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The role of HLA-DQ8 β 57 polymorphism in the anti-gluten T-cell response in coeliac disease

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Abstract

Major histocompatibility complex (MHC) class II alleles HLA-DQ8 and the mouse homologue I-A^g7 lacking a canonical aspartic acid residue at position 57 are associated with coeliac disease^{1,2} and type I diabetes^{3,4}. However, the role of this single polymorphism in disease initiation and progression remains poorly understood. The lack of Asp 57 creates a positively charged P9 pocket, which confers a preference for negatively charged peptides. Gluten lacks such peptides, but tissue transglutaminase (TG2) introduces negatively charged residues at defined positions into gluten T-cell epitopes by deamidating specific glutamine residues^{5,6} on the basis of their spacing to proline residues⁷. The commonly accepted model, proposing that HLA-DQ8 simply favours binding of negatively charged peptides, does not take into account the fact that TG2 requires inflammation for activation⁸ and that T-cell responses against native gluten peptides are found^{9,10}, particularly in children¹¹. Here we show that 57 polymorphism promotes the recruitment of T-cell receptors bearing a negative signature charge in the complementary determining region 3 (CDR3) during the response against native gluten peptides presented by HLA-DQ8 in coeliac disease. These T cells showed a crossreactive and heteroclitic (stronger) response to deamidated gluten peptides. Furthermore, gluten peptide deamidation extended the T-cell-receptor repertoire by relieving the requirement for a charged residue in CDR3. Thus, the lack of a negative charge at position 57 in MHC class II was met by negatively charged residues in the T-cell receptor or in the peptide, the combination of which might explain the role of HLA-DQ8 in amplifying the T-cell response against dietary gluten.

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Author Contributions Z.H. executed most of the studies, participated in conceptual development and in the preparation of the manuscript; A.W., A.M., C.C., S.A.C., K.Y. provided technical assistance and input into data analysis. M.W., S.T., L.M.S. and F.K. generated and characterized human T-cell clones. C.S.D. and J.A.M. provided humanized HLA-DQ8 transgenic mice. F.K. and L.M.S. reviewed the manuscript. L.T. provided input into conceptual development and experimental design. B.J. developed the concept, supervised all investigations and wrote the manuscript.

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A specific assessment of the role of MHC class II alleles in antigen-specific T-cell responses is possible in coeliac disease, because it is caused by a single and well-defined antigen, gluten, which is present in wheat, rye and barley (reviewed in ref. 12). Gluten is rich in glutamine and proline, and the high content of proline renders gluten resistant to intestinal enzymatic digestion, explaining the persistence of intact peptides for immune recognition. Notably, despite the presence of few negatively charged amino acid residues in gluten and the expression of other MHC alleles potentially capable of presenting gluten peptides, all gluten-specific CD4⁺ T cells isolated from the intestinal mucosa of adult patients are restricted by HLA-DQ2 or HLA-DQ8 (refs 10, 13 and 14), and most recognize a dominant peptide with a deamidated Gln—that is, a negatively charged Glu residue^{5,6}.

To study the T-cell response to native and deamidated gluten peptides (Fig. 1a), we immunized humanized HLA-DQ8 mice with the native 24-amino-acid gluten peptide 219–242 from the α -gliadin comprising the commonly recognized DQ8–I epitope (QGSFQPSQQ)¹⁰, or with a peptide deamidated at positions 229 and 237, the known targets of TG2 (ref. 6). As previously reported¹⁵, the deamidated peptide failed to induce superior effector or recall proliferative (Fig. 1b, left panel and Supplementary Fig. 1a, left panel) or cytokine (Supplementary Fig. 1b, left panels) CD4⁺ T-cell responses. Similar responses were also observed for lower, limiting doses of immunizing peptide (data not shown). However, under conditions of competition, when the two peptides were administered simultaneously, we found that the response against the deamidated peptide largely predominated over the native peptide (Fig. 1b, right panel and Supplementary Fig. 1, right panels). When peptides with single Gln to Glu substitutions at position 229 or 237 (Fig. 1a) were compared in the same competition assay against the native peptide, the E237 peptide behaved like the deamidated peptide, whereas the E229 peptide resembled the native peptide (Fig. 1b middle and lower panels). In accordance with previous studies concluding that the Glu residue at position 229 binds to pocket 1 (P1) of HLA-DQ8, whereas Glu at position 237 binds to pocket 9 (P9)^{6,9,16}, these results indicate that an acidic residue at position P9 confers a functional advantage with respect to immunization.

