A ω-secalin contained decamer shows a celiac disease prevention activity

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ABSTRACT

Celiac disease (CD) is an autoimmune permanent enteropathy that is triggered in susceptible individuals after the ingestion of gluten, a storage protein fraction presents in wheat, rye and barley endosperm. Specific gluten peptides can bind to HLA-DQ2/8 and induce lamina propria CD4+ T-cell responses causing damage of the small intestine mucosa. Recent studies suggested that beside immunodominant and toxic epitopes, wheat gluten also contains epitopes capable of preventing the mucosal response in vitro. Among them, a decapetide (QQQPDAVQPF) from wheat was reported to have an antagonist effect on the agglutination of K562(S) cells and celiac T-cell activation, although the corresponding nucleotidic sequence remained unknown. This study was therefore designed to clone the sequence encoding the protein carrying the decapetide with CD protective properties. A ω-secalin gene encoding containing the decapetide QQQRQPOQPF was isolated. Although the decapetide was not identical to the one previously described, QQQRQPOQPF showed the same capability to prevent K562(S) cell agglutination and celiac mucosa immune activation induced by toxic gliadins. The ω-secalin gene was found in wheat carrying the wheat–rye chromosomal translocations 1BL.1RS. The identification of this immunomodulatory gliadin sequence, naturally occurring in cultivars of wheat toxic for celiac patients, might offer new therapeutic strategies for CD.

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1. Introduction

Celiac disease (CD), an intestinal inflammatory disorder induced by prolamins in susceptible individuals, occurs as a result of the interplay between genetic and environmental factors (Sollid, 2000). The environmental factor required to trigger CD in genetically susceptible patients is the ingestion of prolamins, the alcohol-soluble fraction of the proteins accumulated in the endosperm of wheat (gluenins and gliadins), barley (hordeins) and rye (secalins) families located on the short arms of homeologous groups 1 (Gli-1, γ- and ω-gliadins) and 6 (Gli-2, α- and β-gliadins).

CD shows a strong association with human leukocyte antigen (HLA) class II molecules DQ2 and DQ8, and CD4+ T-cells specific for wheat gluten play a major role. The binding of gluten-derived peptides to the HLA molecules is enhanced when gluten is modified by tissue transglutaminase 2 (TG2) that converts specific glutamine residues to negatively charged glutamic acid (Kagnoff, 2007; Wieser and Koehler, 2008). The causative agent of CD resides mainly in the gliadin fraction of gluten where several peptides were shown to elicit a strong and rapid T-cells response in nearly all celiac patients, those immunodominant sequences occurring in α-, β-, γ- and ω-gliadins (Howdle et al., 1984; Shan et al., 2002).

Vader et al. (2003) identified 11 homologous T-cell stimulatory sequences in wheat, barley and rye prolamine, 7 of them were recognized by gluten-specific T-cell lines from CD-patients. The high proline content makes gluten resistant to proteolytic degradation in the gastrointestinal tract since gastric and pancreatic enzymes are deficient in post-proline cleaving activity. The high glutamine content, instead, is the substrate for the TG2 enzyme (Stepniak and Koning, 2006). The majority of the epitopes were...
found to be “immunogenic”, i.e. able to stimulate specific T-cell lines and clones derived from jejunal mucosa or peripheral blood of celiac patients. Moreover, it has been suggested that different portion(s) of gliadin, ostensibly not the ones recognized by T-cells, trigger an innate activation of celiac small intestine and set the tone for the activity of immunodominant gliadin epitopes (Londei et al., 2005; Maiuri et al., 2003; Meresse et al., 2009). The peptides, corresponding to amino acid residues p31-49 (LGQQQPFPPQQPYQPQPQ) of α/β-gliadin, were shown to be “toxic” as confirmed by their ability to agglutinate K562(S) cells (Silano M, personal communication) and induce mucosal damage when added in culture to duodenal mucosal biopsy or administered in vivo on proximal and distal intestine (Maiuri et al., 1996; Sturgess et al., 1994).

