Moustakas-AK; van de Wal-Y; **Routsias-JG**; Kooy-YMC, van Veelen-P; Drigfhout-JW; Koning-F; Papadopoulos-G *Δομή των σχετιζόμενων με την κοιλιοκάκη **HLA-DQ8 και των μη σχετιζόμενων HLA-DQ9 αλληλίων σε σύμπλεξη με ειδικούς για την νόσο επιτόπους.*** Int-Immunol. 2000 Aug; 12(8): 1157-66.

Η κοιλιοκάκη συσχετίζεται με τα αντιγόνα ιστοσυμβατότητας HLA-DQ2 και HLA-DQ8. Η συσχέτιση αυτή οφείλεται στην εκλεκτική αναγνώριση, από τα Τ-λεμφοκύτταρα του εντερικού βλεννογόνου, θραυσμάτων της γλουτένης σε σύμπλεξη με τα προανφερθέντα αλλήλια του απλοτύπου DQ. Πρόσφατα προσδιορίστηκαν, από την ερευνητική μας ομάδα, δύο ειδικά για τα HLA-DQ8 ενεργοποιητικά πεπτίδια που προέρχονται από τα παράγωγα της γλουτένης, γλιαδίνη και γλουτενίνη τα οποία αγνωρίζονται ειδικά από Τ-λεμφοκυτταρικούς κλώνους του λεπτού εντέρου ασθενών με κοιλιοκάκη. Στη μελέτη αυτή εξετάσθηκε λεπτομερώς η εξειδίκευση των πεπτιδίων αυτών για σύμπλεξη με τα HLA-DQ8 μόρια με τη βοήθεια μοριακής μοντελοποίησης και in vitro αναγνώρισης συνθετικών πεπτιδικών αναλόγων από τους ειδικούς Τ-λεμφοκυτταρικούς κλώνους. Οι δύο Τ-λεμφοκυτταρικοί κλώνοι αναγνωρίζουν υποκατεστημένα πεπτίδια στις θέσεις p1 και p9, αλλά όχι στις θέσεις p2-p8 (κυρίως ο ειδικός για την γλιαδίνη κλώνος). Παρατηρήθηκαν διαφορετικοί τρόποι αναγνώρισης των τροποποιημένων p1/9 Gln→Glu πεπτιδίων, τα οποία προβλέπεται από την μοντελοποίηση να συμπλέκονται ισχυρότερα με τα DQ8 μόρια. Έτσι βρέθηκε να ενισχύεται η αναγνώριση για το τροποποιημένο ανάλογο του πεπτιδίου της γλιαδίνης σε αντίθεση με το ανάλογο του πεπτιδίου της γλουτενίνης. Το διπλά υποκατεστημένο πεπτίδιο της γλιαδίνης, το οποίο μπορεί να προκύψει έπειτα από κατεργασία με πεψίνη/οξύ/τρανσ-γλουταμινάση, επειδυκνύει αξιοσημείωτη αύξηση ευαισθησίας αναγνώρισης, σε απόλυτη συμφωνία με την ενίσχυση της ικανότητας δέσμευσης του πεπτιδίου στο DQ8 που προβλέπεται από την ελαχιστοποίηση ενέργειας. Απρόσμενα, τα δύο αρχικά πεπτίδια βρέθηκαν να αναγνωρίζονται από τους αντιστοίχους Τ- λεμφοκυτταρικούς κλώνους και σε σύμπλεξη με τα μόρια HLA-DQ9 (β57Asp). Επίσης αναγνώριση υπήρξε και για το τροποποιημένο πεπτίδιο (p1/9 Gln→Glu) και παρόλη την υποδεκαπλάσια ευαισθησία δέσμευσης, αποτελεί την πρώτη αναφορά δέσμευσης ενός p9Glu σε ένα αλλήλιο MHC-II που εμπεριέχει β57Asp.

Τα αποτελέσματα αυτά παρέχουν σημαντικές πληροφορίες για τους μηχανισμούς που υπεισέρχονται στην παθογένεση της αυτοανοσίας και την πιθανή ρύθμιση της.

International Immunology, Vol. 12, No. 8, pp. 1157–1166

Structure of celiac disease-associated HLA-DQ8 and non-associated HLA-DQ9 alleles in complex with two disease-specific epitopes

Antonis K. Moustakas, Yvonne van de Wal[1], John Routsias[2,3], Yvonne M. C. Kooy[1], Peter van Veelen[1], Jan Wouter Drijfhout[1], Frits Koning[1] and **George K. Papadopoulos**

Laboratory of Biochemistry and Biophysics, Faculty of Agricultural Technology, Technological Educational Institute of Epirus, 47100 Arta, Greece
[1]Department of Immunohematology and Blood Bank, Leiden University Medical Center, RC 2300 Leiden, The Netherlands
[2]Laboratory of Immunology, Department of Internal Medicine, University of Ioannina Medical School, 45110 Ioannina, Greece

[3]Present address: Laboratory of Pathophysiology, The Medical School, University of Athens, Athens 11527, Greece

Keywords: antigens/peptides, autoimmunity, HLA-DQ, MHC, T cells

Abstract

The association of celiac disease (CD) with HLA-DQ2 and HLA-DQ8 is indicative of preferential mucosal T cell recognition of gluten fragments bound to either DQ allele. We have recently identified two gluten-derived, HLA-DQ8-restricted T cell stimulatory peptides, one each from gliadin and glutenin, recognized by specific T cell clones derived from the small intestine of CD patients. We have now performed molecular modeling and examined the fine specificity of these peptides in complex with HLA-DQ8. There is only one binding register for both peptides, with glutamine residues at the p1 and p9 anchor positions. Both T cell clones recognize substituted peptides at p1 and p9, but poorly so at p2–p8, especially the gliadin-specific clone. Contrasting patterns of recognition of p9Gln → Glu peptide variants (both predicted as better DQ8 binders by modeling) were observed: enhancement of recognition for the gliadin peptide, yet complete absence thereof for the glutenin peptide. The double-substituted gliadin peptide variant p1/9Gln → Glu, which can also arise by pepsin/acid/transglutaminase treatment, shows a considerable increase in sensitivity of recognition, consistent with better binding of this peptide to DQ8, as predicted by energy minimization. Surprisingly, the two native peptides are also recognized by their respective T cell clones in the context of the related molecule HLA-DQ9 (β57Asp$^+$). The p1/9Gln → Glu gliadin peptide variant is likewise recognized, albeit with a 10-fold lower sensitivity, the first reported p9Glu binding in a β57Asp$^+$ MHC II allele. Our results have important implications for the pathogenesis of autoimmune disease and the possible manipulation of aberrant responses thereof.

Introduction

It is generally accepted that celiac disease (CD) is caused by aberrant recognition of gluten-derived peptides by intestinal mucosal T lymphocytes. Gluten is the water-insoluble portion of wheat and consists of a complex mixture of glutamine- and proline-rich glutenin and gliadin molecules. The vast majority of patients carry the HLA-DQ2 (DQA1*0501/B1*0201) allele (1,2), while most of the DQ2$^-$ patients share the HLA-DQ8 (DQA1*0301/B1*0302) allele (3–5). Both DQ2- and DQ8-restricted gluten-specific T cells have been isolated from the small intestine of CD patients (6,7) suggesting that the disease-associated DQ alleles play a key role in the pathogenesis by the presentation of gluten-derived peptides to mucosal

Correspondence to: G. K. Papadopoulos

Transmitting editor: J.-F. Bach

Received 19 July 1999, *accepted* 27 April 2000

T lymphocytes. Thus far, three different gluten-derived peptides have been identified that are recognized by T cell clones isolated from small bowel biopsies of CD patients (8–10). Two of these epitopes are derived from gliadin (gda09:198–232 and gdb2:134–153) and the third from glutenin (glt04:707–742). The gliadin 198–232 and glutenin 707–742 fragments are recognized in the context of the disease-associated DQ8 allele, while the gliadin 134–153 epitope is restricted to DQ2. Deamidation of glutamine residues to glutamate, either acid induced or enzyme mediated, has recently been shown to increase the antigenicity of gliadin-derived epitopes, presumably due to an enhanced binding affinity for DQ2 or DQ8 (11,12).

