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Tailoring the immune response to wheat gliadin by enzymatic transamidation

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ABSTRACT

Enzymatic transamidation of wheat gliadin by microbial transglutaminase inhibits IFN-γ secretion by intestinal T cell lines from celiac disease (CD) patients. Here, we analysed its effects on intestinal biopsies from CD patients and studied the underlying mechanisms in HLA-DQ8 transgenic (tg) mice, a model of T-cell mediated gluten sensitivity. *In vitro* challenge with a soluble form of transamidated gliadin (spf) upregulated IL-10 transcript levels in human biopsy samples. Furthermore, the ratio of IL-10/IFN-γ transcripts was significantly increased following treatment with spf. In DQ8 tg mice, recall responses *in vitro* in the presence of dendritic cells pulsed with transamidated gliadin showed that gliadin-specific $CD4^+$ T cells did not produce IFN-γ at any tested dose. On the contrary, spf-specific $CD4^+$ T cells still secreted IFN-γ, but they also produced significant levels of IL-10 with both native and transamidated gliadin. Interestingly, this anti-inflammatory activity was restricted to a specific reverse-phase high-pressure liquid chromatography (RP-HPLC) fraction encompassing α-gliadins. These findings suggested an ability of transamidated gliadin to revert, as well as to prevent, the inflammatory phenotype triggered by native gliadin. This property was intrinsically associated with specific components of the α-gliadin fraction.

1. Introduction

Celiac disease (CD) is the most common food-sensitive enteropathy in humans. This condition is triggered by the absence of oral tolerance to wheat gluten and related proteins of rye and barley. Almost all CD patients express the major histocompatibility complex (MHC) class II molecules HLA-DQ2.5, HLA-DQ8, or HLA-DQ2.2. In particular, HLA-DQ2.5 represents the major genetic risk factor [1]. This genetic link highlights the central role of adaptive immunity in the pathogenesis of CD. In detail, gluten peptides bind to DQ2 and DQ8 heterodimers on the surface of antigen-presenting cells (APCs) in the lamina propria (LP) and trigger $CD4^+$ T cell activation [2]. Furthermore, efficiency in the presentation of gluten peptides influenced the risk of disease development. HLA-DQ2.5 homozygotes had a higher risk of developing CD than heterozygotes [3]. Also, the number of HLA-DQ2.5 heterodimers that are expressed on the surface of the APCs influenced the amplitude of the inflammatory response. HLA-DQ2.5 homozygotes had a stronger proliferative T cell response and a higher IFN-γ expression in comparison with heterozygous individuals [4]. Both gluten components, gliadin and glutenin, had a high content of glutamine and proline residues that played a crucial role in CD pathogenesis. Most of the epitopes were identified after deamidation catalysed by tissue transglutaminase (tTG), which converted specific glutamine residues to glutamic acid [5]. The negative charges introduced by the tTG activity significantly increased the binding affinity of gluten peptides to DQ2 [6–8] and DQ8 [9] molecules. Proline residues protected gliadin and glutenin epitopes against digestive proteolysis and directed the tTG-mediated deamidation [10]. So far, a relatively large number of deamidated peptides have been involved in the development of CD [11]. On the other hand, different studies showed that the number of regulatory $Foxp3^+$ T cells (Tregs) was increased in the LP of individuals with active CD [12,13]. This means that a defect in the recruitment or in the *de novo* generation of

Abbreviations: APCs, antigen presenting cells; CD, celiac disease; DCs, dendritic cells; mTG, microbial transglutaminase; rCD, CD patients in remission; RP-HPLC, reverse phase high-pressure liquid chromatography; spf, soluble form of transamidated gliadin; tg, transgenic; tTG, tissue transglutaminase

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intestinal Tregs was not implicated in CD pathogenesis. Interestingly, exogenous IL-10 could suppress the gliadin-specific IFN-γ expression *in vitro* in intestinal biopsies from treated CD patients [14]. Thus, these data underscored gluten-specific $CD4^+$ T cells as the main target for approaches aiming to recover gluten tolerance. In this sense, a promising modulatory methodology was represented by blocking the peptide-binding site of DQ2/8 heterodimers. So far, many distinct dominant T-cell epitopes have been described for gliadin [11]. Among these, a deamidated α-gliadin peptide (p57–73 QE65) has been identified, containing two nine amino acid sequences (DQ2-α-I: PFPQPELPY; and DQ2-α-II: PQPELPYPQ) recognized by intestinal T cell clones [6].