Beside immunodominant and toxic peptides, wheat gluten can also contain minor variations in the amino acid sequence of epitopes involved in the CD process capable to interfere with the gliadin-induced toxicity (Spaenij-Dekking et al., 2005). A peptide has been shown to be protective when applied alone or as part of a poly(T) primer starting from 1 μg of total RNA, following the manufacturer’s recommendations. The first CDNA strand was used as the template for amplification of the full-length ω-scelin genes. PCR was performed in 25 μl reactions with 50 ng of cDNA, 1X PCR buffer (Promega) with 1.5 mM MgCl₂, 200 nM of each primer, 200 μM dNTPs and 1 U of GoTaQ DNA Polymerase (Promega). The thermal cycling profile consisted of an initial denaturation step at 94 °C for 6 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 30 s, 72 °C for 2 min. A final extension step at 72 °C for 7 min was applied.

2. Experimental

2.1. Plant material and isolation of nucleic acids

The bread wheat cultivar Kavkaz (Triticum aestivum L., genome AABBDD) carrying the 1BL.1RS translocation from rye (Secale cereale L.) was used to isolate total RNA from seed endosperm collected during grain filling (20-day after anthesis) on plants grown in a growth chamber. Total RNA was extracted using the Trizol reagent (Invitrogen). The quality and concentration of the total RNA were checked by agarose gel electrophoresis. The RNA sample was then treated with DNAse to remove DNA contamination. The RNA samples were stored at 20°C prior to reverse transcription. The cDNA was generated using Superscript II RNase H⁻ reverse transcriptase (Invitrogen) and a poly(T) primer starting from 1 μg of total RNA, following the manufacturer’s recommendations. The first CDNA strand was used as the template for amplification of the full-length ω-scelin genes. PCR was performed in 25 μl reactions with 50 ng of cDNA, 1X PCR buffer (Promega) with 1.5 mM MgCl₂, 200 nM of each primer, 200 μM dNTPs and 1 U of GoTaQ DNA Polymerase (Promega). The thermal cycling profile consisted of an initial denaturation step at 94 °C for 6 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 30 s, 72 °C for 2 min. A final extension step at 72 °C for 7 min was applied.

2.2. Isolation of the full-length ω-scelin genes

Published DNA sequences (accessions X60294, X60295 and AF000227) from ω-scelin complete genes (Hull et al., 1991) were used to design a pair of oligonucleotide primers (forward 5'CACAATACAACTAGGACCTCTCC and reverse 5'CCCCATGCC-TATACCTACTACAA3') for the amplification of ω-scelin genes. Primers forward and reverse were defined as sequences that stretched from 12 nucleotides upstream to 8 nucleotides downstream of the annotated start and stop codon of CDS, respectively.

The single-stranded cDNA was synthesized using 200 U of SuperScript™ II RNase H⁻ reverse transcriptase (Invitrogen) and a poly(T) primer starting from 1 μg of total RNA, following the manufacturer’s recommendations. The first CDNA strand was used as the template for amplification of the full-length ω-scelin genes. PCR was performed in 25 μl reactions with 50 ng of cDNA, 1X PCR buffer (Promega) with 1.5 mM MgCl₂, 200 nM of each primer, 200 μM dNTPs and 1 U of GoTaQ™ DNA Polymerase (Promega). The thermal cycling profile consisted of an initial denaturation step at 94 °C for 6 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 30 s, 72 °C for 2 min. A final extension step at 72 °C for 7 min was applied.

2.3. Identification of RPQ peptide using the PCR-based method

Gene specific primers (rpq/F 5'CCTCCACACCAACCTTCCACAC3' and rpq/R 5'CTACTCAAAATTGTTGCTGGGC3') were designed to specifically amplify the gene encoding ω-scelins containing the RPQ tripeptide. The forward primer was designed on the region encoding the tripeptide RPQ. The amplifications were carried out on genomic DNA, following the same PCR conditions above described except for DNA concentration, equal to 80 ng.

