We had recently identified small molecular compounds that are able to accelerate the ligand exchange reactions of HLA-DR molecules. Here we show that this acceleration is due to the induction of a “peptide-receptive” state. Dissociation experiments of soluble HLA-DR2-CLIP (class II-associated invariant chain peptide) complex and peptide-binding studies with “nonreceptive” empty HLA-DR1 and -DR2 molecules revealed that the presence of a small phenolic compound carrying an H-bond donor group (–OH) results in the drastic increase of both off- and on-rates. The rate-limiting step for ligand exchange, the transition of the major histocompatibility complex molecule from a nonreceptive into the receptive state, is normally mediated by interaction with the chaperone HLA-DM. In this respect, the effect of small molecules resembles that of the natural catalyst, except that they are still active at neutral pH. These “chemical analogues” of HLA-DM can therefore modulate the response of CD4+ T cells by editing the antigen composition of surface-bound class II major histocompatibility complex on living antigen-presenting cells.

The invariant chain protein directs delivery of nascent MHC-class II molecules to late endosomal compartments (1, 2). During the transfer, it fulfills a dual function by also blocking the peptide-binding site to prevent premature peptide loading (3). In late endosomal compartments, the protein is proteolytically digested, leaving only a short peptide region (CLIP) that remains associated to the peptide-binding groove of the class II MHC molecule. Prior to peptide loading, CLIP has to be removed. This process is catalyzed by HLA-DM (4), an MHC class II-like molecule with chaperone function (5) that accelerates the dissociation of CLIP and of other low affinity peptides and facilitates loading with peptides with sufficient affinity to the MHC molecule (6–8).

The catalytic mechanism of HLA-DM-mediated ligand exchange is not known yet. It had been postulated (5) that it involves disruption of conserved H-bonds, which are part of a network connecting the peptide to the binding groove (9). Up to now, no crystal structures of HLA-DM-MHC-peptide complexes have been reported, but amino acid substitutions in class II MHC molecules affecting this H-bond network can, at least, indicate a drastic enhancement of spontaneous dissociation rates (10).

However, HLA-DM-mediated ligand exchange is apparently more complex than just accelerating ligand release. It is known that direct loading of peptides onto empty purified class II MHC molecules is a slow process (9). Once they have lost their ligand, class II MHC molecules quickly acquire a conformational state that renders them “nonreceptive” with regard to their capacity to bind peptides (11, 12). Although the conversion to the nonreceptive form seems to take place rapidly, the transition from the nonreceptive to a peptide-receptive state is very slow. HLA-DM apparently catalyzes this process also. By binding to empty class II MHC molecules, it keeps the complex in a state that permits the rapid exchange of peptides (13, 14).

We have recently shown that not only HLA-DM but also certain small molecular compounds are able to accelerate ligand exchange reactions on HLA-DR molecules (15). In contrast to HLA-DM, however, small molecular ligand exchange catalysts are effective at neutral pH. They can therefore mediate ligand exchange directly on the surface of living cells and facilitate loading of class II MHC molecules with peptide antigens (15) or even with full-length proteins (16). Because the presence of an H-bond-forming group appeared crucial for their function, it was assumed that the small molecules, as proposed earlier for HLA-DM, act by disrupting H-bonds. It was still unclear whether their catalytic effect is just because of the accelerated release of bound peptides or whether the functional state of the class II MHC molecule is affected by the small molecule.

To address this important question, we compared the catalytic effect of soluble HLA-DM with that of an active small molecule, parachlorophenol (pCP). Peptide release and peptide loading experiments were carried out with soluble forms of HLA-DR using preloaded complexes, or nonreceptive “empty” molecules, as well as with HLA-DR-expressing APC. The major objective was to determine whether the accelerated ligand exchange triggered by small molecules is caused by the induction of a receptive state.

