Small Organic Compounds Enhance Antigen Loading of Class II Major Histocompatibility Complex Proteins by Targeting the Polymorphic P1 Pocket*†

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Major histocompatibility complex (MHC) molecules are a key element of the cellular immune response. Encoded by the MHC they are a family of highly polymorphic peptide receptors presenting peptide antigens for the surveillance by T cells. We have shown that certain organic compounds can amplify immune responses by catalyzing the peptide loading of human class II MHC molecules HLA-DR. Here we show now that they achieve this by interacting with a defined binding site of the HLA-DR peptide receptor. Screening of a compound library revealed a set of adamantane derivatives that strongly accelerated the peptide loading rate. The effect was evident only for an allelic subset and strictly correlated with the presence of glycine at the dimorphic position β86 of the HLA-DR molecule. The residue forms the floor of the conserved pocket P1, located in the peptide binding site of MHC molecule. Apparently, transient occupation of this pocket by the organic compound stabilizes the peptide-receptive conformation permitting rapid antigen loading. This interaction appeared restricted to the larger Glyβ86 pocket and allowed striking enhancements of T cell responses for antigens presented by these “adamantyl-susceptible” MHC molecules. As catalysts of antigen loading, compounds targeting P1 may be useful molecular tools to amplify the immune response. The observation, however, that the ligand repertoire can be affected through polymorphic sites form the outside may also imply that environmental factors could induce allergic or autoimmune reactions in an allele-selective manner.

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The ligands of class II MHC molecules are generated mostly from exogenous protein sources (1). After internalization, they are fragmented by proteases and loaded onto class II MHC molecules in a process catalyzed by the chaperone HLA-DM (2, 3). Processing and loading take place in a dedicated endosomal compartment (MCII vesicle) (4). After transport to the cell surface the peptide-MHC complexes are presented to CD4+ T cells, which upon recognition induce the antigen-specific immune response.

On the cell surface some of these peptides are displayed for up to 100 h (5). A considerable fraction of the peptide-MHC molecules, however, dissociates during presentation, so that also “empty” MHC molecules are always present on class II MHC expressing cells. They are particularly abundant on immature dendritic cells, where they seem to play a role in cell membrane-associated antigen processing by capturing of extracellular antigens (6, 7). In general, however, the ligand composition of MHC molecules should reflect the protein content of the cell rather than that of the environment. Uncontrolled antigen capture by empty MHC molecules on the cell surface therefore to have been. Presumably as a safeguard mechanism class II MHC molecules inactivate rapidly after dissociation of the ligand. They convert into a “non-receptive” state (8), which is characterized by the inability of the MHC molecule to bind new peptide ligands.

While, in principle, this inactivation is reversible, recovery into the peptide-receptive state is extremely slow. In previous studies we have shown that certain organic compounds, such as aliphatic alcohols and phenol derivatives, have an intrinsic capacity to accelerate the peptide loading of class II MHC molecules (9). Although their catalytic activity is rather weak, the effect is mediated nonetheless by a defined mechanism (10). While we could establish that these organic compounds are able to re-induce the peptide-receptive state, the
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FIGURE 1. Dose-response curves and chemical structure of catalytic adamantyl compounds. Soluble DRB1*0101 was incubated with biotinylated HA306-318 peptide in the presence of indicated amounts of adamantyl compounds. The curves represent the amount of HA306-318/HLA-DR1 complex formed after 1 h of incubation with AdEtOH (filled circles), AdCaPy (open circles), AdBeSA (filled inverted triangles), AdPr (open triangles), AdCDME (filled squares), and 3M-AdCDME (open squares). The dashed line indicates the spontaneous load. The amount of DRB1*0101-HA306-318 complex was determined by ELISA and is expressed as fold increase compared with the non-catalyzed loading reaction. Chemical structures are shown on the right.

molecular basis of this transition remained unknown. A screening of a library of ~20,000 organic compounds has now revealed a number of new catalytic molecules that accelerate peptide loading at substantially higher rates. Most importantly, however, the analysis of their catalytic activity revealed a striking allele selectivity, which allowed identifying their putative binding site.

