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Research Article

Two wheat decapeptides prevent gliadin-dependent maturation of human dendritic cells

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ABSTRACT

Celiac disease (CD) is a small intestinal enteropathy, triggered in susceptible individuals by the ingestion of dietary gluten.

Dendritic cells (DC) are instrumental in the generation and regulation of immune responses and oversee intestinal immune homeostasis promoting and maintaining oral tolerance to food antigens.

The aim of this study was to monitor the effect of peptic-tryptic digest of gliadin (PT-gliadin) on the maturation of human monocyte-derived DC and the impact of pDAV and pRPQ decapeptides in the modulation of PT-gliadin-induced phenotypic and functional DC maturation.

Immature DC (iDC) were challenged in vitro with PT-gliadin. In some experiments iDC were pre-treated with pDAV or pRPQ and after 2 h PT-gliadin was added to the cultures.

We found that PT-gliadin up-regulates the expression of the maturation markers HLA-DR, CD83, CD80 and CD86. The functional consequence of PT-gliadin treatment of iDC is a significant increase in IL-12, TNF-alpha production as well as in their T cell stimulatory capacity. On the contrary, the digest of zein had no effect on DC maturation. Interestingly, we found that pre-treatment of iDC with pDAV or pRPQ decapeptides significantly prevents the functional maturation of DC induced by PT-gliadin. On the other hand, pDAV and pRPQ did not revert the PT-gliadin-induced phenotypic maturation of DC.

Here we report, for the first time, that naturally occurring peptides are able to prevent the gliadin-dependent DC maturation. This finding could have implication for CD, raising the perspective of a potential therapeutic strategy alternative to a gluten free diet.

Abbreviations: CD, celiac disease; DC, dendritic cells; iDC, immature dendritic cells; PT-gliadin, peptic-tryptic digest of gliadin; HLA, PBMC, peripheral blood mononuclear cells. human leukocyte antigen; IL, interleukin; APC, antigen presenting cells; LPS, lipopolysaccharide; mAbs, monoclonal antibodies; MLR, mixed lymphocyte reaction

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Introduction

Celiac disease (CD) is a life-long autoimmune disorder of the small intestine triggered by dietary gluten, the alcohol-soluble protein fraction of wheat, rye and barley. CD is strongly associated with HLA-DQ2 and/or HLA-DQ8, as both genotypes predispose for the disease development [1].

Gluten is a complex mixture of gliadin monomers and polymeric glutenins that are highly resistant to the gastrointestinal enzymatic proteolysis, and this results in the presence of large and potentially immunogenic peptides at the intestinal mucosa surface. Gliadin peptides, after crossing the epithelium into the lamina propria, undergo deamidation by tissue transglutaminase. Once deamidated, the gliadin peptides acquire the negative charges to bind efficiently to the HLA-DQ2 or HLA-DQ8 molecules on the surface of the APC and be presented to $CD4^+$ T lymphocytes. Activated gliadin-specific T cells secrete high levels of pro-inflammatory cytokines which, in turn, stimulate natural killer cells and $CD8^+$ intraepithelial T lymphocytes in intestinal mucosa [2,3] and induce enterocytes apoptosis, leading to the development of the typical celiac mucosal lesion: villous atrophy, crypt hyperplasia and lymphocytes infiltration [4–6].

Dendritic cells (DC), the most potent antigen presenting cells (APC), are well equipped for activation of both the innate and adaptive immune response and are in charge of the balance between tolerance and active immunity in the intestine [7]. Their regulatory properties depend on the signals they receive from the gut environment [8]. DC priming ability is acquired upon maturation and is characterized by the activation of different transcriptional factors, leading to the modulation of genes involved in cytoskeleton rearrangement, antigen processing, control of migration and regulation of inflammatory responses [9].

DC are critical for the development of CD-specific immune responses and are present throughout the intestine, including the lamina propria of the small and large intestine, the isolated lymphoid follicles, the Peyer patches and the mesenteric lymph nodes [10].

