

Interference of Distinct Invariant Chain Regions with Superantigen Contact Area and Antigenic Peptide Binding Groove of HLA-DR¹

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In the endoplasmic reticulum, MHC class II $\alpha\beta$ dimers associate with the trimeric invariant chain (Ii), generating a nine-subunit ($\alpha\beta\text{Ii}$)₃ complex. In the presence of Ii, the peptide binding groove is blocked, so that loading with self or antigenic peptides can only occur after proteolytic removal of Ii in specialized post-Golgi compartments. The class II-associated invariant chain peptide region of Ii (about residues 81–104) is known to mediate binding to class II molecules and blockade of the groove, but this does not exclude additional contact sites for Ii. Using a set of overlapping Ii peptides and recombinant soluble Ii, we demonstrate here that a large segment of Ii encompassing approximately residues 71 to 128 interacts with HLA-DR molecules. The N- and C-terminal regions of this Ii segment appear to bind outside the peptide groove to the contact area for the staphylococcal superantigen *Staphylococcus aureus* enterotoxin B on the $\alpha 1$ domain. The core region of this segment (residues 95–108) prevents binding of antigenic peptides, probably by interaction with the peptide groove. Occupation of the groove with antigenic peptides abolishes binding not only of the core region, but also that of those Ii peptides that bind outside the groove. These findings suggest the existence of distinct conformational states of class II molecules, with Ii binding preferentially to one form. *The Journal of Immunology*, 1995, 155: 4757–4765.

The MHC class II-associated Ii³ is a monomorphic type II transmembrane glycoprotein (1) that decisively influences the quality of Ag presentation by MHC class II molecules (2). In the human system, the Ii gene encodes four polypeptide chains originating from differential splicing of the corresponding mRNA (3): the p33 form as well as the less abundant p41 form are both found without and with N-terminal extensions resulting in the p35 and the p43 forms (4, 5). Ii forms trimers in the ER and chaperones proper assembly of MHC α - and β -subunits, finally generating a nonameric complex ($\alpha\beta\text{Ii}$)₃ (6). In the ER and during transport to endocytic compartments, $\alpha\beta\text{Ii}$ complexes are not accessible for peptides (7, 8). Furthermore, the assembly with Ii prevents $\alpha\beta$ dimers from association with polypeptides (9) or self-aggregation and inactivation (10, 11). A sorting signal in the cytoplasmic tail of Ii is responsible for efficient targeting of the nine-chain complex to post-Golgi compartments of the endocytic pathway (12, 13), where loading with self or antigenic peptides is

thought to occur (14–17). Stepwise proteolytic destruction of Ii, probably by members of the cathepsin family in vesicles of the endosomal-lysosomal system (18, 19), renders $\alpha\beta$ dimers accessible for peptides derived from exogenous (20) and endogenous source proteins (21). $\alpha\beta$:peptide complexes finally travel to the cell surface for subsequent presentation of peptides to T cells (20).

Biochemical studies on several naturally occurring fragments of Ii provided insight into the interaction of Ii with class II molecules; a p25 Ii fragment was found to be associated with $\alpha\beta$ dimers. p25 is a soluble C-terminal Ii fragment (22) starting at position 98 of the p31 form of murine Ii (23) or at positions 91, 93, and 99 of the human p33 form (24). In contrast, treatment of cells with the cysteine protease inhibitor leupeptin leads to accumulation of a 22-kDa membrane-anchored N-terminal leupeptin-induced polypeptide (25) that trimerizes and is still engaged in a nonameric complex with MHC $\alpha\beta$ dimers (26). Both findings are in agreement with the results of systematic exon deletions and N- or C-terminal truncations of recombinant Ii, arguing that the membrane-proximal segment formed by amino acids 81 to 105 of exon 3 is crucial for interaction of Ii with class II molecules (27–29), whereas the region between amino acids 163 and 183 seems to be essential for Ii trimerization (28). Further evidence underlining the capability of Ii region 81 to 105 to bind promiscuously to $\alpha\beta$ dimers comes from a nested set of Ii peptides spanning the above region. These CLIP (30) have been eluted from several murine (31, 32) and human class II molecules extracted from cells (21, 33, 34). They vary in length from 14 to 28 residues (21, 35) and are potent inhibitors of antigenic peptide binding to class II molecules (30, 36).

It is still a matter of discussion, whether CLIP is a residual fragment of Ii proteolysis that remains bound to class II $\alpha\beta$ dimers or whether it rebinds after proteolysis of Ii. However, the finding that the number of $\alpha\beta$:CLIP complexes is drastically reduced during transport from endosomes to the plasma membrane in B cells

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³ Abbreviations used in this paper: Ii, invariant chain; ER, endoplasmic reticulum; CLIP, class II-associated invariant chain peptides; SEB, *Staphylococcus aureus* enterotoxin B; sDR1, soluble DR1; HA, influenza virus hemagglutinin; MBP, myelin basic protein; IM, influenza virus matrix protein; AMCA, 7-amino-4-methyl-coumarin-3-acetic acid; HPSEC, high performance size-exclusion chromatography; rIi, recombinant Ii.

(37) and the fact that purified $\alpha\beta$:CLIP complexes cannot be distinguished from those generated by limited *in vitro* proteolysis of $\alpha\beta$:Ii complexes (34) favor the hypothesis that the CLIP segment of Ii associates with $\alpha\beta$ dimers during formation of the nonameric complex early after biosynthesis in the ER. In human wild-type B cells, a small but significant amount of $\alpha\beta$ dimers of various HLA-DR and -DQ isotypes is associated with CLIP under steady state conditions (21, 38), whereas in HLA-DM-negative Ag-processing mutants, such as T2 transfected with HLA-DR3, -DR4, or -DR11, CLIP was revealed to be the predominant self-peptide bound to DR molecules (30, 34). The presence of HLA-DM appears to prevent the accumulation of $\alpha\beta$:CLIP complexes and leads to proper peptide loading of class II molecules (39, 40). Thus, DM may be directly involved in the removal of CLIP, at least in the HLA-DR-associated Ag presentation pathway (41, 42).

There are findings arguing that $\alpha\beta$:CLIP as well as $\alpha\beta$ Ii complexes behave differently from complexes with Ag-derived peptides. Ii fails to form SDS-stable complexes with $\alpha\beta$ dimers (43), and the same has been found for certain $\alpha\beta$:CLIP complexes (30, 34). CLIP is selectively released at endosomal pH (34, 44–46), $\alpha\beta$:CLIP complexes do not undergo time-dependent maturation (46), and single mutations outside the peptide binding groove of class II molecules prevent CLIP, but not antigenic peptide, binding (36). Conversely, other studies have shown that polymorphic residues of $\alpha\beta$ dimers are involved in CLIP binding comparable to their involvement in antigenic peptide binding (34, 47, 48). However, it should be kept in mind that at present we can exclude neither different binding modes of CLIP for different class II alleles nor the existence of more than one binding mode for CLIP for certain class II molecules.

There exists evidence that class II molecules are flexible entities that can occur in different conformations (49, 50). For example, the recently described short-lived kinetic intermediates of $\alpha\beta$:peptide complexes (51–53) seem to bear alternative conformations compared with the long-lived complexes known from the crystal structure of HLA-DR1 (54). Therefore, the questions arise whether $\alpha\beta$ dimers complexed to Ii or the CLIP region are structurally different from mature class II molecules loaded with antigenic peptides and whether CLIP cooperates with other Ii stretches for binding to $\alpha\beta$ dimers.

In the present study we addressed the question of whether, besides CLIP, other regions of Ii would interact with class II molecules. Using a set of overlapping Ii-derived peptides and recombinant Ii we demonstrate that regions flanking CLIP contribute to the interaction of DR $\alpha\beta$ dimers with Ii. These regions appear to contact the $\alpha 1$ domain outside the peptide binding groove, as superantigen SEB interferes with their binding. Unexpectedly, antigenic and, therefore, bona fide groove binding peptides were also found to inhibit those Ii peptides that bound to the SEB contact area outside the groove, suggesting the existence of two different class II conformations: one stabilized by Ii binding and another that is gradually formed in the presence of antigenic peptides according to principles of the induced fit model.

