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Forced Expression of HLA-DM at the Surface of Dendritic Cells Increases Loading of Synthetic Peptides on MHC Class II Molecules and Modulates T Cell Responses

Abdul Mohammad Pezeshki,* Marie-Hélène Côté,* Georges A. Azar,* Jean-Pierre Routy,† Mohamed-Rachid Boulassem,‡ and Jacques Thibodeau*}

Adoptive transfer of autologous dendritic cells (DCs) loaded with tumor-associated CD4 and CD8 T cell epitopes represents a promising avenue for the immunotherapy of cancer. In an effort to increase the loading of therapeutic synthetic peptides on MHC II molecules, we used a mutant of HLA-DM (DMY) devoid of its lysosomal sorting motif and that accumulates at the cell surface. Transfection of DMY into HLA-DRβ2 cells resulted in increased loading of the exogenously supplied HA307–318 peptide, as well as increased stimulation of HA-specific T cells. Also, on transduction in mouse and human DCs, DMY increased loading of HEL48–61 and of the tumor Ag-derived gp100244–259 peptides, respectively. Interestingly, expression of DMY at the surface of APCs favored Th1 differentiation over Th2. Finally, we found that DMY+ and DMY− mouse APCs differentially stimulated T cell hybridomas sensitive to the fine conformation of peptide–MHC II complexes. Taken together, our results suggest that the overexpression of HLA-DMY at the plasma membrane of DCs may improve quantitatively, but also qualitatively, the presentation of CD4 T cell epitopes in cellular vaccine therapies for cancer. The Journal of Immunology, 2011, 187: 000–000.

Although immunization with tumor Ags can activate CTLs in patients, it has become clear that cancer immunotherapy efforts solely based on class I-specific epitopes will not deliver reliable vaccines. Indeed, the critical role of CD4+ T cells in the immune response against tumors has now been well established in a number of experimental animal models (1). Th cells, more specifically Th1, specific for tumor-associated Ags (TAAs) provide help to CTLs and dendritic cells (DCs). They also mediate important effector functions, even in the case of tumors initially negative for MHC II expression (2). Efforts were made in recent years to identify new tumor Ags and to incorporate MHC II-restricted peptides in vaccines to stimulate CD4+ T cells in cancer patients (3). However, the simultaneous injection of class I- and class II-restricted peptides did not clearly demonstrate the benefit of including class II epitopes, highlighting the importance of carefully controlling and monitoring the quality of the CD4+ T cell response (4).

Autologous DCs are powerful adjuvants for therapeutic cancer vaccines, and they have been pulsed with various sources of tumor Ags ranging from apoptotic bodies to recombinant TAAs (5). Genetic approaches aiming at the endogenous expression of tumor Ags are also being explored. In all cases, tumor Ags must be processed into the endocytic pathway to generate peptides or polypeptides exposing MHC II-binding sequences. At that point, under the control of a chaperone called HLA-DM (DM), the class II-associated invariant chain (II) peptide (CLIP) is removed from the MHC II groove and replaced by an antigenic peptide (6). DM is a highly conserved, nonclassical class II molecule containing a tyrosine-based lysosomal sorting motif (YxxL) in its β-chain cytoplasmic tail (7). DM activity is optimal at the acidic pH of late endosomes, and it edits the repertoire of antigenic peptides following molecular rules that are still under investigation (6).

Of the different protocols being developed to increase the display of tumor T cell epitopes on MHC II, pulsing APCs with synthetic oncoproteptides is the most convenient one (8). Peptides bypass the need for intracellular processing and can be loaded directly onto cell surface MHC II in a DM-independent manner. However, it soon became apparent that such Ags could have serious limitations because of their rather inefficient loading (9). Also, binding of synthetic peptides can create MHC II isomers that are distinguished by T cells. Indeed, Unanue and collaborators have shown that synthetic HEL48–61 peptides generate two distinct isomers when bound to I-Aαβ. The more stable conformers are referred to as type A and the less stable ones as type B. Although some T cells (also called type A) can recognize both conformers, other clones (type B) are specific for the type B peptide–MHC II complexes. Interestingly, the type B-specific effector T cells do not recognize the epitope coming from intracellular processing of the native Ag and that are loaded under the control of H2-DM (10). Thus, it is predicted that a substantial fraction of the T cell repertoire activated by peptide-loaded DC vaccines will not recognize tumor-resident APCs displaying the target native epitopes, as these are loaded on MHC II in the presence of DM.

