Complexes of Two Cohorts of CLIP Peptides and HLA-DQ2 of the Autoimmune DR3-DQ2 Haplotype Are Poor Substrates for HLA-DM

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Complexes of Two Cohorts of CLIP Peptides and HLA-DQ2 of the Autoimmune DR3-DQ2 Haplotype Are Poor Substrates for HLA-DM

Lars-Egil Fallang,* Sujin Roh,‡ Anders Holm,* Elin Bergseng,† Taejin Yoon,‡ Burkhard Fleckenstein,* Arunima Bandyopadhyay, Elizabeth D. Mellins,2,3§ and Ludvig M. Sollid2,3,§†

Atypical invariant chain (II) CLIP fragments (CLIP2) have been found in association with HLA-DQ2 (DQ2) purified from cell lysates. We mapped the binding register of CLIP2 (II 96–104) to DQ2 and found proline at the P1 position, in contrast to the canonical CLIP1 (II 83–101) register with methionine at P1. CLIP1/2 peptides are the predominant peptide species, even for DQ2 from HLA-DM (DM)-expressing cells. We hypothesized that DQ2-CLIP1/2 might be poor substrates for DM. We measured DM-mediated exchange of CLIP and other peptides for high-affinity indicator peptides and found it is inefficient for DQ2. DM-DQ-binding and DM chaperone effects on conformation and levels of DQ are also reduced for DQ2, compared with DQ1. We suggest that the unusual interaction of DQ2 with II and DM may provide a basis for the known disease associations of DQ2.


Materials and Methods

Cell lines

The following EBV-transformed B lymphoblastoid cell lines (B-LCL) were used in this study: CD114 (from a celiac disease patient) expressing DQ2 and DR3; 2.2.93 expressing DP4, DQ4, DR1, DR3, and no DM (7); 8.1.6 expressing DP4, DQ2, and DR3 (8); 9.5.3 expressing DP4, DQ2, DR3, and no DM (8); 3.1.13 expressing DP4 and DQ1 (9); and 9.22.3 expressing DR4 and DQ2 (8). The DR3-expressing cells also express DR52a. The DM transfectants of 2.2.93 and 9.5.3 (2.2.93-DM and 9.5.3-DM, respectively) were generated by retroviral transduction as previously described (7).

Antibodies

Abs used in this study were B8.11 (IgG2b, anti-DR, gift from B. Malissen (Centre d’immunologie de Marseille Luminy, Marseille, France)), L243 (IgG2a, anti-DR), ISCR3 (IgG3, anti-DR), SPV-L3 (IgG2a, anti-DQ, gift from H. Spits (University of Amsterdam, Amsterdam, The Netherlands)), Ia3 (IgG2a, anti-DQ; Biodiagn), 2.12.E11 (IgG1, anti-DQ2), B7/21.2 (IgG3, anti-DP), XD5.A11 (IgG1, anti-class II, gift from P. Cresswell (Yale University School of Medicine, New Haven, CT)), SC1 (IgG1, anti-DM), 16.23 (IgG3, anti-DR3), anti-HLA-DM conjugated with PE (IgG1; BD Pharmingen), CerCLIP (IgG1, anti-human CLIP, gift from P. Cresswell).

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Flow cytometry
For cell surface staining, cells were incubated (40 min on ice) with primary Abs and washed. Bound Ab was detected by incubation (40 min on ice) with goat F(ab')2, anti-mouse IgG (H + L) conjugated with FITC or PE (Caltag Laboratories). For intracellular staining, cells were fixed and permeablized using the Cytofix/Cytoperm kit (BD Pharmingen) and then stained using PE-conjugated Ab. Cells were analyzed using a FACSscan flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (Tree Star).

Pulse-chase immunoprecipitation
Cells were washed three times and starved for 1–2 h in methionine/cysteine-free RPMI 1640 containing 10% dialyzed FBS (Invitrogen). Cells were cultured in RPMI 1640 containing 0.5% FBS and 2 mM-glutamine (37°C, 5% CO2). Aliquots of cells were collected and washed at the indicated time points and lysed in buffer (Tris-HCl (pH 8.0) with MgCl2, 1% Nonidet P-40, and complete protease inhibitors (Roche Diagnostics)) at 4°C for 1–2 h. Lysates were precleared three times with normal mouse serum and Pansorbin (Calbiochem) and then incubated with antibodies against class II molecules coupled to protein-A or protein-G-Sepharose beads according to the manufacturer’s instructions. The water-soluble DQ2 molecules were affinity purified using the mAbs 2.12.E11 or BglII (DR) and BglIII (DQ) restriction enzymes. The oligonucleotides used for the construction of the molecules can be found in Table I. Constructs were verified by DNA sequencing. The sDQ2 and sDR3 molecules were affinity purified using the mAbs 2.12.E11 or B8.11, respectively.

Generation of sDM
sDM molecules were produced in stably transfected S2 cells and purified byisonofluorin affinity chromatography and size exclusion chromatography (11).