Altered versions of p57-73 QE65 peptide, lacking IFN-γ stimulatory activity and retaining most of the IL-10 stimulatory activity of the original peptide, were produced by Anderson et al. [15] to address this issue. Furthermore, cyclic and dimeric peptide analogs of the immunodominant 33-mer peptide, containing a cluster of DQ2.5 restricted α-gliadin epitopes [16], efficiently inhibited antigen presentation in a dose-dependent manner [17]. Nevertheless, treatments should also take into account the heterogeneity of gluten epitopes recognized by reactive T cells in CD [11]. We previously showed that treatment of wheat gliadin in the presence of microbial transglutaminase (mTG) and amine group donors inhibited IFN-γ expression in all intestinal T cell lines of CD patients [18]. This finding indicated that transamidation was effective in neutralizing the inflammatory activity of a large repertoire of epitopes. Furthermore, in a murine model we found that the IL-10/IFN-γ ratio was significantly higher for spleen cells treated with transamidated gliadin compared with cells stimulated with native gliadin [19]. Most importantly, a clinical study highlighted a reduction in the T cell response, as well as an improvement of mucosal lesions in CD individuals who ingested rusks produced with transamidated wheat flour as compared to those who ate rusks produced with unmodified flour [20]. In the present study, we further analysed the mechanisms underlying the immune properties of transamidated gliadin. Our data indicated that this manipulated molecule was effective for inducing an anti-inflammatory response toward wheat gliadin.

2. Materials and methods

2.1. Antigens

Gliadin was extracted from commercial wheat flour purchased from a local market in Avellino, Italy, using a modified Osborne procedure [21]. A soluble form of transamidated gliadin (spf) was produced by the same commercial wheat flour as described in Mazzeo et al. [22] by using microbial transglutaminase (mTG; ACTIVA®WM, specific activity: 81–135 U/g, Ajinomoto Foods Hamburg, Germany). Preparation of peptic-tryptic digests (pt) of gliadin and spf for organ culture studies was performed as previously described [19]. In brief, gliadin or spf (0.1 mg/mL) were suspended in 0.2 N HCl adjusted to pH 1.8. Pepsin (2 mg/mL; Sigma Aldrich srl, Milan, Italy, specific activity: 3260 U/mg) was added and the solutions were stirred for 4 h. Then, the pH was adjusted to 7.4, trypsin (Sigma, 2 mg/mL; specific activity: 10,600 U/mg) was added and the solutions were incubated for an additional 4 h at 37 °C. Solutions were then boiled (10 min) and freeze-dried. Protein content was determined by Bradford analysis [23] and protein pattern was qualitatively analysed by 8–16% SDS-PAGE and Coomassie R-250 blue staining.

2.2. Patients and organ culture analysis

Seven treated $DQ2^+$ CD patients (age range 19–47 years, median age 36 years) were enrolled in this study (Table 1) by the Gastroenterology Department of S.G. Moscati Hospital, Avellino, Italy. All patients had been diagnosed according with the British Society of Gastroenterology diagnostic guidelines [24]. All patients were on a gluten free diet for at least 18 months and found negative for anti-tTG and EMA serum antibodies for at least 1 year. Patients with inflammatory bowel disease, tumors and liver diseases were excluded. This study was registered and approved by the Human Research Ethics Committee of Campania Nord, Italy (CECN/314/2015) and all procedures performed in accordance with the ethical standards described in the World Medical Association's Helsinki Declaration. All individual participants included in the study signed an informed consent letter with detailed descriptions of the aims and procedures of the study. Biopsies from the distal duodenum were obtained during an upper GI endoscopy. Two specimens were used for histology, and the other specimens were cultured for 8 h with pt-gliadin/pt-spf (1 mg/mL; w/v) [19] or medium alone and finally stored in liquid nitrogen for RNA analysis. Histology was conducted adopting a modified Oberhuber-Marsh classification of the intestinal mucosal lesion [25].

Table 1
Demography, morphology and gliadin/spf-specific IFN-γ mRNAs of treated CD patients (rCDs).

Patients	Age	Gender	Marsh degree[a]	IFN-γ mRNA (AU)[b]	
				gliadin	spf
rCD1	19	F	1	1.6 ± 0.3	1.0 ± 0.3
rCD2	47	F	0	1.7 ± 0.2	2.0 ± 0.4
rCD3	32	F	1	1.5 ± 0.2	1.9 ± 0.4
rCD4	38	M	0	1.0 ± 0.1	1.0 ± 0.1
rCD5	22	F	0	5.9 ± 0.2	4.8 ± 0.2*
rCD6	42	F	1	11.8 ± 0.3	16.7 ± 0.2*
rCD7	36	F	0	1.4 ± 0.1	1.5 ± 0.2
rCD8	33	M	0	39.4 ± 0.2	27.8 ± 0.3*

[a] Histology assessment was performed using a modified Oberhuber-Marsh classification of the intestinal mucosal lesion (34): Type 0: normal mucosa; Type 1, normal mucosa but increased number of intraepithelial lymphocytes; Type 2, mild atrophy; Type 3, atrophy.
[b] IFN-γ mRNA levels following challenge with pt-gliadin or pt-spf; mean ± SD. *: $P < 0.05$.