Empty MHC II and functional DM accumulate, albeit at low levels, at the plasma membrane of B lymphocytes and immature DCs (11, 12). This minor peptide loading pathway depends on the presence of extracellular proteases capable of processing Ags. In immature DCs, one can speculate that the caspase-mediated inactivation of AP-2, the adaptor protein complex that mediates...
internalization of proteins with tyrosine-based sorting motifs, causes the accumulation of DM at the plasma membrane (13). On maturation, internalization of DM to endocytic compartments resumes and most MHC II become filled with peptides (11).

In this study, in an effort to increase the efficiency of peptide-based vaccine strategies, we have tested the hypothesis that a DM molecule devoid of its lysosomal sorting signal (DMY) would accumulate by default at the surface of APCs and would improve Ag loading (9). Our results demonstrate that cell surface loading of synthetic peptides in the presence of DMY modulates both quantitatively and qualitatively the T cell response, creating a shift toward a Th1 response and favoring the accumulation of type A peptide-MHC isomers.

Materials and Methods

Plasmids and Abs

Wild-type (pSVL-Dyn2.myc) and dominant-negative (pSVL-DynK44E.myc) dynamic 2 cDNAs were provided by Dr. R. B. Valentine (14). The AdDMY was constructed in pAdenoVator-CMV5 (CuO) from pBud-DMY (15). The pBud-DMY, pBud-CNIIA, pREP4-CD14, pBud-DRα-DR8, pREP4-IV p33, and pREP4-IV p35 plasmids have been described previously (16, 17). The pBud-DMY increases peptide loading (9). Our results demonstrate that cell surface

Peptides, peptide loading, and superantigens

Biotinylated and nonbiotinylated HEL48–61 (PKYKVNQTLKLA) (PKY), HA306–318 (PKYKVNQTLKLA) (PKL), gp100(24–100) (TGRAILGHT-MEVTYYH), CLIP81–105 (LPKPPKPVSKMRMATPLLMQALPMG), and HEL44–61 (DGSTIDGYIQNRS) were purchased from Centre de recherches du Centre Hospitalier de l’Université Laval (Quebec, QC, Canada). Biotin was added to the N terminus using an ε-aminocaproic acid linker. The peptides were purified by HPLC, and purity was >95%. Staphylococcal enterotoxin B (SEB) was from Toxin Technology (Sarasota, FL). For binding assays, cells were incubated for 5 h at 37˚C with peptides in culture medium containing 2% of FBS. Where indicated, n-propanol was added to a final concentration of 2%. Cells were washed four times with PBS and either used directly for functional assays or incubated with streptavidin-PE (Jackson ImmunoResearch, West Grove, PA) for flow cytometry.

Cells, mice, and transfections

HEK 293T, HEK 293T DR1, HEK 293T DR li, HeLa CIITA, HeLa CIITA DO, and HeLa DR1 li cells have been described previously (16, 17). The murine fibroblast cell line DAP (ATCC CRL-1949) was transfected using Fugene6 (Roche Diagnostics, Canada). Cells stably expressing CIITA or murine fibroblast cell line DAP (ATCC CRL-1949) was transfected using Fugene6. The HEK 293T, HEK 293T DR1, HEK 293T DR Ii, HeLa CIITA, HeLa CIITA DR1, and HeLa DR1 Ii cells were isolated by negative selection (Miltenyi Biotec, Auburn, CA). Anti-mouse I-AK and DMY surface expression. Human naive CD4+ T cells were isolated by negative selection (Miltenyi Biotec, Auburn, CA). Anti-mouse IFN-γ, anti-human IFN-γ, anti-human IL-4, and anti-human IL-4 were from BioLegend (San Diego, CA). Cell surface and intracellular staining were performed as described previously (16).