2.4. GATEWAY™ cloning system and sequencing analysis

GATEWAY™ system was used for the directional cloning of ω-scelin PCR products into pDonor221 vector through the BP reaction (Invitrogen). The target cDNA fragments were amplified in a two-step PCR protocol. The first step was performed with the ω-scelin primers described above, modified with the addition of the attB tag at the 5' end (5'-aaaaagctgtcAACAATCCAATGAA-GACCTTCC-3', forward; 5'-agaaagtgttcGGATCCATTACACT- ACTACAA-3', reverse). The second PCR amplification was carried out with attB adaptor primers (5'-ggggacacattttgcttaaaaaagcagct-3' and 5'-ggggacacatttgcagcaaaaaagcagt-3'), which recognize the attB tags. The final PCR product was then cloned into pDonor221 vector through the BP reaction by means of BP clonase enzyme. The reaction was stopped by proteinase K and mixed with DH5α competent cell (Invitrogen) for transformation. Transformed cells were selected on LB plate containing 50 μg/ml Kanamycin. The procedure was according to the manufacturer’s protocol. Cloned genes were sequenced on both strands using the ABI Prism™ Big Dye Terminator Cycle Sequencing kit on an ABI Prism™ 3130 Genetic Analyzer automated sequencer (Applied Biosystem) using M13 forward and M13 reverse primers. The sequences were analyzed and translated into amino acid sequences by Vector NTI Explorer (version 10). The prediction of signal peptides was performed on SignalP 3.0 online accessible (http://www.cbs.dtu.dk/services/SignalP/) program. The sequence data reported in this paper appear in the NCBJ—GenBank nucleotide sequence database under the accession Nos. FJ816032–FJ816047 and FJ823438–FJ823444.
2.5. Peptide preparation

The decamers pRPQ (QQPQRPQQPF) and pDAV (QQPQDAVQPF) from u-secalin, the peptide from human thyroid peroxidase position 536–547 (DPLRGCILLARP, below referred to as pTPO), the peptide from α/β-gliadin position 31–43 (LQQGQFPFPQPPQ, p31-43) and the peptide from α/β-gliadin position 57–68 (QLQFPFPQPLP, pz-9) were synthesized by Primm (Milan, Italy) and purity determined by reverse-phase HPLC. These peptides were used at concentration of 20 μg/mL. Peptic–trypsin digest of gliadin (PT-gl) from bread wheat T. aestivum var. San Pastore was obtained as previously described and used at 2 mg/mL (De Ritis et al., 1979).

2.6. Agglutination test

KS62(S) cells were maintained in RPMI medium (GIBCO, commercialized by Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum and subcultured every 10 days. To perform the agglutination experiments, the cells were harvested by centrifugation and washed twice with calcium- and magnesium-free phosphate-buffered saline solution (PBS). The test was performed with cells resuspended at a concentration of 10^8 cells/ml in calcium- and magnesium-free PBS buffer; 25 μl of cell suspension was added to each well of a 96-well microtitre plate containing PT-gl at concentration of 2 mg/ml and the pDAV, pRPQ and pTPO were used at a concentration of 20 μg/ml. The final total volume was 100 μl. The cell suspension was incubated at room temperature for 30 min. A drop of suspension was then applied to a microscope slide to count clumped and single cells. Control wells were included, as appropriate. The inhibition tests were carried out by mixing the appropriate concentration of test compounds with PT-gl before the addition of the cells. Agglutination activities were measured with a 96-plate reader equipped with a stirrer. The cell suspension turbidity was read at 600 nm (OD 600 nm) under continuous stirring at time 0 and after 30 min. The difference of reading between T0 and T30 × 100 (delta OD) was calculated as a velocity of agglutination (CV).

2.7. Culture of intestinal epithelial cells and PBMCs purification

Human colon carcinoma T84 cells (ATCC catalogue No. CCL-248 http://www.atcc.org) were cultured in 25 cm² culture flasks (Falcon) in Dulbecco’s modified Eagle medium/F-12 (DMEM/F-12) supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin. Cells were kept in an incubator at 37 °C and 5% CO2. The medium was refreshed every 2–3 days and cells were transferred once a week. Human peripheral blood mononuclear cells (PBMCs) from five active celiac patients were isolated using lympholite (Cederlane, UK) density gradient, overlaid in a decapeptide (QQPQDAVQPF) capable of preventing the activation of peripheral B lymphocytes by gliadin peptides. Starting from this knowledge, a bioinformatic research was designed to identify the genes encoding gluten components and containing the 10-mer sequences X60294 and X60295 encode a u-secalin from P. De Vita et al. / Journal of Cereal Science 55 (2012) 234–242