Fluid-phase peptide-binding assay
Detergent-solubilized DQ2 and DR3 molecules were purified from the CD114 cell line and used in a fluid-phase competitive inhibition assay with [125I]-labeled indicator peptides as previously described (12). Briefly, labeled indicator peptides (KPLLIIAEDVEGYE; MB 65-kDa Hsp 243–255Y or EPRAPWIEQEGPWEY; HLA class I α 46–60) for DQ2 and KTIAYDEEARR; MT 65-kDa 3–13 for DR3) and unlabeled peptides were incubated with sDM or DR3 molecules overnight at 37°C in a pH 4.9 citrate phosphate buffer containing 0.05% of peptides of peptide and DQ2/DR3 molecules were separated from unbound peptides by spin column chromatography. Radioactivity was determined, and the concentrations of the competing peptides required for half-maximal inhibition of the binding of the indicator peptide (IC50) were calculated.

Peptide-binding assays measuring the DM effect using water-soluble peptide-linked molecules were done in a similar manner. Molecules (2 μg) were treated with 1/10 U of thrombin (Novagen) for 90 min at room temperature followed by inhibition with 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (Sigma-Aldrich). The cleaved molecules were incubated overnight at 37°C in a pH 4.3 citrate phosphate buffer in the presence or absence of various amounts of sDM (as indicated in the figures) and, in some experiments, noncleaved molecules (0–7 μM) as well, along with the [125I]-labeled indicator peptides and protease inhibitors.

Solid-phase peptide exchange assay
HLA class II molecules captured from cell lysates were used for a peptide exchange assay (13). Briefly, clarified cell lysates of 0.5 × 10^6 cells of B-LCL 8.1.6 and 9.5.3 were added to wells coated with either mAb 2.12.E11 (anti-DQ2), or mAb L243 (anti-DR). After incubation at 4°C overnight, the wells were washed, and 2.5 μM biotinylated indicator peptides were added (identical peptide sequences as in fluid-phase binding assay) in a pH 4.3 citrate phosphate buffer containing protease inhibitors. To adjust for the amount of captured DQ2 and DR3, biotinylated mAb B8.11 (anti-DR) or SPV-L3 (anti-DQ) in pH 7.4 citrate phosphate buffer was added to the relevant wells and incubated for 48 h at 37°C. The wells were washed and streptavidin-europium diluted 1/2000 in assay buffer.

Table I. Oligonucleotides used in the construction of sDQ2-peptide and sDR3-CLIP1 complexes

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>Fwd/Rev</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIP1 oligonucleotides</td>
<td>Fwd^a</td>
</tr>
<tr>
<td>CLIP2 oligonucleotides</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
<tr>
<td>CLIP1 M91A mutagenesis primer</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
<tr>
<td>DRα amplification primers</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
<tr>
<td>DRβ amplification primers</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
<tr>
<td>Fos amplification primers</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
<tr>
<td>Jun amplification primers</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
<tr>
<td>DQB1*0201-CLIP1 + LS amplification primers</td>
<td>Fwd</td>
</tr>
<tr>
<td>Rev</td>
<td>Rev</td>
</tr>
</tbody>
</table>

^a Fwd, Forward; Rev, reverse.
in the presence or absence of 6 mM chloride treatment (1 mM). The cleaved molecules were incubated at 37°C temperature, followed by 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride treatment (1 mM). The cleaved molecules were incubated at 37°C in the presence or absence of 6 mM chloride in a pH 5.0 citrate phosphate buffer for the required length of time. High-affinity competitor peptides were added in excess (35 µM) (MB 65-kDa Hsp 243-255 for DQ2 and MT 65-kDa 3-13 for DR3). The dissociation was stopped by neutralizing the sample with cold citrate phosphate buffer (pH 7.2). Released peptides (CLIP1: RDSGPVSKRMATMPPLMQAGAGSLVPR, CLIP2: RDSGMATPLQALPMGAGLSVPR, CLIP1 M91A: RDSGPVSKMATMPPLMQAGAGSLVPR, and γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γδ γdelta
total eluate from DQ2 is notably high, however. We also confirmed the prior finding that two families of CLIP peptides are associated with DQ2 from B-LCL: conventional CLIP peptides, here termed CLIP1, and unusual CLIP peptides, CLIP2 (see Tables II and III).