Using a panel of T-cell hybridomas derived from immunized lymph nodes, we compared the specificity and T-cell receptor (TCR) repertoire of CD4⁺ T cells elicited by the native and the deamidated peptides. Immunization with native and deamidated peptides induced crossreactive T cells, in addition to T cells recognizing specifically one or the other form of the peptide. Notably, nearly a quarter of the hybridomas generated against the native peptide showed a ‘heteroclitic’ response¹⁷ against the deamidated peptide, in that they responded much better to the deamidated than to the native peptide, with a shift in the dose response curve of up to 100-fold (Fig. 1c and Supplementary Fig. 2). Immunization with the native peptide, however, did not induce T cells that recognized the deamidated peptide exclusively, confirming the absence of *in vivo* deamidation in this experimental setting. Importantly, a heteroclitic response was never observed after immunization with the deamidated peptide.

Analysis of the TCR repertoire (Fig. 1d) revealed that most T cells recognizing the native peptide—whether elicited by immunization with the native or the deamidated peptides—preferentially used a limited set of V chains including V 11, V 5, V 14 and V 2 T cells. T cells that exclusively recognized the native peptide predominantly expressed V 11 (45%) and to a lesser degree V 14 (15%), whereas T cells that equivalently recognized the two peptides mostly expressed V 5 (75%). In marked contrast, T cells specific for the deamidated peptide showed a broad TCR repertoire with no preferential V (Fig. 1d) or V chain use (data not shown).

By studying the response of T-cell hybridomas to amino- and carboxy-terminal deletion mutant peptides, we identified five different patterns. Most (82%) of the T cells specific for

the native peptide responded to the minimal $^{229}\text{QGSFQPSQ}^{237}$ 9-amino-acid determinant (pattern 1), whereas the remaining clones responded to the minimal 9-amino-acid $^{228}\text{GQGSFQPSQ}^{236}$ determinant (pattern 2; Supplementary Table 1, lower panel). Hybridomas responding equally well to native and deamidated peptides also showed a relatively restricted response pattern; that is, pattern 1 and 4 accounted for 78% of these hybridomas (Supplementary Table 1, lower panel). In contrast, T cells specific for the deamidated peptide showed at least four different response patterns, none of which was predominant (Supplementary Table 1, lower panel). Notably, response pattern 2, characterized by a response to $^{228}\text{GEGSFQPSQ}^{236}$, was never observed for T cells specific for the deamidated peptide (Supplementary Table 1, lower panel), indicating a requirement for the acidic residue at position 237. The diversity of response patterns observed with T cells specific for the deamidated peptide might be due to shifts in the peptide register, as the structure of MHC class II molecules allows peptides to frameshift within the MHC groove¹⁸. Alternatively, a single register might be occupied by the deamidated peptide, but the diverse TCRs could contact different residues. Altogether, these results indicate that recognition of the native peptide imposes strict requirements leading to limited TCR use, whereas by displaying several epitopes by a yet undetermined mechanism, the deamidated peptide recruits a far broader TCR repertoire.

Examination of TCR sequences revealed the notable conservation of a negative charge at position 3 of CDR3 in most T cells recognizing the native peptide, including 92% of those specific for the native peptide, and 60–92% of those that responded to both the native and the deamidated peptides (Table 1). Importantly, 91% of the negative charges were non-germline-encoded, indicating an antigen-driven selection process (Supplementary Table 2). During TCR V(D)J recombination, further diversity is generated by the stochastic addition and deletion of palindromic and non-germline nucleotides at the coding joints between the gene segments. Specifically, here more than 35% of the non-germline-encoded negatively charged residues resulted from one non-germline nucleotide addition, 19% from three palindromic nucleotide additions and the rest from two non-germline, three non-germline, or one palindromic nucleotide additions and from the combination of two palindromic and one non-germline nucleotide additions (Supplementary Table 2). Interestingly, published TCR sequences from early islet infiltrates in NOD mice are also characterized by a conserved negative charge at position 3 of the CDR3 loop^{19,20}. In contrast, only 29% of T cells specific for the deamidated gluten peptide had a negative charge at that position (Table 1). A possible model accounting for these findings is that HLA-DQ8- and IA^{g7}-restricted TCRs against peptides lacking acidic residues must use a negative charge in their CDR3 to stabilize peptide–MHC (pMHC) complexes through charge–charge interactions.