Materials and Methods

Cells

EBV-transformed B-LCL LD2B (HLA workshop no. 9083) was used as a source for isolation of DR2. Cells were maintained in RPMI with HEPES (Life Technologies, Grand Island, NY) with 5% calf serum (Life Technologies) at 37°C using roller bottles. *Spodoptera frugiperda* (Sf9) were maintained at 27°C in Grace medium (Life Technologies) in the presence of 10% FCS. For protein production, cells were grown in serum-free SF900 medium (Life Technologies). Virus infection was performed as previously described (55).

Table 1. Dissociation equilibrium constant K_d of Ii peptides for binding to HLA-DR2

Peptide	Residues	Sequence	K_d [nM]
Ii61	61–78	RLDKLTVTSQNLQLENLR	>10 ⁶
Ii71	71–88	NLQLENLRMKLPKPKPKV	1,600
Ii81	81–98	LPKPKPKVSKMRMATPLL	3,000
Ii90	90–108	KMRMATPLLQALPMGALP	35
Ii95	95–108	TPLLQALPMGALP	280
Ii101	101–118	ALPMGALPQGFPMQNAATKY	4,300
Ii111	111–128	PMQNAATKYGNMTEDEVHMV	6,800
Ii121	121–138	MTEDHVMHLLQNALDPLKV	80/10,800
Ii131	131–148	QNADPLKVYPLKGSFPE	>10 ^{6a}
Ii141	141–158	PLKGSFPENLTHLKNTE	>10 ⁶
CLIP	81–105	LPKPKPKVSKMRMATPLLQALPMG	23

^a The Scatchard plot of Ii121 gave a biphasic curve, indicating a low and a high affinity binding site.

Purification of DR2

LD2B cells (5×10^9) were homogenized in hypotonic lysis buffer (20 mM Tris (pH 7.8) containing protease inhibitors (0.2 mM PMSF, 2.5 μ g/ml leupeptin, 5 μ g/ml pepstatin, and 5 μ g/ml chymostatin)). Cytosolic proteins were separated by differential centrifugation ($500 \times g$, 4°C, 10 min; $12,000 \times g$, 4°C, 10 min; $140,000 \times g$, 4°C, 1 h). Pellets containing the membrane fractions were pooled and lysed with lysis buffer containing 1% Nonidet P-40 for 1 h on ice, and nuclear material was removed by centrifugation at $500 \times g$ for 10 min at 4°C. Further purification of the cell extract was performed by centrifugation for 10 min at $12,000 \times g$ at 4°C and ultracentrifugation at $140,000 \times g$ for 1 h at 4°C. DR $\alpha\beta$ molecules were purified from the cell extract using a column of mAb L243 (56) coupled to the manufacturer's protocol. Glycine coupled to Sepharose was used as a precolumn. The cell extract was passed over the glycine-Sepharose and L243 Sepharose columns for 16 h. The L243 column was extensively washed with 100 mM sodium phosphate, pH 8.0, and 0.5% Zwittergent-12, followed by 100 mM sodium phosphate (pH 8.0) and 0.1% Zwittergent-12, and eluted with 100 mM sodium phosphate (pH 11.0) and 0.1% Zwittergent-12. The eluted material was neutralized with 1 M HCl and concentrated by ultrafiltration with a 20-kDa cut-off membrane (Sartorius, Göttingen, Germany). Solubilized DR molecules were kept at 4°C and a concentration of about 0.5 mg/ml.

sDR1 was obtained from Sf9 cells infected with baculovirus as previously described (55).

Purification of p25

The p25 fragment of Ii was isolated from EBV-transformed B-LCL LD2B as described previously (24).

Peptides

The following peptides spanning the region between the transmembrane domain and the putative trimerization domain of Ii were used (sequences are given in Table 1): Ii-(61–78) (Ii61), Ii-(71–88) (Ii71), Ii-(81–98) (Ii81), Ii-(90–108) (Ii90), Ii-(95–108) (Ii95), Ii-(101–118) (Ii101), Ii-(111–128) (Ii111), Ii-(121–138) (Ii121), Ii-(131–148) (Ii131), and Ii-(141–158) (Ii141). Numbering is according to the p33 form of human Ii (5).

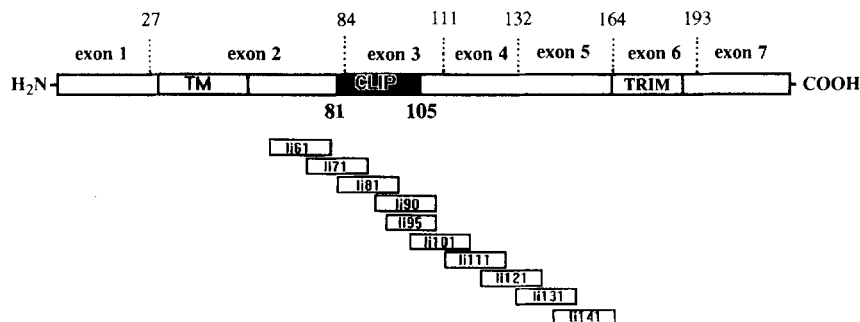
In addition, the following antigenic peptides were used: PKYVKQNTLKLAT, HA-(307–319); PVVHFFKNIVTPRTPPPSQGK, MBP-(85–105); HLVEALYLVCGERGFFYTPKA, INS-(10–30), bovine insulin β -chain; and PLKAEIAQRLEDV IM-(19–31).

Peptides were synthesized on an AMS422 Multiple Peptide Synthesizer (Abimed, Langenfeld, Germany) using F-moc chemistry and purified by reverse phase HPLC. Purified peptides were analyzed by matrix-assisted laser desorption mass spectrometry and shown to be single species. N-terminal labeling with AMCA (Lambda, Graz, Austria) was performed as previously described (57).

Peptide binding assay

Peptide binding to DR2 and sDR1 was measured by HPSEC, as previously described (58). Briefly, purified DR2 (200 nM) or sDR1 (50 nM) molecules were coincubated with AMCA-labeled peptide (10 nM to 50 μ M) in the absence or the presence of inhibitor molecules in 100 mM sodium phosphate buffer (pH 6.0) and 0.1% Zwittergent-12 (Calbiochem, La Jolla, CA).

FIGURE 1. The following peptides spanning the region between the transmembrane domain (TM) and the putative trimerization domain (TRIM) of Ii were used: Ii-(61–78) (Ii61), Ii-(71–88) (Ii71), Ii-(81–98) (Ii81), Ii-(90–108) (Ii90), Ii-(95–108) (Ii95), Ii-(101–118) (Ii101), Ii-(111–128) (Ii111), Ii-(121–138) (Ii121), Ii-(131–148) (Ii131), and Ii-(141–158) (Ii141). Numbering is according to the p33 form of human Ii (5). The exon borders of the Ii gene are indicated (65).



After incubation for 40 h at 37°C, HPSEC was performed on a Superdex 75 HR 5/20 column (Pharmacia) equilibrated with 150 mM sodium phosphate buffer (pH 6.0), 0.1% Zwittergent-12, and 15% acetonitrile. The column run-through passed through a F1080 fluorescence detector (Merck-Hitachi; excitation, 350 nm; emission, 450 nm) and a L4001 UV detector (Merck-Hitachi, Darmstadt, Germany) set up in series. Signals were recorded by a model D2500 integrator (Merck-Hitachi, Darmstadt, Germany). Peptide:DR complexes eluted after 2.5 min with an elution volume of 1.5 ml. K_d values were calculated from the slope of the Scatchard plots of bound/free peptide vs bound peptide. Inhibition was calculated from fluorescence signals in the absence (F_0) and the presence (F_i) of inhibitor: % inhibition = $(F_0 - F_i)/F_0 \times 100$.

Recombinant Ii

The cDNA encoding the human Ii (p33 form) was obtained from the Ii expression vector IiSV51L (12) using the 820-bp fragment, which was flanked by the *NarI* site at the 5' end and the *SacI* site at the 3' end. This fragment and a double stranded synthetic oligonucleotide (TAT GGA TGA CCA GCG CGA CCT TAT CTC CAA CAA TGA GCA ACT GCC CAT GCT GGG CCG G) were introduced into the pET3a vector using the *NdeI* and *SacI* site. The transmembrane region was deleted by removing the fragment from the *SacI* site to the *PstI* site, thereby restoring the 3' C-terminal coding 40 bp with a double stranded oligonucleotide (GGC CGG CTG GAC AAA CTG ACA GTC ACC TCC CAG AAC CTG CA). At the 3' end of the Ii coding region, a 6-His tail was introduced at the *BstYI* site using the oligonucleotide (GAT CTG GGC CCA GTC CCC ATG CAC CAC CAC CAC CAC TGA GCT). The resulting plasmid pET3a.Ii.DTMR-His was used to express rIi in *Escherichia coli* (BL21).