CLIP1 peptides are derived from a sequence around Ii 83–101 and contain the Ii 91–99 core-binding motif. CLIP2 sequences, from around Ii 92–107, overlap with, but are C-terminal to, the sequences of CLIP1 peptides. The sequence differences in CLIP1 vs CLIP2 suggested that the peptides bound DQ2 in different frames. CLIP1 has been found to bind in the same frame to all alleles where this has been examined, with M91 in the P1 pocket (16). To identify the binding register of CLIP2 peptides to DQ2, we examined the binding of five 11-mers (Ii 97–107, Ii 96–106, Ii 95–105, Ii 94–104, Ii 93–103) that scan through Ii in positions 93–107 in comparison to a naturally processed form of CLIP2 peptide (Ii 92–107: RMATPLLMQALPMGAL) (4). We used a peptide-binding assay that measures the IC50 value of the test peptide, which is the amount of peptide needed to give 50% inhibition of the binding of a high-affinity indicator peptide. This analysis

### Table II. Identification of the Ii peptides bound to DQ2 and DR3

<table>
<thead>
<tr>
<th>Residues</th>
<th>Sequence</th>
<th>Detected</th>
<th>Detected</th>
<th>Detected</th>
<th>Length</th>
<th>Molecular Mass (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ii81–104</td>
<td>LKPFPKVPVSKRMATPLLMQALPM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24</td>
<td>2674.5</td>
</tr>
<tr>
<td>Ii81–103</td>
<td>LKPFPKVPVSKRMATPLLMQALP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>23</td>
<td>2543.4</td>
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<tr>
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<td>PKPPKVPVSKRMATPLLMQALP</td>
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<td>+</td>
<td>+</td>
<td>22</td>
<td>2430.4</td>
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<tr>
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<td>LKPFPKVPVSKRMATPLLMQQA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>21</td>
<td>2333.3</td>
</tr>
<tr>
<td>Ii93–109</td>
<td>MATPLLMQALPMGALPQ</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>17</td>
<td>1781.9</td>
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<td>MATPLLMQALPMGALP</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>16</td>
<td>1653.9</td>
</tr>
</tbody>
</table>

*Peptides acid eluted from 50 μg of affinity-purified DQ2 and DR3 from 8.1.6 and 9.5.3 B-LCLs were sequenced using LC-coupled ESI-MS/MS to identify all Ii peptide truncations present in at least one of the elutions. The peptides were present in multiple charged states (2- to 5-fold) and the observed molecular mass was calculated by deconvolution.*
demonstrated that Ii 94–104 and Ii 92–107 had similarly low IC50 values, whereas Ii 95–105 and Ii 93–103 had increased IC50 values, consistent with weaker binding (Fig. 2A). This pattern implied that the core-binding region of CLIP2 was Ii 96–104 (PLLMQALPM), with P96 at the P1 position. This conclusion is compatible with the observation that N-terminally extended peptides bind with increased affinity due to hydrogen bond formation between the MHC II main chain and P-1 and P-2 (17).

To confirm the binding frame, we conducted a competitive inhibition binding assay with peptide variants of Ii 94–104, in which a lysine (K) was introduced in the putative P4, P5, or P6 positions (Ii 94–104;M99K, Ii 94–104;Q100K, and Ii 94–104;A101K, respectively). A positively charged lysine would be expected to prohibit binding to DQ2 when introduced at P4 and P6, but not at P5, as the P5 side chain is predicted to face the solvent. As seen in Fig. 2A, the IC50 value of Ii 94–104;Q100K is low, while the Ii 94–104;M99K and Ii 94–104;A101K values are high, consistent with our conclusion that PLLMQALPM (Ii 96–104) represents the core-binding region of CLIP2. The overlapping, but distinct, binding frames of CLIP1 and CLIP2 are shown schematically in Fig. 2B.

High levels of DQ2-Ii complexes in the presence or absence of DM

The finding of abundant CLIP peptides associated with DQ2 in cell lysates of DM-expressing cells led us to ask whether the DQ2-CLIP complexes found in DM-expressing cells represent a novel repertoire. We compared the peptide elution profile of DQ2 purified from 8.1.6 cells (DM expressing) with the elution profile of DQ2 purified from the DM-deficient mutant 9.5.3 derived from 8.1.6. MS analysis revealed that, like DQ2 from 8.1.6 cells, DQ2 from DM-deficient 9.5.3 cells also bound predominantly Ii-derived peptides, representing both CLIP1 and CLIP2 species. In contrast, and as previously reported (18), peptides eluted from DR3 (and DR52a) were predominantly Ii peptides in the absence of DM, whereas a heterogeneous mixture of self-peptides was associated with DR3 (and DR52a) from DM-expressing cells (data not shown). Since DR52a has very low affinity for CLIP (19), the observed CLIP peptides most probably derive from DR3. An overview of the Ii variants associated with DQ2 and DR3 is presented in Table II.

