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Original Article

T-cell response to different cultivars of farro wheat, *Triticum turgidum* ssp. *dicoccum*, in celiac disease patients

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SUMMARY

Background & aims: Celiac disease is a gluten sensitive disorder that occurs in genetically susceptible individuals. The present study deals with variation in the immune response of mucosal T-cells from celiac children to prolamins extracted from nine landraces of farro wheat (*Triticum turgidum* ssp. *dicoccum*) with contrasting storage protein compositions.

Methods: The prolamin fraction from nine 'dicoccum' wheat landraces was subjected to peptic–tryptic digestion and supplied to T-cells from mucosal explants of four celiac patients. Immune reactions in terms of cell proliferation and INF-γ secretion by intestinal T lymphocytes were then determined.

Results: T-cell lines exposed to digested prolamins from landraces L5563, L5558 and L5540 showed negligible proliferative responses and released INF-γ amounts similar to that of untreated control cells. By contrast, landraces Ersa 6, Ersa 8, Leonessa 4 and Leonessa 5 proved to be very active in triggering the immune responses, whereas landraces Filosini and Prometeo exhibited an intermediate behavior. One-dimensional fractionations by A-PAGE or SDS-PAGE revealed distinctive prolamin patterns amongst the landraces analysed.

Conclusions: 'Dicoccum' wheat represents a heterogeneous species showing a wide variation in both prolamin composition and T-cell immunological activation, some 'dicoccum' landraces being poor in or devoid of noxious gluten proteins.

1. Introduction

Exposure to dietary gluten causes celiac disease (CD), an intestinal inflammatory disorder that occurs in approximately 0.5–1% of individuals.[1,2] This disease is characterized by a rapid cytotoxic effect on the intestinal epithelium, followed by a T-cell-mediated immune response.[3,4] The external factors that trigger CD are prolamins, a heterogeneous group of storage proteins accumulated in the endosperm of cereal seeds.[5] Harmful prolamins include gliadins and glutenin subunits, which are responsible for the unique viscoelastic properties of wheat gluten,[6] as well as hordeins and secalins, the alcohol-soluble proteins of barley and rye, respectively.[7]

The recent literature strongly supports the hypothesis that CD is a multiform disease derived from interaction of two polymorphous factors i.e. the immunological pattern of the CD patients exposed to gluten and the primary structure of prolamins present in the grain of the different cereal species or cultivars.[8,9] A single cultivar of common (bread) wheat *Triticum aestivum*, the hexaploid species of the *Triticum* genus, possesses hundreds of prolamin genes, each expressing multiple alleles responsible of the extremely high level of polymorphism in the prolamin patterns of the thousands of wheat cultivars grown all over the world.[5,6] Polymorphism in prolamin composition has also been observed in other cultivated wheat species such as *Triticum turgidum* ssp. *durum* (durum wheat), *Triticum monococcum* ('monococcum' wheat) and *T. turgidum* ssp. *dicoccum* ('dicoccum' or farro wheat).[10–12] This polymorphism was

Abbreviations: A-PAGE, acid-polyacrylamide gel electrophoresis; CD, celiac disease; HMW-GS, high-molecular-weight glutenin subunit; iTCL, intestinal T-cell line; LMW-GS, low-molecular-weight glutenin subunit; PBMCs, peripheral blood mononuclear cells; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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found to be paralleled by variation in either the present/absence or the amount of T-cell-stimulatory prolamin sequences, indicating that some wheat varieties are likely to be less toxic for celiacs.[13] Moreover, reduced lesions, if any, were observed in cultured intestinal mucosa from CD patients exposed to prolamin peptides from 'monococcum' wheat and some durum wheat cultivars, as well as from common wheat cultivars lacking some gliadin polypeptides.[14–17]

Large differences in the harmfulness profile of prolamins have been found in the *Triticum* genus, which contains diploid, tetraploid and hexaploid species.[9,13,18] Prolamins from *T. turgidum* ssp. *dicoccum* were shown to differ from those of spelta wheat (*T. aestivum* ssp. *spelta*) and bread wheat cv. S. Pastore in lacking cytotoxic effects against human colon cancer Caco-2/TC7 cells grown *in vitro*.[19]

The tetraploid AABB-genome wheat *T. turgidum* ssp. *dicoccum* was the main cereal crop during the roman period under the Latin name of "farrum", and is still cultivated in hilly areas of Italy, Greece, Turkey and Balkan countries. The farro wheat is characterized by hulled (non-free-threshing) kernels and low gluten content compared to *T. turgidum* ssp. *durum*, its naked-kernel counterpart grown in the Mediterranean countries for pasta and cous–cous.[12]

The development of wheat genotypes lacking gluten proteins with harmful sequences has been proposed.[9,13,16] These genotypes could be valuable to select and breed wheat varieties of good breadmaking or pasta-making quality for consumption by CD patients.

In the present work, gliadin-responsive T-cell lines from celiac patients were investigated for their reactions against peptic–tryptic digested prolamins extracted from nine 'dicoccum' landraces with contrasting storage protein compositions. This investigation was aimed at obtaining information about immune reactivity of an ancient wheat crop that has been neglected by the breeding activity in the last century. The hope is that selected cultivars of 'dicoccum' wheat could represent an alternative food for CD patients, and reduce the exposition of the general human population to harmful gluten proteins.