**EXPERIMENTAL PROCEDURES**

**Peptide and Chemicals**—The small molecular compounds pCP, chlorobenzene (CB), and parachloroanisol (pCA) were obtained from Fluka. Stock solutions of these chemicals were prepared in PBS 10% Me2SO at a final concentration of 100 mM. The peptides IC106–120 (CLIP, KM RMAPTLLQALPM) (17), IC106–120_M107–112 (CLIPM107–112) (17), MBP86–100 (NPVHFFKNIIVPT) (18), HA306–318 (PRYVKQNTLK) (19), and CO280–272 (IAGFQGEPGQG) (20) were synthesized using standard solid phase F-moc chemistry. For the biotinylated peptides IC106–120 (bCLIP), MBP86–100 (bMBP86–100), and HA306–318 (bHA306–318), the biotin tag was attached to the N termini of peptides, which were extended by a short amino acid spacer sequence (SGSG).
**Surface Plasmon Resonance Analysis of Peptide-HLA-DR Complex Dissociation**—Preformed HLA-DR2-bCLIP complex was generated by incubating 10 μl of HLA-DR (1 mg/ml) with 0.5 μl of biotinylated CLIP peptide (1 mg/ml) in the presence of 5% ethanol for 18–24 h at 37 °C. The reaction was diluted 1:33 in 100 mM HEPES, 150 mM NaCl, 3 mM peptide (1 mg/ml) in the presence of 5% ethanol for 18–24 h at 37 °C.

**Detection of HLA-DR-Peptide Complexes by ELISA**—The binding reaction was added to ELISA plates coated previously with an α-HLA-DR monoclonal antibody (L243, American Type Culture Collection, Manassas, VA) and were incubated for 2 h at 4 °C. The plates were washed and incubated for 0.5 h at room temperature with Eu3+-labeled streptavidin (DELPIA, Wallac) and developed with a fluorescence enhancing solution (15 μM β-naphthyl)trifluoroacetate, 50 μM tri-o-cresylphosphine oxide, 6.8 mM potassium hydrogen phthalate, 100 mM acetic acid, 0.1% Triton X-100). The Eu3+ fluorescence was measured in a Victor fluorescence reader (Wallac) using the time-resolved mode at an excitation wavelength of 340 nm and an emission wavelength of 614 nm.

**Generation of a Receptive HLA-DR Molecule by Dissociation of Unstable Peptide Complexes**—Unstable peptide-HLA-DR complex was generated by incubating 2.5 μl of HLA-DR1 (1 mg/ml) with 1 μl of the low affinity peptide CLIP<sub>306–318</sub> (3 mg/ml) in the presence of 5% ethanol for 18 h at 37 °C. The formation of short-lived receptive HLA-DR1 was triggered by diluting the HLA-DR1-bCLIP<sub>306–318</sub> complex with PBS/1% bovine serum albumin to a final volume of 165 μl. The complex was incubated at 37 °C, and aliquots of 25 μl were taken at different time points and incubated with 1 μl of bHA306–318 peptide (1 mg/ml) in the absence or in the presence of 10 mM pCP for 1.5 h at 37 °C. Detection of the complex was carried out by ELISA as described above.

**Separation of Small Molecules from HLA-DR Molecules with Spin Columns**—In some experiments, pCP was removed prior to the binding reaction. For this, empty HLA-DR was preincubated with 10 mM pCP for 30 min followed by passage of the solution through a Micro Bio-Gel P-6 chromatography column (Bio-Rad) according to the manufacturer's recommendation.

**Peptide Loading of Empty HLA-DR Molecules**—To follow the kinetics of HLA-DR1-bHA306–318 or HLA-DR2-bMBP86–100 complex formation, 3 μl of HLA-DR (0.1 mg/ml) were incubated for different times with 0.5 μl of biotinylated peptide (0.01 mg/ml) in the absence or presence of 10 μl of pCP at 37 °C. All reactions were carried out in a phosphate/acetate buffer (100 mM Na<sub>2</sub>HPO<sub>4</sub>, 28 mM sodium acetate, 50 mM NaCl, and 1 mg/ml dextran). Increasing concentrations of HLA-DM (0.3–1.6 μM) followed by increasing concentrations of pCP (0.5–20 μM) were washed over the surfaces at a flow rate of 5 μl/min. The experiments were carried out on a BIAcore instrument according to the manufacturer's recommendations.