2.3. Mice and antigen treatments

AB^0DQ8 transgenic mice expressing the HLA-DQ8 molecule [26] were reared on a gluten free diet (Altromin-MT-mod, Rieper SpA, Bolzano, Italy) at our animal facility (accreditation n. 164/99-A). All procedures met the guidelines of the Directive 2010/63/EU of the European Commission and were approved by the Animal Care and Use Committee of Italian Ministry of Health (AUP #285/2016-PR). For parenteral immunization, 6- to 12-week-old mice were primed by intraperitoneal injection with gliadin or spf (300 μg in weight) emulsified in Freund's complete adjuvant (Sigma Aldrich srl, Milan, Italy) (day 0). Booster injections with antigen in incomplete Freund's adjuvant were performed on days 7 and 14. Mice were sacrificed on day 21 to recover their spleens.

2.4. Reverse-phase high-pressure liquid chromatography

Reverse-phase high-pressure liquid chromatography (RP-HPLC) of gliadin or spf sub-fractions was done in a SMART system (Amersham Pharmacia Biotech-GE Life Sciences). Proteins were separated using a μRPC C2/C18 PC 3.2/3 column (Amersham Pharmacia Biotech). The column was eluted with a linear gradient of 30–80% acetonitrile-0.1% trifluoroacetic acid at a flow rate of 0.1 mL/min. Elution was monitored at 220 nm.

2.5. In vitro culture of murine $CD4^+$ T cells

Spleens were disaggregated through a stainless steel wire mesh to dissociate the cells. Erythrocytes were removed by incubating the cell

suspensions with a Tris-buffered ammonium chloride solution. $CD4^+$ T cells were purified by negative magnetic cell sorting (Miltenyi Biotec, Gladbach, Germany). Dendritic cells (DCs) were differentiated *in vitro* from bone marrow cells of naive DQ8 transgenic (tg) mice as previously described [27]. On day 8, non-adherent DCs were harvested by gentle pipetting and placed in 48-well plates (2.5×10^5 cells/well). They were incubated in culture medium (RPMI 1640 containing 10% inactivated foetal calf serum (FCS), 100 U/mL penicillin, 100 µg/mL streptomycin, 1% non-essential amino acids, 2 mM glutamine, and 50 µM 2-mercaptoethanol) with gliadin, spf or their fractions for 18 h. Finally, they were treated with 1 µg/mL lipopolysaccharide (LPS) for 4 h to induce the maturation of DCs [28]. $CD4^+$ T cells (5×10^5) were added to different doses of DCs for 72 h. Supernatants were collected to quantitate IFN-γ and IL-10 secretions by ELISA.

2.6. Cytokine analysis

Total RNA was extracted from intestinal biopsies using TRIzol reagent (Invitrogen) and quantitated as previously described [19]. cDNA was prepared from 1 µg of total RNA by reverse transcription at 42 °C for 60 min [19]. Real-time PCR was then performed on the iCycler iQTM Real-Time Detection System (Bio-Rad Laboratories Inc, Hercules, CA). The amplification mixture contained iQTM SYBR Green Supermix (Bio-Rad), 0.2 µM of each primer and cDNA. The following primer sequences were used for IL-10: forward 5′-CAGTACAGCCGGGAAGACAA TAAC-3′, reverse 5′- AATCACTCTTCACCTGCTCCACTG-3′. The primer sequences for the housekeeping gene L-32 and for IFN-γ have been previously described [19]. The PCR reaction conditions were: 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s (39 cycles). Gene transcription levels were computed using the ΔΔCt method [29] and expressed as absolute units (AU) after normalization to the L-32 housekeeping gene. For cytokine protein assays of murine $CD4^+$ T cells, the culture supernatants were collected after 72 h and analysed for secretion of IFN-γ and IL-10 by in-house sandwich ELISA. The following clones were used as capture and detection antibodies: R4-6A2 and XMG1.2 (IFN-γ), JES5-2A5 and JES5-16E3 (IL-10) (BioLegend, San Diego, CA).

2.7. Statistical analysis

mRNA levels in intestinal biopsies were evaluated by Student's *t*-test or by Wilcoxon signed rank test. In mice, differences in the protein levels of cytokine were determined by the Wilcoxon signed rank test. The dose-response effects were evaluated by one-way analysis of variance (ANOVA) and Tukey test post-hoc analysis. For all tests, $P < 0.05$ was assumed to denote a statistically significant difference.