EXPERIMENTAL PROCEDURES

Compounds and Reagents—100 mM stock solutions of p-chlorophenol (pCP, Fluka) were prepared in phosphate-buffered saline, 10% Me2SO; 2-(1-adamantyl)ethanol (AdEtOH, Sigma), 3-(1-adamantyl)-5-hydrazidocarbonyl-1-N-pyrazole (AdCaPy), 4-(1-adamantyl)benzenesulfonylamine (AdBeSA), 1-propionyladamantane (AdPr), 1-[2-(N,N-dimethylamino)-ethoxycarbonyl]adamantane (AdCDME), and 1-[2-(N,N-dimethylamino)ethoxycarbonyl]-3,5,7-trimethyladamantane (3M-AdCDME), and 1-(2-(N,N-dimethylamino)ethoxycarbonyl)-3,5,7-trimethyladamantane (3M-AdCDME), all from Chemical Diversity) were dissolved in Me2SO (100 mM). IC106-120 (KMRMATPLLMQALPM) (11), HA306-318 (PKYVKQNTLKLAT) (12), and MBP86-100 (NPVHFFKNVTPRT) (13) were obtained from EMC microcollections GmbH (Tübingen, Germany). Biotin tags were attached to the N terminus. Phycocerythrin-(PE) and allophycocyanin-conjugated streptavidin were purchased from Caltag, or FACSCanto instrument (BD Biosciences). Cells were gated using a life-gate after staining with propidium iodide. T Cell Assay—5 × 10^4 HLA-DR-expressing cells/well were loaded overnight with biotinylated IC106-120 peptide, then diluted 1:3 and incubated with 200 µg/ml HA306-318 in the absence or presence of 1 mM AdEtOH.

Cells—L1501 (DRB1*1501) and L1502 (DRB1*1502) were generated by stable transfection of L929 cells (ATCC) with DRA1*0101 and DRB1*0101 or DRB1*1501 (Val^106→Gly), respectively. Epstein-Barr virus-transformed B cell 721.221 (DRB1*0101) was obtained from ATCC, and MGAR cells (“JHW 9014”; DRB1*1501, DRB5*0101) and RML cells (“JHW 9016”; DRB1*1602, DRB5*0202) both were provided by K. Wucherpfennig. EvHA/X5 (DRB1*0101-restricted, HA306-318-specific) were derived from HLA-DR1tg mice (14) and were generated after fusion of the CD4+ EvHA T cell line with the BW cell; 8475/94 (T cell hybridoma, DRB1*0401-restricted HA306-318-specific) and 2E12 (T cell receptor-transfected mouse BW cell, DRB1*1501- and DRB1*1502-restricted, MBP86-100-specific) were provided by L. Fugger.

Peptide Loading of Cell Surface MHC Molecules—5 × 10^4 HLA-DR-expressing cells/well were incubated at 37 °C in Dulbecco’s modified Eagle’s medium, 5% fetal calf serum in a 96 well U-bottom plate with biotinylated peptide in the presence of catalytic compounds. After 4 h cells were washed and stained with streptavidin-PE alone or double-stained with α-HLA-DR/PE-streptavidin-APC and analyzed by FACS on a FACSCalibur or FACSAnalyze instrument (BD Biosciences). Cells were gated using a life-gate after staining with propidium iodide.

T Cell Assay—5 × 10^4 HLA-DR-expressing cells/well were loaded for 4 h with peptides in the presence of catalytic compounds as described above. Cells were then washed, and 5 × 10^4 T cells were added to the culture, which was then incubated for 24 h in Dulbecco’s modified Eagle’s medium, 5% fetal calf serum in 96-well U-bottom plates. The T cell response was determined in a secondary assay with CTL-L cells (ATCC) as described previously (9).

Transfection and Site-directed Mutagenesis of HLA-DR—L929 cells were co-transfected with two pcDNA3.1 plasmids...
containing the HLA-DR α-chain (DRA*0101) and the respective β-chain using Lipofectamine 2000 (Invitrogen). For binding assays, cells were used that transiently expressed the MHC molecules. Stable lines were generated after selection with G418 (Invitrogen). Site-directed mutagenesis of HLA-DR molecules was carried out in a pcDNA3.1 expression vector (Invitrogen) by using the QuikChange site-directed mutagenesis kit (Stratagene). The following mutagenesis primers were used: DRB1*0101 (Gly\textsuperscript{386} → Val) and DRB5*0101 (Gly\textsuperscript{386} → Val), 5'-CACAACACTCAGGGTTTGAGAGC-TCACAGTGCA and DRB1*1501 (Val\textsuperscript{386} → Gly), 5'-CACAACACTCAGGGTTTGAGAGC-TCACAGTGCA.