Expression of rIi was induced by treatment with 0.4 mM IPTG for 1 h at 37°C. Bacterial pellets were obtained by centrifugation (5000 rpm, 5 min, 4°C), resuspended in 50 mM Tris (pH 7.5), 1 mM EDTA, and 1 mM PMSF and disrupted by freezing/thawing in liquid nitrogen. After the addition of 200 μ g/ml lysozyme, the bacterial suspension was incubated for 15 min on ice. One-tenth volume of 5 M NaCl was added and incubated for an additional 15 min on ice. Bacterial extract was clarified by centrifugation at $10,000 \times g$ for 30 min at 4°C. After the addition of 2 mM $MgCl_2$ and 20 mM imidazole, the extract was loaded onto a Ni^{2+} /NTA column (Quiagen, Chatsworth, MA) and washed extensively with the following buffers: 10 column volumes of 50 mM Tris (pH 7.5), 20 mM imidazole, and 500 mM NaCl; 1 column volume of 1 M NaCl; and 20 mM HEPES (pH 7.5), 90 mM KCl, and 2 mM $MgCl_2$. Elution was performed with 250 mM imidazole. The eluted material was concentrated by ultrafiltration with a 10 kDa cut-off filter (Centriprep, Amicon, Danvers, MA) and further purified by ion exchange chromatography on a Mono S Sepharose column (Pharmacia). Elution was performed with a gradient from 90 mM to 1 M KCl. rIi eluted with 200 to 300 mM KCl and was stored at 4°C.

Results

Flanking regions of CLIP bind to DR molecules

Previous studies using Ii deletion and truncation mutants have shown that the CLIP region encoded by exon 3 is essential for binding of Ii to class II molecules (27–29). As this does not rule out additional contact sites, a series of overlapping peptides (mainly 18-mers) was synthesized spanning the region between the transmembrane and the putative trimerization domain of Ii, residues 61 to 158 (Fig. 1). Each Ii peptide was tagged at the N terminus with the fluorescence label AMCA and tested for binding to purified HLA-DR2 molecules using HPSEC and fluorescence de-

tection. The use of peptides to mimic the behavior of defined stretches of Ii seems to be a valid approach, as the Stokes' radius of Ii trimers suggests an extended conformation without globular folding of Ii (3).

As shown in Table I, all Ii peptides except Ii61, Ii131, and Ii141 were found to bind to DR2, with peptides Ii90 and Ii95 from the CLIP region displaying the highest affinities ($K_d = \sim 35$ and 280 nM, respectively). Ii71 and Ii101 that overlap with the N- and C-terminals of the CLIP sequence, respectively, bind with lower, but significant, affinity ($K_d = \sim 1.6$ and 4.3 μ M). Binding of Ii111 and Ii121 together with the lack of binding of Ii131 suggest that residues 111 to about 128 contribute to Ii binding. Likewise, the twofold higher affinity of Ii71 compared with Ii81 implies that at least some of amino acids 71 to 80 are also involved in binding. The Scatchard plot of Ii121 was biphasic, indicating two binding sites: one with low affinity ($K_d = \sim 10.8 \mu$ M) and the other with high affinity ($K_d = \sim 80$ nM). Binding of the Ii-derived peptides was specific, as it was competed out by the respective unlabeled peptide, and no binding to purified HLA class I molecules was observed (data not shown). These data suggest that not only the CLIP region (residues 81–105), but also a larger segment of Ii ranging from approximately positions 71 to 128, can interact with class II molecules.

As class II molecules are peptide receptors, one might argue that all the Ii-derived peptides bind in the antigenic peptide binding groove like conventional peptides. To address this possibility, DR2 was simultaneously incubated with different AMCA-labeled Ii peptides. Examples are presented in Figure 2a. Using AMCA-Ii71 under saturating concentrations, addition of the AMCA-labeled peptides Ii90, Ii101, and Ii111 led to respective additive binding signals. Likewise, unlabeled Ii90, Ii101, and Ii111 did not inhibit AMCA-Ii71 binding (data not shown), and coinubation of the three nonoverlapping labeled peptides Ii71, Ii90, and Ii111 resulted in additive fluorescence signals, but there was no additivity when overlapping peptides such as Ii71 and Ii81 or Ii71 and Ii61 were coinubated (Fig. 2a). Ii121 was an exception, as it displayed only partial additive binding with Ii71. Ii121 appears to bind to an additional site on MHC class II molecules, which may be the peptide binding groove or part of it. This view is consistent with the observation that the Scatchard analysis resulted in two distinct K_d values for Ii121 (see Table I). Corresponding results for Ii peptide binding were obtained with sDR1 molecules produced in insect cells (Fig. 2b). sDR1 is free of detectable peptide (55) and, therefore, represents a homogeneous population. Accordingly, saturating amounts of labeled Ii71 led to 95% occupancy of sDR1 (data not shown). Simultaneous binding of Ii71 with Ii90, Ii101, and Ii101, but not with Ii61, Ii81, or Ii121 confirms the results obtained with DR2 and excludes the possibility that Ii peptides bind to distinct DR subpopulations. Together, these data indicate that the