3. Results

3.1. Ex vivo analysis of transamidated gliadin in CD patients

We previously showed that gliadin challenge enhanced the IFN-γ secretion *in vitro* in some intestinal biopsy samples obtained from CD patients with active disease [19]. In this model, tTG-mediated deamidation is believed to occur *in situ*, thus activating gliadin peptides [30]. Herein, we confirmed that pt-gliadin upregulated IFN-γ transcription in 5 out of 10 samples (CD2, CD3, CD7, CD8, CD10 arbitrary units, AU ≥ 2) and that the peptic-tryptic digest of a soluble form of transamidated gliadin (pt-spf), significantly downregulated the IFN-γ response in two samples (CD2, CD8) [19] (data not shown). Then, we extended the analysis of the immunomodulatory ability of spf in biopsy cultures from CD patients in remission (rCD). Their demographic data and mucosal status were reported in Table 1. We found that, after an 8-h *in vitro* challenge with pt-gliadin, three samples increased their IFN-γ mRNAs levels (rCD5, rCD6 and rCD8; Table 1). Similarly to CD samples, pt-spf significantly downregulated the IFN-γ response in two of them (rCD5 and rCD8; Table 1), but not in rCD6 that resulted upregulated. It has been reported that both the pro-inflammatory IFN-γ and the anti-inflammatory IL-10 mRNAs were induced in CD biopsies following challenge with pt-gliadin [14]. Therefore, we analysed IL-10 transcript levels in all 18 challenged biopsy samples (10 CD and 8 rCD samples). Interestingly, pt-spf challenge significantly upregulated the IL-10 mRNA expression in comparison with samples incubated with pt-gliadin (Fig. 1). In order to better define the immunomodulatory activity of spf, we measured the ratio of IL-10/IFN-γ transcripts in all challenged biopsies. Thus, we found that treatment with transamidated gliadin significantly increased this ratio (Fig. 2).

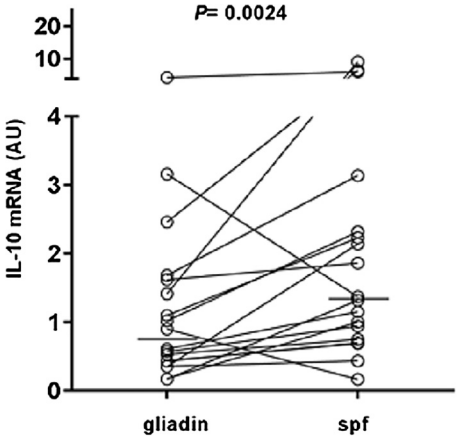

Fig. 1. IL-10 mRNA levels in intestinal biopsies from CD patients. Intestinal specimens from celiac patients with overt disease (n = 10) [19] and in remission (n = 8; Table 1) were subjected to an 8-h incubation with pt digests of native gliadin (gliadin) or spf and IL-10 transcript levels were evaluated by real-time PCR. Values were normalized to L-32 mRNA and were presented as the fold change in gene expression (arbitrary units). Dots represent the mean values from triplicate analyses. Bars represent medians.

3.2. Modulation of the $CD4^+$ T cell response in DQ8 tg mice

To dissect the mechanisms underlying the highlighted immunomodulatory activity of transamidated gliadin, we analysed the recall response of *in vitro* gliadin- and spf-specific splenic $CD4^+$ T cells in DQ8-tg mice. These mice only express a human leukocyte antigen (HLA) class II heterodimer that has been linked to CD [26]. Moreover, mice were from a colony bred on a gluten free diet; therefore, mice lacked a naturally induced oral tolerance. Following parenteral

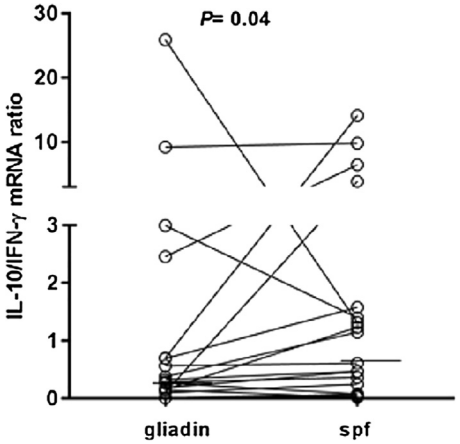

Fig. 2. IL-10/IFN-γ mRNA ratio in intestinal biopsies from CD patients Intestinal specimens from celiac patients with overt disease and in remission were analysed for IFN-γ and IL-10 mRNAs as reported in Materials and Methods. Each dot represents values from a single biopsy sample. Bars represent medians.