**Release of Class II MHC Peptide Ligands by HLA-DM and pCP**—To determine the effect of the small molecule pCP on the dissociation rate of class II MHC-peptide complexes, the release kinetics of bCLIP from soluble HLA-DR2 was analyzed by surface plasmon resonance assays. Preformed HLA-DR2-bCLIP complex was immobilized on streptavidin-coated biosensor chips via the biotin tag of the peptide. Release of HLA-DR was then induced by flushing increasing concentrations of soluble HLA-DM or pCP over the surface to trigger complex dissociation. Loss of surface-bound HLA-DR was detected as a decreased surface plasmon resonance signal and recorded in a sensogram (Fig. 1). Although the spontaneous
dissociation rate of the HLA-DR2–bCLIP complex was determined to be $4.8 \times 10^{-5}$ s$^{-1}$, the off-rate ($k_{off}$) increased in the presence of soluble HLA-DM in a concentration-dependent manner (Fig. 1A). With 0.3 μM HLA-DM, the off-rate is enhanced 5-fold ($2.5 \times 10^{-5}$ s$^{-1}$); with 0.8 μM HLA-DM, 8-fold ($3.8 \times 10^{-5}$ s$^{-1}$); and with 1.6 μM HLA-DM, 12-fold ($5.7 \times 10^{-5}$ s$^{-1}$). A plot of the HLA-DM concentration versus $k_{off}$ is shown in the inset of Fig. 1A.

A similar effect, however, was also observed when HLA-DM was replaced by the small molecule pCP (Fig. 1B). The release of the peptide was accelerated from 3.3-fold at a concentration of 0.5 mM pCP ($1.6 \times 10^{-5}$ s$^{-1}$) up to 43-fold at a concentration of 20 mM pCP ($20.6 \times 10^{-5}$ s$^{-1}$), revealing a linear correlation between the off-rate and the concentration of the small molecule (Fig. 1B, inset). Thus, both HLA-DM and pCP trigger the ligand release and increase the off-rate of complex dissociation in a concentration-dependent fashion.

Loading of Peptides onto Empty HLA-DR Molecules—The previous experiment demonstrated that the release of peptides bound to class II MHC molecules is, in fact, significantly enhanced by pCP. It was still open to question, however, whether the ligand exchange capacity described for pCP was solely because of this effect or whether the compound could also influence the on-rate of peptide binding. To address this question, loading experiments with biotinylated peptides and empty HLA-DR molecules were carried out in which the amount of complex formed during the incubation was determined by ELISA (Fig. 2). Soluble HLA-DR1 (Fig. 2A, left panel) and soluble HLA-DR2 molecules (Fig. 2A, right panel) were incubated with an excess of high affinity peptides bHA(306–318) and bMBP86–100, respectively, in the absence or presence of pCP. In the absence of pCP, the kinetics of the complex formation for both HLA-DR–peptide complexes was rather slow. However, when 10 mM pCP was present, the binding reaction was very rapid. Compared with the noncatalyzed reaction, the relative increase in the amount of peptide complex formed after only 10 min was 9-fold for HLA-DR2–bMBP86–100 and 23-fold for HLA-DR1–bHA(306–318). While 10 min was also the estimated half-time for the two catalyzed reactions, the extrapolation of the spontaneous loading curves suggests that more than 5 h are needed in the absence of pCP for both HLA-DR–peptide pairs to reach half-maximal saturation. Thus, pCP cannot only trigger the release of bound ligands but can also accelerate the loading of peptides onto empty HLA-DR molecules.