In addition to CD, several studies have shown that the DQ8, and to a lesser extent the DQ2, allele is linked to the occurrence of particular autoimmune diseases such as rheumatoid arthritis and juvenile (type 1) diabetes mellitus (14–18). The DQ8 molecule is highly homologous to two other naturally occurring alleles, DQ7 (DQA1*0301/B1*0301) and DQ9 (DQA1*0301/B1*0303). These three alleles share an identical α chain (DQA1*0301) and have nearly identical β chains: the DQB1*0302 allele of DQ8 differs from the DQB1*0301 allele of DQ7 in only six amino acid positions, three of which are in the peptide-binding groove of the molecule, whereas a fourth is on a β-sheet ridge outside the groove, yet in a position influencing the surface electrostatic potential of the particular DQ molecule (19). The DQB1*0303 allele of DQ9 differs in only one amino acid residue from the β chain of DQB1*0302, i.e. position 57, where the DQ8 molecule has an alanine and the DQ9 molecule has an aspartate. Genetic and immunologic studies have implicated position β57 as a dominant residue involved in high-affinity peptide binding and protection from autoimmune type 1 diabetes (20–22). DQ9 is also associated with type 1 diabetes in the Japanese. This allele is very rare in the Caucasian population. Of the major immunogenetic determinants of CD, DQ2 and DQ8, the first is very rare while the latter is found with appreciable frequency in the Japanese population (23). However, CD is unknown in Japan, most probably because the main cereal in the diet of the population there is not wheat, but rice.

We have analyzed unique features of the HLA-DQ8-restricted gluten-specific responses of these two T cell clones by characterizing the glutenin and gliadin epitopes in the context of DQ8. Furthermore, by extensive residue substitution work we have identified key positions in peptide binding and cognate T lymphocyte recognition. To this end, we have modeled the three-dimensional structure of the disease-associated HLA-DQ8 molecule and the closely related DQ9 allele, in association with the two antigenic epitopes and several of their peptide variants. Our modeling reveals the crucial importance of residue β57 with regard to the recognition of the native and the deamidated peptides. Knowledge of the characteristic structural features of T cell stimulatory HLA-DQ–gluten peptide complexes is important to the understanding of CD pathogenesis and by extension to the pathogenesis of other autoimmune diseases where the same HLA-DQ allele is implicated.

Methods

Production of the autoreactive T lymphocyte clones and T cell proliferation assays

Details about the isolation of the two gluten-specific T lymphocyte clones (one specific for a gliadin fragment, called S2, and the other for a glutenin fragment, called S12) have been published elsewhere (8,10,12). In brief, the clones were isolated from a small intestinal biopsy of a Dutch CD patient (DQ2/8$^+$) after informed consent. Both clones were CD3$^+$CD4$^+$ TCRαβ$^+$ and upon antigenic stimulation exhibited T_h1 cytokine secretion profile. Peptides for the various proliferation assays were synthesized using standard *o*-fluorenylmethoxycarbonyl chemistry on a multiple peptide synthesizer (ABIMED AMS 422; Langenfeld, Germany). Proliferation assays to the native peptide and its selected variants were performed in duplicate in 150 μl culture medium in 96-well flat-bottom microtiter plates (Falcon, Oxnard, CA) using 10^4 T cells stimulated with 10^5 irradiated peripheral blood mononuclear cells (3000 rad) in the absence or presence of peptide antigen at the indicated concentrations (10,12). After 48 h, cultures were pulsed with 0.5 μCi of [^{3}H]thymidine, harvested 18 h thereafter and counted.

Homology modeling of HLA-DQ8 and -DQ9

Modeling was based on the coordinates of DR1 complexed to the hemagglutinin peptide HA306–318 (24) as previously described (25–26). The modeling work was performed on a Silicon Graphics Indy workstation using the programs Insight II (version 95.0) and Discover (version 95.0) (Biosym Technologies/Molecular Simulations, San Diego, CA). Proline was chosen in the *trans* configuration, which is by far the most frequent (95%). This amino acid is often found in gliadin and glutenin sequences, and the *trans* configuration is the only one that would yield antigenic peptides which could fit into the groove of HLA-DQ alleles. The ionization state of amino acid side chains was that of pH 5.0, the approximate pH of the endosomal compartment and also the average pH for the crystallization of DR/I-E in several complexes (24,27–29). Modeling performed at pH 7.0 yielded nearly identical conformations for the peptide and the α1β1 domain of the respective DQ molecule. The bound peptides of the modeled DQ molecules were initially positioned using the coordinates of the bound hemagglutinin peptide HA306–318 in its complex with DR1 and by aligning the peptide so that given residues would fall into preferred pockets, as previously identified from binding experiments (30–32). No conflicts or clashes arose from the positioning of the anchor peptide residues in the respective pockets (p1, p4, etc.). Energy minimization was accomplished by 1000 cycles of the steepest gradient method, followed by another 1000 cycles of the conjugate gradient method. No account was taken of the water molecules, as the high-resolution (2.75 Å) structure of HLA-DR1 on which modeling was based identified only two water molecules within the antigen-binding groove. Minimization of the same complex starting from a different atom each time yielded a SD of energy values of 17.51 kcal/mol ($n = 9$). Therefore, we have considered energy differences of $>$44.45 kcal/mol (i.e. 2.54 SD) as significant ($P < 0.05$). Graphical representations of the modeled molecules were obtained on the Silicon Graphics Indy workstation using Insight II. The numbering scheme used for the DQα chain residues is the one adopted previously (22). The coordinates of the complexes of the gliadin and glutenin peptides with DQ8 and DQ9 will be deposited in the Protein Data Bank. They will in the meantime be available to interested researchers from G. K. P.

Results

Antigen-specificity of the two gliadin- and glutenin-specific DQ8-restricted T cell clones

Previously we have reported the sequences of a gliadin and a glutenin peptide that are specifically recognized by two HLA-DQ8-restricted gluten-specific T cell clones isolated from the small bowel of a CD patient (8,10,12). The minimal core region of the T cell stimulatory gliadin 198–232 fragment was defined as residues 206–217 (SGQGSFQPSQQN, referred to as the gliadin peptide), while the minimal core region of the glutenin fragment was defined as residues 723–735 (QQGYYPTSPQQSG, referred to as the glutenin peptide). Based on the published peptide binding motif for DQ8, the gliadin residue 208Q and the glutenin residue 724Q are expected to bind at relative position 1 (p1), whereas the gliadin residue 216Q and the glutenin residue 732Q are expected to occupy p9. These are the only two registers in which no non-preferred residues occur at anchor positions, something also confirmed by molecular modeling (see below). DQ8 favors peptides with negatively charged amino acids at p1 and p9, but can also tolerate Q residues at these positions in the gliadin and glutenin peptides (30–32). Bulky residues can be accommodated at p4, but no basic ones are allowed there, and acidic residues are not well tolerated. Pocket 6 is restricted even further, as in addition to the previous prohibitions bulky hydrophobic residues are not permitted; however, aspartate can be accepted but not glutamate (32). In agreement with this we had previously observed that Q → E replacements at p1 and p9 of the gliadin peptide favor T cell recognition (12). In contrast, such replacements in the glutenin peptide were either neutral to T cell recognition (p1) or abolished it (p9) (see Fig. 2 below) (10).

Functional analysis of gliadin 206–217 and glutenin 723–735 substitution analogues

To identify the amino acid residues that contribute to T cell activation, a series of amino acid substitution analogues of both peptides was tested for T cell stimulatory activity. Each residue was replaced by an A, a K and an additional conservative or non-conservative amino acid. For the gliadin peptide, any substitution in the central part of the core peptide (residues 209–215, p2–p8) completely abolished T cell recognition. By contrast, Q → N and Q → A substitutions at p1 and p9 were well tolerated (albeit with a ~40% reduction of the maximal response by the former substitution at both pockets), while the Q → K substitution abolished T cell proliferation at either position (Fig. 1). For the glutenin peptide, any substitution at p2, p4, p5 and p8 (native residues 725G, 727Y, 728P and 731P respectively) abrogated T cell recognition (Fig. 2). The critical role of the P residues at p5 and p8 of the latter epitope probably reflects the maintenance of the correct conformation of the peptide in the binding groove. However, in contrast to gliadin substitution analogues, amino acid substitutions were accepted at positions p3, p6 and p7 in the glutenin core peptide (such as 726Y to A, F; 729T to A, S; 730S to T). Moreover, Q → A/N/K substitutions at p1 of the glutenin peptide did not influence T cell proliferation, while the p9Q → A/N substitutions reduced the maximal T cell proliferation at the specified peptide dose to 50% of the control value (Fig. 2). As expected from the DQ8 motif, a p9Q → K substitution was not tolerated at all by the glutenin-specific T cell clone. In the gliadin and the glutenin peptides, the substitution at p–2, p–1 (glutenin only), p10, p11 or p12 (wherever appropriate), had little effect on T cell proliferation (Figs 1 and 2); yet the p–1G → A/Y in the gliadin peptide caused a 50% reduction in the T cell proliferative response.