Table III. Quantification of the Ii peptides bound to DQ2 and DR3

<table>
<thead>
<tr>
<th>MHC II Peptide</th>
<th>pmol</th>
<th>SD</th>
<th>MHC II Peptide</th>
<th>pmol</th>
<th>SD</th>
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<tbody>
<tr>
<td>DQ2 (8.1.6)</td>
<td></td>
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</tr>
<tr>
<td>Ii81–104</td>
<td>ND</td>
<td>na</td>
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<tr>
<td>Ii81–103</td>
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<td>31.8</td>
<td>Ii81–103</td>
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<td>Ii82–103</td>
<td>16.5</td>
<td>9.5</td>
<td>Ii82–103</td>
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<td>na</td>
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<td>Ii81–101</td>
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<td>3.2</td>
<td>Ii81–101</td>
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<td>Ii93–109</td>
<td>ND</td>
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<td>Ii93–109</td>
<td>ND</td>
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<tr>
<td>Ii93–108</td>
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<td>1.1</td>
<td>Ii93–108</td>
<td>ND</td>
<td>na</td>
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<tr>
<td>DQ2 (9.5.3)</td>
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<td>DR3 (9.5.3)</td>
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</tr>
<tr>
<td>Ii81–104</td>
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<td>Ii81–103</td>
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<td>14.5</td>
<td>Ii82–103</td>
<td>113.3</td>
<td>22.3</td>
</tr>
<tr>
<td>Ii81–101</td>
<td>88.4 b</td>
<td>35.6</td>
<td>Ii81–101</td>
<td>106.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Ii93–109</td>
<td>3.8</td>
<td>6.5</td>
<td>Ii93–109</td>
<td>ND</td>
<td>na</td>
</tr>
<tr>
<td>Ii93–108</td>
<td>156.3</td>
<td>62.6</td>
<td>Ii93–108</td>
<td>ND</td>
<td>na</td>
</tr>
</tbody>
</table>

*The quantities of the individual Ii peptides as listed above are given, and the data have been summarized in Fig. 3. ND, Not detected; na, not applicable.

b Underestimated due to suboptimal ICPL of eluted peptides from 9.5.3 cells. A rough estimation indicates that about 3 and 8% of total CLIP peptides remained unlabelled when eluted from DQ2 and DR3, respectively.
Spontaneous peptide exchange is equivalent on DQ2 from DM

To more precisely quantify the CLIP peptide pools associated
with DR3 and DQ2 in the presence and absence of DM, both
the eluted peptides and synthetic CLIP peptides of a known amount
were ICPL-labeled and analyzed by nano-LC-coupled Q-TOF-
MS. The peak heights for the light-labeled eluted peptides were
compared with those obtained for the heavy-labeled synthetic
peptides. The results demonstrated that DR3 from DM-deficient
cells had a 25-fold higher level of CLIP peptides compared with
those obtained for the heavy-labeled synthetic peptides. The CLIP level compared with DQ2 from 8.1.6 (Fig. 3). This finding
corroborated that DQ2-CLIP was significantly less affected by
DM than DR3-CLIP.

Spontaneous peptide exchange is equivalent on DQ2 from DM+
or DM− cells

We next investigated the effect of the naturally processed peptide
repertoires associated with DR3 and DQ2 on peptide exchange.
We performed solid-phase peptide-binding assays in which DQ2
and DR3 molecules purified from lysates of 8.1.6 (DM−) and 9.5.3
(DM+) cells were captured by mAbs 2.12.E11 (anti-DQ2) or L243
(anti-DR dimer), and binding of biotinylated peptides to DQ2 or
DR3 was measured. The spontaneous peptide exchange on DR3
originating from DM-null cells was increased >2-fold compared
with DR3 from DM-expressing cells, reflecting the effects of DM
editing of the peptide repertoire for less-exchangeable peptides. In
contrast, peptide exchange on DQ2 from DM-deficient cells was
similar to that observed with DQ2 from the DM-sufficient coun-
terpart (Fig. 4A). These results are consistent with the similarity in
the peptide profiles of DQ2 from 8.1.6 and 9.5.3 cells.

DQ2-CLIP1 and DQ2-CLIP2 display high resistance to DM
action

To more precisely quantify the extent of DM resistance of DQ2-CLIP
compared with DR3-CLIP for peptide exchange, we measured DM-
mediated peptide exchange using a fluid-phase peptide exchange as-
say. Recombinant complexes (sDR3-CLIP1, sDQ2-CLIP1, sDQ2-
CLIP2) were initially generated with tethered peptides attached to the
N terminus of the class II β-chain with a linker including a thrombin
cleavage site (see Materials and Methods). Thrombin-treated, sHLA
molecules were incubated with high-affinity, allele-specific indicator
peptides and increasing DM concentrations before measurement of
binding of the indicator peptides. This in vitro assay allowed us to
increase the DM:DQ2 ratio beyond that achieved in cells (including
the DM transfectants). sDR3-CLIP1 was significantly more sensitive
to DM, showing 50% of maximum peptide exchange at 10- and 20-
fold lower concentrations of DM compared with sDQ2-CLIP1 and
sDQ2-CLIP2, respectively (Fig. 4B).

DQ2 shows reduced binding interaction with DM compared with DQ1

To further explore the interaction of DQ2 and DM, we compared
DQ2 and DQ1 molecules using an in vitro association assay in which
bead-bound, immunoprecipitated DQ was incubated with soluble re-
combinant DM. DQ/DM binding is determined by isolating bead-
associated DQ-DM complexes and detecting DM by immunoblotting
of boiled complexes. With similar input amounts of DQ1 and DQ2 by
silver stain (data not shown) and anti-class II

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sDQ2-CLIP2, respectively (Fig. 4B).
CREASED INTRINSIC STABILITY MAKES A CONTRIBUTION TO THE REDUCED
MORE STABLE THAN sDR3-CLIP1, RAISING THE POSSIBILITY THAT IN-
WAS CALCULATED BY DENSITOMETRIC ANALYSIS OF THE IMAGES IN
IMAGE OF AT LEAST THREE EXPERIMENTS.