2. Materials and methods

2.1. Plant materials

Seeds of farro wheat (*T. turgidum* ssp *dicoccum*, genome AABB, $2n = 28$) landraces Filosini, Prometeo, Leonessa 4, Leonessa 5, Ersa 6, Ersa 8, L5540, L5558 and L5563 were used in the present study. Common wheat (*T. aestivum*, genome AABBDD, $2n = 42$) cv. S. Pastore and durum wheat (*T. turgidum* ssp. *durum*, genome AABB, $2n = 28$) cv. Simeto were used as well. All the plant material was from the collection maintained by the CRA-QCE, Research Unit for Cereal Quality, Rome, Italy.

2.2. Extraction of prolamin, gliadin and total protein

After removing the hull, seeds (100 g) were ground on a Cyclotec 1093 mill (0.5 mm screen) and added to 400 ml (w/v) of 0.5 M NaCl. Extraction of prolamin was performed as described previously.[20] Gliadin polypeptides were extracted from single crushed seeds (25 mg) with 75 μl of 70% (v/v) ethanol for 1 h at room temperature under constant agitation. After centrifugation at 15,000*g* for 10 min, the protein suspension (25 μl) was mixed with 25 μl of an aqueous solution containing 50% glycerol and 0.1% (w/v) pyronine Y. An aliquot (25 μl) of the suspension was fractionated by A-PAGE. Total proteins from individual crushed seeds (25 mg) were extracted with 0.5 ml of a solution containing 0.25 M Tris–HCl buffer (pH 6.8), 0.12% (w/v) SDS, 10% (v/v) glycerol, 0.2% (w/v) pyronine Y and 5% 2-mercaptoethanol and shaken for 1 h at room temperature. After incubation at 80 °C for 20 min and centrifugation at 15,000*g* for 10 min, an aliquot (20 μl) of the protein suspension was fractionated by SDS-PAGE.

2.3. Peptic–tryptic digestion

The alcohol-soluble protein fraction was extracted from whole flour and subjected to sequential digestion as described previously.[21] Prolamin peptic–tryptic digests were freeze-dried, lyophilized and stored at −20 °C until used. The different farro wheat digests were freshly prepared by suspending them in RPMI 1640 and filtered at 0.45 μm. Gliadin preparations were assayed for endotoxin by using the QCL-100 reagent kit (Biowhittaker, Walkersville, MD, USA) and found to have an endotoxin level lower than 0.5 E.U./ml.

2.4. Fractionation by A-PAGE and SDS-PAGE

Protein fractionation by acid-polyacrylamide gel electrophoresis (A-PAGE) was performed at pH 3.1 in a Hoefer SE 600 apparatus (Amersham) at 450 V constant voltage until 1 h after the dye reached the bottom of the gel. The separating gel (16 cm × 18 cm), 1.5 mm thick, was prepared by mixing 7.5% acrylamide ($T = 7.5\%$ and $C = 0.37\%$) with 80 mg of ascorbic acid, 8 ml of sodium lactate buffer (3.4 g/l of 97% NaOH adjusted to pH 3.1 with lactic acid) and 120 μl of 1% ferrous sulphate, brought to 80 ml with distilled water. After cooling at 4 °C, the acrylamide solution was mixed with 24 μl of 2.5% hydrogen peroxide and immediately poured into the gel cassette. The electrophoretic buffer was 0.17 g/l of 97% NaOH adjusted to pH 3.1 with lactic acid.

SDS-PAGE running gels were prepared with 15% (w/v) acrylamide ($T = 15\%$ and $C = 0.5\%$), 0.375 M Tris–HCl (pH 8.4) and 0.1% (w/v) SDS. Stacking gels contained 4.5% (w/v) acrylamide ($T = 4.5\%$ and $C = 0.06\%$), 0.08 M Tris–HCl (pH 6.8) and 0.1% (w/v) SDS. The electrophoresis buffer was 0.025 M Tris–glycine (pH 8.3) and 0.1% (w/v) SDS. The gels (160 × 180 × 1.5 mm) were run at 18 mA/gel. Electrophoresis was stopped 1 h after the tracking dye had reached the bottom of the gel. A 0.25% (w/v) solution of Coomassie Brilliant Blue R250 in 6% trichloroacetic acid was used to stain both A-PAGE and SDS-PAGE gels.

2.5. Patients

Four patients (3F and 1M, mean age at enrollment 4.5 years) were included in this study. Celiac disease was diagnosed according to the revised ESPGHAN criteria.[22] The study was approved by the National Institute of Health Committee (CE-ISS-05/111, January 9th, 2006) and written informed consent was obtained from the parents of the patients. Biopsy specimens from distal duodenum were obtained during upper gastrointestinal endoscopies performed for diagnostic purposes in subjects under study for suspected or known CD at the diagnosis. All patients were consuming gluten containing food and exhibited a positive titre of anti-transglutaminase antibody.

2.6. tTG deamidation

Deamidation mixes were set up as follows: 100 μg/ml Guinea pig liver tTG, 2 mM $CaCl_2$, 1 mg/ml of peptic–tryptic digest of the different farro wheats. Incubation was carried out for 4 h at 37 °C. All reagents were purchased from Sigma Chemical (St. Louis, MO, USA).