Immunogenetics (2019) 71:665–667
https://doi.org/10.1007/s00251-019-01138-5

SHORT COMMUNICATION

Two novel HLA-DQ2.5-restricted gluten T cell epitopes in the DQ2.5-glia-γ4 epitope family

Shuo-Wang Qiao [1,2] · Ludvig M. Sollid [1,2]

Received: 15 October 2019 / Accepted: 15 October 2019 / Published online: 1 November 2019

Abstract

Celiac disease is a chronic inflammatory condition of the small intestine caused by aberrant adaptive immune response to gluten protein from wheat and related cereal plants. Over 90% of celiac disease patients carry the HLA-DQ2.5 allotype and HLA-DQ2.5 presents gluten peptides to gluten-reactive CD4+ T cells in celiac disease patients. A large number of HLA-DQ2.5-restricted gluten T cell epitopes have been identified over the years. These epitopes are in general proline-rich and contain at least one glutamic acid residue that is generated from glutamine in the native gluten protein by deamidation. The deamidation is mediated by the enzyme transglutaminase 2 (TG2). It has been shown that the same T cell could recognize several different HLA-DQ2.5-restricted gluten T cell epitopes due to sequence similarities. In this paper, we demonstrate that three T cell clones derived from duodenal biopsies of different celiac disease patients are able to respond to at least five different gluten T cell epitopes within the DQ2.5-glia-γ4 epitope family, including two novel epitopes.

Keywords Celiac disease · T cell epitope · Gluten · T cell clone

The T cell clones were generated in vitro according to previously published protocols (Molberg et al. 2000). In short, duodenal biopsies were obtained from adult celiac disease patients undergoing routine gastroduodenoscopy, and all had given written consent. The project was approved by the regional ethics committee. The biopsy specimens were incubated with gluten antigen during the first 24 h. The gluten antigen was then removed and remained absent in subsequent T cell culture and expansions. T cell clones were generated by limited dilution and expanded free of specific antigen, with irradiated PBMC as feeder cells in the presence of PHA, IL-2 and IL-15. In T cell proliferation assays, irradiated EBV-transformed HLA-DQ2.5 homozygous cells were incubated overnight with various deamidated gluten peptides (Table 1). Chymotrypsin-digested gluten was used either as native protein, or after TG2-deamidation. T cell clones were added on the following day and ^{3}H-thymidine was added after another 48 h. The plates were harvested and incorporation of the radioactive ^{3}H-thymidine was quantified as counts per minute (CPM) 16 h later. Samples were tested in either duplicates or triplicates.

Three T cell clones derived from three different celiac disease patients all responded to the peptide WPQQQPFPQPQQPFCQPQPQR where the putative binding register to HLA-DQ2.5 is underlined, after deamidation with TG2 where the glutamine residue in bold is targeted. In addition to the clear proliferative response to this novel epitope, all three clones showed response to at least one other DQ2.5-glia-γ4 epitope (Sollid et al. 2012). Two of these clones responded to all three members of the DQ2.5-glia-γ4 epitope family (Fig. 1). Based on these results, we have named this new epitope DQ2.5-glia-γ4d with minimal sequence PQP**E**QPFCQ.

For all three clones, variable responses were observed to the TG2-treated peptide P1317, which was a 20-mer peptide containing overlapping copies of the DQ2.5-

✉ Shuo-Wang Qiao
s.w.qiao@medisin.uio.no

[1] KG Jebsen Coeliac Disease Research Centre, University of Oslo, Oslo, Norway

[2] Department of Immunology, Oslo University Hospital, Oslo, Norway

Table 1 Sequences of synthetic gluten peptides used in T cell proliferation assays. The peptide sequences are aligned according to the presumed 9-amino acid core sequence (underlined) as bound to HLA-DQ2.5. Some peptides are already deamidated containing glutamic acid residues (E in bold) made during peptide synthesis. Other peptides that contain native gluten sequences were subjected to TG2-mediated deamidation. Glutamine residues in the QxP motif that are expected to be targets for TG2-deamidation, are denoted as Q in bold. *: new epitopes defined in this paper. n.d. not determined

Peptide ID	Epitope	Aligned sequence
P1269	α1a	QLQPFPQPELPY
P1314	α1b	PQLPYPQPELPY
P1260	α1b	PQPQLPYPQPELPY
P1313	α2	PQPELPYPQPQL
P1274	α2	PQPELPYPQPQLPY
P1501/TG	α1/α2	LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF
P1502	α1/α2	LQLQPFPQPELPYPQPELPYPQPELPYPQPQPF
P1212	γ1	QPQQSFPEQERP
P1222	γ1	YQQLPQPEQPQQSFPEQERPF
P1298	γ2	GIIQPEQPAQL
P1626	γ3	FPQQPEQPYPQQP
P1317/TG	γ3/ γ5	LQPQQPFPQQPQQPYPQQPQ
P1571	γ5	PEQPFPEQPEQ
P1396	γ4a	FSQPEQEFPQPQ
P1642/TG	γ4b	FPQPQQQFPQPQ
P1380/TG	γ4b	PQQPFPQPQQQFPQPQQPQQ
P1838/TG	γ4c	FLQPQQPFPQQPQQPYPQQPQQPFPQ
P1375/TG	γ4c	QFPQTQQPQQPFPQPQQTFP
P1653	γ4c	TEQPEQPFPQP
P1317/TG	γ4e*	LQPQQPFPQQPQQPYPQQPQ
P1370/TG	γ4d*	WPQQQPFPQPQQPFCQPQPQR
P1381/TG	n.d.	QFPQPQQPQQSFPQQQQPAI