FIGURE 5. DQ2 SHOWED REDUCED BINDING INTERACTION WITH DM COMPARED
WITH DQ1. DQ2 AND DQ1 WERE IMMUNOPRECIPITATED WITH SPV-L3 FROM
DM-POSITIVE (DR-NULL) B-LCLs 9.22.3 (DQ2) OR 3.1.3 (DQ1) AND DM-NULL
CELLS 9.5.3 (DQ2) AND 2.2.93 (DQ1), RESPECTIVELY, AND THEN INCUBATED
WITH SOLUBLE RECOMBINANT DM TO ALLOW MOLECULAR INTERACTION. A. INPUT CLASS II
AMOUNT WAS MEASURED BY IMMUNOBLOTTING WITH ANTI-CLASS II β-CHAIN AB
XD5.A11 (TOP) FOLLOWED BY MEASURING THE AMOUNT OF INTERACTING DM BY
IMMUNOBLOTTING WITH ANTI-DMα mAb SC1. SHOWN IS ONE REPRESENTATIVE
IMAGE OF AT LEAST THREE EXPERIMENTS. B. RATIO OF sDM:CLASS II BAND INTENSITY
WAS CALCULATED BY DENSITOMETRIC ANALYSIS OF THE IMAGES IN A: DM+ CELL
LINES: 3.1.3 (DQ1) AND 9.22.3 (DQ2) AND DM-NULL CELLS: 2.2.93 (DQ1) AND
9.5.3 (DQ2).

DQ2-CLIP1/2 COMPLEXES SHOW HIGH INTRINSIC STABILITY AND REDUCED DM EFFECT

Effects of DM ON VARIOUS CLASS II-PEPTIDE COMPLEXES AND CHANGES IN THE PEPTIDE REPERTOIRES OF CELLS EXPRESSING OR LACKING
DM HAVE LED TO THE CONCLUSION THAT INTRINSICALLY MORE STABLE COMPLEXES ARE, IN GENERAL, MORE RESISTANT TO DM (21, 22). THE DIFFERENTIAL EFFECT OF DM ON DQ2-CLIP AND DR3-CLIP COULD REFLECT DIFFERENCES IN THE INTRINSIC STABILITY OF THESE COMPLEXES. ALTERNATIVELY, THE DIFFERENCES IN PEPTIDE EXCHANGE COULD REFLECT REDUCED EFFICIENCY OF DM INTERACTION WITH THE DQ2 ALLELE. THESE TWO POSSIBILITIES ARE NOT MUTUALLY EXCLUSIVE. TO ASSESS THE INTRINSIC STABILITY OF THE DR3 AND DQ2 COMPLEXES WITH CLIP PEPTIDES, WE MEASURED THE SPONTANEOUS DISSOCIATION RATE OF THE CLIP1 AND CLIP2 PEPTIDES FROM sDQ2 AND CLIP1 FROM sDR3 AT pH 5 WITHOUT DM USING THROMBIN-TREATED, SOLUBLE MOLECULES. THE DISSOCIATION WAS MEASURED BY ELUTING THE BOUND PEPTIDES AND QUANTIFYING THEM BY MALDI-TOF MS ANALYSIS, COMPARE THE INTENSITY OF THE ISOTOPIC PEAKS TO AN ADDED INDICATOR PEPTIDE. THE CLIP1 AND CLIP2 PEPTIDES FROM sDQ2 IN THE ABSENCE OF DM DISPLAYED NEARLY IDENTICAL DISSOCIATION RATES (t1/2 = ~140 h), COMPARED WITH THE 2-FOLD FASTER RELEASE OF CLIP1 FROM DR3 (t1/2 = ~61.5 h). Thus, both CLIP complexes with sDQ2 are more stable than sDR3-CLIP1, raising the possibility that increased intrinsic stability makes a contribution to the reduced efficacy of DM-mediated peptide exchange of the DQ2-CLIP complexes compared with DR3-CLIP1. IN THE PRESENCE OF DM, USING A CLASS II:DM MULAR RATIO OF 1:3.8, THE DISSOCIATION OF CLIP1 FROM sDR3 INCREASED 246 TIMES (t1/2 = 0.25 h). DM ALSO ACCELERATED THE RELEASE OF CLIP1 AND CLIP2 FROM sDQ2 (t1/2 = 9.0 h AND t1/2 = 5.0 h, RESPECTIVELY); HOWEVER, THE DM-MEDIATED ENHANCEMENTS OF CLIP1/2 DISSOCIATIONS FROM DQ2 WERE MODEST (16–28 TIMES).