2.8. Transwell co-culture model

T84 cells were seeded, 3 weeks in advance, at a density of 80 · 10^3 cells cm⁻² on 0.4 μm, 1 cm² tissue culture inserts (BD Falcon, USA). Upon confluence, the resistance was approximately 800 Ω cm². Transwell cultures (12-well) with confluent T84 monolayers were used for co-culture with 1 ml PBMCs (1.5 · 10⁸ cells/ml) using PBMC medium and kept in an incubator at 37 °C and 5% CO2. Cells were allowed to settle for 1 h before the onset of the experiment. T84 cells were apically pre-treated for 1 h with pDAV or pRPQ (40 μg/ml), then p31-43 (40 mg/ml) was added for another 3 h, washed, and treated with pz-9 peptide (40 mg/ml). After 24 h, culture supernatants from the basolateral compartment were collected, centrifuged and stored at –20 °C until cytokine measurement. IFN-γ was measured with an Elisa kit (Biowended).

2.9. Immunolocalizations in biopsy specimens

Duodenal biopsy specimens were taken from untreated CD patients undergoing endoscopy and biopsy for diagnostic purposes as a part of routine diagnostic procedures. The intestinal samples were cultured in vitro for 3 h or 24 h cultured with medium alone, PT-gl (500 mg/ml), p31-43 (20 mg/ml) with or without pRPQ (10 mg/ml), pTPO (536-547) (10 mg/ml) was used as control peptide.

The micrometer frozen tissue sections of biopsy samples from each patient before and after in vitro cultures were fixed in acetone for 10 min. The sections were individually incubated for 2 h at room temperature with the following antibodies: anti-phospho-tyrosine HLA-DR mAb (1:80, mouse IgG2b; Santa Cruz Biotechnology, Santa Cruz, CA), ICAM1 (1:500, mouse monoclonal Ig; Abcam, Cambridge, MA, USA), HLA-DR (1:500, mouse monoclonal Ig; Abcam, Cambridge, MA, USA). The antigen expression and distribution were visualized by 2-color and indirect immunofluorescence. Isotype control mAb (IgG1 or IgG2) or isotype-matched nonimmune Igs was used as control (Mauri et al., 1996). Data were analyzed under fluorescence examination by an LSM510 Zeiss confocal laser scanning unit (Carl Zeiss, Germany).

The study was performed according to the World Medical Association Declaration of Helsinki for ethical principles in medical research involving human subjects, and approved by the “Independent Ethic Committee on Pediatric Research and Clinical Studies”, (DIMED) University of Foggia, reference no. 110610-01/2010 and by the “Ethic Committee” of the Istituto Superiore di Sanità, reference no. CE/ISS 05-112.

2.10. Statistical analysis

All experiments were performed at least in triplicate. Data distribution was analyzed, and statistical differences were evaluated using ANOVA test and SPSS 12 software with a P value <0.05.

3. Results

3.1. Database analysis

The studies of De Vincenzi et al. (1997) and of Silano et al. (2008) have identified in the alcohol-soluble protein fraction of wheat a decapeptide (QQQPODAVQPF) capable of preventing the activation of peripheral B lymphocytes by gliadin peptides. Starting from this knowledge, a bioinformatic research was designed to identify the genes encoding gluten components and containing the 10-mer QQPQDAVQPF. The search of the gluten database (http://appliedbioinformatics.wur.nl/glutendb/tree.html) did not yield any sequences encoding an ORF fully matching the query peptide sequence. Nevertheless, the analysis of the database retrieved five gluten sequences with ORFs showing a partially conserved QQPQDAVQPF 10-mer motif (Fig. 1). The accession numbers AAT74547 and AAG17702 encode α-gliadin proteins of Aegilops tauschii (Hassania et al., 2008) and of T. aestivum (Hisia and Anderson, 2001), respectively. Both sequences contain a copy of the QQPOQQQQPF motif. Three additional sequences were characterized by the presence of a single copy of the QQPQQQQPF 10-mer motif: the sequences X60294 and X60295 encode α-secalin from...
Secale cereale (Hull et al., 1991), while the sequence AF000227 encodes a u-secalin isolated from a wheat genotype carrying the wheat–rye 1BL.1RS translocation (Clarke et al., 1996).