Taken together, our results suggest that T cell recognition of the DQ8–gliadin peptide complex is highly dependent on the original peptide sequence, whereas some flexibility in T cell recognition of the respective glutenin peptide complex was observed.

Modeling of the gliadin and glutenin peptides in HLA-DQ8

Energy minimization of a MHC–peptide complex leads to its most probable conformation in solution (33–35). The energy

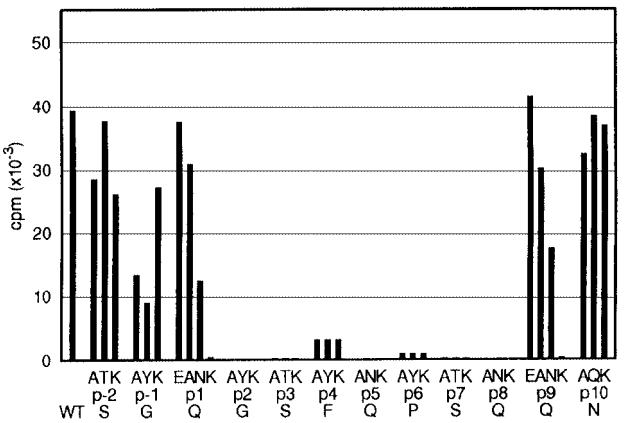

Fig. 1. Substitution analysis of the gliadin epitope (amino acid sequence 206–217) in the context of HLA-DQ8, as measured by the response of the S2 T cell clone. T cells (10^4) were stimulated with 10^5 peripheral blood mononuclear cells (irradiated with 3000 rad) from an HLA-DQ8$^+$ donor in the presence of 13.4 μg/ml of the indicated peptide sequences. Amino acids are shown in single letter codes. Values are shown as mean c.p.m. ($\times 10^3$) of triplicate cultures. SD $<$ 10%. The wild-type (WT) sequence is shown at the bottom line.

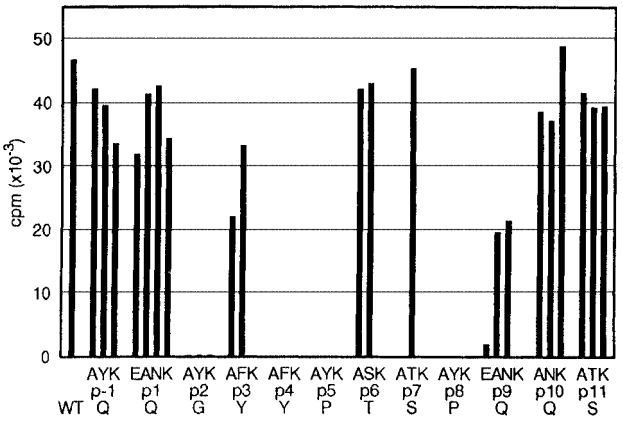

Fig. 2. Substitution analysis of the glutenin epitope (amino acid sequence 723–735) in the context of HLA-DQ8, as measured by the response of the S12 T cell clone. All conditions are the same as in Fig. 1.

Table 1. Differences in minimized energy between the DQ8–native gliadin peptide complex and the peptide variants

Peptide sequence/anchor position[a]	Designation	Minimized energy difference (kcal/mol)[b]
1 4 67 9		
SG**Q**GS**F**Q**PS**Q**Q**N	1p	0
SG***E***GS**F**Q**PS**Q**Q**N	1p1E	–64
SG**Q**GS**F**Q**PS**Q***E***N	1p9E	–80
SG***E***GS**F**Q**PS**Q***EN***	1p1/9E	–137
SG***A***GS**F**Q**PS**Q**Q**N	1p1A	+34
SG***N***GS**F**Q**PS**Q**Q**N	1p1N	–16
SG***K***GS**F**Q**PS**Q**Q**N	1p1K	–8
SG**Q***A*S**F**Q**PS**Q**Q**N	1p2A	–6
SG**Q***Y*S**F**Q**PS**Q**Q**N	1p2Y	+25
SG**Q**G***K*****F**Q**PS**Q**Q**N	1p3K	+42
SG**Q**GS***A*****Q**PS**Q**Q**N**	1p4A	–95
SG**Q**GS***Y*****Q**PS**Q**Q**N**	1p4Y	–19
SG**Q**GS**F***N***PS**Q**Q**N	1p5N	–19
SG**Q**GS**F**Q**PA**Q**Q**N	1p7A	–16
SG**Q**GS**F**Q**PT**Q**Q**N	1p7T	–41
SG**Q**GS**F**Q**PS***N***Q**N	1p8N	–19
SG**Q**GS**F**Q**PS**Q***A***N	1p9A	–3
SG**Q**GS**F**Q**PS**Q***N***N	1p9N	–13

[a]Anchors in bold. The substituted residue in each sequence from p1 to p9 is designated in italics. The native peptide is designated 1p.
[b]The total energy for the DQ8–1p complex as a homodimer of heterodimers was 3624 kcal/mol.

Table 2. Differences in minimized energy between the DQ8–native glutenin peptide complex and the peptide variants

Peptide sequence/anchor position[a]	Designation	Minimized energy difference (kcal/mol)[b]
1 4 67 9		
Q**Q**GY**Y**P**TS**P**Q**QS	2p	0
Q***E***GY**Y**P**TS**P**Q**QS	2p1E	+47
Q**Q**GY**Y**P**TS**P***E***Q***S***	2p9E	–137
Q***K***GY**Y**P**TS**P**Q**QS	2p1K	+16
Q**Q**G***A***Y**Y**P**TS**P**Q**QS	2p3A	–117
Q**Q**G***F***Y**Y**P**TS**P**Q**QS	2p3F	+24
Q**Q**GY***F***P**TS**P**Q**QS	2p4F	+13
Q**Q**GY**Y**P***AS***P**Q**QS	2p6A	+9
Q**Q**GY**Y**P**TT**P**Q**QS	2p7T	+3
Q**Q**GY**Y**P**TA**P**Q**QS	2p7A	–14
Q**Q**GY**Y**P**TS**P***A***QS	2p9A	+29

[a]Anchors in bold. The substituted residue in each sequence from p1 to p9 is designated in italics. The native peptide is designated as 2p.
[b]The total energy for the DQ8–2p complex as a homodimer of heterodimers was 3665 kcal/mol.

values of various complexes of the same MHC molecule bound to a peptide or any of its variants (all in the same register), are generally correlated with the binding affinities of these peptides (26,34–36). As shown in Tables 1 and 2, most preferred substitutions at p1 and p9 for DQ8 lead to significantly lower energy values.

The alignment of the gliadin and glutenin peptides into the groove of HLA-DQ8 was carried out according to the published binding motif for HLA-DQ8 (28–31), resulting in preferred residues at relative positions 1, 4, 6, 7 and 9. For both peptides only one binding register was found that fulfilled the motif at all five pockets. These binding registers are (anchors in bold):

```
            p1 p4 p6 p9
             :  :  :  :
Gliadin     SG**Q**GS**F**Q**PS**Q**Q**N
Glutenin     Q**Q**GY**Y**P**TS**P**Q**QSG
```

Therefore, in both DQ8–peptide complexes, identical amino acids are found at p1, p2, p7 and p9, whereas an aromatic amino acid is found at p4. The conformation of the glutenin peptide, however, is rather different from that of the gliadin peptide as a result of the presence of two prolines (at p5 and p8) in the former, while the latter contains only one proline, at p6 (Fig. 3).