Docking and Energy Minimization—AdCaPy was docked by hand, with the support of the “Dock” module in Sybyl 6.92 software (Tripos Inc.), into the P1 pocket of the x-ray structures HA306-318-DRB1*0101 and of MBP86-100-DRB1*1501 (Protein Data Bank entry codes 1DLH and 1BX2, respectively). The minimizations were made with MOE (Chemical Computing Group Inc.) using the MMFF94x force field. The default protonation state and the charges were calculated. Water molecules in the x-ray structures were removed. All atoms within 9 Å from containing the HLA-DR α-chain (DRA*0101) and the respective β-chain using Lipofectamine 2000 (Invitrogen). For binding assays, cells were used that transiently expressed the MHC molecules. Stable lines were generated after selection with G418 (Invitrogen). Site-directed mutagenesis of HLA-DR molecules was carried out in a pcDNA3.1 expression vector (Invitrogen) by using the QuikChange site-directed mutagenesis kit (Stratagene). The following mutagenesis primers were used: DRB1*0101 (Gly\textsuperscript{386} → Val) and DRB5*0101 (Gly\textsuperscript{386} → Val), 5'-CACAACACTCAGGGTTTGAGAGC-TCACAGTGCA and DRB1*1501 (Val\textsuperscript{386} → Gly), 5'-CACAACACTCAGGGTTTGAGAGC-TCACAGTGCA.

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FIGURE 3. Allele-specific effect of adamantyl compounds on peptide loading. A, catalytic effect on the loading of HLA-DR transfected fibroblast cells. Fibroblast cells expressing either no HLA-DR (left panel), DRB1*0101 (middle panel), or DRB1*0401 (right panel) were incubated for 4 h with 2 μg/ml biotinylated HA306-318. Incubation was carried out in the presence of pCP (filled circles), AdEtOH (open circles), or AdCaPy (filled triangles). After staining with streptavidin-PE the amount of peptide loaded within 4 h onto the cells was determined by FACS analysis and is expressed as geometric mean (geo. mean). The horizontal solid line represents the background fluorescence detected in the absence of the peptide; the dashed line represents spontaneous peptide loading detected in the absence of catalysts. B, allele-selective influence of adamantyl compounds on the peptide loading. MGAR cells expressing the two allelic variants DRB1*1501 and DRB5*0101 were incubated with 1.25 μg/ml biotinylated IC106-120 (left panel) or 2.5 μg/ml biotinylated MBP86-100 (middle panel). In parallel, DRB1*1602-expressing RML cells were incubated with the same amount of MBP86-100 (right panel). Loading was carried out for 4 h in the presence of indicated amounts of pCP (filled circles), AdEtOH (open circles), or AdCaPy (filled inverted triangles). C, effect of AdEtOH on DRB1*1501 and DRB5*0101. The fibroblast cell lines L466 expressing DRB1*1501 (left panels) and L416 expressing DRB5*0101 (right panels) were incubated with 2.5 μg/ml biotinylated MBP86-100 (upper panels) or 5 μg/ml biotinylated IC106-120 (lower panels). Expression was carried out in the presence of the indicated amounts of pCP (filled circles) or AdEtOH (open circles).

TABLE 1

| Residue β86 | Adamanthal-susceptible | Natural HLA-DRβ allele | β86-mutated HLA-DRβ |
|-------------|------------------------|------------------------|---------------------|
| Gly         | Yes                    | 1*0101, 1*0202, 1*0401, 1*0801 | 1*1501 (Val → Gly) |
| Val         | No                     | 1*101.1*102.1*104.1*1502 | 1*1501 (Val → Gly) |

ment, but molecules carrying the bulky adamantyl group were frequently found to be active. Some examples are shown in Fig. 1. Within this set highest activity was detected for AdEtOH and AdCaPy. Nearly the same activity was determined for a adamantyl compound substituted with benzene-sulfonamide (AdBeSA) followed by AdCDME and AdPr. Although the chemical nature of the side chain had apparently some influence, the catalytic activity was largely determined by the adamantyl group itself. The introduction of additional methyl substitutions at the adamantyl core structure could completely abrogate its activity (3M-AdCDME).

We have shown before that the interaction of the MHC molecule with organic compounds is fully reversible and based on the induction of a peptide receptive state (10). The same applies also for the activity of adamantyl compounds. AdEtOH can therefore efficiently trigger the exchange of MHC-bound peptides with free peptides of higher affinity (Fig. 2A). IC106-120 was removed from soluble DRB1*0101 in less than 6 h, while almost no exchange is observed in the absence of the catalyst. No removal of IC106-120 was evident in the absence of free HA306-318, indicating that IC106-120 ligand was indeed replaced by the high affinity peptide.