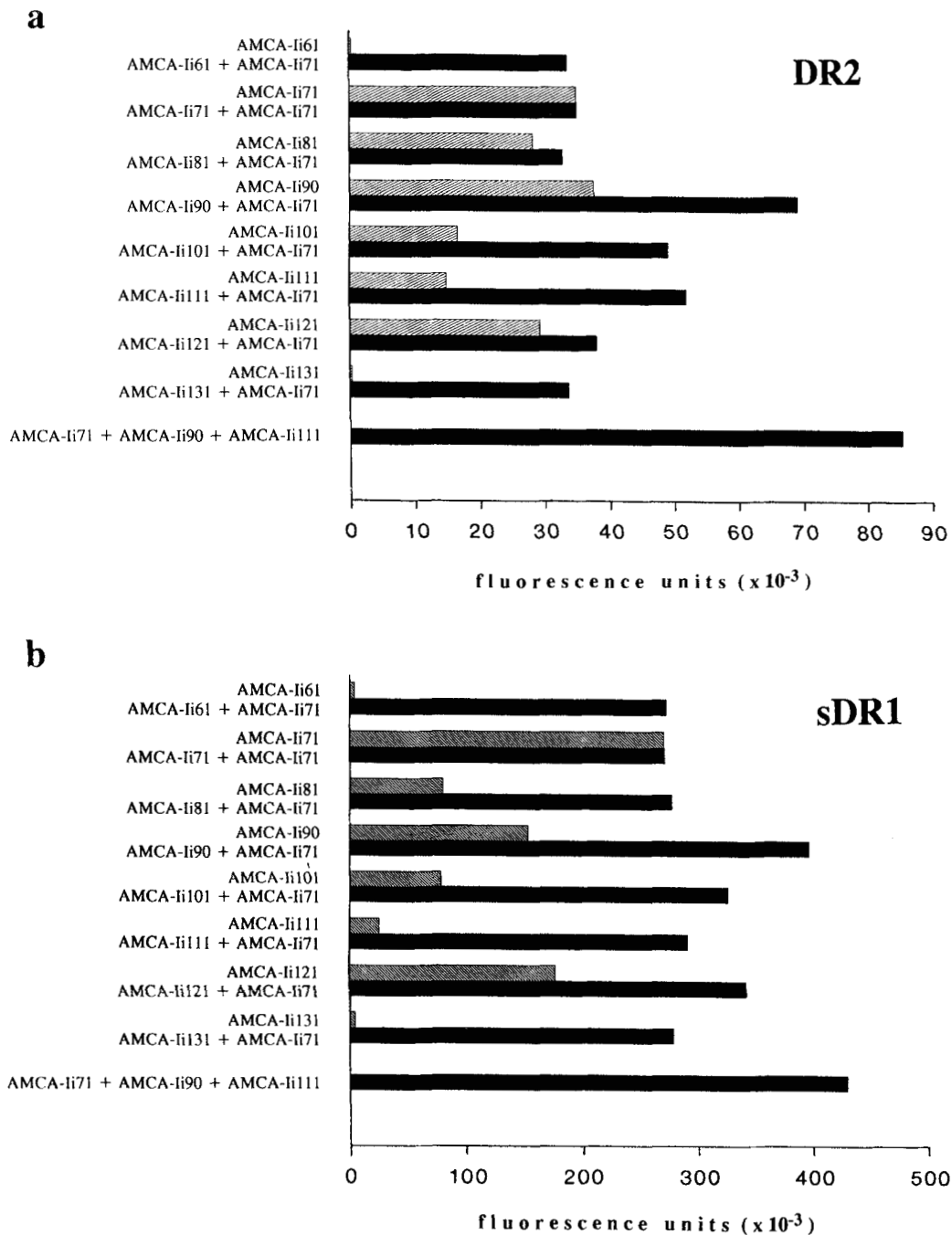


FIGURE 2. Cobinding of Ii-derived peptides. *a*, DR2 (200 nM) was coincubated with different AMCA-Ii peptides (5 μ M) in the absence (hatched bars) and the presence (black bars) of saturating concentrations of AMCA-Ii71 (50 μ M) for 40 h in HPSEC binding assay. *b*, sDR1 (50 nM) was coincubated with different AMCA-Ii peptides (5 μ M) in the absence (hatched bars) and presence (black bars) of saturating concentrations of AMCA-Ii71 (50 μ M) for 40 h in HPSEC binding assay.

nonoverlapping Ii peptides bind to distinct sites on $\alpha\beta$ dimers and rule out that all peptides bind in the groove.

Ii-derived peptides reflect binding of intact *Ii*

To determine whether the Ii-derived peptides mirror the behavior of the corresponding regions in the Ii polypeptide chain and bind to the respective contact sites, inhibition studies with human rIi lacking the transmembrane domain were performed. As depicted in Figure 3, coincubation of rIi with the respective AMCA-labeled Ii peptides in a 2:1 molar ratio moderately reduced binding of Ii90

and Ii95 and very efficiently reduced binding of the other Ii peptides, reflecting the different affinities of the respective Ii peptides for DR2 (Table I). At higher concentrations of rIi, binding of Ii90 and Ii95 and also that of Ii121 were completely blocked (data not shown). rIi was also able to prevent binding of AMCA-labeled antigenic peptides MBP-(85–105) from MBP and HA-(307–319) from HA (Fig. 3), but failed to block the control peptide INS-(10–30) from the bovine insulin B chain that has been suggested to occupy an alternative binding site on class II molecules (P. Jensen, personal communication). Additional evidence for correct binding

FIGURE 3. Inhibition of Ii peptide binding by rIi. DR2 (200 nM) was incubated together with the indicated peptides in its AMCA-labeled form (1 μ M) in the absence (black bars) or the presence (cross-hatched bars) of rIi (2 μ M) and analyzed after 40 h in the HPSEC binding assay.

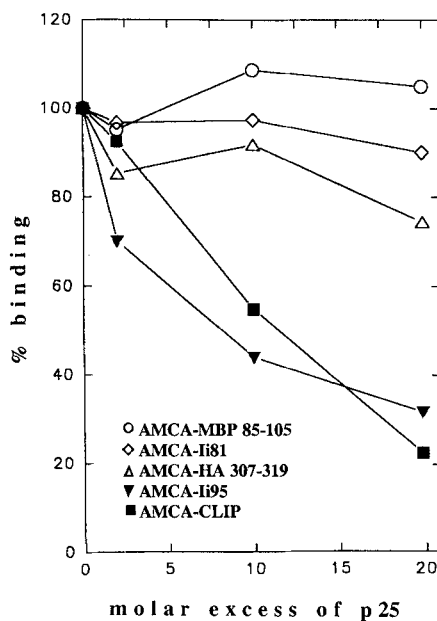
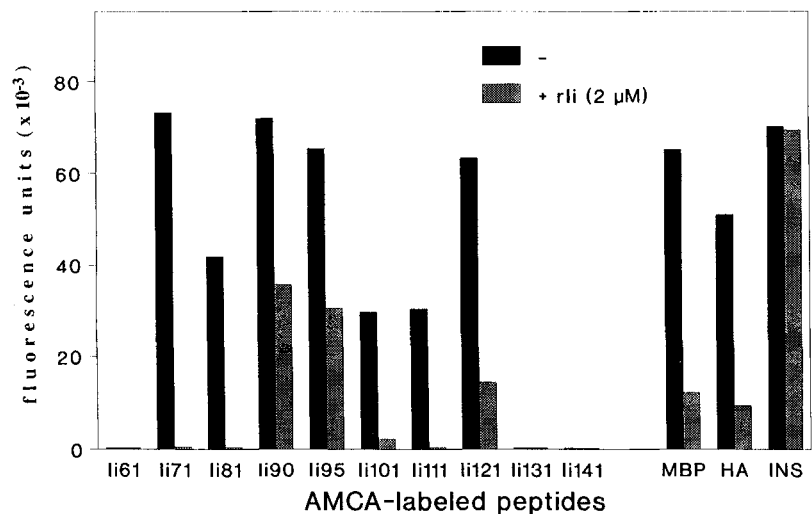


FIGURE 4. Selective inhibition of Ii peptide binding by Ii fragment p25. Titration of the inhibition by p25 (2–20 μ M) of AMCA-labeled peptides Ii81, Ii95, CLIP-(81–105), MBP-(85–105), and HA-(307–319), 1 μ M each, on DR2 (200 nM). p25 starts with Ala¹⁰¹, as shown by Edman sequencing, and contains the complete C terminus of Ii.

of the Ii peptides was derived from the C-terminal Ii fragment p25, which is found associated with class II molecules *ex vivo* and *in vitro* (22, 24). p25 was isolated from the EBV-transformed B cell line LD2B. Sequencing of p25 by Edman degradation showed that its N-terminal amino acid uniformly was Ala¹⁰¹. Thus, it shares the same N terminus with peptide Ii101. p25 selectively blocked binding of Ii peptides Ii95 and CLIP-(81–105), which overlap with the p25 sequence, but did not inhibit binding of nonoverlapping Ii81 or antigenic peptides (Fig. 4). These data for p25 are in good agreement with the binding of Ii101, Ii111, and Ii121, in qualitative and in quantitative terms. Taken together, the additive binding and the selective inhibition by rIi and p25 strongly indicate that the Ii-derived peptides are oriented on sDR1 and DR2 molecules in the same way as the corresponding regions in intact Ii.

Interaction of Ii segments with the SEB contact area

For identification of the contact region of class II molecules for Ii-derived peptides we employed SEB. The x-ray structural analysis of the co-crystal of SEB with HLA-DR1 showed that SEB binds to the α 1 domain of $\alpha\beta$ dimers exclusively outside the groove without interference with peptide binding in the groove (59). The results shown in Figure 5 confirm that SEB does not prevent groove binding of antigenic peptides; neither AMCA-MBP-(85–105) nor AMCA-HA-(307–319) binding to DR2 (Fig. 5a) or sDR1 (Fig. 5b) was influenced by SEB, even at a 20-fold molar excess of SEB (data not shown). In contrast, a twofold molar excess of SEB strongly or moderately blocked binding of all Ii peptides except Ii95 (Fig. 5, a and b). Ii95 was not inhibited even at high SEB concentrations (see *inset*, Fig. 5a). Only in the case of Ii101 and Ii111 were allelic differences in SEB inhibition observed; both peptides were efficiently inhibited by SEB on sDR1 (Fig. 5b), but only moderately on DR2 molecules (Fig. 5a). Inhibition of CLIP-(81–105) binding by SEB followed competitive kinetics, suggesting that both molecules competed for the same site (46). Together, these data imply that regions covering about amino acids 71 to 90 and 109 to 128 interact with sites outside the peptide binding groove on the SEB contact area on the α 1 domain of DR molecules. The exact boundaries of these segments are not clear from these studies.