The p1 and p9 pockets both accommodate a Q residue. The p1 pocket can also accept E, A, N or even K which is an indication of the remarkable versatility of this pocket in HLA-DQ8 and other -DQ alleles (Tables 1 and 2) (30–32, 35–38). Pocket p9 is mostly favorable to acidic and to polar groups, essentially due to the presence of α79R and β57A. The native Q in the gluten peptides is well accommodated, as is A and N (Tables 1 and 2). A Q $\rightarrow$ E substitution at either p1 or p9 in the gliadin peptide leads to a significant decrease in minimized total energy (Table 1). A double Q $\rightarrow$ E substitution at p1 and p9 results in a remarkable decrease in energy (137 kcal/mol) of the modeled DQ8–gliadin-p1/9E complex as compared to the native peptide–DQ8 complex (Table 1), consistent with the increased sensitivity of the T cell clone to the Q $\rightarrow$ E substituted gliadin peptide analogues (8,12) (Fig. 4A and B).

Surprisingly, the glutenin peptide with the p9Q $\rightarrow$ E substitution that is predicted both from motif considerations and energy minimization to result in better binding (Table 2), fails to induce T cell proliferation (Fig. 2). Comparison of the orientation of the p9Q and p9E variants of the glutenin peptide indicated no significant movements of any of the putative TCR contact residues of DQ8 or the glutenin peptide. However, a slight movement by 1.15 Å of Oε2 and 0.67 Å of Oε1 carboxylate oxygens relative to the nitrogen and oxygen respectively of the glutamine amide was observed within the p9 pocket. This could account for the non-stimulatory properties of the peptide analogue.

The p4 pocket is spacious because of the β13G residue in DQ8 (25,30) and here it accommodates a F (gliadin) or a Y (glutenin). In fact, a Y residue is predicted as rather preferred at this position (Tables 1 and 2), but a F $\rightarrow$ Y substitution in the gliadin peptide or a Y $\rightarrow$ F substitution in the glutenin peptide prohibits T cell recognition (Figs 1 and 2). The minimized conformation shows considerable residue rearrangement in this pocket depending on the presence of either a F or a Y. In the case of p4Y, the TCR contact residues β70R and β74E approach the more hydrophilic p4Y, with the γ-carboxylate of β74E forming a hydrogen bond with the hydroxyl group of p4Y, while at the same time maintaining the β70R–β74E salt bridge (Fig. 4D). By contrast, in the case of p4F, the β74E carboxylate points away from the hydrophobic benzyl ring of p4F (Fig. 4C). In fact, in this case

the methylene groups of β28T, β70R and β71T make contact with the benzyl ring of p4F, maximizing the extent of hydrophobic interactions. Thus, the different set of interactions among the p4 anchor (F/Y) and DQ8 residues of the p4 pocket (β28T, β70R, β71T, β74E and β78V) result in a different overall conformation of the DQ8–peptide complex, which is likely to influence T cell recognition.

The sixth and the seventh pockets of the two epitopes are occupied by either a P and an S (gliadin) or by a T and an S (glutenin) respectively. Whereas substitutions of the P and the S in the gliadin peptide prohibit T cell recognition (Fig. 1), the p6T and the p7S in the glutenin peptide can be replaced by A/S and T respectively without loss of T cell recognition (Fig. 2). The p6T of glutenin is partly covered by the α chain residues of DQ8 (not shown). Therefore, the semi-conservative substitutions by S and A allow the same relative orientation and do not yield any changes in the minimized energy (Table 2 and Fig. 3B and D). By contrast, the p7S of the same peptide is exposed and thus a potential TCR contact (Fig. 3B). While the p7S → A substitution yields a lower energy and consequently predicts favorable binding (Table 2), the resulting complex is not recognized by the T cell clone. On the contrary, the p7S → T substitution is favorable both for predicted binding (Table 2) and T cell proliferation (Fig. 2), indicating that the β-hydroxyl group of S/T at p7 is necessary for recognition by the glutenin-specific T cell clone.

DQ allelic polymorphism and T cell recognition

The CD-associated DQ8 molecule is structurally highly similar to the non-associated DQ9 allele. This DQ9 allele differs from the DQ8 allele by only one amino acid residue (D versus A respectively at position β57) which changes the nature of the preferred residue at pocket 9 [acidic or uncharged polar for DQ8, hydrophobic for DQ9 (30–32,36,37)]. The peptide binding motif of DQ9 is therefore predicted to be identical to that of DQ8 at p1, p4, p6 and p7 but to differ at p9 (30–32,36), which was confirmed by modeling of the gliadin peptide in DQ9 (data not shown). Consequently, DQ9 can present both the gliadin and the glutenin peptide to the peptide-specific T cell clones (Fig. 5 and data not shown). To analyze the influence of D at position β57 in DQ9 we examined the dose-dependence of T cell responses against the gliadin peptide and its p1 and p9 Q → E analogues. We observed that, as with DQ8, the p1Q → E analogue is more efficiently recognized in the context of DQ9 compared to the wild-type peptide (Fig. 5). As expected, significantly reduced T cell activation was found for the p9Q → E analogue in the context of DQ9 at all peptide concentrations. Surprisingly, the negative effect of the p9Q → E substitution can be compensated by a simultaneous p1Q → E substitution. We note, however, that despite the improved recognition of the p1E- and p1/9E-gliadin analogue–DQ9 complexes by the S2 clone, compared to the wild-type peptide counterparts, these complexes are recognized with 10-fold lower sensitivity as compared to the DQ8–gliadin-p1E or -p1/9E complexes (Fig. 5 and fig. 1 of ref. 12). By contrast, the native peptide is recognized with the same sensitivity by the S2 clone in the DQ8 and the DQ9 contexts.

Minimization of the DQ9–gliadin-p1/9E complex yielded a stable arrangement of p9E and the DQ9 residues constituting pocket 9, with the carboxylate group of the p9E forming a salt bridge with α79R and a hydrogen bond with β9Y (Fig. 6). This is most probably a case where the very good fit of the peptide residues at p1 and p4 of DQ9 is able to overcome the poor accommodation at p9, leading to a complex recognized by the gliadin-specific T cell clone. No differences were found in T cell recognition of the glutenin peptide in the context of DQ8 versus DQ9 (data not shown).

While the gliadin and the glutenin peptides fit with the same unique alignment into the grooves of DQ8 and DQ9, there seems to be no possible favorable alignment for the gliadin peptide, or any of its mutants listed in Fig. 1, in the groove of DQ7, making it essentially impossible for this to be recognized productively by the DQ8-restricted S2 T cell clone in such a context (proliferation and modeling data not shown).

Discussion

The peptide binding motif for HLA-DQ8 is characterized by the preference for negatively charged and, secondarily, polar residues at p1 and negatively charged residues at p9 (30–32,36). The preference for a negatively charged residue at p9 is a unique feature of DQ8, while other β57Asp$^-$ DQ alleles show additional preferences for other residues at this pocket (26,37). The highly homologous molecules HLA-DQ7 and -DQ9 have a negatively charged aspartate at position β57 that most probably interacts with the oppositely situated α79Arg (24–29,31,34,38–41). HLA-DQ8 carries instead an alanine at β57, leaving the α79Arg free to interact with a negatively charged p9 residue. In accordance with this preference for a negatively charged residue at p9, the p9Q → E variant of the gliadin peptide has a strongly increased DQ8-restricted T cell stimulatory capacity (12 and this work), while only a poor T cell response was seen for this variant peptide in the context of DQ9.

By contrast, the glutenin p9Q → E analogue activated the glutenin peptide-specific T cell clone only marginally, both in the DQ8 and the DQ9 context. The p9E glutenin peptide analogue is predicted by modeling to bind to DQ8 with high affinity and to result in little change in the overall structure of the complex. The poor T cell stimulatory capacity of the DQ8–glutenin-p9E peptide complex must therefore affect T cell recognition in subtle ways. Similar results have been observed in other cases where a change in the anchor residue (still resulting in a binding peptide) yielded a complex not recognized by the cognate T cell clone (42–44). The implications for disease pathogenesis of the contrasting recognition patterns of the gliadin- and glutenin-specific T cell clones regarding the p9Q → E substitution remain to be established. Essentially, the degree to which these two clones contribute to the pathogenesis of CD in DQ8$^+$ patients will determine the importance of the epitope transformation to the disease process.