The induction of the receptive state was also evident in enhanced peptide loading (Fig. 2B). Without any catalysts more than 20 h were needed to reach half-maximal loading of DRB1*0101 with the HA306-318 peptide. The presence of AdEtOH or AdCaPy, however, reduced $t_{1/2}$ to ~30 min. The enhancement of the on-rate was clearly dose-dependent (Fig. 2C) and up to a concentration of 1 mM compound did not show saturation (Fig. 2D).

Allele Selectivity of Adamantyl Compounds—Catalytic organic compounds can be used not only with recombinant MHC proteins but also with living cells to mediate loading with peptides and T cell antigens (9, 10, 15). As shown in Fig. 3A, AdEtOH and AdCaPy enhanced the loading of two cell surface HLA-DR molecules DRB1*0101 and DRB1*0401 with HA306-318. Loading was in fact much more effective than with pCP, an aromatic compound previously shown to exhibit some catalytic activity on HLA-DR molecules (10). No loading was detected on fibroblast cells that do
not express any class II MHC molecules, demonstrating HLA-DR specificity of the adamantyl-mediated loading enhancement.

Extension of the experiments to cells expressing other allelic variants of HLA-DR, however, produced unexpected results (Fig. 3B). MGAR cells are Epstein-Barr virus-transformed B cells which naturally express the HLA-DR variants DRB1*0101 and DRB5*0101 and IC106-120 and MBP86-100 are known to bind to these cells. When loading MGAR cells with the two peptides enhancement by AdEtOH and AdCaPy was indeed observed for IC106-120 (left panel). Notably, however, the two adamantyl compounds completely failed to enhance loading with MBP86-100, while pCP was able to increase the loading of both peptides (middle panel). The effect was not due to the MBP86-100 peptide, since loading of DRB1*1501-expressing RML cells could be improved by AdEtOH (right panel).

To dissect this phenomenon, experiments were repeated with fibroblast cells each expressing only one of the two HLA-DR molecules present on MGAR cells (Fig. 3C). IC106-120 binds with higher affinity to DRB5*0101, since virtually no binding was detected on DRB1*1501 expressing cells. MBP86-100, on the other hand, preferentially binds to DRB1*1501 and only weakly to DRB5*0101. pCP enhanced the binding of both peptides to the respective HLA-DR molecule but AdEtOH enhanced only the binding of IC106-120 on DRB5*0101. No enhancement was detected for the binding of MBP86-100 to DRB1*1501, suggesting that DRB1*1501 was “non-susceptible” to adamantyl-mediated catalysis.

**Adamantyl Susceptibility Correlates with Residue Gly\(^{86}\)**—The result of the previous experiment implicated that adamantyl compounds exhibit catalytic activity only on a subset of HLA-DR molecules. A comparison of catalytic activity with the allelic variations in fact suggested a correlation of susceptibility with a well known dimorphism at position 86 of the \(\beta\)-chain (16). In HLA-DR molecules this position is represented either by glycine (Gly\(^{86}\)) or valine (Val\(^{86}\)) (17). All MHC molecules susceptible to adamantyl compounds expressed glycine at this position, while DRB1*1501, the only non-susceptible molecule identified so far, expressed valine at \(\beta86\) (Table 1).

To confirm this putative linkage, \(\beta86\) residues of DRB1*0101, DRB5*0101, and DRB1*1501 were replaced by site-directed mutagenesis with the corresponding dimorphic alternate (Fig. 4 and Table 1). After transfection of fibroblast cells the influence of organic compounds was tested again in a peptide loading experiment. While with pCP the enhancement was evident on all wild type and mutant forms of HLA-DR, AdEtOH was active only on variants expressing Gly\(^{86}\) (DRB1*0101wt, DRB5*0101wt, and DRB1*1501 Val\(^{86}\) → Gly). Substitution of this residue by valine rendered the Gly\(^{86}\) variants non-susceptible to AdEtOH (DRB1*0101 Gly\(^{86}\) → Val, DRB5*0101 Gly\(^{86}\) → Val), while Val\(^{86}\)-expressing DRB1*1501, normally not affected by AdEtOH, is strongly susceptible when Val\(^{86}\) is replaced by glycine (DRB1*1501 Val\(^{86}\) → Gly).