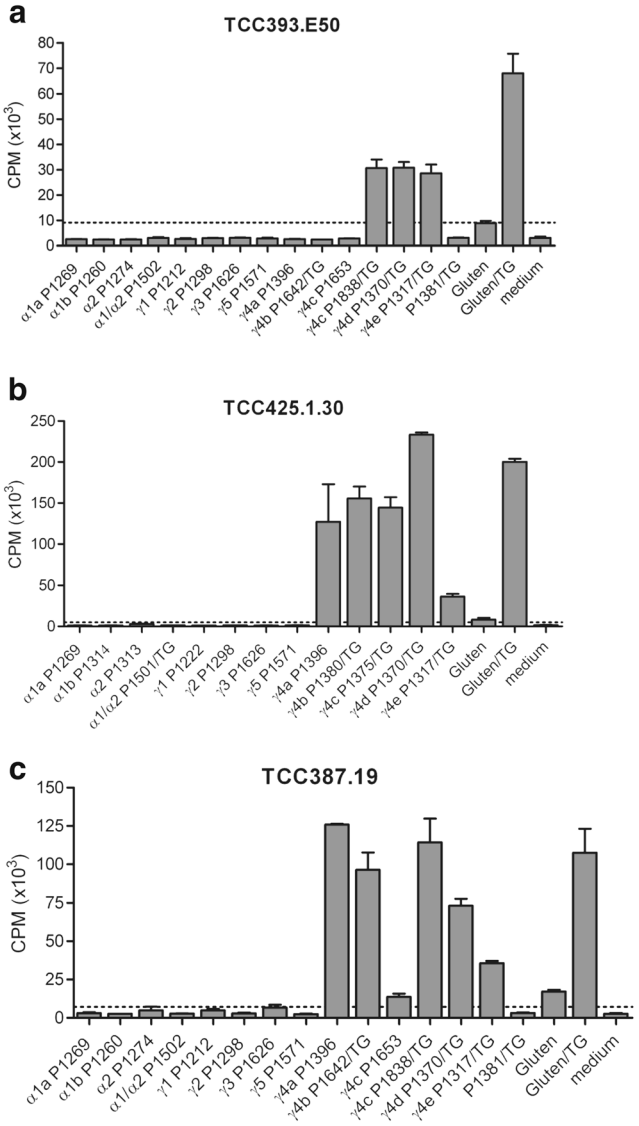

Fig. 1 T cell proliferation response of three different celiac disease patient derived T cell clones to peptides containing HLA-DQ2.5-restricted gluten epitopes. All peptides were tested at 10 μM except the potent P1501/P1502 peptides that were used at 2 μM. Gluten was tested at 100 μg/ml. Peptides were either synthesized in the deamidated version, or were deamidated by 2-h preincubation with 10 μg/ml recombinant TG2 (denoted with /TG). The bars show average CPM, the error bars show the standard error of the mean. Dotted line shows 3x CPM with medium control which is the threshold level used for response. **a** TCC425.1.30. **b** TCC387.19. **c** TCC393.E50

glia-γ3 and DQ2.5-glia-γ5 epitopes. However, none of the T cell clones responded to the shorter P1626 and P1571 peptides containing the DQ2.5-glia-γ3 and DQ2.5-glia-γ5 epitopes, respectively. Based on sequence alignment, we infer that the T cell responses were directed against the 9-mer core sequence LQP**Q**QPFP**Q** located at the N-terminal end of the P1317 peptide. This 9-mer core sequence is highly similar to the DQ2.5-glia-γ4c 9-mer sequence **Q**QP**Q**QPFPQ. We have named this novel epitope with minimal sequence LQP**E**QPFPQ DQ2.5-glia-γ4e. To note, none of the three clones showed proliferative response to any of the peptides containing other HLA-DQ2.5-restricted glia-α or glia-γ epitopes tested (Fig. 1).

In conclusion, based on universal cross-reactivity to highly similar gluten sequences observed in several T cell clones derived from different celiac disease patients, we identified two novel HLA-DQ2.5-restricted gluten T cell epitopes that were both members of the DQ2.5-glia-γ4 epitope family. In both cases, although we did not formally prove by designated experiments, we are confident that these novel epitopes are HLA-DQ2.5-restricted as the same T cells responded to peptides harboring the DQ2.5-glia-γ4a, DQ2.5-glia-γ4b and DQ2.5-glia-γ4c epitopes that shared highly similar sequences. The HLA-DQ2.5 binding and the binding register of these other three DQ2.5-glia-γ4-restricted epitopes have all been thoroughly demonstrated in a previous study (Qiao et al. 2005).

References

Molberg Ø, McAdam SN, Lundin KEA, Sollid LM (2000) Studies of gliadin-specific T cells in celiac disease. In: Celiac disease - methods and protocols. Humana Press, Totowa, pp 105–124

Qiao SW, Bergseng E, Molberg O, Jung G, Fleckenstein B, Sollid LM (2005) Refining the rules of gliadin T cell epitope binding to the disease-associated DQ2 molecule in celiac disease: importance of proline spacing and glutamine deamidation. J Immunol 175:254–261

Sollid L, Qiao SW, Anderson R, Gianfrani C, Koning F (2012) Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. Immunogenetics 64: 455–460