Promiscuous Binding of Synthetic Copolymer 1 to Purified HLA-DR Molecules

Masha Fridkis-Hareli and Jack L. Strominger

*J Immunol* 1998; 160:4386-4397; http://www.jimmunol.org/content/160/9/4386

---

**References**

This article cites 68 articles, 36 of which you can access for free at:
http://www.jimmunol.org/content/160/9/4386.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Promiscuous Binding of Synthetic Copolymer 1 to Purified HLA-DR Molecules

Masha Fridkis-Hareli and Jack L. Strominger

Copolymer 1 (Cop 1) is a random synthetic amino acid copolymer of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine, effective both in suppression of experimental allergic encephalomyelitis and in the treatment of relapsing forms of multiple sclerosis. Cop 1 binds promiscuously and very efficiently to living APCs of various HLA haplotypes. In the present study, a substantial part of the whole mixture of random polypeptides that compose Cop 1 was shown to bind to purified human HLA-DR1, DR2, and DR4 with high affinity in a temperature- and time- and, in the case of DR4, pH-dependent manner, and was competitively inhibited by DR-restricted peptides, but not by peptide derivatives that bind with low affinity. Bacterial superantigens inhibited Cop 1 binding only at very high concentrations. The formation of the Cop 1-DRI complex was also shown by SDS-PAGE. These findings represent the first direct evidence for interactions of Cop 1 with purified DR molecules, and suggest that its effectiveness in experimental allergic encephalomyelitis and multiple sclerosis may be directly related to its binding in the groove of HLA-DR proteins.


Maj or histocompatibility complex molecules play a central role in regulation of immune responses through their ability to bind and present processed peptides to T cells (1). Crystallographic analysis of class I (2, 3) and class II (4, 5) MHC molecules has shown that, whereas both ends of the binding groove are open in the latter, in class I MHC molecules both ends are closed and involved in peptide binding. These structural differences result in distinct models of peptide binding that have been further elucidated by acid extraction and sequencing of natural MHC-associated ligands (6–12). The majority of peptides eluted from class I molecules have an allele-specific binding motif and are 8 to 11 amino acids long. In contrast, class II MHC-bound peptides vary in length (10 to 34 amino acids) and also exhibit distinct sequence motifs (12–17).

Copolymer 1 (Cop 1) is a synthetic amino acid copolymer effective both in suppression of experimental allergic encephalomyelitis (18–22) and in the treatment of relapsing forms of multiple sclerosis (MS) (23, 24). The possible mechanisms proposed for the activity of Cop 1 in experimental allergic encephalomyelitis and MS involve, in the first step, binding to class II MHC molecules, following which two pathways may be activated: 1) induction of Ag-specific suppressor cells (21, 25), and 2) competition with myelin Ags for activation of specific effector T cells (26). Indeed, Cop 1 inhibited the response to myelin basic protein (MBP) of various MBP-specific T cell lines and clones, irrespective of their MHC restriction, only in the presence of APC, suggesting that the site of competition between MBP and Cop 1 is most probably the class II MHC peptide-binding site (27). Cop 1 bound promiscuously and very efficiently to living APCs of various HLA haplotypes, as well as to mouse APC (28). Cop 1 competed with the autoantigens MBP, proteolipid protein, and myelin oligodendrocyte glycoprotein for APC binding, and displaced these bound autoantigens, whereas it could not be displaced by these substances (29, 30). The binding of Cop 1 was immediate and very efficient, regardless of the incubation temperature (and even at 0°C) or treatment of the APC (fixation, protease inhibitors), suggesting that proteolysis is not required for Cop 1 interaction with the class II MHC proteins (31).

The present study was undertaken to attempt to resolve some of the anomalies encountered in studies of binding to intact cells, particularly the instantaneous kinetics and the independence of temperature. Moreover, in those studies, evidence of binding to class II MHC proteins was indirect and based on inhibition of binding by an anti-HLA-DR mAb, which could have several interpretations. Thus, in spite of the accumulated data on immunologic recognition of Cop 1 and its effects in vivo and in vitro, direct evidence for Cop 1 binding to class II MHC molecules and the precise analysis of these interactions are still missing. This characterization is of a great importance in view of the therapeutic applications of Cop 1 in MS patients. In this study, Cop 1 was shown to interact with purified HLA-DR1, DR2, and DR4 molecules. Using detergent-solubilized HLA-DR and biotinylated Cop 1, Cop 1 binding was inhibited significantly by DR-restricted peptides, but not by peptide derivatives that bind with low affinity. Various characteristics relating to the affinity, kinetics, temperature, and pH dependence of the binding, as well as the effect of superantigens will be described. These characteristics are substantially different from those observed previously using APC.

