Cutting Edge: Selective Deamidation by Tissue Transglutaminase Strongly Enhances Gliadin-Specific T Cell Reactivity

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**References**

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Celiac disease (CD) is a common malabsorption syndrome that is precipitated upon exposure to the dietary wheat gluten or its active component gliadin (1). The occurrence of the disease is strongly associated with HLA-DQ2 (2, 3), while most DQ2-negative patients express the DQ8 molecule (4). Gliadin-specific DQ2- and DQ8-restricted T cells have been found at the site of the lesion in the gut (5, 23). Taken together, these findings suggest that the intestinal damage in CD is caused by aberrant mucosal T cell recognition of gliadin-derived peptides in the context of disease-associated DQ2 and DQ8 molecules.

Next to the T cell response against gliadin, IgA Abs against gliadin and endomysium are specific indicators of the disease (6, 7). Recently, tissue transglutaminase (tTG) has been identified as the autoantigen for antiendomysium Abs (8). tTG belongs to a family of calcium-dependent enzymes that catalyzes the cross-linking of proteins by introducing a covalent bond between lysine (K) and glutamine (Q) residues (9). Whereas several K-containing proteins can serve as acceptor substrates, only a limited number of Q-containing donor substrates exist. Since mucosal tTG activity is increased in CD patients (10), and since gliadin is a preferred substrate of the enzyme (10), it has been speculated that tTG might be important in the generation of gliadin-gliadin or gliadin-tTG complexes, giving rise to novel antigenic epitopes (8). The putative formation of gliadin-tTG complexes could explain the appearance of antiendomysium Abs upon gliadin exposure in vivo and in vitro (11) as has been suggested previously (12).

The association of CD with DQ2 and DQ8 is indicative of the preferential mucosal recognition of gliadin fragments bound to these DQ alleles (13). However, gliadin-derived peptides display only a low affinity for DQ molecules (14). The peptide-binding motifs of both DQ2 (15–17) and DQ8 (18, 19) indicate a preference for negatively charged residues at several positions of DQ-bound peptides. Interestingly, because of the large proportion of Q residues in gliadin (≤40%), the conversion of Q to E (glutamic acid) residues by deamidation would result in relatively large numbers of negatively charged E residues in gliadin. Since tTG is capable of the deamidation of Q residues (20), we have now examined whether tTG might increase the mucosal T cell response against a gliadin digest and a previously identified, DQ8-restricted, gliadin-derived epitope (23).

Materials and Methods

Ags and peptides

A pepsin/trypsin digest of gliadin (Fluka, Buchs, Switzerland) was prepared as described previously (23). Peptides were synthesized by standard 9-fluorenylmethoxycarbonyl chemistry on a multiple peptide synthesizer (Abimed AMS 422; Abimed, Langenfeld, Germany).

Isolation of gluten-specific T cells

The isolation of the gliadin-specific HLA-DQ8-restricted T cell clone (TCC) S2 from a small intestine biopsy of patient S (HLA-DQ2/8 heterozygous) has been described previously (23). Gluten-specific TCCs of a...
biopsy of patients P (HLA-DQ2/8 heterozygous) and Po (HLA-DQ2/6 heterozygous) were established in a similar fashion, with the exception that autologous PBMCs were used as APCs instead of IL-4/granulocyte-macrophage CSF-cultured monocytes. TCC S2, TCC P1, and TCC Po27 are all CD8+CD4- TCCs, not CD8+CD4+ TCRαβ+.

**tTG treatment**

The pepsin/trypsin-digested gliadin and the gliadin 202–219 peptide (at concentrations of 500 and 250 μg/ml, respectively) were incubated with 100 μg/ml of guinea pig tTG (T-5398; Sigma, St. Louis, MO) at 37°C for 2 h in PBS with 1 mM CaCl2 and subsequently used in T cell proliferation assays.

**T cell proliferation assays**

Proliferation assays were performed in duplicate or triplicate in 150 μl of culture medium in 96-well flat-bottom plates (Becton Dickinson, Lincoln Park, NJ) using 10^5 T cells that had been stimulated with 10^7 irradiated (3000 rad) HLA-DQ-matched PBMCs in the absence or presence of Ag at the indicated concentrations. The cultures were pulsed with 0.5 μCi of [3H]thymidine after 48 h and were harvested 18 h thereafter.