Interference with antigenic peptide binding

It is known that CLIP and antigenic peptides interfere with each other's binding. We addressed the question of which of the Ii-derived peptides would block antigenic peptides and vice versa. When AMCA-labeled Ii peptides were incubated for 48 h in the presence of antigenic peptide, it was observed on both sDR1 and DR2 that all Ii peptides were inhibited by antigenic peptide (Fig. 6, a and c). The inhibition was dose dependent, as 50 μ M MBP-(85–105) (50-fold excess) was more efficient than 2 μ M (twofold molar excess) of the same peptide. In addition, the inhibition was time dependent, as very little blockade was observed after 2 h of incubation (data not shown). The interference of groove binding peptides with those Ii peptides binding to the SEB contact area outside the groove (Ii71 and Ii111; Fig. 5) suggests different conformational states of DR molecules. Further evidence for at least two alternative conformations was obtained when the reverse experiment was performed, namely inhibition of labeled antigenic peptides by unlabeled Ii peptides. After 48-h coinubation, only Ii90 and Ii121 (the peptide with the exceptional behavior; Table I)

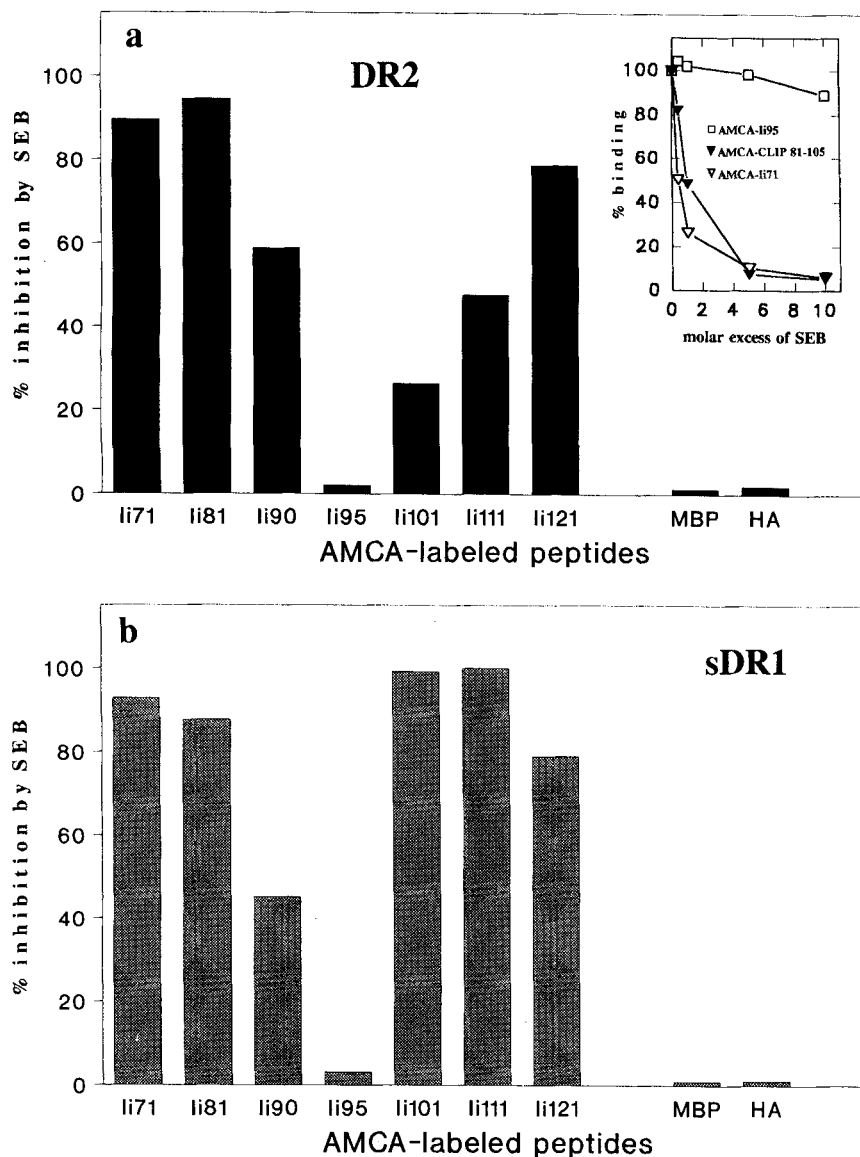


FIGURE 5. Selective inhibition of Ii peptide binding by SEB. *a*, Binding of the indicated AMCA-labeled Ii peptides (1 μ M) to DR2 (200 nM) in the absence or the presence of SEB (2 μ M) was analyzed by HPSEC. *Inset*, Titration of the inhibition by SEB (0.4–10 μ M) of peptides AMCA-Ii71, AMCA-Ii95, and AMCA-CLIP(81–105) on DR2 (K_d values; cf Table 1). *b*, Binding of the indicated AMCA-labeled Ii peptides (1 μ M) to sDR1 (50 nM) in the absence or the presence of SEB (2 μ M) was analyzed by HPSEC.

were able to decrease AMCA-MBP-(85–105) binding to DR2 and AMCA-HA-(307–319) binding to sDR1, but not the other Ii peptides despite a 100-fold molar excess (Fig. 6, *b* and *d*). The same amount of unlabeled MBP-(85–105) or HA-(307–319) led to quantitative inhibition on DR2 and sDR1, respectively. This result demonstrates that the region Ii90–108 of CLIP is essential for efficient inhibition of antigenic peptide binding to DR1 and DR2 molecules.

However, when the kinetics of inhibition were studied in more detail, we found that after short time incubation, antigenic peptide binding was also inhibited by Ii71, which binds outside the groove (Fig. 7). In the absence of Ii71, AMCA-HA-(307–319) attained half-maximal binding to sDR1 after a $t_{1/2}$ of approximately 4 h, reaching maximal binding after approximately 30 h (Fig. 7*a*). Under steady state conditions, about 85% of sDR1 molecules were occupied by AMCA-HA-(307–319). However, in the presence of Ii71, the strong inhibition observed after 1 h (75%) gradually decreased to 15% after 48 h. The time dependency of inhibition, as calculated from the data in Figure 7*a*, is depicted in Figure 7*b*. This effect is not due to different kinetics of AMCA-HA and AMCA-Ii71 binding, as the two association curves are almost superimposable (Fig. 7*a*). For ligands and inhibitors binding to the same site and stabilizing the same conformation of $\alpha\beta$ dimers, the inhibitory

potential is expected to be independent of the incubation time. Indeed, when the groove binding influenza matrix peptide IM-(19–31) was used as an inhibitor for AMCA-HA-(307–319), no time dependency of the inhibitory potential was observed. Together, these data indicate distinct binding sites for Ii71 and antigenic peptides and the existence of at least two distinct conformational states of $\alpha\beta$ dimers.

Discussion

Previous studies using recombinant Ii truncation and deletion variants provided indirect evidence that the CLIP region (residues 81–104) is essential for promiscuous binding to class II molecules. Especially residues 96 to 104 were found to mediate binding to DR1 (28), and residues 90 to 104 were sufficient for inhibition of loading of DR1, DR2, and DR3 with antigenic peptides (33, 46, 48). The present study shows that Ii peptide Ii90 (residues 90–108) binds not only to DR1 and DR2, but also to peptides from the flanking regions (amino acids 71–89 and 109–128), albeit with lower affinity (Table I and Fig. 8). The moderate or low affinity of these flanking regions might explain why they were not found in studies involving coprecipitation of certain Ii truncation constructs