Materials and Methods

Cell lines and Abs

Homozygous EBV-transformed human B lymphocyte lines used for immunoaffinity purification of HLA-DR1, DR2, and DR4 molecules were
LG-2 (DRB1*0101), MAGE-1 (DRB1*1501), and Priess (DRB1*0401/DRB4*0101), respectively. The cells were grown in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 50 μg/ml penicillin G, and 50 μg/ml streptomycin in roller bottles and stored as pellets at −80°C. The anti-DR hybridoma LB3.1 (IgG2b) (32) was grown in serum-free medium (Macrophage-SFM; Life Technologies, Gaithersburg, MD).

### Protein purification

Immunoadfinity purification of HLA-DR1, DR2, and DR4 molecules was performed as previously reported (33), with minor modifications. Briefly, detergent-soluble membrane preparations from LG-2, MAGE-1, and Priess cells were passed at a flow rate of approximately 11 ml/h through a series of columns in the following sequence: Sepharose CL-6B (30 ml), normal mouse serum Affi-gel 10 (10 ml), protein A-Sepharose CL-4B (5 ml), and the LB3.1 protein A-Sepharose CL-4B (5 ml). DR2a (DRB5*0101) and DR53 (DRB4*0101) allele-linked molecules were not removed from the LB3.1 protein A-Sepharose CL-4B (5 ml). DR2a (DRB5*0101) and DR53 (DRB4*0101) were captured with LB3.1, and biotin-peptide binding was measured. Insets are Lineweaver-Burk plots of the binding data.

### Peptides and proteins

Cop 1 is a synthetic random copolymer prepared by polymerization of the N-carboxyamidocarboxylic acid of t-alanine, γ-benzyl-l-glutamate, ε-N-trifluoroacetyl-l-lysine, and β tyrosine (18) (the end product is a mixture of acetate and 2-acylaminobenzyl-l-glutamate, as described (28)). Unreacted biotin was removed by dialysis (Spectra/Por membrane MWCO 5000; Spectrum Medical Industries, Laguna Hills, CA).

#### Class II peptide-binding assays

**Binding reactions**

Detergent-solubilized HLA-DR1, DR2, or DR4 molecules (0.5 μg/sample) were incubated with biotinylated peptides at various concentrations for 40 h at 37°C in 50 μl of the binding buffer, transferred to prepared microtiter assay plates, and incubated for 1 h at 37°C for capture of peptide

### Table I. **Affinity of Cop 1 binding to purified HLA-DR molecules**

<table>
<thead>
<tr>
<th>Cop 1</th>
<th>HA 306-318</th>
<th>MBP 84-102</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (μM)</td>
<td>IC₅₀ (μM)</td>
</tr>
<tr>
<td>DR1</td>
<td>10.0 × 10⁻⁸</td>
<td>7.5</td>
</tr>
<tr>
<td>DR2</td>
<td>13.0 × 10⁻⁸</td>
<td>10</td>
</tr>
<tr>
<td>DR4</td>
<td>4.5 × 10⁻⁸</td>
<td>16</td>
</tr>
</tbody>
</table>

aCop 1 with average m.w. of 8800, 7800, and 8150, and HA 306-318 were incubated at a range of concentrations with purified HLA-DR molecules at pH 5.0 followed by capture with class II-specific mAb and peptide detection with alkaline phosphatase-streptavidin. KG is the dissociation constant at equilibrium, was calculated using the Lineweaver-Burk equation (40). IC₅₀, inhibitory concentration giving 50% inhibition, was calculated based on the competitive binding assays (Fig. 2A).