To test whether the negative charge at position 3 of CDR3 is required for recognition of the native peptide, we generated a TCR mutant lacking the conserved negative charge (Fig. 2a), as well as a negatively charged HLA-DQ8 mutant in which an Asp was introduced at position 57 (Fig. 2b). The TCR was obtained from a representative V 5.1/V 17 hybridoma that responded equivalently to the native and deamidated peptides and was retrovirally transferred into 58 – – T cells. Notably, mutation of the conserved negative charge at position 3 of CDR3 (V 5.1 (E – Q)) resulted in complete loss of recognition of the native peptide, whereas the response to the deamidated peptide was preserved (Fig. 2a, middle panel). As a control, mutation of a non-conserved negative charge at position 8 of CDR3 (V 5.1 (D – N)) did not alter the response to either native or deamidated peptides (Fig. 2a, right panel). We next performed the reciprocal experiment in which HLA-DQ8 was mutated to introduce an Asp at position 57. Using T-cell hybridomas responding equally well to native and deamidated peptides, we observed a marked loss of response to the native peptide (Fig. 2b, left panel). Loss of recognition of the native peptide with the Asp 57 DQ8 mutant was also confirmed with three hybridomas recognizing selectively native peptides (data not

shown). In contrast, recognition of the deamidated peptide was only moderately affected (Fig. 2b, right panel), consistent with previous studies showing that MHC alleles carrying an Asp at position 57 can bind peptides harbouring acidic residues. These results support the hypothesis that HLA-DQ8 57 polymorphism can elicit strong responses against non-negatively charged peptides by recruiting TCRs that express a negative charge at position 3 of CDR3.

The amplitude of the T-cell response results from the combined strength of the binding of peptide to MHC and the binding of TCR to pMHC complexes. The strong response elicited by the native peptide was surprising given the lack of a negative charge to match the positive P9 pocket in HLA-DQ8. Indeed, whereas the deamidated peptide readily competed with the binding of indicator peptide to HLA-DQ8, binding of the native peptide was below detection (Supplementary Fig. 3a). When indirectly measured by hybridoma stimulation using a clone responding to both peptides, the binding of the native peptide exhibited similar on-rate but increased off-rate (Supplementary Fig. 3b). Overall these results are in accordance with previous observations showing strong *in vivo* and *in vitro* T-cell responses against peptides for which no binding to MHC could be detected by direct binding assays^{21,22}. Furthermore, our data suggest that the instability of complexes between native peptide and HLA-DQ8 is compensated by the presence of a negatively charged residue in the CDR3 loop. In agreement with these findings, the presence of T cells during the short incubation time (1 h) of antigen-presenting cells with gluten peptides influenced the magnitude of their response against the native but not the deamidated peptide (Supplementary Fig. 3c).

Because the native gluten peptides recruit a T-cell population that is not only largely crossreactive but also substantially heteroclitic against the deamidated peptides, these cells could be recruited and continuously activated once inflammation is triggered and TG2 is activated to produce deamidated peptide. Consistent with this model, quantification of the CD4⁺T-cell response by enzyme-linked immunospot (ELISPOT) showed that the frequency of T cells recognizing the deamidated peptide was consistently highly increased after immunization with a mixture of native and deamidated peptides (Fig. 3).