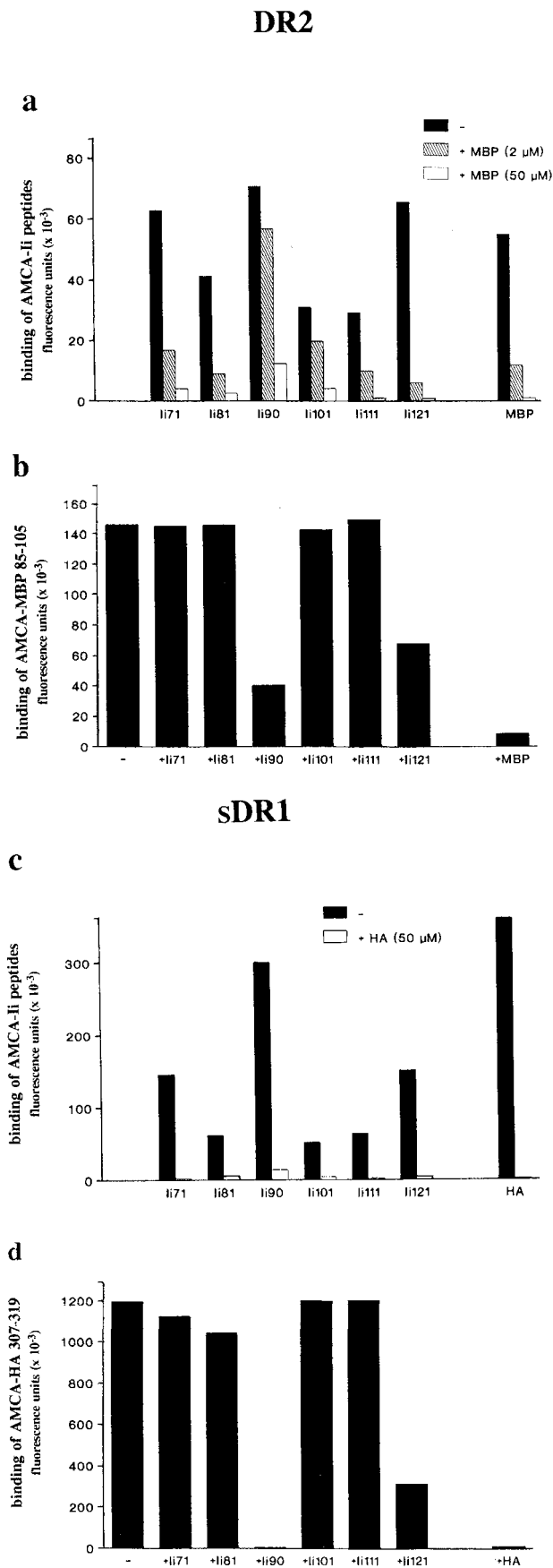


FIGURE 6. Interference of Ii peptides with antigenic peptides on DR molecules. *a*, Inhibition of Ii peptide binding by MBP-(85–105) on DR2. DR2 (200 nM) was incubated with various AMCA-labeled Ii pep-

with $\alpha\beta$ dimers. Several complementary findings strongly suggest that the Ii peptides spanning the region 71 to 128 bound to the DR molecule in the same manner as in the context of the intact Ii. 1) Nonoverlapping Ii peptides displayed additive binding and did not compete with each other, whereas overlapping peptides did not bind simultaneously (Fig. 2). 2) Soluble rIi prevented binding of all Ii peptides (Fig. 3). 3) The C-terminal Ii fragment p25 interfered only with those Ii peptides that overlap with the p25 sequence (Fig. 4). Thus, it is likely that in the context of whole Ii, not only CLIP but also the flanking regions contribute to the assembly of $\alpha\beta$:Ii complexes.

Inhibition by the superantigen SEB provided information on the respective contact sites of Ii peptides on the DR1 and DR2 $\alpha\beta$ dimers and suggested that the flanking regions covering residues 71 to 94 and 109 to 128 interact with the SEB contact area on the $\alpha 1$ domain. These results are schematically compiled in Figure 8. It should be kept in mind that determination of the precise boundaries of the respective segments awaits further studies. For example, it is not clear whether all residues of the segment 71 to 81 interact with the DR2 molecules, but the slightly higher affinity of Ii71 compared with that of Ii81 (Table I) indicates a contribution of at least some residues. Our SEB data seem to be in contrast to the earlier finding that Ii expression on the cell surface does not inhibit SEB binding to DR1⁺ cells (60), but in agreement with our data, inhibition of SEB binding to cells by rIi was recently reported (61).

The only peptide not affected by SEB was Ii95 (Fig. 5). However, the Ii95 sequence is contained in Ii90, which is a powerful competitor of antigenic peptide binding on both DR alleles (Fig. 6, *b* and *d*). The most straightforward explanation for both findings is that peptide Ii90 binds directly to the peptide binding groove. This would be consistent with the recent suggestion that CLIP binds to class II molecules like conventional antigenic peptides, with Met⁹¹ of CLIP being part of a supermotif and an important anchor residue for pocket P1 of the DR groove (47, 48). Indeed, the x-ray structure of a DR3:CLIP complex visualizes residues Pro⁸⁷ to Ala¹⁰¹ in the binding groove, with Met⁹¹ being one of the anchor residues (P., Gosh, M. Amaya, E. Mellins, and D. C. Wiley, personal communication). With Ii90 being located in the groove, it has to be explained how inhibition of Ii90 by SEB from outside the groove is accomplished (Fig. 5). Two possibilities come to mind. Assuming that the crystal data describing binding of CLIP to DR3 can also be extended to DR1 and DR2 molecules with Lys⁹⁰ and Met⁹¹ lying in the groove, then SEB binding outside the groove could be accompanied by small structural changes inside the peptide groove that would prevent binding of Ii90, but not binding of Ii95 or the antigenic peptides HA and MBP. This model would be

ides (1 μ M) in the absence (black bars) or the presence of 2 μ M MBP-(85–105) (hatched bars) or 50 μ M MBP-(85–105) (white bars) for 48 h in HPSEC binding assay. *b*, Inhibition of AMCA-MBP 85–105 binding by Ii peptides on DR2. DR2 (200 nM) was coincubated with AMCA-MBP-(85–105) (100 nM) in the absence and the presence of the indicated Ii peptides (10 μ M) or MBP-(85–105) (10 μ M) for 48 h in the HPSEC binding assay. *c*, Inhibition of Ii peptide binding by HA-(307–319) on sDR1. sDR1 (50 nM) was incubated with various AMCA-labeled Ii peptides (1 μ M) in the absence (black bars) or the presence of 50 μ M HA-(307–319) (white bars) for 48 h in the HPSEC binding assay. *d*, Inhibition of AMCA-HA-(307–319) binding by Ii peptides on sDR1. sDR1 (50 nM) was coincubated with AMCA-HA-(307–319) (100 nM) in the absence and the presence of the indicated Ii peptides (10 μ M) or HA-(307–319) (10 μ M) for 48 h in the HPSEC binding assay.

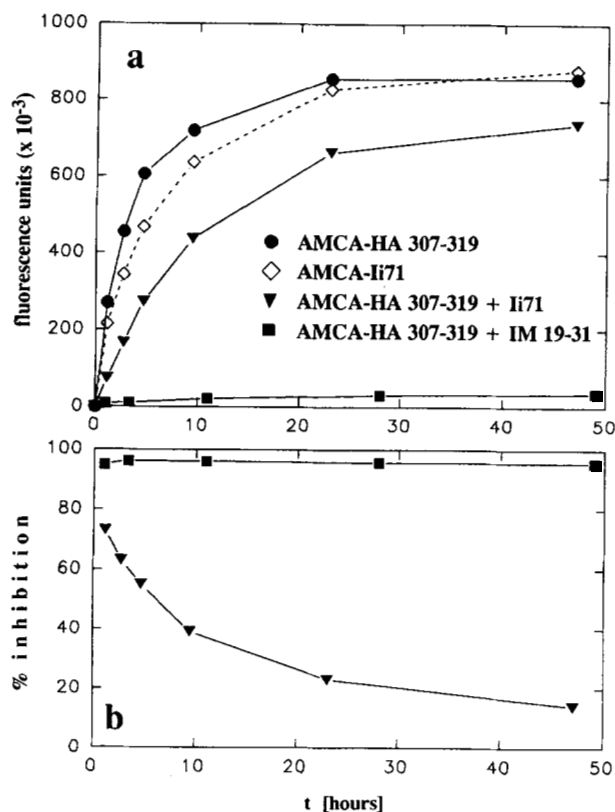


FIGURE 7. Kinetics of the inhibitory effect of Ii71 on AMCA-HA-(307-319) binding to sDR1. *a*, sDR1 (50 nM) was coincubated with 50 nM AMCA-HA-(307-319) (●), 10 μ M AMCA-Ii71 (◇), 50 nM AMCA-HA-(307-319) in the presence of 10 μ M Ii71 (▼) or with 50 nM AMCA-HA-(307-319) in the presence of 10 μ M IM-(19-31) (■) and analyzed by HPSEC after the indicated incubation times. *b*, Inhibitory capacity of Ii71 (▼) or IM 19-31 (■) on AMCA-HA-(307-319) (50 nM) binding to sDR1 at different time points, calculated from values in Figure 7*a*.

consistent with the hypothesis that some, but not all, groove binding peptides might interfere with SEB binding (62). Alternatively, our data raise the possibility that Ii90 binds differently to DR1 and DR2 than described for DR3:crystal. On DR1 and DR2 molecules residues 90 to 94 of Ii90 might reach out of the groove, thereby directly competing with SEB for an overlapping binding area on top or outside of the binding cleft. The latter model is consistent with distinct binding modes for antigenic peptides and Ii90, as suggested by the observation that antigenic peptides preclude the binding of Ii71 and Ii111 (Fig. 6), whereas Ii90 displayed simultaneous binding to both Ii peptides (Fig. 2).