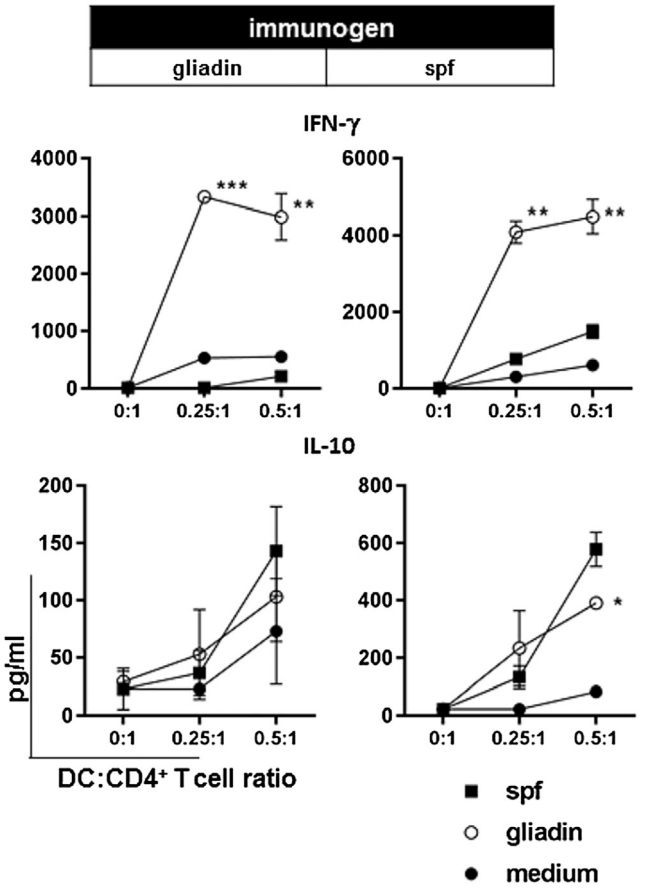

Fig. 3. Modulation of the adaptive immune response by transamidated gliadin. Cytokine production of splenic CD4$^+$ T cells from mice immunized with native gliadin or spf. CD4$^+$ T cells were added to two different doses of antigen-pulsed mature DCs for 72 h. Supernatants were analysed for IFN-γ and IL-10 protein levels by ELISA. Each dot represents mean values from three mice; each value was calculated as the difference between the means of triplicate cultures containing antigen and triplicate cultures with medium alone. The results are representative of three independent experiments. *, $P < 0.05$; **, $P < 0.01$.

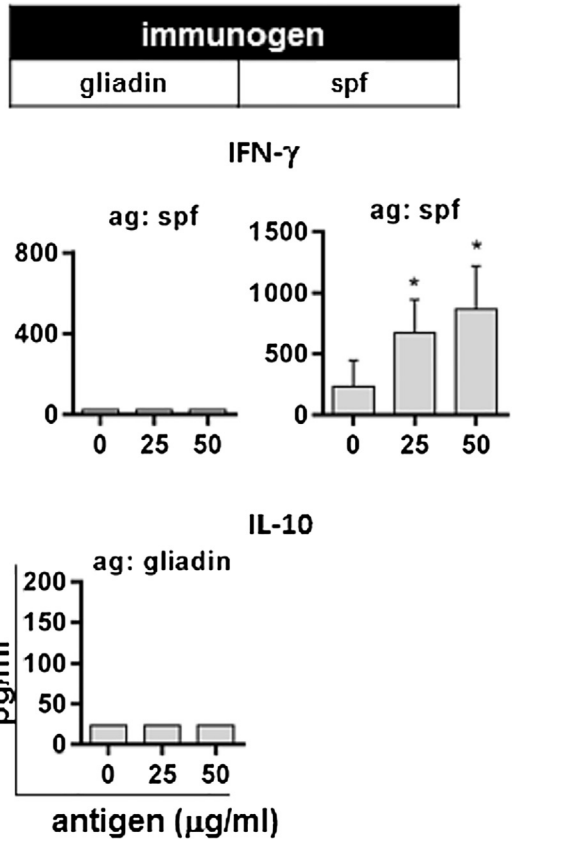

Fig. 4. Dose-response effect of transamidated gliadin. IFN-γ expression assessed after culturing gliadin- or spf-specific CD4$^+$ T cells with unpulsed or pulsed DCs at two different doses (25 and 50 µg/ml). Gliadin-specific CD4$^+$ T cells were similarly analysed for IL-10 expression. Columns represent mean ± SD (pg/mL) of triplicate cultures from a single mouse. The results are representative of three independent experiments. *, $P < 0.05$.

immunization with gliadin or spf, splenic CD4$^+$ T cells were recovered and stimulated *in vitro* with antigen-pulsed syngeneic DCs. The immune response was analysed by cytokine expression after a 72-h culture. Gliadin-pulsed DCs stimulated high levels of IFN-γ in gliadin-specific CD4$^+$ T cells (Fig. 3, top left panel), whereas IL-10 was not induced (Fig. 3, bottom left panel), as expected [31]. The latter result was found independent of the dose of antigen (Fig. 4, bottom panel). On the contrary, gliadin-specific CD4$^+$ T cells did not produce IFN-γ when challenged with spf-pulsed DCs (Fig. 3, top left panel). spf-specific CD4$^+$ T cells secreted IFN-γ in the presence of gliadin-pulsed DCs (Fig. 3, top right panel). Notably, they also produced significant levels of IL-10 following challenge with either antigen (Fig. 3, bottom right panel). The dose-response analysis of the systemic response confirmed the inability of transamidated gliadin to reproduce the IFN-γ levels induced by native gliadin. In particular, only spf-specific CD4$^+$ T cells showed a slight increase of the IFN-γ production by increasing the antigen dose (Fig. 4, top right panel). On the contrary, the suppressive effect of spf observed in gliadin-specific CD4$^+$ T cells was dose-independent (Fig. 4, top left panel).