To assess the pathogenic relevance of our findings further, we examined the TCR structure of human T-cell clones specific for the native DQ8- I epitope (QGSFQPSQQ). Notably, six out of six human T-cell clones, obtained from two independent research centres in Leiden and Oslo, expressed a TCR with a non-germline-encoded acidic residue precisely at position 3 of their CDR3 (Table 2). All these negatively charged residues were non-germline encoded and resulted from one non-germline (16.7%), two non-germline (33.3%), two palindromic (33.3%) and three non-germline (16.7%) nucleotide additions (Supplementary Table 3). In contrast, none of the three non gluten-specific T-cell clones derived from the same patients had this signature charge in their CDR3 (Table 3). To integrate these results in a more general context, we analysed 125 published TCR sequences obtained from HLA-DQ8 individuals with type I diabetes ($n = 116$), Birch ($n = 5$) and nickel ($n = 1$) allergy, or HIV infection ($n = 3$) and found that only 17 had a negatively charged residue at position 3 of the CDR3 loop. Interestingly, all of these 17 TCRs originated from the type I diabetes patients (data not shown).

This study reveals a new mechanism of peptide recognition in the context of HLA-DQ8, the MHC class II allele that predisposes to both type I diabetes and coeliac disease. The P9 pocket of HLA-DQ8, lacking the canonical Asp 57 found in other alleles, has the potential to interact not only with peptides harbouring an acidic residue at the P9 position, but also with TCRs harbouring a negative charge in their CDR3 loop. The potent response generated *in vivo* against native peptides with extremely low affinity for HLA-DQ8 may be

explained by the ability of these TCRs to stabilize pMHC complexes. A large proportion of the TCRs recruited by native peptides display a cross-reactive heteroclitic response to the deamidated peptide, which binds strongly to HLA-DQ8. Furthermore, because deamidated peptides do not impose charge constraints on the TCR, they can recruit an additional TCR repertoire. Consequently, the magnitude of the response against the deamidated peptide is increased under conditions in which native and deamidated peptides are both present. This may be particularly relevant in the context of coeliac disease, because TG2 is probably unevenly activated once inflammation is initiated. Furthermore, the magnitude of the T-cell response is viewed as a limiting factor for disease development^{23,24}. In the absence of a disease model of coeliac disease, it is at present not possible to assess whether this process is important for disease onset, disease progression or other aspects of the disease. It also remains to be determined whether DQ 57 polymorphism also contributes to the strong association of coeliac disease with HLA-DQ2.

It is tempting to speculate that similar mechanisms may explain the association of type I diabetes with HLA-DQ8. The TCR repertoire of early pancreatic islet infiltrate is also characterized by the frequent presence of a negative charge at position 3 of CDR3^{19,20,25}. Furthermore, our preliminary studies suggest that TG2 activity is significantly increased in the pancreas, but not in the draining pancreatic lymph node and the spleen of 12-week-old NOD mice that have ongoing pancreatic inflammation (B.J. and L.T., unpublished observations). The ability to present native and deamidated peptides and recruit crossreactive TCR repertoires directly linked to the polymorphism at position 57 may underlie the remarkable association of HLA-DQ8 with severe immunopathology in mouse and human.

METHODS SUMMARY

Detailed methods are found in the Supplementary Information. Humanized HLA-DQ8 transgenic mice¹⁵ were immunized in the footpad with immunodominant native (Q) and deamidated (E) 2-219–242 gluten peptide versions of this peptide with glutamates in position 229 and/or 237. T-cell proliferation, cytokine enzyme-linked immunoabsorbent (ELISA) and IFN- ELISPOT assays were performed to analyse the immune response to native and deamidated peptides. T-cell hybridomas were generated after immunization with native and deamidated gluten peptides and their specificity was characterized by measuring IL-2 release²⁶. TCR CDR3 sequencing was performed after TCR α -chain amplification with a set of TCR variable region (V α) sense and a TCR constant region (C α) antisense consensus primers as described²⁷. TCR α -chain sequences were identified as TCR gene segments using BLAST and aligned/analysed using International Immunogenetics Database (<http://imgt.cines.fr>). The nomenclature is according to the World Health Organization-International Union of Immunological Societies (IUIS) nomenclature²⁸. Transfectants expressing wild-type and mutated V 17 and V 5.1 TCR of hybridomas that responded equally well to native and deamidated peptides were generated by retroviral transduction of T-cell hybridoma variant 58⁻ (ref. 29). The soluble versions of wild-type and mutant DQ8 molecules were produced as previously described³⁰. Human T-cell clones specific for the 2-219–242 epitope were established from intestinal biopsies of DQ8-positive coeliac disease patients as previously described^{10,13}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Native and deamidated gluten 2-219–242 peptides recruit distinct, yet overlapping TCR repertoires

