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Original article

Evaluation of the safety of ancient strains of wheat in coeliac disease reveals heterogeneous small intestinal T cell responses suggestive of coeliac toxicity

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SUMMARY

Background & aims: Coeliac disease is a chronic small intestinal immune-mediated enteropathy triggered by dietary gluten in genetically predisposed individuals. Since it is unknown if all wheat varieties are equally toxic to coeliac patients seven *Triticum* accessions showing different origin (ancient/modern) and ploidy (di-, tetra- hexaploid) were studied.
Materials and methods: Selected strains of wheat were ancient *Triticum monococcum precoce* (AA genome) and *Triticum speltoides* (BB genome), accessions of *Triticum turgidum durum* (AABB genome) including two ancient (Graziella Ra and Kamut) and two modern (Senatore Cappelli and Svevo) durum strains of wheat and *Triticum aestivum compactum* (AABBDD genome). Small intestinal gluten-specific T-cell lines generated from 13 coeliac patients were tested with wheat accessions by proliferation assays.
Results: All strains of wheat independent of ploidy or ancient/modern origin triggered heterogeneous responses covering wide ranges of stimulation indices.
Conclusion: Ancient strains of wheat, although previously suggested to be low or devoid of coeliac toxicity, should be tested for immunogenicity using gluten-specific T-cell lines from multiple coeliac patients rather than gluten-specific clones to assess their potential toxicity. Our findings provide further evidence for the need for a strict gluten-free diet in coeliac patients, including avoidance of ancient strains of wheat.

1. Introduction

Coeliac disease (CD) is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals.[1] Treatment involves a long-life gluten-free diet (GFD) which excludes wheat, rye, barley and possibly oats. Gluten is however difficult to avoid because of its widespread use in food processing. It is used in the production of soups, sauces, meat products, potato chips, candies, ice cream and as excipients in medicines and vitamins supplements, etc.[2] Therefore the GFD is restricted, unpalatable and causes social disadvantage for the individuals resulting in poor compliance amongst CD patients. The symptoms include diarrhea, weight loss and fatigue, although a broad spectrum of clinical presentations occur. The condition is diagnosed by the presence of some degree of small intestinal villous atrophy with raised number of intra-epithelial

Abbreviations: CD, coeliac disease; GFD, gluten-free diet; TCL, T-cell line; tTG, tissue transglutaminase; Tmp_AA, *Triticum monococcum precoce* (AA genome); Ts_BB, *Triticum speltoides* (BB genome); K_AABB, *Triticum turgidum durum* Kamut (AABB genome); GR_AABB, *Triticum turgidum durum* Graziella Ra (AABB genome); SC_AABB, *Triticum turgidum durum* Senatore Cappelli (AABB genome); S_AABB, *Triticum turgidum durum* Svevo (AABB genome); Tac_AABBDD, *Triticum aestivum compactum* (AABBDD genome); IEL, intraepithelial lymphocytes; PTG, peptic-tryptic digest of gluten; SI, stimulation index.

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lymphocytes which normalise when gluten is removed from the diet.

In the submucosa of the small intestine the enzyme tissue transglutaminase (tTG), generally involved in tissue repair, deamidates gluten peptides, that allows high-affinity binding to the human leucocyte antigen class molecules HLA-DQ2 and HLA-DQ8, subsequently triggering an inflammatory reaction in patients with CD.[3] The activation of gluten-sensitive CD4+ T-cells is central to the inflammatory reaction, although innate immune mechanisms are also thought to be involved.[4]

Gluten is a term that describes storage proteins in wheat endosperm and related cereals. Wheat storage proteins are divided into two major groups of proteins: gliadins and glutenins. Gliadins are further divided into α-, γ- and ω-gliadins and glutenins into high- and low-molecular weight glutenins. The α-gliadins are proteins encoded by a gene family at the Gli-2 loci, which may contain from 25 to 35 to even 150 α-gliadin genes per haploid genome,[5] although most of these (>70%) are presumably pseudogenes. The immunogenicity of many gluten peptides was assessed by activation of gluten-specific T-cells isolated from duodenal biopsies of CD sufferers.[6] Epitopes from gliadins,[7,8] in particular those that cluster within the N-terminal, including a stable 33mer fragment formed by physiologic digestion of α-gliadins,[9] are considered to have by far the highest clinical relevance with regard to the development of CD.

It is unknown if all wheat varieties are equally toxic to individuals with CD. Since large variations exist in the amount of T-cell stimulatory peptides present in different wheat cultivars,[10] numerous accessions have been studied to identify those with a lower coeliac toxic profile.[2,10–12] It has been suggested that a diet based on wheat varieties reduced in T-cell stimulatory epitopes may help in prevention of CD since the amount and duration of gluten consumption are associated with the initiation of CD. The argument then goes that this would especially benefit children in which the onset of CD may be delayed or even prevented and in undiagnosed coeliac patients (which are the vast majority of all coeliac sufferers) might strongly reduce their symptoms.[2] Nevertheless this assumption is controversial and still subject to much debate and investigation.