As previously reported (15), small molecular compounds seem to require an H-bond-forming group (e.g., –OH) to catalyze peptide exchange reactions. To evaluate the role of this group on peptide loading, two aromatic compounds were tested where the –OH group was either missing (CB) or blocked by a methyl group (pCA). Compared with the control (i.e., spontaneous complex formation), pCP enhances 7.4-fold the amount of HLA-DR1–bHA(306–318) complex generated after 1 h of incubation. In the absence of the –OH function (CB), the amount of complex produced is very close to the control, indicating that the catalytic activity of the molecule is impaired by the loss of the –OH group (CB). The same applies when the –OH function is substituted by a methoxy group (pCA), suggesting that the –OH function is required for peptide release as well as for the loading of empty HLA-DR molecules.

Induction of a Peptide-receptive State—A slow binding kinetic of peptides to empty HLA-DR molecules is caused by the nonreceptive conformation of the MHC molecule. Up to now, no specific probes or conformation-specific antibodies are available that allow discrimination between nonreceptive and receptive conformers. Induction of receptive forms can only be demonstrated indirectly by rapid binding kinetics as observed, for instance, in the presence of pCP. However, preparations of empty HLA-DR1 molecules from insect cells are potentially, to some extent, contaminated with HLA-DR1 molecules already loaded with peptides. To exclude the theoretical possibility that the apparent rapid loading is influenced by the release of such “endogenous” ligands, kinetic experiments were performed in which the HLA-DR1 preparation was preincubated with the catalysts to remove these ligands prior to the loading reaction (Fig. 3A). Although the loading in the noncatalyzed reaction always remains slow (Fig. 3A, left panel), the presence of HLA-DM results in rapid kinetics of HLA-DR1–bHA(306–318) complex formation irrespective of the time point when the bHA(306–318) was added (middle panel). Likewise, the same rapid complex formation is also observed when preincubation of the HLA-DR1 preparation was carried out with pCP (Fig. 3A, right panel). Release of ligands apparently does not play a role in the small molecule-mediated increase of the on-rate.

“Nascent” receptive MHC molecules can be generated by the dissociation of low affinity peptides (11, 12). The receptive conformation of the empty MHC molecule, however, is short-lived and switches rapidly into a nonreceptive form. To demonstrate that pCP, in fact, converts nonreceptive MHC molecules back into the receptive form, a low affinity peptide–HLA-DR complex was produced by loading HLA-DR1 with an analogue of CLIP, in which the side chain of the first anchor residue was removed ($t_{1/2} \sim 2$ h at pH 7.0 for HLA-
Chemical Analogues of HLA-DM Molecules

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FIG. 3. Induction of receptive state. A, loading of empty HLA-DR1 preparations after preincubation with HLA-DM or pCP. Preparations of soluble empty HLA-DR1 were preincubated without any catalyst (left panel), with 1.6 μM HLA-DM (middle panel), or with 10 mM pCP (right panel) to release any ligands potentially already bound to MHC molecules. After 0, 60, or 150 min, bHA306–318 peptide (40 μg/ml) was added to start the loading reaction (indicated by the arrows). The amount of HLA-DR1-bHA306–318 complex formed was determined by an ELISA reaction at the indicated time points. Incubation was carried out at pH 5 for HLA-DM and at pH 7.3 for pCP. B, recovery of nonreceptive HLA-DR1 by pCP. Nonreceptive HLA-DR1 was generated by spontaneous conversion of receptive HLA-DR1 after dissociation of the low affinity peptide complex HLA-DR1-CLIPMαβ. Dissociation was started by diluting the complex formation reaction (0.7 mg/ml HLA-DR1, 0.9 mg/ml CLIPMαβ) 1:30 in PBS/1% bovine serum albumin. After 0, 3, or 6 h, 40 μg/ml bHA306–318 peptide was added in the absence (black bars) or presence (gray bars) of 10 mM pCP. The amount of HLA-DR1-bHA306–318 complex produced after 1.5 h of loading was determined in an ELISA reaction. C, reversible conversion of receptive and nonreceptive HLA-DR. Receptive HLA-DR was generated by incubation of soluble HLA-DR1 (12 μg) with 10 mM pCP in 30 μl PBS. pCP was then removed by a rapid separation (separ.) in a micro Bio-Gel P-6 spin column (Bio-Rad), and the kinetic of peptide loading was determined immediately after separation by incubation with bHA306–318 (0.033 μg/ml) in the absence (open squares) or presence (filled squares) of 10 mM pCP. As the control, the peptide loading was carried out with HLA-DR1, from which pCP had not been separated by spin columns (filled circles) and with HLA-DR1 alone (open circles). The amount of HLA-DR1-bHA306–318 complex formed at the indicated time points was determined by an ELISA reaction.