bIC₅₀ values for unlabeled MBP 84-102 in the competitive binding assays with tetanus toxin (see Fig. 2A).
FIGURE 2. Inhibition of binding of Cop 1 (A), MBP 84–102, and HA 306–318 (B) to HLA-DR molecules by different competitors. Purified HLA-DR1, DR2, or DR4 was incubated with A, biotinylated Cop 1 (7800) and Cop 1 (8150) (1.5 μM), or B, biotinylated HA 306–318 and MBP 84–102 (1.5 μM) alone or in the presence of unlabeled Cop 1, MBP 84–102, HA 306–318, HA 306–318 (K308), or HA 306–318 (D308) at a range of concentrations. All incubations were conducted in duplicate at pH 5 for 40 h at 37°C. For methods, see legend for Figure 1. Specific binding is expressed as percentage of inhibition using the formula: percentage of inhibition = 100% − [(signal with competitor − background/signal without competitor − background) × 100].
class II complexes. Water-soluble HLA-DR1 molecules, produced in insect cells infected with baculoviruses (35), were obtained from Dr. D. C. Wiley (Harvard University, Cambridge, MA). Binding of biotinylated peptides to water-soluble HLA-DR was conducted in the binding buffer with no n-octyl β-D-glycopyranoside or in PBS under similar conditions as for detergent-soluble DR molecules.

Inhibition reactions
Biotinylated Cop 1, MBP 84–102, or HA 306–318 at a final concentration of 1.5 μM in 50 μl of the binding buffer was coincubated with unlabeled inhibitors (Cop 1, HA 306–318, HA 306–318 (D308), HA 306–318 (K308), MBP 84–102, SEA, SEB, or TSST-1) and DR molecules for 40 h at 37°C. The inhibition assay of Cop 1 binding by SEA was performed in the presence of 10 μM zinc chloride in the binding buffer (36).

Detection of peptide class II complexes
Biotinylated peptide-biotin was detected using streptavidin-conjugated alkaline phosphatase, as follows. Plates were washed three times with TTBS and incubated with 100 μl of streptavidin-conjugated alkaline phosphatase (1:3000; Bio-Rad, Richmond, CA) for 1 h at 37°C, followed by addition of p-nitrophenyl phosphate in triethanolamine buffer (Bio-Rad). The absorbance at 410 nm was monitored by a microplate reader (model MR4000; Dynatech, Chantilly, VA).

FIGURE 2. (continued)

FIGURE 3. Binding of Cop 1 to HLA-DR1, DR2, and DR4 at pH 3 to pH 11. Purified HLA-DR1, DR2, or DR4 (0.5 μg/sample) was incubated in duplicate with the three preparations of biotinylated Cop 1 (1.5 μM) for 40 h at 37°C. For methods, see legend for Figure 1.
PAGE and Western blot

SDS-PAGE was conducted with the NOVEX mini cell electrophoresis system. Separation gel was 10% in acrylamide, and stacking gel was 5%. DR1-Cop 1 complexes were run under nonreducing conditions for 200 V, stained with Coomassie brilliant blue, fixed for 3 h in 10% methanol/10% acetic acid, and dried on Cellophane paper (Bio-Rad) at 25°C. For blotting, samples were transferred to nitrocellulose paper (PROTRAN). Detection of Cop 1-DR1 complexes was by either streptavidin-conjugated horseradish peroxidase (HRP) and ECL (Amersham, Arlington Heights, IL) (using biotinylated Cop 1 in the assay), or polyclonal DR antiserum, followed by HRP-conjugated anti-rabbit IgG Ab and ECL.

Results

Binding of Cop 1 to purified HLA-DR1, DR2, and DR4 molecules

Detergent-soluble DR1, DR2, and DR4 proteins were purified from homozygous EBV-transformed B cell lines LG-2, MGAR, and Priess (DRB1*0101, *1501, and *0401, respectively). The total protein extracted from 50 g of cells was 35 to 40 mg in each case (70–80% of the yields previously reported for DR1 (33)). They were stable αβ complexes (>95%), as measured by SDSPAGE (data not shown). To determine the affinity of Cop 1 for HLA-DR proteins, binding assays were conducted with biotinylated Cop 1 with an average m.w. of 5800 (Cop 1 (5800)), 7800 (Cop 1 (7800)), and 8150 (Cop 1 (8150)), and biotinylated HA 306–318 peptide, a promiscuous epitope (37–39). Cop 1 and HA 306–318 were incubated at a range of concentrations with purified HLA-DR molecules at pH 5, followed by capture with class II-specific mAb and peptide detection with alkaline phosphatase-streptavidin. Cop 1 and HA 306–318 bound in a dose-dependent manner, and the binding was saturable (Fig. 1, A–C). Double-reciprocal plots of the binding data were linear, allowing the calculation of apparent $K_d$ values using the Lineweaver-Burk equation (40). The affinity of the three preparations of Cop 1 tested to HLA-DR1 was somewhat lower than that of HA 306–318 (Table I), whereas their affinity to DR2 was higher and to DR4 was similar to HA 306–318, and within the range of values for antigenic peptides that bind to the peptide-binding groove (41–43, 44).