The decapeptide QQPQDAVQPF originally described by De Vincenzi et al. (1997) and the amino acid sequence QQPQRPQQPF found in the database share some structural homologies although the central tripeptide "DAV" is replaced by "RPQ". Arginine (R) and aspartic acid (D) are both polar amino acids, although with opposite charge (negative for aspartic acid, positive for arginine), they have the ability to form H-bonds. Proline (P), glutamine (Q), alanine (A) and valine (V) are all characterized by a neutral charge in physiological cell conditions. After these findings, further studies were undertaken to characterize the gene carrying the QQPQRPQQPF motif and to verify the activity of the new peptide in the celiac response.

3.2. Molecular characterization of the u-secalin gene family

The results of the database analysis have indicated the u-secalin as the only prolamine gene family encoding a peptide with some homology with the QQPQDAVQPF sequence reported by Silano et al. (2008). Therefore, a set of PCR primers was designed on two highly conserved regions of the u-secalin sequences available in the database and used to clone all u-secalins expressed in the endosperm of the bread wheat Kavkaz, a variety carrying one of the original wheat–rye translocation events. Amplified cDNAs were cloned and more than 100 randomly selected clones from the u-secalin specific library were sequenced. These sequences identified 23 non-redundant u-secalin genes.

The ORFs of the cloned u-secalin sequences ranged from 115 to 357 amino acid residues, the longest corresponding to the sequence already available in the database with the exception of a few amino acid substitutions. This variability in u-secalin sequence length is in agreement with what has been previously found (Chai et al., 2005). The primary sequences of the u-secalins cloned in the present study confirm the presence of repeat motifs rich in glutamine and proline residues (PQQPFPQF and QQPFQF) very common also in u-gliadins and C-hordeins (Tatham and Shewry, 1995).

Among the 23 u-secalins identified in this work, 7 deduced proteins contained the QQPQRPQQPF motif. The ORF of these sequences ranged from 200 to 357 amino acids and shared a high identity level with the u-secalins published in the NCBI gene bank. The selected 7 sequences were compared with the u-secalins described by Hull et al. (1991) and four main structural regions were identified: (a) a conserved 19 amino acids signal peptide; (b) an N-terminal region of 27 amino acids; (c) a repetitive region rich in glutamine and proline and (d) a short C-terminal region of 12 amino acids.
amino acids ending with 4 valine amino acid residues (Fig. 2). The signal sequence, the C-terminus and the N-terminus regions were conserved among all cloned ω-secalins with little differences due to single amino acid substitutions. The N-terminal sequences started with RQL, one of the known variants already described in ω-secalins, ω-gliadins and C-hordeins (Kasarda et al., 1983). Based on available literature (Ciccocioppo et al., 2005; Vader et al., 2003), two antigen peptides, Glia-γ2 (QQPFPQQPQFPQFPQ) and Sec-γ1 (PQQPQPSSFPQQPQ), the latter corresponding to the minimal epitopes of the Glia-γ1, were identified within the sequences of cloned ω-secalins genes. Glia-γ2 was present in 8 ω-secalins, while Sec-γ1 was present in 5 amino acid sequences. Furthermore, 2 ω-secalin genes with RPQ motif (FJ823444 and FJ8234438) contained peptides that align with both gluten epitopes (Glia-γ2 and Sec-γ1).

3.3. RPQ-specific marker

A forward primer (rpqF) designed on the region spanning the RPQ motif of ω-secalin sequence FJ823444, was paired with the reverse primer rpqR and used to selective amplify the alleles containing the RPQ motif. The RPQ genotype produced a 200 bp fragment while no amplicons were yielded in non-RPQ genotypes (Fig. 3). This dominant marker available for selection at the Sec-1 locus, allows the discrimination of alleles containing RPQ versus non-RPQ.