FIGURE 6. RELATIONSHIP BETWEEN INTRINSIC AND DM-CATALYZED DISSOCIA-
TION. DISSOCIATION RATE CONSTANTS, k, CALCULATED FROM THE t1/2 BY THE EQUATION
t1/2 = ln2/k, ARE PLOTTED ON A LOG10 SCALE IN UNITS OF HOURS−1. THE x-axis SHOWS DISSOCIATION IN THE ABSENCE OF DM; THE y-axis SHOWS DISSOCIATION IN THE PRESENCE OF sDM. THE SOLID BEST-FIT STRAIGHT LINE THROUGH THE DATA HAS A SLOPE OF 0.47 (95% CONFIDENCE INTERVAL, 0.3–0.64; THIN DASHED CURVES); THE EXTREMELY STABLE DR*0401 COMPLEX AND THE COMPLEXES STUDIED IN THIS REPORT WERE EXCLUDED FROM THE CORRELATION ANALYSIS. THE SOLID LINE OF SLOPE 1 THROUGH THE ORIGIN INDICATES NO DM EFFECT; THE VERTICAL DISTANCE OF EACH DATA POINT FROM THIS LINE IS A MEASURE OF DM SUSCEPTIBILITY. THESE DATA HAVE BEEN PUBLISHED WITH THE EXCEPTION OF THE DATA FROM THE DQ2 AND DR3 COMPLEXES (21). TO COMPARE CURRENT RESULTS TO PREVIOUS WORK ON 36 COM-
PLEXES, IT WAS NECESSARY TO CORRECT VALUES FOR THE DIFFERENCES IN THE CONCENTRATION OF DM USED; PREVIOUS WORK INDICATED THAT EFFECTS OF DM IN THE DM CONCENTRATION RANGE OF BOTH OF THESE ASSAYS ARE LINEAR (E. D. MELLINS, UNPUBLISHED DATA).

DM EFFECT ON OTHER DQ2-PEPTIDE COMPLEXES IS ALSO REDUCED

To explore the possibility that altered DM-DQ2 interaction contributed to the modest effect of DM on DQ2-CLIP1/2 half-lives, we assessed four additional sDQ2-CLIP2 complexes; sDQ2-γ′L GLIADIN, sDQ2-αL GLIADIN, and two mutated forms of CLIP1 WHERE THE RESIDUE BURIED IN MHC CLASS II POCKET 1 IS MUTATED FROM METHIONINE TO EITHER ALANINE (M91A) OR PROLINE (M91P). COMPARED WITH sDR3-CLIP1, THE CLIP1 M91P PEPTIDE HAD A SLIGHTLY LONGER SPONTANEOUS DISSOCIATION TIME (t1/2 = 93.6 h), THE γ′L GLIADIN PEPTIDE HAD A SIMILAR DISSOCIATION RATE (t1/2 = 55.2 h), WHILE THE INTRINSIC STABILITY OF THE CLIP1 M91A AND αL GLIADIN PEPTIDES WERE BOTH LOWER THAN sDR3-CLIP1 (t1/2 = 13.1 h AND 8.3 h, RESPECTIVELY). IN THE PRESENCE OF DM, ALL FOUR OF THESE sDQ2-CLIP2 PEPTIDE COMPLEXES SHOWED ONLY MODERATE ENHANCEMENT IN PEPTIDE DISSOCIATION (5–19 TIMES).

To further evaluate the DM susceptibility of these seven complexes, we sought to assess DM effects in relationship to their intrinsic stability. We compared our current results to previous findings where we measured peptide dissociation in the presence and absence of DM for a panel of complexes of varying
respectively) as described in Materials and Methods. Cell lysates (5 × 10⁶ cell equivalents/lane) were immunoprecipitated with anti-DQ mAb SPV-L3 and of two monomorphic anti-DQ Abs to the DM-null cell 9.5.3 and its DM-transfectant, 9.5.3-DM. At saturating amounts of Ab, the binding of each of these Abs was comparable in the DM-expressing and nonexpressing cells, suggesting that cell surface abundance of DQ2 was not influenced by DM. In contrast, the level of DQ1 expression in the DM-null B-LCL 2.2.93 was increased by expression of DM in the 2.2.93 transfectant. The difference in effects on DQ1 and DQ2 was not due to cell line differences, because the behavior of DP4 and DR3 molecules was consistent across DM transfectants of both cell lines (Fig. 7).

Conformation of DQ1 but not DQ2 is altered by DM expression
DM interaction with nascent class II molecules influences their conformation, probably in both peptide-dependent and -independent manners (28). We assessed whether DQ2 was susceptible to DM effects on molecular maturation, in comparison to DQ1. After pulse-chase metabolic labeling of the same cell lines used in Fig. 7, we immunoprecipitated DQ1 and DQ2 with the monomorphic anti-DQ Ab SPV-L3. We observed increased binding of SPV-L3 (Fig. 8) or the DQ2-specific Ab 2.12.E11 (data not shown). This result does not depend on the cell lines tested since DP4 undergoes DM-dependent increases in precipitable molecules in 9.5.3-DM and 2.2.93-DM (data not shown; Roh et al., submitted for publication). We conclude that DQ2 resists DM-mediated chaperoning as well as peptide exchange.