To further characterize the affinity of Cop 1 and HA 306–318 binding, competitive binding assays were conducted with biotinylated Cop 1 and unlabeled inhibitors (Cop 1, MBP 84–102, HA 306–318 peptide, and its derivatives) (Fig. 2A). The binding of biotinylated Cop 1 to DR1 molecules for each preparation of Cop

FIGURE 4. Time course of Cop 1 and HA 306–318 binding to HLA-DR1, DR2, and DR4. A. At different temperatures for 6 h; B, time course at 37°C up to 72 h; or C, stability of Cop 1-DR complexes at 37°C up to 72 h. Purified HLA-DR1, DR2, or DR4 (0.5 µg/sample) was incubated in duplicate with biotinylated Cop 1 (5800) (1.5 µM) at pH 5 for various time intervals (A, B), or with biotinylated Cop 1 (5800), Cop 1 (7800), and Cop 1 (8150) for 24 h, followed by removal of the excess of Cop 1 and reincubation (C). For methods, see legend for Figure 1.
1 was inhibited similarly by unlabeled Cop 1 and by HA 306–318 peptide, but 10-fold less by MBP 84–102, as expressed by the 50% inhibitory dose (Table I). In the case of DR2, unlabeled Cop 1 was a better competitor than both peptides, whereas the binding to DR4 molecules was somewhat better inhibited by MBP 84–102 and HA 306–318 than by Cop 1. In contrast, HA 306–318 derivatives with the substitution of Y at position 308 to either D or K, which were previously shown to bind HLA-DR1 with low affinity (13), did not have any effect on Cop 1 binding to HLA-DR1, DR2, or DR4 (Fig. 2A). Furthermore, the binding of biotinylated HA 306–318 to DR1 and DR4, and of biotinylated MBP 84–102 to DR2 was inhibited efficiently by the three preparations of unlabeled Cop 1 (Fig. 2B), suggesting that the binding site of the antigenic peptides HA 306–318 and MBP 84–102 overlaps with that of Cop 1. Thus, Cop 1 binds promiscuously to DR1, DR2, and DR4 molecules, with an affinity range similar to that of antigenic peptides for which it is an effective competitor.

**pH dependence of Cop 1 binding**

Peptide-binding experiments have suggested that class II molecules may differ with respect to the pH at which they prefer to bind antigenic peptides (41–43). In general, peptide binding is enhanced at acidic pH compared with neutral pH (41, 42, 45, 46). To examine whether Cop 1 binding is affected by pH, biotinylated Cop 1 was incubated with purified HLA-DR1, DR2, or DR4 at pH range from 3 to 11. The results (Fig. 3) showed similar levels of Cop 1 binding to DR1 and DR2, but lower binding to DR4 at neutral pH (probably due to slower binding (see below)).