a, Humanized HLA-DQ8 transgenic mice were immunized with native (Q) and deamidated (E) versions of the 24-amino-acid gluten peptide 2-219-242 and two E peptide analogues with a Glu residue either in position 229 (E229) or 237 (E237). The draining lymph nodes were collected 8 days after immunization and CD4⁺ T cells were purified to perform functional assays and derive 2-219-242 gluten-peptide-specific HLA-DQ8-restricted T-cell hybridomas. **b**, Purified CD4⁺ T cells were tested for proliferative *in vitro* responses with the indicated peptide at the specified concentrations in the presence of irradiated (30 Gy) spleen cells from non-immunized humanized HLA-DQ8 mice. Responses are given in c.p.m. from the mean ³H-thymidine incorporation in triplicate cultures $\pm$ s.d. $n = 3$ mice per each condition of immunization. The data are representative of three independent experiments for upper panels, and two independent experiments for middle and lower panels. **c**, A panel of 2-219-242 gluten-peptide-specific CD4⁺ T hybridomas was derived after immunization with native (Q) or deamidated (E) peptide alone. Hybridoma reactivity was defined by measuring IL-2 secretion. Q and E hybridomas responded exclusively to native and deamidated peptides, respectively. Q = E, hybridomas responded equally well to native and deamidated peptides; Q > E, hybridomas responded 10–100 times better to native than to deamidated peptide; E > Q, hybridomas responded 10–100 times better to deamidated than to native peptide. The percentage of T-cell hybridomas obtained in each class is indicated. Importantly, assessing the quality of the immunization, no E-specific hybridomas were obtained after Q immunization, and conversely no Q-specific hybridomas were obtained after E immunization **d**, TCR α -chain variable region usage of individual hybridomas was defined by flow cytometry after immunization with Q (open circles, left panel) and E peptides (filled circles, right panel).

Figure 2. Negative charge at position 3 of the TCR CDR3 loop is critical for native gluten peptide reactivity

a. Generation of TCR-V 5.1-chain mutants from a representative Q = E hybridoma with either substitution of Glu to Gln at position 3 (V 5.1 (**E** $\rightarrow$ **Q**)) or Asp to Asn at position 8 (V 5.1 (**D** $\rightarrow$ **N**)). The mutated amino acids are indicated in bold. Wild-type (WT) V 17 chain in association with wild-type or mutant TCR V 5.1 chains was retrovirally transduced in the CD4 transfected 58 β T-cell hybridoma variant²⁹. Hybridoma transfectants expressing wild-type V 5.1/V 17 (as a control, left panel), and mutated V 5.1(**E** $\rightarrow$ **Q**)/V 17 (middle panel) or V 5.1(**D** $\rightarrow$ **N**)/V 17 (right panel) TCR heterodimers were stimulated with either Q (filled diamonds) or E (open circles) peptide at the indicated concentrations. The IL-2 release values are the mean of triplicate cultures $\pm$ s.d. The data are representative of three independent experiments. **b.** A representative Q = E hybridoma was stimulated with Q (left panel) and E (right panel) peptides in the presence of wild-type HLA-DQ8 (WT DQ8, filled diamonds) or mutant HLA-DQ8 (D57 DQ8, open circles) with an Ala to Asp substitution at position 57. The level of IL-2 was measured using mouse cytotoxic T lymphocyte lines (CTLL) bioassay. The mean of duplicate cultures are shown, and data are representative of three separate experiments.