Fig. 2. Multiple sequence alignment by ClustalW2 program of 7 ω-secalin proteins containing the QQPQRPQQPF motif (bold and underlined). The main structural features of the ω-secalin (signal peptide, N-terminal region, repetitive region and C-terminal region) are indicated. Asterisks indicate sequence identity among all amino acid sequences.
3.4. The decamer RPQ peptide prevents gliadin-induced K562(S) cell agglutination

K562(S) is a gliadin-sensitive leukemia cell line, that undergoes a massive agglutination when exposed to gliadin preparations from cereals with toxic activity in CD (Silano and De Vincenzi, 1999). Although it is still not clear why these unrelated cell lines agglutinate when exposed to gliadin peptides, K562(S) agglutination is a powerful, economic and rapid tool to screen the toxicity of the gluten fractions and to study the protective effect of peptides (or other preparations) towards the gluten toxicity in CD. The decamer QQPQDAPQPF has been previously shown to control the gliadin-induced agglutination of K562(S) cells (De Vincenzi et al., 1997). To investigate whether the decamer QQPQDAPQPF was also effective in controlling gliadin-induced K562(S) agglutination, K562(S) cells were challenged with 2 mg/ml of PT-gl in presence or absence of 20 μg/ml pQQPQRPQPF or pTPO irrelevant peptide. The decamer QQPQDAPQPF, but not the control peptide, was able to prevent gliadin-induced K562(S) cell agglutination, whereas pTPO was devoid of any inhibitory effect on gliadin-induced K562(S) cell agglutination (Fig. 4).

3.5. pRPQ down-regulates the release of IFN-γ by celiac PBMCs in a bi-dimensional cellular model of duodenal mucosa

The in vitro pre-incubation of celiac duodenal mucosa with the toxic p31-43 enables immunodominant gliadin peptides, such as pz-9, to induce the activation of mucosal T-cells. The signature of celiac activated T-cells is the production of interferon-γ (IFN-γ) (Maiuri et al., 2003). In order to mimic the micro-environment of duodenal mucosa, a bi-dimensional cellular model was set up, consisting of intestinal T84 cells cultivated onto microporous inserts where PBMCs from celiac patients have been introduced in the basolateral compartment. As shown in Fig. 5, pre-treatment with both pDAV and pRPQ peptides is able to prevent the release of IFN-γ from PBMCs stimulated with p31-43 and pz-9. This finding proves that pRPQ is able to control the downstream events leading to the Th1-mediated inflammation in celiac mucosa upon exposure to gliadin, including the very precocious events mediated by p31-43.

3.6. The pRPQ controls the gliadin-induced mucosal response in celiac duodenum

The pDAV has been previously shown to reduce T-cell activation in celiac patients (Silano et al., 2008). Therefore, we tested whether the pRPQ was also effective in controlling gliadin-induced immune response in celiac intestine. Celiac biopsies were challenged with 500 mg/ml of a PT-gl in the presence or absence of 10 mg/ml pRPQ or pTPO irrelevant peptide. The pRPQ, but not the control peptide, was able to control gliadin-induced increase of CD25+ cells within the lamina propria (Fig. 6A) as well as the expression of ICAM1 (Fig. 6B) and HLA-DR (Fig. 6C), markers already used in celiac duodenum to assess gliadin-induced mucosal activation. Control peptide was devoid of any inhibitory effect on gliadin or p31-43 induced activation (data not shown). Peptide RPQ was devoid of any stimulatory effects on celiac and control duodenum.

4. Discussion

CD is a frequently occurring food intolerance causing inflammation in the small intestine and a range of related symptoms after ingestion of gluten proteins from wheat and homologous proteins from rye and barley. While gluten-free food products are available on the market, there is considerable interest in the development of non-dietary therapies that improve the health and quality of life of celiac sprue patients (Blagi et al., 1999; Schuppan et al., 2009). For instance, a linear copolymer of hydroxyethylmethacrylate and sodium 4-styrene sulfonate prevented in vitro gliadin-induced epithelial toxicity and intestinal-barrier dysfunction in HCD4/Dk8 mice (Xia et al., 2006).

Natural variants of gluten peptides, mainly resulting from point mutations, are present in the wheat germplasm and some of them can have an impact on the T-cell stimulatory activity (Spaenij-Dekking et al., 2005). The accumulation of these mutation events has resulted in a large number of CD and CD-like epitopes where a few amino acid differences significantly alter the T-cell stimulatory properties of the glutenin epitopes. Key amino acids substitution can either completely or partially abrogate the T-cell stimulatory activity of the gluten peptides (Vader et al., 2003). For instance, mutations determining single amino acid substitution in amino acids flanking the target Q residues (i.e. the replacement of a proline residue) are sufficient to prevent CD peptides from stimulating the T-cells (Vader et al., 2003).