The interaction of DM with DQ2 is competitively inhibited by DR3
Given that DR3 and DQ2 are tightly linked alleles and are generally expressed concurrently, they likely are competing ligands for DM within peptide-loading compartments. To model this situation in vitro, we examined whether DR3 could competitively inhibit DQ2-DM interaction. We used sDR3 or sDQ2 with tethered CLIP1 as a competitor for DQ2 or DR3, respectively, with thrombin-cleaved CLIP1, in a DM-mediated peptide exchange reaction. We varied the ratios of thrombin-treated

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**FIGURE 7.** Cell surface expression level of DQ2 is not enhanced by coexpression of DM. Fold change in MFI of staining of 2.2.93-DM compared with 2.2.93 (anti-DR (L243), anti-DP (B7/21.2), anti-DQ (SPV-L3 and *Ia*3 staining), or of 9.5.3-DM compared with 9.5.3 (anti-DR (L243), anti-DP (B7/21.2), anti-DQ (SPV-L3 and *Ia*3), anti-DQ2 (2.12.E11), and anti-DR3 (16.23) staining). Mean values from multiple experiments are shown as horizontal bars. Dotted line indicates no change (MFI ratio = 1). Analyses using *t* tests showed that all of the changes except DQ2*, DQ2b, and DR3 are significant (*p* < 0.05).

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**FIGURE 8.** DQ2 does not show DM-dependent conformational change during biosynthesis. 2.2.93, 9.5.3, and their DM transfectants (2.2.93-DM and 9.5.3-DM, respectively) were pulsed for 15 min with [³⁵S]methionine/cysteine and then chased for 2, 5, 3, 5, 4, and 4.5 h (*lanes 1, 2, 3, 4, 5, 6,* respectively) as described in Materials and Methods. Cell lysates (5 × 10⁶ cell equivalents/lane) were immunoprecipitated with anti-DQ mAb SPV-L3 and then analyzed by SDS-PAGE. Representative images from one of five independent experiments are shown. *, (1 = 2.2.93; 2 = 2.2.93-DM; 3 = 9.5.3; and 4 = 9.5.3-DM) at 0 h.
to thrombin-untreated complexes and measured binding of labeled, high-affinity peptides. At equimolar, 2- and 10-fold molar excess concentrations of DR3, the peptide exchange on DQ2 fell by 15, 27, and 61%, respectively. DQ2 was a less effective inhibitor, reducing the peptide exchange on DR3 by 2, 6, and 38% at the same concentrations (Fig. 9). These results argue that DR3 is a more potent inhibitor of DM-mediated peptide exchange of DQ2 than vice versa. At 10-fold molar excess of DR3 compared with DQ2, which roughly approximates physiological intracellular conditions (29), the inefficient DQ2-CLIP-DM interaction is further hampered by the presence of a successful, competitive ligand for DM.

Discussion

For most MHC class II alleles, biosynthesis and peptide loading is regulated by the actions of two cofactors: Ii and DM. Full-length Ii typically associates with class II with its core CLIP region (CLIP1; Ii 91–99) bound to the class II peptide-binding groove, and nested peptides from this region are left in the groove after Ii typically associates with class II with its core CLIP region regulated by the actions of two cofactors: Ii and DM. Full-length Ii and may allow an alternate register of association in the peptide exchange assay (Fig. 4B), the dose-response curve with DM is shifted to the right for sDQ2-CLIP2 compared with sDQ2-CLIP1. However, in Fig. 6, off-rate measurements of the two forms of CLIP demonstrate comparable values in the absence of DM and a longer half-life of the sDQ2-CLIP1 complex in the presence of DM. These findings can be rationalized, because the data in Fig. 4B reflect the net effect of off-rate and on-rate of the CLIP variants in the presence of DM, while the assay in Fig. 6 includes excess competitor peptide, precluding any rebinding of the CLIP peptides and therefore any influence of peptide on-rates.

HLA haplotypes with the DR3-DQ2 segment are associated with many autoimmune disorders, including celiac disease, type 1 diabetes, systemic lupus erythematosus, Graves’ disease, and Addison’s disease (5, 6). Notably, for many of these diseases, the major HLA effects have been mapped to the DR3-DQ2 interval (43, 44). The direct comparison of DR3 and DQ2, as we have done, is therefore of particular interest. The unusual interactions between DQ2 and Ii and DM could be one reason for the widespread predisposition to autoimmunity of DR3-DQ2 haplotypes.

The Q2-related effects may act at two levels: at the level of similar to DR3-peptide complexes; prolonged retention of either class II-CLIP or more DM-resistant complexes does not appear to occur (34). With linkage disequilibrium in MHC class II haplotypes and coregulated expression of class II isotypes, DQ2 is almost always expressed concurrently with DR3, which is capable of out-competing DQ2 for limiting amounts of DM, as evident in Fig. 9.