Results

DMY increases peptide loading

Using cells in which a small subset of DM resides at the plasma membrane, it has been shown that MHC II Ag processing can occur extracellularly (11, 12). We have redressed the issue of DM-mediated peptide exchange at the cell surface by using a dominant-negative form of dynamin (Dyn K44E). As opposed to DR, DM reaches the endosomes after its rapid clathrin-mediated internalization from the cell surface (20, 21). Thus, transient expression of Dyn K44E causes the selective accumulation of DM at the plasma membrane (Fig. 1A). HEK 293T cells stably expressing DR1 and Ii (HEK293T DR1 Ii) were transiently transfected with DM and either the wild-type dynamin (Dyn) or the Dyn K44E mutant. In line with a functional role for cell surface DM, there was a 2-fold increase in the binding of the biotinylated HA307–318 synthetic peptide (HA-PKY) on cells expressing the Dyn K44E mutant (Fig. 1A). These results suggest that DM-assisted peptide loading occurred directly at the plasma membrane and not in the endocytic pathway.

To further increase the levels of DM at the plasma membrane without the pleiotropic effects associated with the expression of the DynK44E, we used a DM variant (DMY) that bears a point mutation destroying its YxxL lysosomal sorting motif. On transient transfection into HEK293T DR1 li cells, DMY was found mostly at the plasma membrane (Fig. 1B, surface counts) but is still detectable in the endocytic pathway by confocal microscopy (data not shown). In contrast, the control wild-type DM could only be detected after membrane permeabilization (Fig. 1B, total counts). Interestingly, DM and DMY appeared equally effective in removing CLIP from DR (Fig. 1B). Then, we compared the capacity of these DM isoforms to assist the loading of HA-PKY on live cells. Peptide loading was greatly increased (>6-fold at 50 μM HA-PKY) in the presence of DMY, in line with the results obtained earlier with the DYN K44E mutant (Fig. 1C, 1D). This translated also into a more efficient stimulation of the HA-specific Jurkat T cells, confirming that the peptide–MHC II complexes are properly folded (Fig. 1E). Conversely, peptide binding was only marginally enhanced (<2-fold) after expression of wild-type DM (Fig. 1C, 1D). Although this small increase could be caused by the very transient cell surface expression of few recycling wild-type DM molecules, it is most likely indirect and the result of the intracellular editing of the peptide repertoire. Increased peptide loading was also observed on HeLa DR1 cells transduced with an adenoviral vector encoding DMY (Fig. 2A; compare mock and adDMY conditions). Peptides loaded with or without DMY remained stably associated over a period of 24 h after shifting the cells to 4˚C (Supplemental Fig. 1). To confirm the direct role of DMY in mediating peptide exchange, we conducted inhibition experiments using a DM-specific mAb or by cotransduction with another adenovirus encoding HLA-DO (DO). Presence of the DM-specific mAb during peptide loading almost completely...
inhibited DM-mediated peptide loading, presumably by preventing the interactions with DR. Although its exact mechanism of action remains to be identified, DO associates in the endoplasmic reticulum with DM and is a very potent inhibitor of peptide loading at near neutral pH values (20). Indeed, cotransduced DO totally inhibited the DMY-assisted loading of exogenous synthetic peptides on HeLa DR1 cells, further demonstrating the functionality of DMY (Fig. 2A, right panel).

The effect of DMY was tested using a second peptide-MHC II combination. HeLa cells transfected with the MHC CIITA (HeLa CIITA) express the product of the homozygous DRB*0102 allele and bind more efficiently a HA307–318 peptide variant with a tyrosine-to-leucine substitution at position 3 (HA-PKL) (22, 23). As expected, in the absence of DMY, binding of HA-PKY was slightly decreased by Ii, most likely because CLIP engages into more stable complexes at neutral pH or because it prevents the loading of low-affinity ligands (peptides or polypeptides) in the groove of DR (27). Altogether, our results demonstrate that expression of DM at the plasma membrane increases the loading of exogenously supplied synthetic peptides.

**Modulation of DMY function**

Because the main physiological role of DM is the removal of CLIP from the groove of classical MHC II, we have addressed the impact of Ii on peptide exchange. The binding of HA-PKY was measured on HEK293T DR1 cells transfected with Ii, DMY, or both (Fig. 3A). Because Ii slightly increased DR expression at the plasma membrane, peptide binding results were normalized to account for this fact (Fig. 3A, right panel). Loading of HA-PKY was slightly decreased by Ii, most likely because CLIP engages into more stable complexes at neutral pH or because it prevents the loading of low-affinity ligands (peptides or polypeptides) in the groove of DR (27).