The observation that antigenic peptides were able to prevent not only binding of Ii90, but, after long-term incubation, also binding of those Ii peptides whose contact sites are outside the groove on the SEB contact area, such as Ii71 and Ii111 (Fig. 6), suggests the existence of at least two conformational states of class II molecules. One state (designated here as S2) is described by the conformation we know from the crystallographic studies with HLA-DR1 (63); it is stabilized by groove binding peptides that bind with slow on and off rates and excludes simultaneous stable binding of Ii peptides under steady state conditions (Fig. 7). The other conformational state (S1) is compatible with Ii binding and characterized by fast on and off rates of its ligands explaining the fast on rate of Ii in vivo (64) and the fast off-rate of CLIP on DR1 (44), DR3, DR4, and DR11 (34). Since antigenic peptides can compete

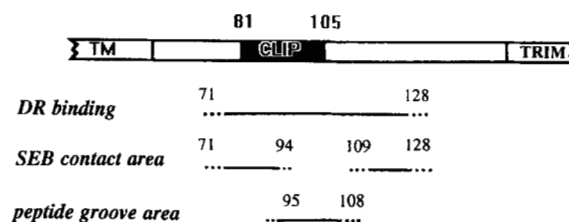


FIGURE 8. Functional domains of invariant chain.

with Ii peptides binding to the SEB contact area (e.g., Ii71; Fig. 7), they appear to be able to bind to the S1 state (or a similar intermediate conformation). With time, however, antigenic peptides (but not Ii peptides) induce a gradual shift toward the stable S2 state. Such a model would explain that inhibition of AMCA-HA by Ii71 decreases with time (Fig. 7), and inhibition of AMCA-Ii71 by antigenic peptides increases with time (data not shown). This model is consistent with previous kinetic studies also suggesting distinct conformational states of class II molecules (51-53). According to this, initial binding of antigenic peptide by a fast on rate and low affinity is followed by a conformational change resulting in a slow on rate and high affinity. Such a maturation of DR2 molecules could not be observed when CLIP was used as a ligand (46).

In conclusion, the present study adds further evidence that class II molecules are dynamic entities that can occur in distinct conformational states. These conformational modulations could be subtle, but sufficient to influence the respective binding sites. It would be advantageous if a conformation of $\alpha\beta$ favoring Ii binding were to be generated during biosynthesis, as this would facilitate rapid association with the trimeric Ii scaffold in the ER. Since CLIP has a fast off rate, it seems reasonable to assume that stabilization of the $\alpha\beta$ Ii complex could be mediated by additional Ii contact sites recruiting flanking regions of the CLIP segment, as described here. These additional contact sites may also compensate for the poor binding of the CLIP peptide to certain class II alleles, such as DR4 (34) (H. Kropshofer, A. Vogt, and G. J. Hämmerling, unpublished observations) or A^k (47).

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References

- Strubin, M., B. Mach, and E. O. Long. 1984. The complete sequence of the mRNA for the HLA-DR-associated invariant chain reveals a polypeptide with an unusual transmembrane polarity. *EMBO J.* 3:869.
- Germain, R. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76:287.
- Cresswell, P. 1992. Chemistry and functional role of the invariant chain. *Curr. Opin. Immunol.* 4:87.
- O'Sullivan, D. M., D. Noonan, and V. Quaranta. 1987. Four invariant chain forms derive from a single gene by alternate splicing and alternate initiation of transcription/translation. *J. Exp. Med.* 166:444.
- Strubin, M., C. Berte, and B. Mach. 1986. Alternate splicing and alternate initiation of translation explain the four forms of the Ia-antigen associated invariant chain. *EMBO J.* 5:3483.
- Roche, P. A., M. S. Marks, and P. Cresswell. 1991. Formation of a nine-subunit complex by HLA class II glycoproteins and the invariant chain. *Nature* 354:392.
- Roche, P. A., and P. Cresswell. 1990. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 345:615.
- Newcomb, J., and P. Cresswell. 1993. Characterization of endogenous peptides bound to purified HLA-DR molecules and their absence from invariant chain-associated $\alpha\beta$ dimers. *J. Immunol.* 150:499.
- Busch, R., I. Y. Vturina, J. Drexler, F. Momburg, and G. J. Hämmerling. 1995. Poor loading of MHC class II molecules with endogenously synthesized short peptides in the absence of invariant chain. *Eur. J. Immunol.* 25:48.