3.3. The anti-inflammatory response of gliadin fractions

To associate the reported immunomodulatory properties to a specific protein component, spf constituents were separated by RP-HPLC. As shown in Fig. 5A (top panel), spf gave 3 main peaks (fractions 1–3). The electrophoretic analysis identified 4 protein bands of 40 kDa (fraction 1), 38 kDa (fraction 2), 48 kDa, and 42 kDa (fraction 3) (Fig. 5A, bottom panel). The immunological assessment of separated spf fractions showed that only fraction 3 partially retained the ability to stimulate the production of IL-10 in spf-specific CD4$^+$ T cells (Fig. 5B). The RP-HPLC elution profile of native gliadin classically identified 7 majority peaks belonging to ω- (1), α- (2 through 6), and γ-gliadins (7) (Fig. 6A) [32]. As expected, gliadin-specific CD4$^+$ T cells only produced IFN-γ when stimulated with different fractions (Fig. 6B). On the contrary, spf-specific CD4$^+$ T cells still secreted significant IL-10 with various fractions. Notably, spf-specific CD4$^+$ T cells mainly increased the IL-10/IFN-γ ratio when stimulated with fraction 3, which included some α-gliadins (Fig. 6B).

4. Discussion

Our studies demonstrated the ability of transamidated gliadin (spf) to increase the ratio of IL-10/IFN-γ transcripts in challenged intestinal biopsies of CD patients as well as to induce an anti-inflammatory phenotype in a mouse model of CD4$^+$ T cell mediated gluten sensitivity. This immunomodulatory property was intrinsically associated to specific components of the α-gliadin fraction. We previously showed that *in vitro* challenge of gliadin-specific Th1 cell lines from intestinal biopsies of CD patients with transamidated gliadin inhibited IFN-γ expression [18]. More recently, abrogation of gliadin immunogenic properties by mTG was confirmed by analysing intestinal T cell clones from CD patients [33]. Structural analyses of celiacogenic epitopes showed that isopeptide bond formation involved the same glutamine