The dramatically improved responsiveness of the S2 T cell clone to the DQ9–gliadin-p1E or -p1/9E peptide complexes was still 10 times lower than what is observed with the corresponding DQ8 complexes. This is probably due to the presence of β57Asp in DQ9 that probably influences recognition of such complexes by the specific DQ8-restricted TCR. An analogous situation to the double recognition in the

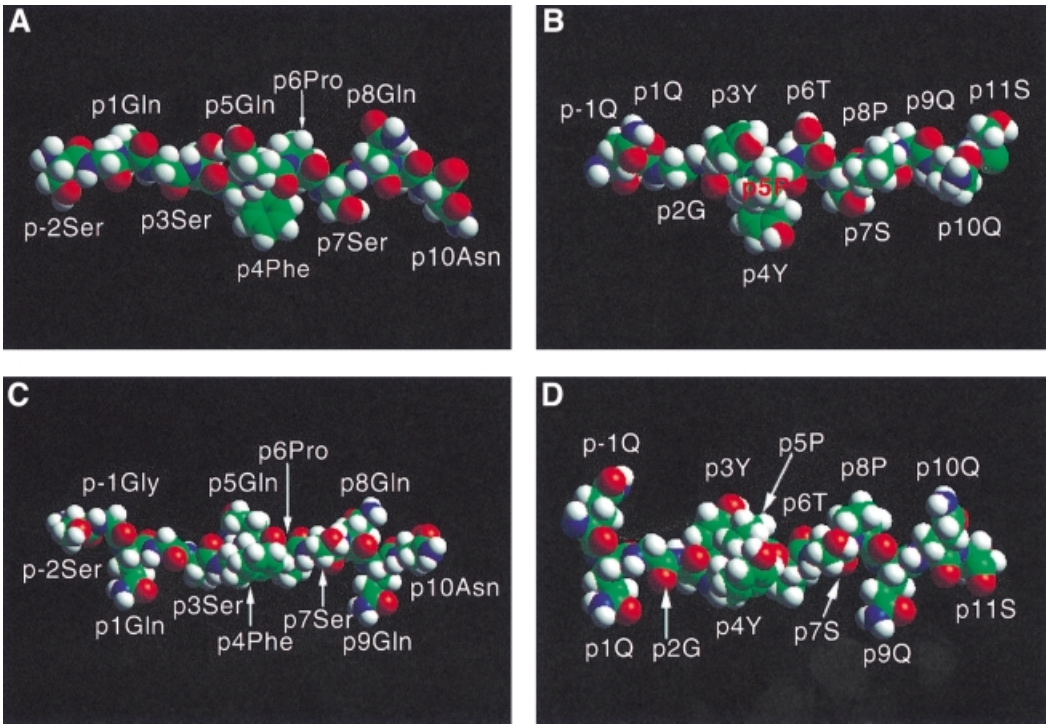
A
p1Gln
p5Gln
p6Pro
p8Gln
p-2Ser
p3Ser
p4Phe
p7Ser
p10Asn
B
p-1Q
p1Q
p3Y
p6T
p8P
p9Q
p11S
p5P
p2G
p4Y
p7S
p10Q
C
p6Pro
p-1Gly
p5Gln
p8Gln
p-2Ser
p10Asn
p3Ser
p1Gln
p4Phe
p7Ser
p9Gln
D
p-1Q
p5P
p3Y
p8P
p10Q
p6T
p11S
p1Q
p2G
p4Y
p7S
p9Q

Fig. 3

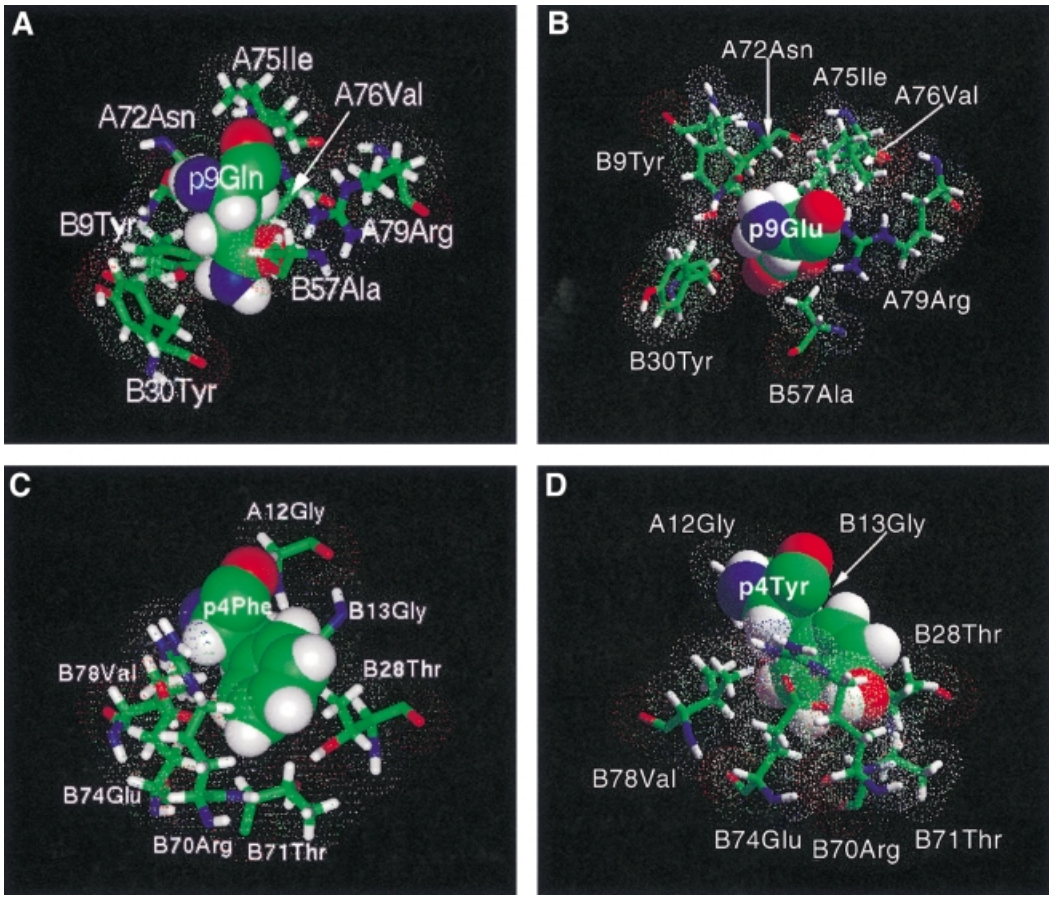
A
A75Ile
A76Val
A72Asn
p9Gln
B9Tyr
A79Arg
B57Ala
B30Tyr
B
A72Asn
A75Ile
A76Val
B9Tyr
p9Glu
A79Arg
B30Tyr
B57Ala
C
A12Gly
p4Phe
B13Gly
B78Val
B28Thr
B74Glu
B70Arg
B71Thr
D
A12Gly
B13Gly
p4Tyr
B28Thr
B78Val
B74Glu
B70Arg
B71Thr

Fig. 4

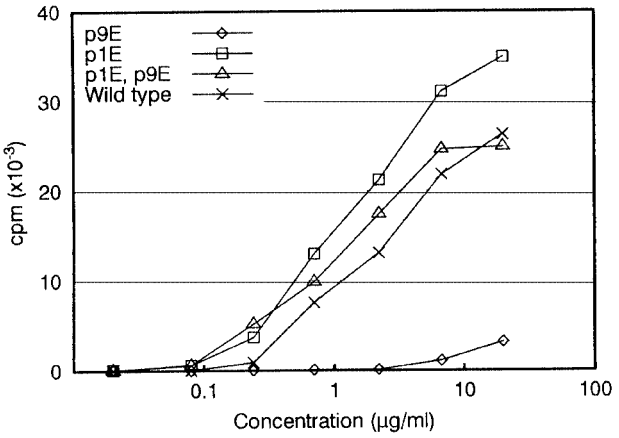

Fig. 5. Dose–response curves of wild-type (WT) gliadin 206–217 peptide, and three of its substituted analogues (p1E, p9E and p1/9E), in the context of DQ9. Conditions are identical to those in Fig. 1.