**Time course and temperature dependence of Cop 1 binding to HLA-DR molecules**

Antigenic peptides bind to class II proteins, either purified or on the surface of APC, with slow association rates (40, 47, 48). Similar kinetics of binding to living APC was described for a synthetic multichain polypeptide (49). In contrast, Cop 1 binding to both murine and human APC was immediate, regardless of the incubation temperature (31). Therefore, the time course of biotinylated Cop 1 and HA 306–318 binding to purified DR1, DR2, and DR4 molecules was measured by incubation for various time intervals at different temperatures (Fig. 4A). In contrast to the previously reported nearly instantaneous and promiscuous binding of Cop 1 to intact APC even at 0°C (28, 31), incubation of Cop 1 (5800) with DR1 and DR4 molecules at different temperatures showed no binding at 0°C, very low levels at 25°C, and higher binding at 37°C, which increased over a period of 6 h in a manner similar to the HA 306–318 peptide (although the binding to DR4 was unusually slow even at 37°C). By contrast, a fraction of the DR2 molecules appeared to bind Cop 1 very rapidly even at low temperature (the binding was specific since it was inhibited by unlabeled Cop 1 (data not shown)); this fraction may represent a fraction of the total, which is either empty or binds very low affinity self peptides. Throughout the incubation period, the levels of Cop 1 binding to DR1 and DR2 were higher than to DR4 (Fig. 4A). The time course of Cop 1 (7800) and Cop 1 (8150) binding to DR1, DR2, and DR4 molecules was similar to that of Cop 1 (5800) (data not shown). The complex of Cop 1 with all DR allotypes used was very stable, since the binding was detected after 72 h of incubation at 37°C, even when the excess Cop 1 was removed from the reaction mixture (Fig. 4, B and C).

**Effect of superantigens on Cop 1 binding to HLA-DR molecules**

Bacterial superantigens SEA, SEB, and TSST-1 are globular proteins that do not require processing to be presented to T cells and that bind outside the peptide-binding groove (50–53). However,
peptides may influence the interaction of class II proteins with bacterial exotoxins, since a mutation in the peptide-binding groove was shown to interfere with SEB and TSST-1 binding (54). Different effects of SEA and SEB on Cop 1 interaction with class II molecules on intact APC have previously been seen. Cop 1 and SEA do not seem to compete for the same binding site on the MHC molecule, since no effect of Cop 1 was found on SEA-induced proliferation of an Ag-restricted mouse T cell clone, in contrast to the strong inhibition of the Ag-specific T cell response (29). However, on the other hand, partial inhibition of Cop 1 binding to living APC was detected in the presence of SEB (unpublished observations). Competitive binding assays were, therefore, conducted with unlabeled SEA, SEB, and TSST-1. Cop 1 binding to DR1, DR2, and DR4 was only inhibited by the superantigens at very high molar ratios of superantigen:Cop 1 (50:1), and with somewhat higher levels of inhibition by SEB than by SEA or TSST-1 (Fig. 5). Similarly, binding of biotinylated SEB was inhibited extensively only by unlabeled SEB, with a modest effect by Cop 1 or HA 306-318 (Fig. 6). Thus, Cop 1 and superantigen compete only slightly for binding to HLA-DR molecules.

Determination of Cop 1 fraction bound to HLA-DR molecules

Cop 1, which is a mixture of polypeptides, composed of hydrophobic (Ala), aromatic (Tyr), as well as charged amino acids (Lys and Glu), may contain different sequences that are able to bind different MHC determinants. Therefore, it was of particular importance to determine the fraction of Cop 1 that binds to each DR allele. For that purpose, biotinylated Cop 1 (5800) was incubated with detergent-soluble DR1, DR2, or DR4 molecules over a large dose range up to a fivefold excess (Fig. 7). The binding of 0.15 μM Cop 1 to DR1, DR2, or DR4 reached saturation at 1:1 ratio (data not shown), suggesting that Cop 1 displaced endogenously bound peptides (also supported by data on Fig. 1, showing higher saturation levels of Cop 1 than of HA 306–318). The exact
proportion of the whole Cop 1 mixture and the nature of the epitope bound in each case are under continuing investigation.