In this study, a gluten peptide capable of inhibiting the pathogenic immune response was identified in a α-secalin gene isolated from a bread wheat cultivar. During wheat breeding, a number of useful traits (i.e. disease resistance) were introgressed from related species. The wheat–rye chromosomal translocations 1BL.1RS and 1AL.1RS were used to introgress in wheat cultivars several genes conferring resistance to diseases and other yield related-traits (Mater et al., 2004; Weng et al., 2007). Hundreds of commercial wheat cultivars carrying either 1AL.1RS or 1BL.1RS wheat–rye translocation have been developed (Rabinovich, 1998). Despite the huge number of wheat genotypes carrying 1RS from rye, most wheat cultivars with rye translocations derive from few original translocation events. The original 1BL.1RS translocation event was selected in Germany from crosses between several wheat genotypes and the rye cultivar Petkus carrying a resistance to fungal diseases. From these progenies, two widely used Russian cultivars (Kavkaz and Aurora) were selected. Besides the target traits, the wheat–rye translocations also introgressed a number of other linked loci, all located on the short arm of the chromosome 1 of rye, among them, the Sec-1 locus encoding the α-secalins (Clarke et al., 1996) and, along with this locus, the protective decapptide QQPQDAPQPF.

The studies reported above demonstrate that the decapptide QQPQDAPQPF is capable of interfering with K562(S) cell agglutination and intestinal mucosa immune activation induced by gliadin peptides exposure in the same manner as reported for the QQPQDAPQPF (De Vincenzi et al., 1997). Despite the partially different amino acid composition, these data suggest that the tripeptide, either RPQ or DAV, plays a relevant role in the biological activity of these peptides.

CD is the results of a sequential cascade of events involving both the innate and adaptive branches of the immune response to gluten. The activation of an early innate response to gliadin peptides has been identified as central to CD pathogenesis (Maiuri et al., 2003). Small non-T-cell stimulatory peptides, as the
α-gliadin-derived p31-43, are able to induce early epithelial and dendritic cell activation in celiac but not control duodenum and set the tone for the mucosal response to the immunodominant gliadin peptides. Such a priming effect of the “innate” peptides is required for the mucosal T-cell response in celiac patients (Maiuri et al., 2003). It has been recently reported that the gliadin p31-43 accumulates within the lysosomes leading to increased Reactive Oxygen Species (ROS) generation that in turn activate the tissue TG2 in gliadin-sensitive intestinal epithelial cells and celiac duodenum (Luciani et al., 2010). This further indicates that a deregulation of the cellular homeostasis is rapidly induced by gliadin after ingestion. The identification of peptides with putative protective activity against the early mucosal response to gliadin may open a new scenario in CD. Notably, pRPQ is able to control the precocious inflammatory events in celiac mucosa upon exposure to p31-43. This finding could open the way for a new therapy of CD, alternative to gluten-free diet, since pRPQ blocks the initial events of the downstream mucosal inflammation pathogenetic of CD.

A search in the gluten database has found only five sequences, out of 2091, carrying a peptide sharing some homologies with the protective decapeptide described by Silano et al. (2008) suggesting that these are rare events probably deriving after a 9-bases insertion into a more common QQPQQPF motif (present in 90 sequences listed in the gluten database).
When diploid, tetraploid and hexaploid *Triticum* accessions were tested for the presence of T-cell stimulatory epitopes in gliadins and glutenins, different sets of CD epitopes were identified and some ancient wheat and emmer genotypes have been found to possess a lower CD-toxicity compared to modern wheat cultivars (Molberg et al., 2005; Spaenij-Dekking et al., 2005; Vincentini et al., 2009). The identification of the gene encoding the protective QQPQRQQPF peptide might contribute to explain a component of this large genetic diversity in the amount of T-cell stimulatory and/or toxic peptides present in the wheat germplasm and might help to breed genotypes with low CD-toxicity. Although further studies are required to assess whether the protective peptide also exerts its activity following the digestion process, an immunomodulatory gliadin sequence naturally occurring in a cultivar of cereal toxic for

![Diagram showing the expression of ICAM1 and HLA-DR](image-url)
celiac patients, may represent a new challenge for the development of innovative strategies to dampen gluten toxicity in celiac patients.

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