DQ8 and DQ9 context of the gliadin peptide and some of its p1 and/or p9 analogues by the S2 clone has not been reported before, to our knowledge, for any other peptide. Testing of p9Ala and Leu variants of the herpes simplex virus 2, VP-16-derived peptide (native p9Asp) showed that these variants could achieve recognition by the cognate T cell clone in the DQ9 but not in the DQ8 context, despite adequate binding of these peptides to DQ8 (31). All peptide extraction and binding and T cell proliferation work with mouse I-A/E and human HLA-DQ/R alleles has shown that acidic residues are favored at pocket 9 only when the relevant MHC class II allele is β57Asp$^-$ (13,20,30–32,36–38). A close examination of the crystal structure of several MHC class II alleles (thus far all β57Asp$^+$), shows that the β57Asp residue is positioned in such a way as to maximize its charge–charge interaction with the oppositely positioned α76Arg, and in most cases to form a hydrogen bond with the p10 amide from the bound antigenic peptide (25,29,34,39,40). In order to fit into the p9 pocket of a β57Asp$^+$ MHC II allele, a negatively charged D/E residue would have to get past the small 'opening' of the pocket that contains the β57Asp barrier, an energetically very

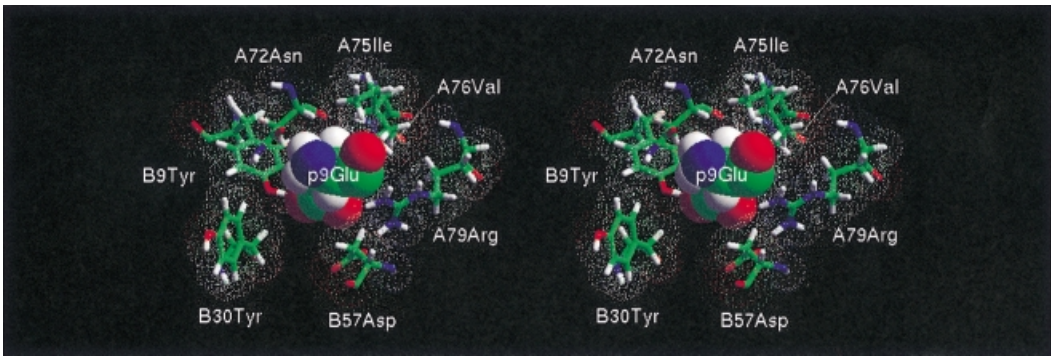

Fig. 6. The arrangement of p9Glu of the double-substituted gliadin peptide, p1/9E, in pocket 9 of DQ9, shown in stereo mode in order to better appreciate this unique setting. View from the β1 helix at the level of the β-sheet floor. There is very close contact between the pairs of p9Glu and α79Arg, p9Glu and β9Tyr, β57Asp and α79Arg, and minimal repulsion (maximum distance) between the side-chain carboxylates of p9Glu and β57Asp. This is the first reported fit of an acidic residue in this pocket in any MHC II β57Asp$^+$ allele. Color code as in Fig. 3.

Fig. 3. (A and B) The two antigenic peptides, as bound to DQ8 after energy minimization and viewed from above (TCR view). The glutamine residues (Q) in the p1 and p9 anchors are not visible because they point into the groove. (A) The gliadin peptide. (B) The glutenin peptide. The residues are in van der Waals solid-rendered form. In the gliadin peptide–DQ8 complex the surface from DQα residues covers completely the p2Gly, and p3Ser residues of the antigenic peptide (not shown). Both peptides are viewed from the same angle. In the glutenin peptide p6Thr is partly buried while p–1Gln, p3Tyr, p5Pro, p7Ser, p8Pro and p10Gln are completely exposed and potentially accessible to TCR. (C and D) The two antigenic peptides as bound to DQ8 and viewed from the side of the β1 helix, at the level of the β-sheet floor. (C) The gliadin peptide. (D) The glutenin peptide. Note the nearly identical orientation of identical residues in the same pockets. Color convention: carbon, green; oxygen, red; nitrogen, blue; hydrogen, white.

Fig. 4. Fitting of gliadin and glutenin peptide residues (native or mutated) into individual DQ8 pockets. (A and B) Pocket 9 of the DQ8–gliadin peptide complex in the native (A), and p9Gln → Glu form (B) as viewed from the side of the β1 helix at the level of the β-sheet floor. The introduction of a negatively charged carboxylate in place of the amide group at this pocket strengthens the peptide–DQ8 interactions. In particular, the presence of the glutamate at p9 causes the formation of a salt bridge between one of its carboxylate oxygens and the guanidine group of α79Arg. The other carboxylate oxygen of p9Glu forms a hydrogen bond with the hydroxyl group of β9Tyr. (C and D) Pocket 4 of the gliadin and glutenin peptides complexed with DQ8. (C) The gliadin peptide (p4F). (D) The glutenin peptide (p4Y). The peptide residue is in space filling form, while the DQαβ residues are in stick and dot-surface representation. Note that in (C) the aliphatic part of β70Arg makes contact with the aromatic ring of p4Phe and so does the methyl group of β28Thr. By contrast, this hydrophobic benzyl ring of p4Phe is far away from the charged ends of β70Arg and β74Glu, while in (D) these two groups make contact with the hydroxyl group of p4Tyr. This is a classic case where a bulky residue at p4 will fit well in DQ8, but hardly at all in DQ7. The main reason for this is the presence of β13Gly and β26Leu in contrast to the bulkier Ala and inflexible Tyr respectively, at these two positions in DQ7. Color conventions as in Fig. 3.

unfavorable process. This is so both in the artificial situation of binding given peptides to purified MHC class II molecules, as well as the natural situation where the loading of peptides to such molecules in the endosome is aided by the HLA-DM and -DO molecules (45,46). Evidence regarding the latter situation is provided by the many peptide extraction studies, where in no β57Asp$^+$ MHC class II alleles are there detectable acidic residues at p9 (reviewed in 37). The binding of the doubly substituted p1/9Q → E gliadin peptide to DQ9, in contrast to the p9Q → E peptide, is most probably due to the favorable set of interactions at p1 (E) and p4 (F) that are capable of overcoming the unfavorable interaction arising from p9E. The lower sensitivity of the S2 clone to the DQ9–gliadin-p1E and -p1/9E complexes, however, indicates that these complexes would not cause as much proliferation as their DQ8–peptide counterparts *in vivo*. Assuming that S2 is one of the major clones involved in CD pathogenesis, the lower sensitivity could be part of the reason for the non-association of DQ9 with CD.

The energy minimization studies used in this work to deduce the binding propensities of various peptide variants of the gliadin and glutenin epitopes have been validated in a number of binding/modeling studies in the past few years (26,33,35,47–49). Indeed it is possible to detect the subtleties of the binding propensities of the major pockets of MHC II molecules and also to generally correlate good peptide fit into a specific groove with the lower minimized energy value of the corresponding complex. Recently the crystal structures of two mouse I-A alleles (highly homologous to HLA-DQ) were published and the respective coordinates became available (39,40). It was thus of interest to compare these crystal structures to the modeled structures of HLA-DQ. The β chain of HLA-DQ resembles more the corresponding chain of HLA-DR than that of H-2/I-A, as evidenced by the absence of the β65–66 deletions (seen in about half of all I-A alleles), as well as absence of the β84a insertion (evident in the I-A^d and I-A^k crystal structures and most probably present in the same position in all other I-A alleles) in any HLA-DQB alleles thus far. By contrast, the α chain of HLA-DQ is more similar to that of I-A, as evidenced by the existence of the β bulge at α9, predicted by Brown *et al.* (50), but apparently ignored by most modeling studies of mouse I-A and human HLA-DQ alleles. Because of the β bulge and the small side chains occupying the β sheet on the first strand of the β chain, the antigenic peptide bends slightly into the groove (25,26 and this work). By not incorporating this β bulge structure into our modeling, we have obtained a larger than expected bend of the antigenic peptide into the groove. There are also two hydrogen bonds that were missed by our modeling (α9 carbonyl to p4NH and α68His to p9 carbonyl). However, these changes do not alter the character of any of the pockets, nor the nature of the interactions between peptide anchor residues and DQ8/9 allelic residues, or the conclusions reached based on modeling with HLA-DR1 as the reference structure.