Figure 3. Coimmunization with native and deamidated peptides amplifies the T-cell response to deamidated peptide

Humanized HLA-DQ8 transgenic mice were immunized with Q or E gluten peptides alone, or in combination. Eight days after immunization CD4⁺T cells were purified and re-challenged *in vitro* with 32.3 μ M Q or E peptides. The frequency of IFN- γ -producing cells was assessed using ELISPOT. Each bar represents the mean spot number of triplicates $\pm$ s.d. ** $P < 0.001$; data are representative of three independent experiments, each comprising three mice for each type of immunization.

Table 1

Negative charge in the CDR3 loop of native gluten-reactive T-cell hybridomas

Hybridoma	Specificity	V	J	Length	CDR3	
QR17 (FJ410957)	Q	3	J1-1	6	CAS SsdIEV	FFGKG
QP13 (FJ410958)	Q	11	J2-7	6	CAS SLdSEQ	YFGPG
QN17 (FJ410959)	Q	11	J2-7	6	CAS SLdIEQ	YFGPG
QQ21 (FJ410960)	Q	11	J1-1	6	CAS SLdTEV	FFGKG
QO14 (FJ410961)	Q	11	J2-1	7	CAS SLenAEQ	FFGPG
QN19 (FJ410962)	Q	11	J2-1	7	CAS SLenAEQ	FFGPG
QR5 (FJ410963)	Q	11	J1-3	8	CAS SLdSGNTL	YFGEG
QR11 (FJ410967)	Q	14	J2-5	8	CAW SlpGGDTQ	YFGPG
QR6 (FJ410965)	Q	5.1	J2-1	9	CAS SLevNYAEQ	FFGPG
QR28 (FJ410964)	Q	11	J2-1	9	CAS SLELGnAEQ	FFGPG
QR38 (FJ410966)	Q	5.1	J2-4	9	CAS SLeTQNTL	YFGAG
QR18 (FJ410969)	Q	1	J2-7	9	CAS SQdtGagEQ	YFGPG
QR19x (FJ410968)	Q	1	J2-7	9	CAS SQdtGagEQ	YFGPG
EQ5 (FJ410970)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
QO3 (FJ410971)	Q=E	5.1	J1-2	9	CAS SLeiaNSDY	TFGSG
EQ3 (FJ410972)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
EQ18 (FJ410973)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
QN8 (FJ410974)	Q=E	5.1	J1-2	9	CAS SLeiaNSDY	TFGSG
EQ18 (FJ410975)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
EQ5S1 (FJ410976)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
QR18 (FJ410977)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
EQ15 (FJ410978)	Q=E	5.1	J1-2	9	CAS SLeTANSDY	TFGSG
QR4 (FJ410979)	Q=E	5.1	J1-2	9	CAS SLevaNSDY	TFGSG
QQ34 (FJ410980)	Q=E	5.1	J2-4	9	CAS SLeSQNTL	YFGAG
EQ1 (FJ410981)	Q=E	8.1	J2-5	10	CAS gdlTGGQDTQ	YFGPG
QC10 (FJ410982)	Q=E	11	J2-2	10	CAS SLDRgdTGQL	YFGEG