**Allele-selective Enhancement of the CD4+ T Cell Response**—The major function of class II MHC molecules is to present peptide antigens to CD4+ T cells. Improved antigen loading by organic compounds therefore directly increases the sensitivity of the antigen-specific CD4+ T cell response (9, 10, 15). On DRB1*0101 the presence of AdEtOH during antigen loading can lead to shifts of the dose-response curves of almost 2 orders of magnitude (Fig. 5A). Without any catalyst the threshold concentration for the in vitro T cell response against the influenza virus-derivated HA306-318 antigen was slightly above 10 ng/ml peptide. 50 \(\mu\)M AdEtOH reduced the detection limit to 1 ng/ml peptide, and 250 \(\mu\)M AdEtOH further shifted the threshold to almost 0.1 ng/ml.

The effect by AdEtOH, however, is evident only when the antigen is presented by a susceptible HLA-DR molecule. As shown in Fig. 5B, enhancement is evident for the HA306-318-specific CD4+ T cell response when presented by Gly\(^{86}\)-expressing DRB1*0101 and DRB1*0401 molecules. The same applies also for the MBP86-100-specific response restricted by DRB1*1502. Absol-
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**FIGURE 5.** Allele-selective effect on the CD4+ T cell response. A, influence of AdEtOH on the antigen dose response. DRB1*0101-expressing 721.221 cells were incubated for 4 h with the indicated amounts of HA306-318 peptide alone (open circles) or in the presence of 50 μM (filled circles), 100 μM (filled inverted triangles), 150 μM (filled squares), 200 μM (filled diamonds), or 250 μM AdEtOH (filled triangles). After incubation cells were washed and used to stimulate HA306-318-specific EvHA/X5 T cell hybridoma. Dose response (left panel) is expressed as counts/min (cpm); the dashed line represents the background signal detected in the absence of any antigen. The relative enhancement (right panel) was determined by calculating the ratio of the peptide concentration required for half-maximal response (EC50). B, influence of adamantyl susceptibility of HLA-DR molecules on the T cell response. Dose-response curves are shown after incubating APC with the specific T cell antigen in the absence (filled circles) or presence of 50 μM AdEtOH (open circles). T cell response is shown for the AdEtOH-susceptible HLA-DR alleles DRB1*0101 (upper left panel), DRB1*0401 (upper right panel), and DRB1*1502 (lower left panel) and for the non-susceptible DRB1*1501 (lower right panel). The DRB1*0101- and DRB1*0401-restricted response was determined with HA306-318 peptide on L57.23 fibroblasts with EvHA/X5 T cell hybridoma 2E12, which was able to recognize the peptide on both allelic variants. Fibroblast cells L1502 (DRB1*1502) and L1501 (DRB1*1501) were used. Dose-response curves were generated after loading the fibroblast cells for 4 h in the absence (filled circles) or presence of 250 μM AdEtOH (open circles).

Lately no enhancement is observed when the antigen is presented by DRB1*1501. The two DRB1*15 variants differ only in the substitution at β86 (DRB1*1502, Gly<sub>β86</sub>; DRB1*1501, Val<sub>β86</sub>) and both are able to present the antigen to the 2E12 T cell hybridoma. Due to the allele selectivity of AdEtOH, however, enhancement is evident only when the T cell recognizes the antigen on the susceptible DRB1*1502 molecule.

**DISCUSSION**

In this study we showed that low molecular weight compounds exist, which are able to enhance the immune response in an allele-specific way. By targeting a polymorphic binding site on the class II MHC molecule they induce a conformational transition that allows the rapid ligand exchange. The presence of these compounds during antigen loading can therefore result in dramatic enhancements of antigen-specific T cell responses. The effect is limited to immune responses restricted by “susceptible” MHC molecules. Site-directed mutagenesis revealed that this susceptibility correlates with the presence of a glycine residue at position β86 of the class II MHC molecule. The residue is located in the peptide binding site and forms the floor of the conserved pocket P1 (18) (Fig. 6A). At least for the adamantyl compounds P1 therefore seems to be the target site to exhibit their catalytic effect.