In summary, we report here on the T cell clonal recognition properties of two DQ8-restricted CD-associated epitopes, one from gliadin and the other from glutenin. Both peptides can fit into the HLA-DQ8 groove in one unique register consistent with the binding motif of this allele. The gliadin epitope is very sensitive to any substitution in the p2 to the p8 positions, yet Q → E substitutions at p1 or p9, or both positions, lead to an increase in sensitivity of the cognate S2 T cell clone. By contrast, the glutenin epitope allows some degeneracy of recognition in positions p3, p6 and p7, shows equally good recognition for a p1Q → E analogue, yet exhibits no proliferation for the p9Q → E analogue. Since both we and others have recently established that transglutaminase can selectively change the p1 and p9 Q residues into E for these two epitopes (10–12), these substitution studies attain special significance for disease pathogenesis. The molecular modeling studies performed on these DQ8–peptide complexes indicate that all substitutions into TCR contact residues, as well as conservative substitutions into anchor residues, result in energies that signify binding of the relevant peptide to the DQ8 molecule. Therefore, the extensive non-recognition by the T cell clone noted, especially for the gliadin peptide analogues, must rest with the exquisite recognition requirements of this particular TCR for the peptide–DQ8 complex. The recognition of these antigenic peptides and their analogues by the very closely related DQ9 allele runs a parallel course with DQ8-restricted recognition of these peptides. However, the gliadin p9Q → E analogue is not recognized by the S2 T cell clone in the DQ9 complex, while the double-substituted p1/9Q → E analogue stimulates the S2 clone in the same context. The binding of this peptide analogue to DQ9 is in sharp contrast to what has been observed with several β57Asp$^+$ MHC II alleles, and it is allowed in this case probably because of the very favorable interactions at p1E and p4F. The extensive analysis of T cell recognition and the molecular conformation of the DQ8/9–peptide complexes allow for further understanding of the pathogenesis of DQ8-linked CD, at the level of these two T cell clones. This is especially significant as other DQ8-restricted T cell clones established previously from CD patients by another laboratory also recognize the gliadin peptide (8,12). As the HLA-DQ8/9 alleles are also associated with susceptibility to another autoimmune disease (type 1 diabetes), insight gained from the HLA-DQ8/9–peptide interactions here may be of benefit in the understanding of the role of these alleles in the new disease setting as well (19,51,52).

While this manuscript was under revision the first crystal structure of an MHC II peptide–TCR complex was published (53), indicating an orthogonal instead of the diagonal mode of interaction between MHC II–peptide and TCR. The diagonal mode was established for several MHC I–peptide–TCR complexes (reviewed in 54). In the structure published there is little if any contact between p–1 or p10 residues and TCR (53). While the orthogonal mode of interaction between MHC II–peptide and TCR may not always hold (54), it is also possible that changes of peptide residues in such positions cause slight alterations in the MHC II–peptide structure, thus affecting TCR recognition.

Acknowledgements

The studies reported in this work have been supported in part by a grant from the Biotech program of the European Union (contract no. BIO4 CT95 0263) to F. K. and G. K. P., and a matching grant from the General Secretariat of Research and Technology of Greece to G.

K. P. We also thank Dr Nicolaos A. Papandreou for his work in comparing the alignment of the DQ8-p9E–glutenin complex to that of the DQ8–native glutenin complex.

Abbreviations

CD	celiac disease
DQ2	DQA1*0501/B1*0201
DQ7	DQA1*0301/B1*0301
DQ8	DQA1*0301/B1*0302
DQ9	DQA1*0301/B1*0303

References

1 Tighe, M. R., Hall, M. A. and Barbado, M. 1992. HLA class II alleles associated with celiac disease susceptibility in a Southern European population. *Tissue Antigens* 40:90.

2 Sollid, L. M., Markussen, G. M. and Ek, J. 1989. Evidence for a primary association of coeliac disease to a particular HLA-DQ alpha/beta heterodimer. *J. Exp. Med.* 169:345.

3 Spurkland, A., Ingvarsson, G., Falk, E. S., Knutsen, I., Sollid, L. M. and Thorsby, E. 1997. Dermatitis herpetiformis and celiac disease are both primarily associated with the HLA-DQ(α1*0501, β1*02) or the HLA-DQ(α1*03, β1*0302) heterodimers. *Tissue Antigens* 49:29.

4 Spurkland, A., Sollid, L. M., Polanko, I., Vardal, F. and Thorsby, E. 1992. HLA-DR and -DQ genotypes of celiac disease patients serologically typed to be non-DR3 and non-DR-5/7. *Hum. Immunol.* 35:188.

5 Tighe, M. R., Hall, M. A., Ashkenazi, A., Siegler, E., Lanchbury, J. S. and Ciclitira, P. J. 1993. Celiac disease among Ashkenazi Jews from Israel: a study of the HLA class II alleles and their associations with disease susceptibility. *Hum. Immunol.* 38:270.

6 Lundin, K. E. A., Scott, H., Hansen, T., Paulsen, G., Halstensen, T. S., Fausa, O., Thorsby, E. and Sollid, L. M. 1993. Gliadin-specific HLA-DQ2(α1*0501, β1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J. Exp. Med.* 178:187.

7 Lundin, K. E. A., Scott, H., Fausa, O., Thorsby, E. and Sollid, L. M. 1994. T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognise gliadin when presented by DQ8. *Hum. Immunol.* 41:285.

8 van de Wal, Y., Kooy, Y. M. C., van Veelen, P. A., Peña, A. S., Mearin, L. M., Molberg, Ø., Lundin, K. E. A., Sollid, L. M., Mutis, T., Benckhuijsen, W. B., Drijfhout, J. W. and Koning, F. 1998. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc. Natl Acad. Sci. USA* 95:10050.

9 Sjöström, H., Lundin, K. E. A., Molberg, Ø., Körner, R., McAdam, S. N., Anthonsen, D., Quarsten, H., Norén, O., Roepstorff, P., Thorsby, E. and Sollid, L. M. 1998. Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand. J. Immunol.* 48:111.

10 van de Wal, Y., Kooy, Y. M. C., van Veelen, P., Vader, W., August, S. A., Drijfhout, J. W., Peña, A. S. and Koning, F. 1999. Glutenin is involved in the gluten-driven mucosal T cell response. *Eur. J. Immunol.* 29:3133.

11 Molberg, Ø., McAdam, S. N., Körner, R., Quarsten, H., Kristiansen, C., Madsen, L., Fugger, L., Scott, H., Norén, O., Roepstorff, P., Thorsby, E., Lundin, K. E. A., Sjöström, H. and Sollid, L. M. 1998. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat. Med.* 4:713.

12 van de Wal, Y., Kooy, Y. M. C., van Veelen, P., Peña, S., Mearin, L., Papadopoulos, G. K. and Koning, F. 1998. Cutting edge: selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. *J. Immunol.* 164:1585.

13 Janeway, C. A., Jr and Travers, P. 1997. *Immunobiology*, 3rd edn, p. 410. Current Biology Press, London.

14 Thorsby, E. and Rønningen, K. S. 1993. Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:371.

15 Sanjeevi, C. B., Höök, P., Landin-Olsson, M., Kockum, I., Dahlquist, G., Lybrand, T. P. and Lernmark, Å. 1996. DR4 subtypes and their molecular properties in a population-based study of Swedish childhood diabetes. *Tissue Antigens* 47:275.

16 Papadopoulos, G. K. 1998. The genetics of type 1 diabetes. *Immunology Today* 19:288.

17 Nabozny, G. H., Baisch, J. M., Cheng, S., Cosgrove, D., Griffiths, M. M., Luthra, H. S. and David, C. S. 1996. HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. *J. Exp. Med.* 183:27.

18 Zanelli, E., Krco, C. J. and David, C. S. 1997. Critical residues on *HLA-DRB1**0402 HV3 peptide for HLA-DQ8-restricted immunogenicity: implications for rheumatoid arthritis predisposition. *J. Immunol.* 158:3545.

19 Sanjeevi, C. B., Lybrand, T. P., DeWeese, C., Landin-Olsson, M., Kockum, I., Dahlquist, G., Sundqvist, G., Stenger, D., Lernmark, Å. and the members of the Swedish Childhood Diabetes Study, 1995. Polymorphic amino acid variations in HLA-DQ are associated with systemic physical property changes and occurrence of IDDM. *Diabetes* 44:125.