**Binding of Cop 1 to water-soluble HLA-DR1**

Less than 20% of isolated class II molecules binds antigenic peptides added either in vitro (13, 55) or in vivo (63). The production of soluble HLA-DR1 with empty peptide-binding site in insect cells infected with baculoviruses made it possible to bind significantly more antigenic peptide with faster kinetic rates (35). To examine whether the binding of Cop 1 increases the stability of HLA-DR1 to denaturation by SDS, Cop 1 was incubated with soluble DR1 in PBS for 40 h at 37°C, and the resulting complexes were analyzed by SDS-PAGE (Fig. 8). Preincubation with either Cop 1 or HA 306–318 caused part of the soluble DR1 to become resistant to SDS-induced dissociation. The HA 306–318-DR1
complex migrated as a strong band that corresponds to the αβ heterodimer, whereas the Cop 1 mixture formed higher m.w. complexes with HLA-DR1 that were observed as numerous bands on the polyacrylamide gel. The shift of the bands at approximately 33 and 22 kDa was due to the presence of unbound Cop 1, which interfered with the migration of free DR1 chains. On the other hand, HA 306–318 derivatives with low affinity to HLA-DR1 (K308 or D308) did not form stable complexes (Fig. 8). To clarify whether the bands correspond to the αβ heterodimer complexed with Cop 1, Western blot was performed on the three preparations of biotinylated Cop 1 with or without HLA-DR1 molecules (Fig. 9A). In parallel, the nitrocellulose was probed with polyclonal DR antiserum (Fig. 9B). For each preparation of Cop 1 incubated with HLA-DR1, the band of the size of approximately 50 kDa contained both the αβ heterodimer and Cop 1, in contrast to the case when no DR1 was added (Fig. 9, A and B). The saturation-binding experiment (Fig. 10) showed that Cop 1 and HA 306–318 bound in a dose-dependent manner, but Cop 1 affinity to water-soluble empty HLA-DR1 was lower than that of HA 306–318 (Kₐ = 3 × 10⁻⁸ M for Cop 1, Kₐ = 1.7 × 10⁻⁸ M for HA 306–318 using the Lineweaver-Burk equation, similar to values obtained for DR1 isolated with bound peptides shown in Table I).

**FIGURE 8.** SDS-PAGE of soluble Cop 1-DR1 complexes. Water-soluble HLA-DR1 (5 µg/sample) molecules were incubated with excess unlabeled HA 306–318, HA 306–318 (K308), HA 306–318 (D308), and two different preparations of Cop 1, in PBS for 40 h at 37°C. Separation gel was 10% in acrylamide, and stacking gel was 5%. DR1-peptide complexes were run under nonreducing conditions for 1 h at 200 V, stained with Coomassie brilliant blue, and treated as described in Materials and Methods. The smear in the lanes loaded with Cop 1-DR1 complexes corresponds to the unbound Cop 1 itself, which migrates at 20 to 30 kDa, slower than its actual m.w. due to its linear conformation.

**FIGURE 9.** Western blot of soluble Cop 1-DR1 complexes. Water-soluble HLA-DR1 (5 µg/sample) molecules were incubated with excess biotinylated (A) or unlabeled (B) Cop 1 (5800), Cop 1 (7800), and Cop 1 (8150), in PBS for 40 h at 37°C. Following gel electrophoresis (see legend for Fig. 8), samples were transferred to a nitrocellulose paper. Detection of Cop 1-DR1 complexes was either by streptavidin-conjugated HRP and ECL (A), or by polyclonal DR antiserum, followed by HRP-conjugated anti-rabbit IgG Ab and ECL (B).
Discussion

In this study, direct evidence for binding of a synthetic random copolymer Cop 1 to purified HLA-DR molecules is provided. The binding site of Cop 1 overlaps that of the antigenic peptide HA 306–318, since 1) they are cross-inhibitory in competitive peptide-binding experiments, 2) they have similar kinetics and temperature dependence of binding, and 3) each stabilizes the empty DR1 αβ heterodimer against SDS denaturation.

Promiscuous binding of Cop 1 to intact APC of both mouse and human origin has previously been shown (28). The binding was immediate and very efficient, regardless of the incubation temperature (28, 31). In this study, Cop 1 was observed to bind to three different purified HLA-DR proteins in a manner similar to the antigenic peptide HA 306–318, exhibiting binding only at 37°C that increased over the period of at least 6 h of incubation. The discrepancy between these data may be due to 1) different mechanisms operating in the course of Cop 1 binding to purified HLA-DR molecules as compared with living APC, or 2) nonspecific interaction of Cop 1, which is a positively charged copolymer, with the cell surface. However, the latter was previously ruled out by several lines of evidence (28). On the other hand, using multichain polypeptide (Tyr,Glu)-Ala–Lys and the fluorometric binding assay to living cells, it was suggested that the affinity of peptide binding to intact cells may be higher than to purified soluble MHC molecules (49). Moreover, this possibility is also supported by the observed enhanced binding of peptides to class II MHC molecules in the presence of natural phospholipids from B lymphoma cells; the authors suggest that lipids may induce conformational changes in class II proteins that favor peptide binding (56).