**Mass spectrometry**

Electrospray ionization mass spectrometry was performed using a hybrid quadrupole-time of flight (TOF) mass spectrometer, the Q-TOF (Micromass, Manchester, U.K.), as described previously (23). Briefly, precursors were selected with the quadrupole, and fragments were collected with high efficiency with the orthogonal TOF mass spectrometer. Argon was applied as the collision gas (pressure was 4 x 10^-3 mbar), and the collision voltage was ~30 V.

**Results**

Q208 → E and Q216 → E substitutions increase the antigenicity of the gliadin 206–217 peptide

Previously we have identified a peptide derived from gliadin that is specifically recognized by an HLA-DQ8-restricted TCC (TCC S2) that was isolated from the small bowel of a patient suffering from CD (23). The minimal core region of the T cell-stimulatory gliadin 198–232 fragment was defined as residues 206–217 (sequence SGQGSFOPSQQN of gda09; SwissProt accession number P18573). We and others (17, 21) have suggested that the conversion of Q to E at these positions in the peptide, including the Q residues at positions 212 and 219 (data not shown), corresponds to the conversion of Q to E at these positions in the peptide. To test this hypothesis, substitution analogues of the gliadin 206–217 epitope, in which individual Q residues were replaced by an E, were tested for their T cell-stimulatory capacity. The observed T cell responses against a concentration range of the gliadin preparation and the peptide demonstrated a clear increase in the antigenicity of the Ag due to the tTG treatment (Fig. 2). Similarly, tTG treatment of the gliadin digest enhanced the reactivity of a gliadin-specific DQ8-restricted TCC (TCC P1) and a gliadin-specific DQ2-restricted TCC (TCC Po27) that had been derived from other CD patients (Fig. 3B). The peptide specificity of these latter two TCCs is not known.

Selective deamidation of the gliadin 202–219 peptide by tTG

Next, we determined the number and location of potentially modified residues in the gliadin 202–219 peptide upon tTG treatment. For this purpose, the peptide was subjected to tandem mass spectral analyses. Such an analysis yields a peptide fragmentation pattern that contains information on the nature and order of the amino acids in the peptide. A comparison of the fragmentation patterns of the tTG-treated and untreated peptide revealed a shift of 1 Da at Q202/203, Q208, Q215, and Q216 (data not shown); the shift corresponded to the conversion of Q to E at these positions in the peptide. No significant shifts were observed at any other position in the peptide, including the Q residues at positions 212 and 219 (data not shown). The highest percentage of deamidation was found for Q202/203 and Q216 (~70% of these residues were converted into E), whereas the tTG treatment resulted in 50% deamidation of Q208 and 11% of Q215 (Fig. 3). Thus, tTG treatment selectively deamidates Q residues in the gliadin 202–219 peptide. In the core of the peptide, the most dramatic deamidation occurred at Q208 and Q216 (see Discussion).

**Discussion**

It has been well-established that CD is caused by gliadin ingestion in susceptible individuals. The presence of a T cell infiltrate at the
site of the lesion in the gut is characteristic of the disease. Whereas the T cell response is directed against gliadin, IgA Abs to endomysium correlate well with disease (6, 7). Recently, tTG was identified as the autoantigen that is recognized by these antiendomysium Abs (8). Although such Abs are not likely to play a direct role in the pathogenesis of CD (22), the increased tTG activity in the intestine. No tTG-specific T cell responses were found (data not shown). Values show the mean cpm (× 10^3) of duplicate cultures. T cell responses in the absence of gliadin were <150 cpm. The SD was <10%.

FIGURE 3. Selective deamidation of Q residues in gliadin peptide 202–219. The percentages of deamidation of the individual Q residues in the gliadin 202–219 peptide were calculated on the basis of the mass shifts observed in the fragmentations patterns as obtained by tandem mass spectrometry. An average overall mass shift of ∼2 Da was found for the tTG-treated peptide (data not shown). The mass profiles did not allow discrimination between the conversion of a Q to E at either position 202 or 203, so % deamidation at Q202/Q203 is shown collectively. The residues at positions 208 and 216 constitute the p1 and p9 anchors (see Discussion).
results support the hypothesis that the mucosal presentation of (modified) gliadin-derived peptides is the mechanism underlying the association of CD with DQ2 and DQ8 and suggest that enzymatic deamidation may amplify the disease process. Which other currently unknown gliadin-derived peptides are similarly modified remains to be determined and will to a large extent depend upon the sequence specificity of tTG, which will be the subject of future research.

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References