The structural basis of the unusual interaction of DQ2 with DM is likely to involve polymorphic residues of DQ2. DM is thought to disrupt H bonds between the peptide backbone and residues around pocket 1 of the class II molecule (α53, β81) or stabilize a class II conformation in which these hydrogen bonds are disrupted, along with more global changes effecting pocket structure (7, 35–40). Mutational analysis has demonstrated the critical role of the protruding α51F in DR3 for interaction with DM (37, 38). This residue is predicted to participate in hydrophobic interactions with DM and may serve as a lever to alter the location of the extended strand including α51–53 (37, 38) and its distance from the bound peptide. In DQ2, the α44–53 stretch (numbering as in Ref. 41) contains several polymorphic residues and deletion of α53. Further work should identify whether this region indeed is of significance and which residues are involved.

It is striking that CLIP2 is a prominent component of the DQ2 peptide repertoire. We show that CLIP2 binds to DQ2 by placing a proline at the P1 position. As part of a polypeptide chain, the proline side chain is unable to engage in amide hydrogen bonding because of its ring structure. In most MHC class II molecules, there is a hydrogen bond from residue 53 of the α-chain to the amide nitrogen of the P1 residue. Not only does DQ2 have a deletion of residue α53, but there is evidence that DQ2 is unable to form the P1-related hydrogen bond (42). Thus, proline can be accommodated by DQ2 at P1 without energetic penalty, perhaps explaining the unusual association of DQ2 with CLIP2 peptides. An interesting question is how these CLIP2 fragments come to be associated with DQ2. The CLIP region is unstructured within the full-length Ii and may allow an alternate register of association in the endoplasmic reticulum. Alternatively, CLIP2 peptide associates with DQ2 after some proteolysis of Ii in endosomes, either through reption or rebinding.

On first glance, it may appear that our data on the DM susceptibility of DQ2-CLIP1 compared with DQ2-CLIP2 complexes are inconsistent. Among naturally processed peptides, the proportion of DQ2-CLIP2 is modestly increased in DM-expressing compared with nonexpressing cells, suggesting increased resistance to DM editing. In the peptide exchange assay (Fig. 4B), the dose-response curve with DM is shifted to the right for sDQ2-CLIP2 compared with sDQ2-CLIP1. However, in Fig. 6, off-rate measurements of the two forms of CLIP demonstrate comparable values in the absence of DM and a longer half-life of the sDQ2-CLIP1 complex in the presence of DM. These findings can be rationalized, because the data in Fig. 4B reflect the net effect of off-rate and on-rate of the CLIP variants in the presence of DM, while the assay in Fig. 6 includes excess competitor peptide, precluding any rebinding of the CLIP peptides and therefore any influence of peptide on-rates.
presentation of peptide epitopes in the periphery and at the level of T cell selection in the thymus.

In the periphery, immunodominant T cell epitopes are mainly selected based on the (high) kinetic stability of the peptide-MHC II interaction (45–47), a process generally enhanced by the presence of HLA-DM (47–49). Exceptions have been found in autoimmune models, where peptides with low kinetic stability proved immunodominant (46, 50–52). The presentation of these low-affinity peptides may be augmented by a reduced DM interaction and by being in high abundance. The vigorous immune responses of celiac disease patients to gluten peptides that bind DQ2 with relatively low affinity can thus relate to both reduced DM interaction of DQ2 and the presence of high amounts of gluten peptides in the gut mucosa.

In the thymus, the overabundance of unexchanged CLIP peptides may affect T cell selection. Studies in mice have demonstrated that a narrow spectrum of MHC class II ligands profoundly alters the repertoire of CD4+ T cells (53–56). The effects include reduction in both number and diversity of CD4+ T cells, thought to be due to inefficiency of thymic-positive selection (53, 54). In addition, T cells within this population are negatively selected on a limited self-peptide repertoire and show broad reactivity to self-peptides, increasing their potential for autoreactivity (54, 56).

Another aspect of escaping DM editing is that the residual CLIP1/2 peptides, although of relatively higher affinity for DQ2 than CLIP1 peptides for many other alleles, nonetheless may be less ideal than a DM-edited peptide repertoire for stabilizing class II surface expression and inhibiting exchange with extracellular peptides, including potential autoantigens (9, 57). Our previous work on the half-life of complexes that survive DM editing shows that CLIP1/2 peptides, although of relatively higher affinity for DQ2 and the presence of high amounts of gluten peptides in the gut mucosa.

To the best of our knowledge, the authors have no financial conflict of interest.

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Disclosures

The authors have no financial conflict of interest.

References


15. Chicz, R. M., R. G. Urban, J. C. Gorga, D. A. Vignali, W. S. Lane, and J. L. Strominger. 1993. Specificity and promiscuity among naturally processed peptides sensitive? One possibility is that, under certain circumstances, this characteristic will lead to presentation of numerous antigenic epitopes instead of a few immunodominant epitopes (49), and this may provide a selective advantage in combating some infectious diseases. However, a deleterious side effect could be an increased susceptibility to autoimmune diseases. Studies in humanized mice models should shed light on the in vivo relevance of the phenomena described here.

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