Hybridoma	Specificity	V	J	Length	CDR3
Q _{N5} (FJ410983)	Q>E	2	J2-4	8	CTC SAeaGNTL
Q _{P9} (FJ410984)	Q>E	14	J2-3	8	CAW SIIsAETL
Q _{R31} (FJ410985)	Q>E	14	J2-3	8	CAW SIpSAETL
Q _{N13} (FJ410986)	Q>E	14	J1-5	8	CAW SIeagQAP
Q _{Q18} (FJ410987)	Q>E	2	J1-6	10	CTC SAdSGVNSPL
E _{F12} (FJ410988)	E	8.1	J1-2	8	CAS gDsaNSDY
E _{R7} (FJ410989)	E	8.1	J1-2	8	CAS gDsaNSDY
E _{T18} (FJ410990)	E	14	J2-5	8	CAW SIpGGDTQ
E _{K2} (FJ410991)	E	7	J2-7	9	CAS SLgGQGYEQ
E _{E6} (FJ410992)	E	5.1	J2-5	9	CAS SLrgNQDTQ
E _{E18} (FJ410993)	E	6	J2-4	9	CAS SpaGSQNTL
E _{P1x} (FJ410994)	E	5.1	J2-7	9	CAS SIwDRdYEQ
E _{T11} (FJ410995)	E	5.1	J2-3	9	CAS SLeIAETL
E _{P4} (FJ410996)	E	6	J1-3	10	CAS SITGSGNTL
E _{A16s1} (FJ410997)	E	6	J2-7	10	CAS SIppqSYEQ
E _{S1} (FJ410998)	E	5.1	J1-2	10	CAS SIGTGkdSDY
E _{Q10} (FJ410999)	E	11	J1-2	10	CAS SLeaGtNSDY
E _{H6} (FJ411000)	E	11	J1-2	10	CAS SLeaGtNSDY
E _{A39s2} (FJ411001)	E	2	J2-2	11	CTC SA d DRGNTGQL YFGEG
Q _{Q31-2} (FJ411002)	E>Q	11	J2-7	7	CAS SL d gYEQ
E _{E9} (FJ411003)	E>Q	11	J2-1	8	CAS SLeGGAEQ
E _{B4s2} (FJ411004)	E>Q	11	J1-6	8	CAS SLEGSPL
E _{C2s3} (FJ411005)	E>Q	14	J1-1	9	CAW SrGTINTEV
Q _{R34} (FJ411006)	E>Q	11	J1-5	10	CAS SL d GrNQAP
Q _{R15} (FJ411007)	E>Q	11	J2-2	11	CAS SL d GrNTGQL
E _{C33s3} (FJ411008)	E>Q	11	J2-3	12	CAS SLeaGGrgAETL
E _{F3} (FJ411009)	E>Q	11	J2-3	12	CAS SLeaGGrgAETL
E _{C42s1} (FJ411010)	E>Q	11	J2-3	12	CAS SLnGGrgAETL

CDR3 sequences from T-cell hybridomas characterized in Fig. 1 are presented. Hybridomas names are indicated in capital letter Q when they were derived from immunization with native peptide, and in capital letter E when they were derived from immunization with deamidated peptide. GenBank accession numbers are in parentheses. Junctionally encoded amino acids are shown as lower-case. Amino acid residues at position 3 are bold when they are negatively charged.

Table 2

Negative charge in the CDR3 loop of human native gluten reactive T-cell clones

Clone	Specificity	V	J	Length	CDR3
Spec16 (FJ410946)	Q	7.1	J2-3	10	CAS SQdlrNTGEL
Spec12 (FJ410947)	Q=E	9	J1-5	7	CAS SQddQPQ
Spec12 (FJ410948)	Q=E	1	J2-5	10	CAS SVydgRGETQ
Spec13 (FJ410949)	Q=E	9	J1-5	7	CAS SQddQPQ
Spec13 (FJ410950)	Q=E	1	J2-5	11	CAS sttPGTgTETQ
TCC360.11 (FJ410951)	E>Q	3	J1-6	7	CGs svdaSPL
TCC360.8 (FJ410952)	E>Q	1	J1-1	9	CAS SVeamNTEA
TCC489.2.1.4 (FJ410953)	E Q	1	J2-3	11	CAS SVeaGvsTDTQ
Spec22 (FJ410954)	Non-gluten	22	J1-5	10	CAS SlgpgdNQPQ
Spec1 (FJ410955)	Non-gluten	1	J2-3	11	CAS SVAGGIFTDTQ
TCC360.10 (FJ410956)	Non-gluten	13	J1-6	8	CAS reGvQETQ

DR3 sequences of gluten-specific HLA-DQ8-restricted and non-gluten-reactive control T-cell clones are presented. T-cell clones were obtained from research centres in Leiden (Spec16, 12, 13, 22 and 1) and Oslo (TCC360.11, TCC360.8, TCC489.2.1.4 and TCC360.10). Spec12 and Spec13 expressed two functional V chains, which can be explained by the absence of allelic exclusion. GenBank accession numbers are in parentheses. Junctionally encoded amino acids are presented in lower-case. Amino acid residues at position 3 are bold when they are negatively charged.