In complex with the peptide, P1 accommodates an anchor side chain of the peptide ligand (19–21). Residue β86 restricts the depth of P1 (18, 22), so that Val<sub>β86</sub>-containing pockets can bind only small aliphatic anchor side chains (Ile, Leu, Val, and Met), whereas deeper Gly<sub>β86</sub>-containing pockets accommodate also larger aromatic residues (Phe, Tyr, and Trp) (Fig. 6B). Compared with the smaller pCP molecule, which enhances peptide loading irrespective of β86, the spherical adamantyl group requires significantly more space (Fig. 6C). Allele-selective enhancement by adamantyl compounds might therefore be due to the increased space requirements. Computational docking and energy minimization calculations indicated that only the Gly<sub>β86</sub>-containing P1 pocket is in fact large enough for the bulky adamantyl group. While in a simulation the adamantyl group of AdCaPy was pushed out of the shallow Val<sub>β86</sub>-P1 pocket of DRB1*1501 by 1.7 Å (data not shown), it remained stably inside the Gly<sub>β86</sub>-pocket of DRB1*0101 (Fig. 6D). The van der Waals radius of the adamantyl group is only slightly bigger than the inner surface of a Gly<sub>β86</sub>-P1 pocket harboring an aromatic anchor side chain. Energy minimization of the DRB1*0101-AdCaPy complex resulted in a root mean square deviation of 0.34 Å for the backbone of both α-helices lining the P1 pocket and of only 0.56 Å for the side chains surrounding the docked adamantyl group (Fig. 6E).

Thus, ligand-exchange catalysis by adamantyl compounds seems indeed to require the occupation of P1. The catalytic effect of organic compounds is mediated by the induction of the
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Peptide-receptive state (10), and other groups have shown that mutant DRB1*0101 molecules, which express permanently filled P1 pockets due to a Gly → Tyr substitution, are in fact mostly in the receptive state (23). Hence, occupation of P1 by the adamantyl group seems to have the same effect except that transient binding allows replacement by a peptide to form the stable ligand complex. In a mechanistic model, the effect could be explained by assuming that the receptive form is correlated with an open P1 pocket while the non-receptive state is linked to a collapsed P1 pocket (Fig. 7). Based on this assumption occupation of P1 by adamantyl compounds should stabilize the receptive state, resulting in fast loading rates as the result of an increased pool of peptide-accessible receptive MHC molecules.

Similar interactions might also be the basis of the catalysis by other organic compounds such as pCP. Invariance of their catalytic activity with regard to β86, however, leaves it open whether P1 is in fact the target. Although previous studies suggested that H-bond donor groups are crucial for certain organic compounds (9, 10), this does apparently not apply for adamantyl derivatives. The structural dissimilarity of the substituents of AdEtOH (hydroxy-ethyl group) and AdCaPy (substituted pyrazole ring) rather suggests that the hydrophobic adamantyl group by itself is largely responsible for the effect.

In contrast to noble metal complexes (24), which seem to strip peptides from class II MHC molecules by irreversibly inactivating the receptor complex, the interaction with organic compounds is reversible (10). This applies also for some other ethanamine) and “amantadine” (adamantane-1-amine), which are both used to treat influenza A virus infections. Their mechanism is not fully understood, but involvement of MLE activity seems unlikely, since in particular “amantadine” has low ligand-exchange capacity (data not shown). While in this case MLE activity is probably not involved in their primary effect, catalytic antigen loading could still be a cause of unwanted side effects.

Autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, and multiple sclerosis (MS) are induced by the “accidental” recognition of self-antigens. The addition of the weak catalyst pCP to preparations of crude spinal cord homogenate can activate encephalitogenic T cells specific for the self-antigen MBPβ6-100 (15). MBPβ6-100 has been implicated with human MS and is known to induce an MS-like autoimmune disease in mice, and as shown here, AdEtOH amplified the T cell response against this epitope even more effectively. Most importantly, however, enhancement of the in vivo autoimmune response is evident only on susceptible HLA-DR alleles.

In principle, environmental factors acting similar to AdEtOH could provoke the induction of autoimmune diseases in an allele-selective manner. It is in fact a typical characteristic of most autoimmune diseases that the prevalence is correlated with particular allelic variants of class II MHC (27). In some cases the susceptibility could be even linked to polymorphic P1-like pockets. This applies for type 1 diabetes, which is correlated with position β57 in pocket P9 (28), for rheumatoid
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![Diagram of Peptide Loading](image)

**FIGURE 7. Mechanistic model.** Peptide loading of class II MHC molecules is a multistep process, in which the conversion of the non-receptive state into the unstable peptide-receptive state is rate-limiting (33, 34). In this model the peptide-receptive conformation needed for antigen loading is correlated with an open P1 pocket, while the pocket is collapsed in the non-receptive state. A filled P1 pocket should therefore result in the stabilization of the receptive state. A filled P1 pocket should increase the number of peptide-accessible MHC molecules by preventing the re-conversion into the non-receptive state. Inactivation of the complex so that free peptides can reach the face, J. J., Davis, M. M., and McConnell, H. M. (1998) *Immunity* 9, 699–709

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