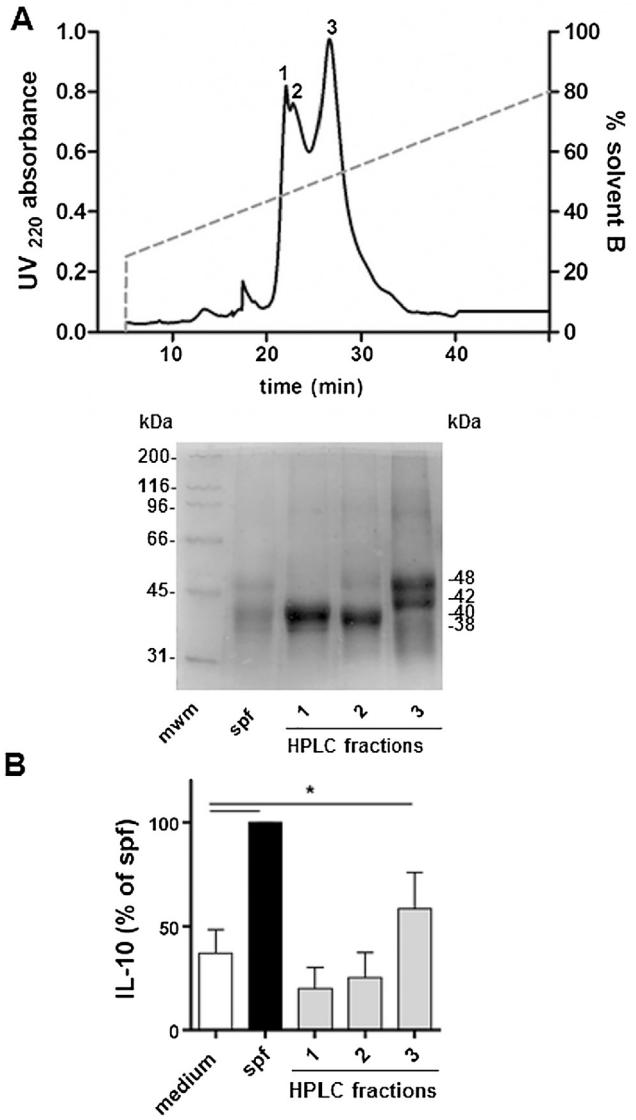

Fig. 5. Analysis of transamidated gliadin components. (A) RP-HPLC profile of purified spf showing three major peaks (upper panel). SDS-PAGE of purified fractions showing distinct Coomassie-stained bands (bottom panel). (B) IL-10 production of spf-specific $CD4^+$ T cells incubated in the presence of DCs pulsed with spf fractions. Values were expressed as % of IL-10 induced by spf. Columns represents mean values ± SD from three mice; each value was calculated as the difference between the means of triplicate cultures containing antigen and triplicate cultures with medium alone. The results are representative of three independent experiments. *, $P < 0.05$.

residues that are deamidation sites for tTG. Therefore, the transamidation reaction successfully prevented the formation of immunodominant gluten peptides [22]. Herein, we confirmed this activity in a more complex model, the organ culture of an intestinal biopsy from CD patients. Specifically, pt-spf challenge reduced IFN-γ mRNA levels in 4 out of 8 reactive CD (untreated and treated) samples. Notably, we found that IL-10 mRNA was significantly upregulated in biopsies treated with pt-spf in comparison to its expression in biopsy samples incubated with pt-gliadin. Consequently, pt-spf induced an anti-inflammatory effect (*i.e.* increased IL-10/IFN-γ ratio) in challenged biopsies. In the same *ex vivo* model, past studies showed that exogenous IL-10 could suppress the gliadin-driven IFN-γ response [14]. Also, gliadin-specific T cell lines generated from biopsy cultures following incubation with gliadin plus IL-10 did not secrete IFN-γ [14]. Together, these findings highlighted the usefulness of strategies that restore IL-10 secretion for downregulating the gliadin-specific Th1 response. To gain further insight about the anti-inflammatory activity of spf, we tested these molecules in DQ8 tg mice, a model of Th1-driven gluten sensitivity [31]. In agreement with previous findings [19], gliadin-specific $CD4^+$ T cells drastically reduced IFN-γ secretion after *in vitro* challenge with spf-pulsed DCs. This happened independently of the spf dose, confirming that transamidation influenced the binding of immunodominant gliadin epitopes to the HLA molecule [18]. Therefore, we evaluated whether transamidation might favor HLA binding of low-affinity epitopes, thus influencing the outcome of the adaptive immune response toward gliadin. To this aim, mice were immunized with spf. Isolated $CD4^+$ T cells produced lower levels of IFN-γ along with consistent secretion of IL-10 when stimulated *in vitro* with the cognate antigen. On the other hand, IFN-γ production partially recovered by increasing the dose of spf. Furthermore, challenge of these cells with native gliadin completely restored IFN-γ secretion, further confirming that transamidation reduced the number of epitopes activating Th1 $CD4^+$ T cells. Notably, spf sensitization also generated $CD4^+$ T cells that secreted IL-10 in response to gliadin. Taken together, these data revealed the ability of transamidated gliadin to produce a distinct T cell repertoire in DQ8 mice shifted toward a regulatory/anti-inflammatory phenotype. We previously showed that transamidation is not a random process, but it effectively targets distinct glutamine residues [22]. Moreover, quantitative RP-HPLC resolved spf in 4 discrete proteins. However, when isolated, spf components failed in stimulating comparable IL-10 secretion. This suggested that spf treatment generated different anti-inflammatory epitopes broadly distributed in the transamidated gliadin. Furthermore, *in vitro* challenge of spf-specific $CD4^+$ T cells with seven discrete HPLC fractions of native gliadin showed that only fraction 3 significantly increased the IL10/IFN-γ ratio. Fraction 3 encompasses various α-gliadins [32]. Therefore, modulatory epitopes could be restricted to a few α-gliadin molecules in native gliadin.

Traditional gluten-free products are devoid of gluten proteins that confer better baking properties to wheat based-products. Furthermore, an inappropriate gluten-free diet can cause nutritional deficiencies [34]. Therefore, transamidation of gluten proteins would be a suitable approach to overcome such limitations if definitively confirmed to be safe for CD patients. From this point of view, it has been shown that transamidated gluten can be used to produce wheat flour [35] and bread with less immunoreactive gluten [36]. More recently, we showed that wheat flour treatment based on the use of filtered *Streptomyces mobaraensis* mTG-active culture supernatant led to the acquisition of an immunomodulatory activity for gliadin [37]. Our new data suggested that further beneficial properties could be related to the enzymatically-treated gliadin.