**FIGURE 1.** DMY increases peptide binding to MHC II. A, HEK293T DR Ii cells were transfected with DM together with wild-type dynamin (Dyn) or the dominant-negative mutant (Dyn K44E). After 48 h, cells were stained for DM or DR, and biotinylated HA-PKY peptide (50 μM) was loaded for 4 h before flow cytometry. Mean fluorescence values (MFV) are plotted. B, HEK293T DR Ii cells (DRB1*0101) were transfected with empty vector (mock), DM, or DMY and stained for cell surface expression of DR, CLIP, and DM (upper panels). Alternatively, cells were permeabilized and stained for total expression of Ii and DM (lower panels). Filled curves represent the fluorescence background of cells incubated with the secondary Ab alone. C, Cells from B were loaded with bio-HA-PKY peptides and analyzed by flow cytometry. Filled curves represent the fluorescence background of cells incubated with the PE-coupled avidin alone. D, MFV from C were plotted as bar charts. E, Functional assay using cells described in B and Jurkat HA 1.7 T cells.

**FIGURE 2.** DMY increases peptide binding of low-affinity peptides. A, HeLa DR1 cells (mock) were transduced with a DMY-expressing adenoviral vector (adDMY) and loaded with HA-PKY peptide (50 μM) in the presence or absence of the DM-specific Map.DM1 mAb (left panel). Alternatively, HeLa DR1 cells were cotransduced with a DMY-expressing adenoviral vector and a second adenovirus encoding DO (adDO, right panel). Filled curves represent the fluorescence background of cells incubated with the PE-coupled avidin alone. B, HeLa CIITA cells (DRB1*0102) were transduced or not with adDMY and loaded with biotinylated HA-PKY or HA-PKL peptides. Cells were analyzed by flow cytometry. All conditions were done in triplicate, and error bars indicate the SDs to the means. Each experiment was done at least twice and a representative experiment is shown.
endoplasmic reticulum or endosomes (6, 24). In contrast, DMY was more efficient on cells devoid of Ii, suggesting again that low-affinity peptides are associated with DR1 in these conditions. Altogether, our results demonstrate that DMY acts as a general editor of the peptide repertoire, independent of Ii expression (25).

Because DM is most functional at pH values ranging from 5 to 6, we reasoned that lowering the pH during the assay may potentiate peptide loading (6). We tested the resistance of APCs to acidic pH using propidium iodide. Although >50% of the cells died after a 4-h incubation period at pH 5, viability was not affected at pH 6 (9) (Supplemental Fig. 3). Thus, we repeated peptide loading and found that HA-PKY binding was increased ~2-fold when performed at pH 6, but only in the presence of DMY (Fig. 3B).

We tested the possibility of combining DMY with small molecules capable of forming H-bonds and that act as chemical analogs of DM (26). Such MHC-loading enhancers, like n-propanol, modulate both off and on rates of peptides on soluble and cell-bound DR (27). Fig. 3C shows that addition of 2% n-propanol to HEK293 DR Ii cells increased >3-fold HA-PKY loading, in line with the ability of such compounds to induce a peptide-receptive state of MHC II. Interestingly, the effects of n-propanol and DMY were additive. The peptide–MHC II complexes generated in all these conditions were properly folded and functional, as judged by their ability to stimulate the HA-specific Jurkat T cells (Fig. 3D).

Transduction of DMY in DCs

The capacity of DMY to increase peptide loading was tested on DR1+ MoDCs after adenoviral transduction and maturation with LPS. Expression of DM at the plasma membrane was increased dramatically in these conditions and CLIP disappeared from the cell surface (Fig. 4A). Peptide loading of HA-PKY was increased (Fig. 4B) and so was the T cell stimulation of HA-specific Jurkat T cells (Supplemental Fig. 3). We also tested the binding of a synthetic melanoma oncopeptide, gp100174–190, to DCs prepared from another donor expressing the DR7 allotype (15). Again, the presence of DMY at the plasma membrane was sufficient to eliminate all traces of CLIP and resulted in increased peptide loading (Fig. 4C, 4D).