The peptide-binding motifs of various human class II molecules have been identified (12, 57, 58). Anchor position 1, which accepts only aromatic or aliphatic peptide side chains, is essential for a high affinity interaction between peptides and all DR molecules tested to date. Other major anchor positions are found at peptide positions 4, 6, 7, and 9. The anchors tend to be flanked by clusters of charged residues; small residues, especially Ala, are frequent in the center of the motif. Several promiscuous peptides, capable of binding to many different class II alleles, have been identified (57–62). They were found to contain either overlapping class II-binding motifs or supermotifs (58, 59). Cop 1, which is a mixture of polypeptides, composed of hydrophobic (Ala), aromatic (Tyr), as well as charged amino acids (Lys and Glu), may contain different sequences that are able to bind to different MHC determinants. An alternative explanation for indiscriminate Cop 1 binding is that it contains promiscuous class II-binding motif(s). The promiscuous binding of Cop 1 to different DR proteins is of great interest in relationship to its effectiveness in treatment of an unselected group of MS patients. Although MS is linked genetically to DR2 (the DRB1*1501 allele), only about 60% of MS patients carry this allele.

The binding of Cop 1 to DR4 was faster and greater at acidic than at neutral pH, in contrast to the pH-independent DR1 and DR2 binding. Preferential binding to most class II molecules at low pH was observed previously, consistent with their exposure to Ag in acidic endosomal compartments (41–43, 45, 46). Higher levels of Cop 1 binding to DR4 at pH 5 are also consistent with the enhanced peptide binding observed at acidic pH, through exchange with the class II-associated invariant chain (CLIP) and exogenous peptides (59, 63–65).

Recently, a peptide from the B chain of insulin was shown to bind with high affinity to class II molecules through a site that is distinct from the peptide-binding groove (66). It weakly inhibited the binding of antigenic peptides, and peptides with high affinity for the peptide-binding groove did not inhibit binding of the insulin-derived peptide. The rate of association with class II was very rapid, reaching saturation in 2 h, and the binding was inhibited by SEB. However, Cop 1 efficiently inhibited the binding of HA 306–318, but not the low affinity peptides; conversely, HA 306–318 inhibited the binding of Cop 1. Moreover, the time course of Cop 1 binding was similar to that of HA 306–318. SEB, SEA, and TSS-T inhibit only at very high concentrations.

These data all suggest that the effectiveness of Cop 1 in experimental allergic encephalomyelitis and MS is the result of binding to class II MHC proteins within the peptide-binding groove. At this site it may act as either a blocking peptide or as an antagonist or partial agonist, resulting in suppression of autoimmune T cell responses (e.g., by induction of T suppressor cells) or anergy, or both. Since many known HLA-DR-binding motifs contain an aromatic or hydrophobic residue in anchor position 1, followed by several hydrophobic or aromatic anchors that are flanked by clusters of charged and small residues (5, 11, 12, 14, 17, 67–69), the presence of Y, E, K, and A in Cop 1 may well account for its promiscuous binding to HLA-DR molecules. In the present work, for DRB1*0101, anchor positions 1, 4, 6, and 9 can accommodate Y, A; A; A; and A, Y, respectively. For DRB1*0401, the first anchor can be Y, the second (position 4) A or E, followed by charged amino acids at positions 7 and 9 (K or E). Similarly, Y is found in the first anchor of DRB5*0101 (DR2a) molecules (70, 71), which are present in small amounts in the DR2 preparation. However, for DRB1*1501 (DR2b), no aromatic residue was found in the first anchor, while the second could have Y (70, 71). Regarding the latter, surprisingly Y, however, was also found in the mixture of peptides eluted from empty DR2 (unpublished data). Determination of the actual peptide sequence(s) from Cop 1 found in the binding sites of HLA-DR proteins, together with the identification of the potential T cell epitope within the mixture of Cop 1 polypeptides, is an important future goal.

Acknowledgments

We thank Mrs. Anastasia Haykov for expert technical assistance. This work is part of a collaboration with Dvora Teitelbaum, Ruth Arnon, and Michael Sela, Weizmann Institute (Israel), on Cop 1 binding to class II major histocompatibility complex molecules and its functional consequences.

References