20 Marshall, K. W., Liu, A. F., Canales, J., Perahia, B., Jorgensen, B., Gantzos, R. D., Aguilar, B., Devaux, B. and Rothbard, J. B. 1994. Role of polymorphic residues in HLA-DR molecules in allele-specific binding of peptide ligands. *J. Immunol.* 152:4946.

21 Todd, J. A., Bell, J. I. and McDevitt, H. O. 1987. HLA-DQ_β gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329:599.

22 Mantovani, V., Corazza, G. R., Bragliani, M., Frisoni, M., Zaniboni, M. G. and Gasbarrini, G. 1993. Asp57-negative HLA-DQ beta chain and DQA1*0501 allele are essential for the onset of DQw2-positive and DQw2-negative coeliac disease. *Clin. Exp. Immunol.* 91:153.

23 Ohmori, M., Yasunaga, S., Maehara, Y., Sugimachi, K. and Sasazuki, T. 1997. DNA typing of HLA class I (HLA-A) and class II genes (HLA-DR, -DQ and -DP) in Japanese patients with gastric cancer. *Tissue Antigens* 50:277.

24 Stern, L. J., Brown, J. H., Jardetzky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L. and Wiley, D. C. 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* 368:215.

25 Paliakasis, K., Routsias, J., Petratos, K., Ouzounis, C., Kokkinidis, M. and Papadopoulos, G. K. 1996. Novel structural features of the human histocompatibility molecules HLA-DQ as revealed by modeling based on the published structure of the related molecule HLA-DR1. *J. Struct. Biol.* 117:145.

26 van de Wal, Y., Kooy, Y. M. C., Drijfhout, J. W., Amons, R., Papadopoulos, G. K. and Koning, F. 1997. Unique peptide-binding features of the disease-associated DQ(α1*0501, β1*0201) molecule versus the non-disease-associated DQ(α1*0201, β1*0202) allele. *Immunogenetics* 46:484.

27 Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L. and Wiley, D. C. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364:33.

28 Fremont, D. H., Hendrickson, W. A., Marrack, P. and Kappler, J. 1996. Structures of an MHC class II molecule with covalently bound single peptides. *Science* 272:1001.

29 Ghosh, P., Amaya, M., Mellins, E. and Wiley, D. C. 1995. The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. *Nature* 378:457.

30 Kwok, W. W., Nepom, G. T. and Raymond, F. C. 1995. HLA-DQ polymorphisms are highly selective for peptide binding interactions. *J. Immunol.* 155:2468.

31 Kwok, W. W., Domeier, M. E., Johnson, M. L., Nepom, G. T. and Koelle, D. M. 1996. HLA-DQB1 codon 57 is critical for peptide binding and recognition. *J. Exp. Med.* 183:1253.

32 Kwok, W. W., Domeier, M. E., Raymond, F. C., Byers, P. and Nepom, G. T. 1996. Allele-specific motifs characterize HLA-DQ interactions with a diabetes-associated peptide derived from glutamic acid decarboxylase. *J. Immunol.* 156:2171.

33 Johnson, M. S., Srinivasan, N., Sowdhamini, R. and Blundell, T. L. 1994. Knowledge-based protein modeling. *Crit. Rev. Biochem. Mol. Biol.* 29:1.

34 Dessen, A., Lawrence, C. M., Cupo, S., Zaller, D. M. and Wiley, D. C. 1997. X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with peptides from human collagen II. *Immunity* 7:473.

35 Nelson, C. A., Viner, N. J., Young, S. P., Petzold, S. J. and Unanue, E. R. 1996. A negatively charged anchor residue promotes high affinity binding to the MHC class II molecule I-A^k. *J. Immunol.* 157:755.

36 Godkin, A., Friede, T., Davenport, M., Stevanovic, S., Willis, A., Jewell, D., Hill, A. and Rammensee, H.-G. 1997. Use of eluted peptide sequence data to identify the binding characteristics of peptides to the insulin-dependent diabetes susceptibility allele HLA-DQ8 (DQ3.2). *Int. Immunol.* 9:905.

37 Rammensee, H.-G., Friede, T. and Stevanovic, S. 1995. MHC ligands and peptide motifs: a first listing. *Immunogenetics* 41:178. Also on the net: http://www.uni-tuebingen.de/uni/kxi/.

38 Ettinger, R. A. and Kwok, W. W. 1998. A peptide binding motif for HLA-DQA1*0102/B1*0602, the class II MHC molecule associated with dominant protection in insulin-dependent diabetes mellitus. *J. Immunol.* 160:2365.

39 Fremont, D. H., Monnaie, D., Nelson, C. A., Hendrickson, W. A. and Unanue, E. R. 1998. Crystal structure of I-A^k in complex with a dominant epitope of lysozyme. *Immunity* 8:305.

40 Scott, C. A., Peterson, P. A., Teyton, L. and Wilson, I. A. 1998. Crystal structures of two I-A^d-peptide complexes reveal that high affinity can be achieved without large anchor residues [*Immunity* 8:531]. *Immunity* 8:319.

41 Nepom, B. S., Nepom, G. T., Coleman, M. and Kwok, W. W. 1996. Critical contribution of beta chain residue 57 in peptide binding ability of both HLA-DR and -DQ molecules. *Proc. Natl Acad. Sci. USA* 93:7202.

42 Kersch, G. J. and Allen, P. M. 1996. Structural basis for T cell recognition of altered peptide ligands: a single T cell receptor can productively recognize a large continuum of related ligands. *J. Exp. Med.* 184:1259.

43 Reay, P. A., Kantor, R. M. and Davis, M. M. 1994. Use of global aminoacid replacements to define the requirements for MHC binding and T cell recognition of moth cytochrome *c* (93–103). *J. Immunol.* 152:3946.

44 Evavold, B. D., Sloan-Lancaster, J., Hsu, B. L. and Allen, P. M. 1993. Separation of T helper 1 clone cytolysis from proliferation and lymphokine production using analog peptides. *J. Immunol.* 150:3131.

45 Morris, P., Shaman, J., Attaya, M., Amaya, M., Goodman, S., Bergman, C., Monaco, J. J. and Mellins, E. 1994. An essential role for HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature* 368:551.

46 Kropshofer, H., Vogt, A. B., Thery, C., Armandola, E. A., Li, B.-C., Moldenhauer, G., Amigorena, S. and Hammerling, G. J. 1998. A role for HLA-DO as a co-chaperone of HLA-DM in peptide loading of MHC class II molecules. *EMBO J.* 17:2971.

47 Vartdal, F., Johansen, B. H., Friede, T., Thorpe, C. J., Stevanovic, S., Eriksen, J. E., Sletten, K., Thorsby, E., Rammensee, H.-G. and Sollid, L. M. 1996. The peptide binding motif of the disease associated HLA-DQ (α1*0501, β1*0201) molecule. *Eur. J. Immunol.* 26:2764.

48 Gregori, S., Trembleau, S., Penna, G., Gallazzi, F., Hammer, J., Papadopoulos, G. K. and Adorini, L. 1999. A binding motif for I-E^{g7}, the MHC class II molecule which protects NOD-Eα transgenic nonobese diabetic mice from autoimmune diabetes. *J. Immunol.* 162:6630.

49 Moustakas, A. K., Routsias, J. and Papadopoulos, G. K. 2000. Modelling of the MHC II allele I-A^{g7} of NOD mouse: pH-dependent changes in specificity at pockets 9 and 6 explain several of the unique properties of this molecule. *Diabetologia*, 43:609.

50 Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J. and Wiley, D. C. 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* 332:845.

51 Routsias, J. and Papadopoulos, G. K. 1995. Polymorphic structural features of modelled HLA-DQ molecules segregate according to susceptibility or resistance to IDDM. *Diabetologia* 38:1251.

52 Nepom, G. T. and Kwok, W. W. 1998. Molecular basis for HLA-DQ associations with IDDM. *Diabetes* 47:1177.

53 Reinherz, E. L., Tan, K., Tang, L., Kern, P., Liu, J.-h., Xiong, Y., Hussey, R. E., Smolyar, A., Hare, B., Zhang, R., Joachimiak, A., Chang, H.-C., Wagner, G. and Wang, J.-h. 1999. The crystal structure of a T cell receptor in complex with peptide and MHC Class II. *Science* 286:1913.

54 Wilson, I. A. 1999. Class-conscious TCR? *Science* 286:1867.