DMY polarizes T cell responses

Synthetic CLIP peptides have been used in the past to modulate T cell responses. For example, loading of CLIP at the surface of human DCs induced a Th2 bias in vitro in response to the superantigen SEB (28). In addition, injection of mice with the CLIP peptide and a conventional Ag caused a shift from a Th1 to a Th2 type of response (29). We tested the hypothesis that DMY, by removing CLIP from the cell surface and by increasing the loading of exogenously added peptides, would polarize T cells toward Th1. First, we used HeLa CIITA cells transduced with DMY to stimulate primary naive human T cells with SEB. DMY transduction removed most of the CLIP at the plasma membrane (Fig. 5A). SEB-pulsed APCs were then incubated with T cells, and cytokine production was measured by flow cytometry after intracellular staining. The results demonstrated that DMY expression caused a Th1 shift in the T cell population responding to SEB (Fig. 5B). Although 27.3% of the cells produced IL-4 in the mock-transduced population, the proportion of these cells declined to

FIGURE 3. DMY activity is modulated by Ii, pH, and n-propanol. A, HEK293T DR cells (mock) were transfected with either Ii, DMY, or a combination of both molecules. Binding of biotinylated HA-PKY was measured by flow cytometry (left panel), and the mean fluorescence values (MFV) were plotted as a ratio (HA/L243) to account for variations in the levels of DR expression (right panel). B, HEK293T DR1 Ii cells were transfected with DMY, and peptide loading was performed at the indicated pH values. C, HEK293T DR1 Ii cells (mock) were transfected with DMY, and peptide loading was performed in the presence or absence of 2% n-propanol. D, Functional assay using peptide-loaded cells cocultured with Jurkat HA 1.7 T cells. All conditions were done in triplicate, and error bars indicate the SDs to the means. Each experiment was done at least twice and a representative experiment is shown.

FIGURE 4. DMY increases peptide binding on primary DCs. A, Human DR1+ MoDCs (DRB1*01) were transduced with adDMY or mock virus (Ad0) in the presence of LPS. After 48 h, cells were stained and analyzed by flow cytometry for surface expression of CLIP, DR, and DM. B, Binding of HA-PKY (50 μM) was measured on nontransduced DCs (DR1), DCs transduced with an empty adenovirus (ad0), or with DMY (adDMY). C, Human DR7+ MoDCs (DRB1*07) were treated as in A. D, Binding of the gp100174–190 peptide (50 μM) was measured on nontransduced DCs (DR7), DCs transduced with empty adenovirus (ad0), or transduced with DMY (adDMY). An independent experiment gave similar results.
13.5% on DMY expression. However, the percentage of cells producing IFN-\(\gamma\) was increased from 50.9 to 59.2% in these conditions.

To confirm the role of CLIP in T cell polarization (28), we loaded DR-transfected HeLa cells with exogenous CLIP before pulsing with SEB (Fig. 5C, 5D). SEB binding was not affected by the presence of cell surface DR/CLIP complexes, but we observed that the Th1/Th2 ratio in responding cells was biased by CLIP (Fig. 5E). Although five times more T cells produced solely IFN-\(\gamma\) as compared with IL-4 when stimulated with HeLa DR1 cells, the presence of CLIP resulted in almost equal numbers of cells producing IFN-\(\gamma\) (12%) or IL-4 (9%).

To further investigate the effect of DMY on Th1/Th2 polarization, we used a second approach based on the recognition of the HEL\(_{48-61}\) peptide by mouse primary T cells. We generated APCs using a stable mouse cell line, DAP CIITA, transfected or not with DMY. Although we did not monitor for mouse CLIP expression, it has been shown previously that human CD4\(^+\) T cells from the 3A9 TCR transgenic BALB.k mouse (Fig. 5F, 5G). The 3A9 TCR is specific for the HEL\(_{48-61}\) peptide presented in the context of I-A\(^\alpha\). As observed earlier for SEB, the presence of DMY at the plasma membrane during peptide loading polarized the HEL\(_{48-61}\) T cell response toward a Th1 phenotype (Fig. 5G). The proportion of cells secreting IFN-\(\gamma\) augmented from 29.5 to 45.5% when APCs were transfected with DMY. Although it is difficult to dissociate the relative contribution of CLIP removal and increased peptide loading, these results suggest that DMY will preferentially polarize toward a Th1 response.