DR1-CLIPMαβ, data not shown) (Fig. 3B). This complex was allowed to dissociate for extended periods of time and was subsequently probed for the presence of nonreceptive HLA-DR1 with an excess of the high affinity bHA306–318 peptide. Upon dissociation of the low affinity peptide, the amount of receptive HLA-DR1 that could be loaded with HA306–318 gradually decreased over time. Without any previous dissociation, the amount of HLA-DR1-bHA306–318 complex produced was equivalent to 49,000 cpm; after 2 h, equivalent to 30,000 cpm; and after 6 h of dissociation, the signal even dropped to 14,000 cpm, corresponding to 30% of the initial value. However, when loading with bHA306–318 was carried out in the presence of pCP, the signal of the HLA-DR1-bHA306–318 complex formed was always ~90,000 cpm, so that the fraction of nonreceptive HLA-DR generated after the dissociation of the low affinity peptide did not reduce the rate of HLA-DR1-bHA306–318 complex formation. Thus, pCP seems to be able to recover nonreceptive HLA-DR molecules, which are usually not accessible for immediate peptide loading.

The reconversion of receptive class II MHC molecules back into the nonreceptive form is reportedly very fast and takes place within minutes (11, 12, 23). This was also evident when pCP was separated from empty HLA-DR molecules by a rapid chromatographic step by spin columns, which required <4 min (Fig. 3C). The fast binding kinetics of bHA306–318 observed in the presence of pCP was completely lost after removal of the small molecule, resulting in the slow binding typical to the reaction of nonreceptive HLA-DR. Importantly, the process is completely reversible, because the addition of pCP resulted again in the fast binding kinetics typical for receptive class II MHC.

Influence of pCP on the T Cell Response—Because small molecular ligand exchange catalysts are active at neutral pH (15, 16), antigen-release and -loading experiments with pCP were carried out with living APC (Fig. 4). The impact on the T cell response was tested with two antigens that bind to HLA-DR4 with different affinity. In addition to HA306–318 (the high affinity antigen derived from the influenza virus (Fig. 4, right panels)) CO260–272 (an autoantigen derived from human type II collagen that binds to HLA-DR4 with lower affinity (left panels)) was used. The latter is also described as an immunodominant antigen recognized by CD4+ T cells in the collagen-induced rheumatoid arthritis model (20).

To test the ability of the small molecule to trigger the release of peptide antigens from the surface of an APC, HLA-DR4-expressing fibroblasts were pulsed with the peptide antigen followed by an incubation with different concentrations of pCP (Fig. 4, upper panels). In a second series of experiments, the impact on antigen loading was tested by incubating APC with pCP together with the peptide (Fig. 4, lower panels). After loading or release of the peptides, the T cell response was determined in an in vitro assay with T cell hybridomas specific for the two peptide antigens.

As shown in the upper panels of Fig. 4, the low affinity autoantigen CO260–272 can be removed from HLA-DR4-expressing fibroblast cells by pCP, whereas virtually no release was detected for the high affinity antigen HA306–318. Vice
versa, loading of APC with high affinity HA306–318 was greatly enhanced when pCP was present during the incubation with the peptide, whereas little or no effect was observed for CO260–272. Some increase was observed already at a concentration of 0.25 mM pCP, whereas maximal response was reached at a concentration of 1 mM. Apparently, the rapid release of the low affinity peptide prevents efficient loading of the APC. Thus, small molecular compounds can in fact influence the response of CD4<sup>+</sup>H11001 T cells by affecting the peptide composition on the surface of APC. Similar to HLA-DM, they are able to "edit" the peptide content of HLA-DR molecules according to the intrinsic affinities of their peptide ligands.

