergies and autoimmune diseases in developing countries where nutrition was originally based on gluten free staples or more thoroughly fermented and processed gluten containing products and where consumption of modern, high yielding wheat, often without thorough fermentation and traditional baking has increased steeply in the last decades. Finally, based on these novel findings, NCWS should be redefined as a syndrome with mainly extra-intestinal symptoms and/or exacerbation of pre-existing disease upon consumption of gluten (ATI) containing foods. Importantly, the condition may affect a substantial proportion of subjects with chronic disease who are not aware of this connection, since wheat products are consumed on large scale and on a daily basis. Therefore, after the clear demonstration that wheat and other gluten containing cereals worsen disease via costimulatory activity of ATIs, the next steps are clinical studies 1) to establish markers, preferably serum tests that would aid in positively diagnosing patients with NCWS, e.g. after a short-term challenge, and 2) to perform gluten/ATI challenge studies in well-defined patients with autoimmune, chronic inflammatory intestinal or metabolic diseases, and in subjects with common but ill-defined inflammatory diseases like chronic fatigue syndrome and fibromyalgia.

Practice points

- Non-celiac wheat sensitivity (NCWS) is currently a diagnosis of exclusion, mainly exclusion of celiac disease, wheat allergy and non-inflammatory food intolerances.
- Diagnosis is strongly supported by worsening of extraintestinal symptoms, including autoimmune diseases, when patients are on a gluten-containing diet, and a relatively quick improvement (within days) once patients are on a gluten-free diet
- The pathogenesis is likely linked to activation of the intestinal and extraintestinal innate immune system (monocytes, macrophages, dendritic cells) by nutritional amylase-trypsin-inhibitors (ATIs) from wheat, rye, or barley

Research agenda

- There is a need to establish diagnostic markers, preferably serum tests that support a positive diagnosis of NCWS after a short-term challenge, in patients who are already on a gluten (wheat)-free diet
- There is a need to carry out challenge studies in well-defined patients with autoimmune, chronic inflammatory intestinal and extraintestinal, or metabolic diseases; patients on a gluten (ATI)-free diet should receive a placebo-controlled, single or double blind wheat (gluten/ATI) challenge for several weeks, to confirm NCWS via an association of symptoms, biomarkers with wheat (gluten/ATI) consumption

Conflict of interest

The authors declare no conflict of interest in relation to this manuscript.

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