In an attempt to identify grains which were less toxic to patients suffering from coeliac disease, several scientists strongly focused on the analysis of grains considered forerunners of modern grains including *Triticum monococcum* (bearing the AA genome) and *Triticum speltoides* (considered the progenitor or otherwise very close to the ancestor of the BB genome). In particular, some ancient, that is ancestral, strains of wheat have been suggested to be less toxic for coeliac patients or even lack CD toxicity. For example gliadins of *T. monococcum* were reported to lack coeliac toxicity in an in vitro organ culture system suggesting new dietary opportunities for CD patients.[13] Some ancient wheat varieties including *T. monococcum* and *T. speltoides* were shown to be low in α-gliadin T-cell epitopes[5,10,11] or other gliadin and glutenin epitopes involved in the pathogenesis of CD.[10,11] More recently it has been suggested that some *monococcum* lines (Monlis and ID331) are to be considered toxic for coeliac patients.[14] Misinterpretations of the existing literature and contradictory evidence on the safety of *T. monococcum* accessions could potentially influence CD sufferers to consume ancient cereals that are unsafe for them.

In the last decades, a huge number of durum wheat cultivars have been obtained by artificial selection, generally based on high yield, disease resistance and technological qualities. On the other hand, to preserve genetic variability and reduce genetic erosion it is extremely important to develop and maintain local crops, including old cultivars and landraces, which were not subjected to massive selective breeding or genetic modifications. Examples of this are Graziella Ra, an accession (not a cultivar) of durum wheat that recently appeared on the market as Graziella Ra®, and Kamut®. The latter is considered ancient relative of modern durum wheat. In their paper testing the hypothesis of a potentially reduced or absent coeliac toxicity of these strains of wheat, Gregorini et al.[15] reported that both Graziella Ra and Kamut, are not only putatively as CD toxic as the modern durum accessions analysed, but also contain greater amounts of α-gliadin.

In the present study we sought to test whether the low immunogenicity hypothesis of ancient vs modern wheat varieties is confirmed or not, since any cereal from the tribe Triticeae has to be considered toxic unless thorough in vitro and in vivo evidence to the contrary is produced. The analysis was carried out by using 13 polyclonal gluten-sensitive T-cell lines (TCL) to assess their overall reactivity in relation to their (i) origin (ancient/modern) and (ii) ploidy. Wheat accessions were as follows: the diploid *T. monococcum precoce* and *T. speltoides*, representing potential ancient progenitors that might have hybridised into tetraploid strains of wheat[5,16]; four accessions of the tetraploid (AABB genome) *Triticum turgidum durum* including two ancient (Graziella Ra and Kamut) and two modern (Senatore Cappelli and Svevo) durum wheat varieties[17,18]; and the hexaploid (AABBDD genome) *Triticum aestivum compactum*.

2. Materials and methods

2.1. Subjects

Small intestinal biopsies were obtained at endoscopy performed for diagnostic or clinical management purposes in subjects with suspected or known CD. Thirteen volunteer subjects diagnosed according to British Society of Gastroenterology guidelines[19] included twelve females and one male, median age 35 years, range 23–72 (Table 1). Biopsy specimens were taken from the second part of the duodenum. All participating subjects provided written informed consent. The study was approved by the St Thomas' Hospital Research Ethics Committee (reference number 05/Q0207/167).

2.2. Cereal sources of gliadins

Ancient and modern wheat accessions with their correspondent ploidy, genomes and abbreviated names used in this manuscript

Table 1
Details of CD volunteers included in the study: sex, age, time on a gluten-free diet, DQ status, and histology results according to Marsh-Oberhuber classification of duodenal histological lesions in CD.[20]

Subject	Sex	Age at time of biopsy	GFD (in years)	DQ status	Histology
1	F	35	0	DQ2	1
2	F	28	0	DQ2	3b
3	F	72	7	DQ2	3a
4	F	51	0.33	DQ2	3a
5	F	23	0	DQ2	3a
6	F	60	17	DQ2	1
7	F	32	1	DQ2	0
8	F	23	2	DQ2	0
9	F	23	0.42	DQ8	3a
10	F	53	9	DQ2	3a
11	F	49	0	DQ2	3b
12	M	60	0	DQ2	3a
13	F	28	1.75	DQ2	3a

GFD, gluten-free diet; histology 0, normal mucosal architecture; 1, normal mucosal architecture with an increased number of intraepithelial lymphocytes (IEL); 3a, increased IEL with hyperplastic crypts and mild villous atrophy; 3b, increased IEL with hyperplastic crypts and moderate villous atrophy. Subject number 1 had positive CD serology 3 months prior to endoscopy and family history of CD.