### DISCUSSION

In this study, we investigated the mechanism by which small molecular compounds mediate peptide exchange reactions on HLA-DR molecules. Kinetic analysis revealed that both dissociation of the bound peptide, as well as the binding of the free peptide to the empty binding site, are accelerated. In this respect, the effect of the small molecule indeed resembles that of the natural catalyst HLA-DM.

The loss of a single H-bond reduces the binding energy of a peptide ligand by ~2 kJ/mol, sufficient to trigger release of a low affinity peptide (10). It was therefore proposed that HLA-DM triggers dissociation of peptide-MHC complexes by disrupting such H-bond interactions (5). Apparently, functional groups capable of forming H-bonds are also required by the small molecules (15). Although the surface plasmon resonance experiments revealed that the off-rate of low affinity peptide ligands is increased, the true mechanism seems to be more intriguing. In a series of experiments, it could be demonstrated that, independently of peptide dissociation, the on-rate (i.e. the velocity of peptide binding to empty HLA-DR molecules) also is accelerated. Thus, the simple disruption of H-bonds between peptide and MHC by the small molecule is apparently not the driving force behind the accelerated ligand exchange.

It has been known for more than 10 years that the formation of a stable class II MHC-peptide complex is preceded by the formation of a short-lived complex characterized by fast on- and off-rates (24, 25). A conformational transition, evident for instance in the loss of SDS sensitivity, converts this complex into the long-lived form with half-lives that can range on the order of hours or days. More recently, a similar transition was also reported for empty class II MHC molecules (11, 12). Here the short-lived molecule represents the receptive form, whereas the long-lived form represents the nonreceptive form. Ligand exchange therefore seems to take place according to the following scheme.

![Scheme 1](image-url)

**Fig. 4. Release and loading of T cell antigens by pCP.** The effect of pCP on antigen release (upper panels) and loading (lower panels) was tested in in vitro T cell assays using the low affinity peptide CO260–272 (left panels) and the high affinity peptide HA306–318 (right panels). For release experiments, HLA-DR<sub>4</sub>-expressing fibroblast cells L243.6 were pulsed for 1 h with 10 μg/ml peptide (filled squares), 3.3 μg/ml (open triangles), 1.1 μg/ml (filled triangles), 0.37 μg/ml (open circles), or without any peptide antigen (filled circles). The pulsed APCs were then incubated for 4 h with the indicated amounts of pCP to trigger release of the peptides. The APCs were then washed and used to challenge the T cell hybridomas hCII23.5 and 8475/94 specific for CO260–272 and HA306–318, respectively. For the loading experiments (lower panels), L243.6 was incubated for 4 h with 0.37 μg/ml (filled squares), 0.12 μg/ml (open triangles), 0.04 μg/ml (open circles), or without any peptide antigen (filled circles) in the presence of the indicated amounts of pCP prior to the challenge of the T cell hybridomas. The antigen-specific T cell response was determined in a secondary assay by quantifying the IL2 release using IL2-dependent CTL-L cells.
To exchange the peptide P1 with P2, the stable MHC-P1 complex first has to convert into the unstable intermediate complex (MHC recP1) before P1 can dissociate. This dissociation leaves behind the peptide-receptive empty MHC molecule (MHC rec) that can either convert into the nonreceptive state (MHC nonrec) or acquire the peptide P2 to form the intermediate molecule (MHC recP2). A second conformational transition finally “locks” the peptide complex and generates the stable MHC-P2 form.