10. Germain, R., and A. G. Rinker, Jr. 1993. Peptide binding inhibits protein aggregation of invariant-chain free class II dimers and promotes selective cell surface expression of occupied molecules. *Nature* 363:725.
11. Viville, S., J. Neeffjes, V. Lotteau, A. Dierich, M. Lemeur, H. Ploegh, C. Benoist, and D. Mathis. 1993. Mice lacking the MHC class II-associated invariant chain. *Cell* 72:635.
12. Bakke, O., and B. Dobberstein. 1990. MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. *Cell* 63:707.
13. Lotteau, V., L. Teyton, A. Peleraux, T. Nilsson, L. Karlsson, S. L. Schmid, V. Quaranta, and P. Peterson. 1990. Intracellular transport of class II molecules directed by invariant chain. *Nature* 348:600.
14. Amigorena, S., J. R. Drake, P. Webster, and I. Mellman. 1994. Transient accumulation of new MHC class II molecules in a novel endocytic compartment in B lymphocytes. *Nature* 369:113.
15. Tulp, A., D. Verwoerd, B. Dobberstein, H. L. Ploegh, and J. Pieters. 1994. Isolation and characterization of the intracellular MHC class II compartment. *Nature* 369:120.
16. Qiu, Y., X. Xu, A. Wandinger-Ness, D. P. Dalke, and S. K. Pierce. 1994. Separation of subcellular compartments containing distinct functional forms of MHC class II. *J. Cell. Biol.* 125:595.
17. West, M. A., J. M. Lucoq, and C. Watts. 1994. Antigen processing and MHC class II loading compartments in human lymphoblastoid cells. *Nature* 369:147.
18. Nowell, J., and V. Quaranta. 1985. Chloroquine affects biosynthesis of Ia molecules by inhibiting dissociation of invariant (c) chains from α -B dimers in B cells. *J. Exp. Med.* 162:1371.
19. Maric, M. A., M. D. Taylor, and J. S. Blum. 1994. Endosomal aspartic proteinases are required for invariant chain processing. *Proc. Natl. Acad. Sci. USA* 91:2171.
20. Unanue, E. R. 1992. Cellular studies on antigen presentation by class II MHC molecules. *Curr. Opin. Immunol.* 4:63.
21. Chicz, R. M., R. G. Urban, J. C. Gorga, D. A. A. Vignali, W. S. Lane, and J. L. Strominger. 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *J. Exp. Med.* 178:27.
22. Thomas, L. J., Q. V. Nguyen, W. L. Elliott, and R. E. Humphreys. 1988. Proteolytic cleavage of Ii to p25. *J. Immunol.* 140:2670.
23. Mehlinger, J., M. Harris, C. S. Kindle, D. W. McCourt, and S. E. Cullen. 1991. Characterization of fragments of the murine Ia-associated invariant chain. *J. Immunol.* 146:920.
24. Demotz, S., and C. Danieli. 1993. Release of DR molecules from complexes with invariant chain through the formation of a C-terminal 25 kDa invariant chain fragment. *Mol. Immunol.* 30:1623.
25. Blum, J. S., and P. Cresswell. 1988. Role for intracellular proteases in the processing and transport of class II HLA antigens. *J. Immunol.* 85:3975.
26. Newcomb, J., and P. Cresswell. 1993. Structural analysis of proteolytic products of MHC class II-invariant chain complexes generated in vivo. *J. Immunol.* 151:4153.
27. Freiswinkel, I. M., K. Schenck, and N. Koch. 1993. The segment of invariant chain that is critical for association with major histocompatibility complex class II molecules contains the sequence of a peptide eluted from class II polypeptides. *Proc. Natl. Acad. Sci. USA* 90:9703.
28. Bijlmakers, M. E., P. Benaroch, and H. L. Ploegh. 1994. Mapping functional regions in the luminal domain of class II-associated invariant chain. *J. Exp. Med.* 180:623.
29. Romagnoli, P., and R. N. Germain. 1994. The CLIP region of invariant chain plays a critical role in regulating major histocompatibility complex class II folding, transport and peptide occupancy. *J. Exp. Med.* 180:1107.
30. Riberdy, J. M., J. R. Newcomb, M. J. Surman, J. A. Barbosa, and P. Cresswell. 1992. HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides. *Nature* 360:474.
31. Rudensky, A. Y., P. Preston-Hurlburt, S. C. Hong, A. Barlow, and C. A. Janeway, Jr. 1991. Sequence analysis of peptides bound to MHC class II molecules. *Nature* 353:622.
32. Hunt, D. F., H. Michel, T. A. Dickinson, J. Shabanowitz, A. L. Cox, K. Sakaguchi, E. Appella, H. M. Grey, and A. Sette. 1992. Peptides presented to the immune system by murine major histocompatibility complex molecule I-Ad. *Science* 256:1817.
33. Chicz, R. M., R. G. Urban, W. S. Lane, J. C. Gorga, L. J. Stern, D. A. A. Vignali, and J. L. Strominger. 1992. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. *Nature* 358:764.
34. Avva, R., and P. Cresswell. 1994. In vivo and in vitro formation and dissociation of HLA-DR complexes with invariant chain-derived peptides. *Immunity* 1:763.
35. Vogt, A. B., H. Kropshofer, H. Kalbacher, M. Kalbus, H.-G. Rammensee, J. E. Coligan, and R. Martin. 1994. Ligand motifs of HLA-DRB5*0101 and DRB1*1501 molecules delineated from self-peptides. *J. Immunol.* 153:1665.
36. Gautam, A., C. Pearson, V. Quinn, H. O. McDevitt, and P. Milburn. 1995. Binding of an invariant chain peptide, CLIP, to I-A major histocompatibility complex class II molecules. *Proc. Natl. Acad. Sci. USA* 92:335.
37. Max, H., T. Halder, H. Kropshofer, M. Kalbus, C. A. Müller, and H. Kalbacher. 1993. Characterization of peptides bound to extracellular and intracellular HLA-DR1 molecules. *Hum. Immunol.* 38:193.
38. Chicz, R. M., W. S. Lane, R. A. Robinson, M. Trucco, J. L. Strominger, and J. C. Gorga. 1994. Self-peptides bound to the type I diabetes associated class II molecules HLA-DQ1 and HLA-DQ8. *Int. Immunol.* 6:1639.
39. Morris, P., J. Shaman, M. Attaya, M. Amaya, S. Goodman, C. Bergman, J. J. Monaco, and E. Mellins. 1994. An essential role for HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature* 368:551.
40. Fling, S. P., B. Arp, and D. Pious. 1994. The HLA-DMA gene is required for the formation of stable class II/peptide complexes. *Nature* 368:554.
41. Sandersson, F., M. Kleijmeer, A. Kelly, D. Verwoerd, A. Tulp, J. Neeffjes, H. J. Geuze, and J. Trowsdale. 1994. Accumulation of HLA-DM, a regulator of antigen presentation, in MHC class II compartments. *Science* 266:1566.
42. Karlsson, L., A. Peleraux, R. Lindstedt, M. Liljedahl, and P. A. Peterson. 1994. Reconstitution of an operational MHC class II compartment in nonantigen-presenting cells. *Science* 266:1569.
43. Germain, R., and L. R. Hendrix. 1991. MHC class II structure, occupancy and surface expression determined by post-endoplasmic reticulum antigen binding. *Nature* 353:134.
44. Urban, R. G., R. M. Chicz, and J. L. Strominger. 1994. Selective release of some invariant chain-derived peptides from HLA-DR1 molecules at endosomal pH. *J. Exp. Med.* 180:751.
45. Monji, T., A. L. McCormack, J. R. Yates III, and D. Pious. 1994. Invariant-cognate peptide exchange restores class II dimer stability in HLA-DM mutants. *J. Immunol.* 153:4468.
46. Kropshofer, H., A. B. Vogt, and G. J. Hämmerling. 1995. Structural features of invariant chain CLIP region controlling rapid release from HLA-DR molecules and inhibition of peptide binding. *Proc. Natl. Acad. Sci. USA* 92:8343.
47. Sette, A., S. Southwood, J. Miller, and E. Appella. 1995. Binding of major histocompatibility complex class II to the invariant chain-derived peptide, CLIP, is regulated by allelic polymorphism in class II. *J. Exp. Med.* 181:566.
48. Malcherek, G., V. Gnau, G. Jung, H.-G. Rammensee, and A. Melms. 1995. Supermotifs enable natural invariant chain-derived peptides to interact with major histocompatibility complex class II molecules. *J. Exp. Med.* 181:527.
49. Peterson, M., and J. Miller. 1990. Invariant chain influences the immunological recognition of MHC class II molecules. *Nature* 345:172.
50. Anderson, M. S., and J. Miller. 1992. Invariant chain can function as a chaperone protein for class II major histocompatibility complex molecules. *Proc. Natl. Acad. Sci. USA* 89:2282.
51. Sadegh-Nasseri, S., and H. M. McConnell. 1989. A kinetic intermediate in the reaction of an antigenic peptide and I-E^d. *Nature* 337:274.
52. Sadegh-Nasseri, S., L. J. Stern, D. C. Wiley, and R. N. Germain. 1994. MHC class II function preserved by low-affinity peptide interactions preceding stable binding. *Nature* 370:647.
53. Beeson, C., and H. M. McConnell. 1994. Kinetic intermediates in the reactions between peptides and proteins of major histocompatibility complex class II. *Proc. Natl. Acad. Sci. USA* 91:8842.
54. Stern, L. J., J. H. Brown, T. S. Jardetzky, J. C. Gorga, R. G. Urban, J. L. Strominger, and D. C. Wiley. 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* 368:215.
55. Stern, L. J., and D. C. Wiley. 1992. The human class II MHC protein HLA-DR1 assembles as empty $\alpha\beta$ heterodimers in the absence of antigenic peptides. *Cell* 68:465.
56. Gorga, J. C., V. Horejsi, D. R. Johnson, R. Raghupathy, and J. L. Strominger. 1987. Purification and characterization of class II histocompatibility antigens from a homozygous human B cell line. *J. Biol. Chem.* 262:16087.
57. Kropshofer, H., I. Böhlinger, H. Max, and H. Kalbacher. 1991. Self and foreign peptides interact with intact and disassembled MHC class II antigen HLA-DR via Trp pockets. *Biochemistry* 30:9177.
58. Kropshofer, H., H. Max, and H. Kalbacher. 1993. Evidence for cobinding of self- and allopeptides to human class II major histocompatibility antigen DR1 by energy transfer. *Proc. Natl. Acad. Sci. USA* 90:403.
59. Jardetzky, T. S., J. H. Brown, J. C. Gorga, L. J. Stern, R. G. Urban, Y. Chi, C. Stauffacher, J. L. Strominger, and D. C. Wiley. 1994. Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen. *Nature* 368:711.
60. Karp, D. R., R. N. Jenkins, and E. O. Long. 1992. Distinct binding sites on HLA-DR for invariant chain and staphylococcal enterotoxins. *Proc. Natl. Acad. Sci. USA* 89:9657.
61. Ericson, M. L., M. Sundström, D. M. Sansom, and D. J. Charron. 1994. Mutually exclusive binding of peptide and invariant chain to major histocompatibility complex class II antigens. *J. Biol. Chem.* 269:26531.
62. Thibodeau, J., I. Cloutier, P. M. Lavoie, N. Labrecque, W. Mourad, T. S. Jardetzky, and R.-P. Sekaly. 1994. Subsets of HLA-DR1 molecules defined by SEB and TSST-1 binding. *Science* 266:1874.
63. Brown, J. H., T. S. Jardetzky, J. C. Gorga, L. J. Stern, R. G. Urban, J. L. Strominger, and D. C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364:33.
64. Kvist, S., K. Wiman, L. Claesson, P. A. Peterson, and B. Dobberstein. 1982. Membrane insertion and oligomeric assembly of HLA-DR histocompatibility antigens. *Cell* 29:61.
65. Kudo, J., L. Y. Chao, F. Narni, and G. F. Saunders. 1985. Structure of the human gene encoding the invariant γ -chain of class II histocompatibility antigens. *Nucleic Acids Res.* 13:8827.