Besides transamidation, different enzyme strategies have been explored for CD so far. Treatment of immunogenic gliadin peptides by prolyl endopeptidases rapidly decreased their T cell stimulatory activity, thus highlighting the potential of oral peptidase supplement therapy [16]. Interestingly, oral treatment with ALV003, a mixture of glutenases that causes gluten degradation in the gastrointestinal tract, attenuated small intestinal mucosal lesions in CD patients [38]. Furthermore, combination of prolyl endopeptidases and selected sourdough lactic acid bacteria were successfully tested in the gluten free food industry [39]. Together, these activities testify that the gluten modification strategy is a hot topic in the field nowadays.

In conclusion, our human and murine data pointed out that transamidated gliadin could target a different repertoire of T cells recognizing gliadin epitopes. The output was an increased gliadin-specific anti-inflammatory response. Accordingly, an innovative approach might be explored that is based on the use of transamidated gliadin for boosting oral tolerance to wheat gliadin.

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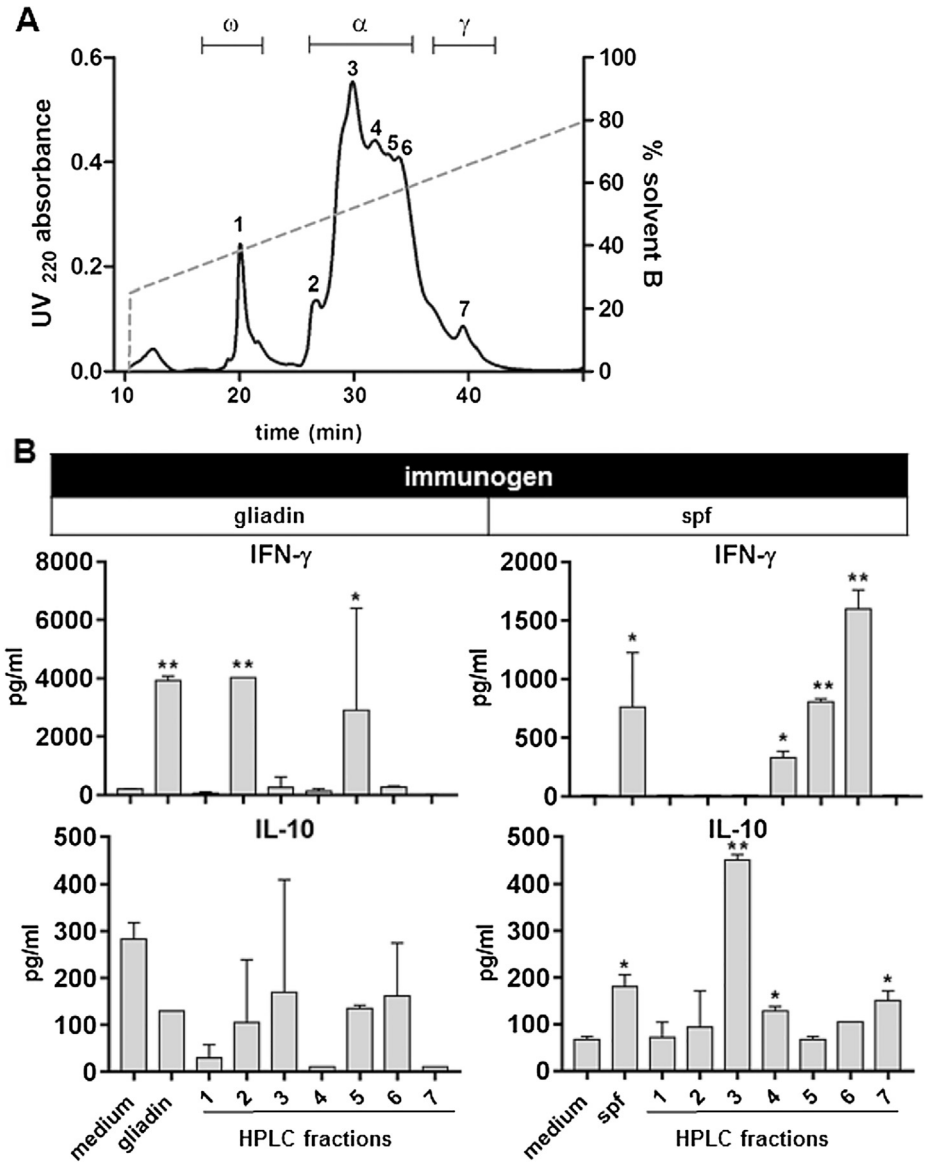

Fig. 6. Identification of the anti-inflammatory components in native gliadin. (A) RP-HPLC profile of gliadin showing seven major peaks. (B) IL-10 and IFN-γ levels of gliadin-specific or spf-specific CD4$^+$ T cells incubated in the presence of DCs pulsed with gliadin fractions. Columns represent mean ± SD from three mice. The results are representative of three independent experiments. *, $P < 0.05$; **, $P < 0.01$.

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Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this Article.

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