The rate-limiting steps in the exchange reaction are apparently the conformational transitions. A recent kinetic study demonstrated that, in particular, the transitions between intermediate and stable peptide complexes are catalyzed by HLA-DM (26). There are several other reports indicating that HLA-DM is also able to recover the receptive structure of empty class II MHC molecules (13, 14), although according to the study by Zarutskie et al. (26), conversion rates of empty MHC molecules are actually not affected by HLA-DM.

It is undisputed, however, that loading of empty HLA-DR1 is drastically enhanced in the presence of HLA-DM. The same enhancement is also observed with the small molecule pCP. Both ligand release and loading of empty MHC molecules is accelerated, implying that here also conformational transitions are catalyzed. So far, inactivation times of receptive class II MHC molecules have been reported for soluble mouse I-E^k (12) and soluble human HLA-DR1 (26) as well as for cell surface I-E^k (23). They were determined by comparing dissociation rates of prebound low affinity ligands with the association rates of high affinity peptides, replacing them and producing t values ranging between 4 and 14 min. Rapid and reversible inactivation was also observed after removal of the small molecule, although binding experiments with high affinity ligands did not reveal any detectable delay in the loss of receptiveness.

At present, the specific structural characteristics of intermediate and stable forms of the class II MHC molecule are not known yet. The molecular mechanisms by which HLA-DM or small molecules are driving the transition are therefore still speculative. In the case of HLA-DM, they are likely the result of conformational shifts caused by broad protein/protein interactions between HLA-DM and HLA-DR (27–29). Small molecules, on the other hand, have to induce these transitions by very specific interactions with defined molecular trigger points. Such trigger points could be located in the hydrophobic pockets of the MHC binding site. It has already been shown that point mutations filling the pocket 1 in the peptide-binding cleft induce a state of permanent receptiveness (11, 30), and occupation of such a pocket by the hydrophobic aromatic part of the pCP might cause a similar effect. In that case, the –OH group would only have a secondary influence by mediating solubility or assisting the binding. However, alternative trigger points could also be located outside the binding site. For instance, “His buttons,” such as a His-Leu bridge connecting the α1- with the α2-domain, had been described, which control pH-dependent conformational transitions of the MHC molecule (31). If such a His residue would be the target of the H-bond donor group of the small molecule, the –OH group would play a very active role in the catalytic process, because it would act on the pH sensor similar to an “immobilized” proton.

Although HLA-DM and the small molecules share many features with regard to their kinetic effects, a major difference is the influence of pH on their catalytic activity. The activity of HLA-DM almost completely diminishes at neutral pH, whereas the small molecules are still active (15). They can therefore alter the peptide repertoire presented by class II MHC molecules on the cell surface. As demonstrated with two peptides (a low affinity autoantigen and a high affinity virus-derived peptide antigen), the small molecule mediates release and loading of T cell antigens according to the peptide repertoire presented by the HLA molecule. Thus, they can actually edit the peptide composition presented by an APC, an effect that directly translates into modified T cell responses.

Small molecular ligand exchange catalysts might therefore play a role in therapeutic settings as enhancers of antigen loading. They could be employed, for instance, to establish effective tumor-specific immune responses by mediating loading of APC with peptides, proteins, or whole cell lysates derived from the transformed tissue. On the other hand, compounds that influence the antigen repertoire of APC, as described for the model compound pCP, might also represent the environmental risk factors of autoimmune diseases. In vitro, it was already demonstrated that pCP can enhance the presentation of the encephalitogenic MBP protein and induce MBP-specific T cell responses upon incubation with crude spinal cord homogenate (16). It therefore does not appear unlikely that incorporated chemicals, drugs, or metabolites that mediate such an “accidental” loading of autoantigen onto activated APC could trigger destructive autoimmune responses.

Taken together, small molecular compounds, such as pCP, represent chemical analogues of HLA-DM that mimic its catalytic effect on class II MHC-peptide complexes. By accelerating the dissociation of low affinity ligands and stabilizing the receptive state of the MHC molecule, they are able to promote the rapid ligand exchange of class II MHC molecules according to their intrinsic affinity and modulate T cell responses by altering the peptide repertoire presented by APC.

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