Currently, the only available therapy for CD is a life-long gluten-free diet, which has been proven to clear the symptoms and prevent the CD potential complications [11,12]. However, the costly and restrictive aspect of complying with a life-long gluten-free regimen may have a significant adverse impact upon the quality of life of the patients [13,14]. Therefore, the development of novel treatment options that allow CD patients to consume gluten is needed. We have previously reported that the decapeptide QQPQDAVQPF (pDAV), naturally occurring in durum wheat, exerts in vitro an antagonistic effect against gliadin toxicity preventing celiac peripheral blood lymphocytes from activation by gliadin peptides [15]. More recently, we identified the decapeptide QQPQRPQQPF (pRPQ), homologous to pDAV, that is capable to prevent the activation of innate immunity in small bowel mucosa specimens challenged in vitro with gluten [16]. This study was set up to monitor the effect of the peptic-tryptic digest of gliadin (PT-gliadin) on human monocyte-derived DC phenotypical and functional developmental program and the impact of two wheat decapeptides pDAV and pRPQ in the modulation of PT-gliadin-induced phenotypic and functional DC maturation.

Materials and methods

Gliadin extraction and digestion and peptides

The alcohol-soluble protein fraction from whole cereal flour of bread wheat (*Triticum aestivum,* variety S. Pastore) was extracted with ethanol 70% and subjected to peptic-tryptic digestion (PT), as previously described [17] and then lyophilized. In all experiments PT-gliadin was added at the beginning of the culture period and left throughout at the concentration of 500 μg/ml. This dose was chosen since it gave the optimal stimulation. In addition, we boiled for 10 min the gliadin preparations before the incubation with the DC to inactivate pepsin and trypsin and to mimic the cooking of dietary gliadin.

Decapeptides QQPQRPQQPF (pRPQ) and its homologous QQPQDAVQPF (pDAV), and from human thyroid peroxidase (hTPO) (LDPLIRGILLARPAKLQV) peptide were synthesized by Inbios (Napoli, Italy) by HPLC (431C model; Applied Biosystems, Foster City, CA) and 99% purified with reverse phase HPLC (5020 system; Varian Inc, Walnut Creek, CA).

The decapeptides pDAV or pRPQ and negative control hTPO were used at a concentration of 100 μg/ml. PT digest of zein, the prolamin of maize, a cereal not toxic for CD patients, was obtained in the same way and used at 500 μg/ml as negative control. The PT-gliadin, PT-zein, hTPO, and the decapeptides pDAV and pRPQ were monitored for endotoxin contamination using the Pyrotell Limulus amebocyte Lysate assay (Cape Cod Inc. Falmouth MA. USA). Endotoxin levels were <0.03 endotoxin units/ml.

Cell culture and treatments

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (Flow Laboratories, Hornby, Ontario) gradient separation of buffy coats obtained from healthy volunteer blood donors by the Transfusion Center of Università degli Studi "La Sapienza" Rome. DC were generated from monocytes purified from PBMC by positive selection using magnetic cell separation columns and CD14 Microbeads (Milteny Biotec, Bergisch Gladbach, Germany). Highly enriched monocytes ($>95\%$ $CD14^+$) were cultured at 6×10^5/ml in RPMI 1640 medium supplemented with 15% heat-inactivated foetal calf serum, L-glutamine and penicillin-streptomycin and 250 ng/ml granulocyte macrophage-colony stimulating factor (GM-CSF) (PeproTech, London, England) and 500 U/ml interleukin (IL)-4 (R&D System) at 37 °C for 5 days. Differentiation to DC was assessed both by morphologic observation and the detection of specific surface markers by flow cytometry. These cells were $CD14^-$, $CD1a^+$, $HLA\text{-}DR^{intermediate}$, $HLA\text{-}ABC^{intermediate}$, $CD80^{low}$, $CD86^{low}$ consistent with an iDC phenotype. Untreated iDC were used as controls. After 5 days of culture PT-gliadin and/or 200 ng/ml lipopolysaccharide (LPS) (*Escherichia coli* serotype 0111:B4, Sigma, St Louis, MO) were added to iDC. LPS-treated DC were $CD83^+$, $HLA\text{-}DR^{high}$, $HLA\text{-}ABC^{high}$ consistent with a mature DC phenotype. The purity of the monocytes was verified as $>90\%$ by direct staining for membrane expression of CD14 (anti-CD14, monoclonal antibody (mAb) were purchased from Pharmingen, San Diego, CA).