DMY edits peptide–MHC II conformation

Recently, Unanue and collaborators (10) have demonstrated that peptide loading in the presence of DM in late endosomes prevents formation of type B peptide–MHC II conformers. To determine whether DMY can affect peptide–MHC isomerization, we tested the response of type A and B mouse T cells specific for HEL\(_{48-61}\). As observed earlier for SEB, the presence of DMY at the plasma membrane during peptide loading polarized the HEL\(_{48-61}\) T cell response toward a Th1 phenotype (Fig. 5G). The proportion of cells secreting IFN-\(\gamma\) augmented from 29.5 to 45.5% when APCs were transfected with DMY. Although it is difficult to dissociate the relative contribution of CLIP removal and increased peptide loading, these results suggest that DMY will preferentially polarize toward a Th1 response.
increased in the presence of DMY (Fig. 6B). We cocultured DMY+ or DMY− DAP CIITA cells with T cell hybridomas in the presence of the HEL48–61 peptide. Fig. 6C shows that DMY-expressing DAP cells stimulated the type A 3A9 T cells more efficiently, in line with the observed increase in peptide loading. Interestingly, the presence of DMY had the opposite effect on both type B-specific T cell lines. (Fig. 6D, 6E).

The impact of DMY on the presentation of HEL48–61 was also tested using mouse BMDCs. Although the expression of DMY did not affect the level of I-Ak at the plasma membrane, it enhanced the binding of the HEL peptide (Fig. 7A, 7B). As observed earlier for DAP CIITA cells, the presence of DMY on BMDCs increased the stimulation of 3A9 whereas reducing the response of both type B T cell lines (Fig. 7C–E). These results demonstrate that DMY edits the conformation of peptide–MHC II complexes, preventing accumulation of type B isomers in favor of type A. Also, because DMY operates at the plasma membrane, the data suggest that the acidic pH of late endosomal vesicles is not a prerequisite for DM to impact on the fine structure of MHC II–peptide complexes and to eliminate type B conformers.

Discussion

The possibility of loading MHC II exogenously with synthetic peptides or Ags is attractive in the context of cellular vaccine
development. Although experimental conditions may greatly affect peptide loading, little optimization has been done to standardize the procedures (32). The capture of short synthetic epitopes is inefficient, and it has been estimated that only 1% of the MHC II on the surface of a B cell line can be successfully loaded (9, 33). Exogenously pulsed peptides bind mostly to cell surface MHC II, but there are examples of internalization and loading inside various compartments of the endocytic pathway (12, 33–36). On a mechanistic basis, DM most likely operates similarly at the plasma membrane and in the endocytic pathway. Indeed, peptide loading was more efficient when cells were maintained at pH 6, a value optimal for DM activity (6). However, we have not thoroughly investigated the impact of endogenous DM expression or the cellular background on the activity of DMY. For example, although DMY clearly increased peptide binding on DCs, the loading appears more efficient on DR1-transfected HEK 293T cells. Also, some MHC II isotypic or allelic polymorphisms may affect the efficiency of DMY. Future experiments should shed light on the impact of peptide affinity for MHC II molecules. Our initial analysis using PKY and the substituted PKL peptides suggest that empirical studies will be needed to assess the efficiency of binding to different alleles in the presence of DMY. The impact of DMY on low-affinity peptides will need to be addressed to rule out the possibility that these are edited out of the groove or that they simply do not persist in the body because of peptide exchange. We have shown that the high-affinity HA307–318 peptide remained stably associated over a period of 24 h at 4°C (Supplemental Fig. 1), but the fate of low-affinity peptides in the presence of competitor peptides may well be different. In this context, the effect of DMY on the loading of low-affinity tumor peptides could be detrimental. Such peptides would necessitate modifications of their anchoring amino acids to increase the affinity for MHC II molecules.

APCs expressing DMY polarize specific T cells toward a Th1 phenotype. Our results using superantigens suggest that the mechanism of action of DMY is independent of a trivial role in increasing avidity of superantigen–TCR interactions. Indeed, SEB binding to MHC II was not affected by DMY (Supplemental Fig. 4). The fact that DMY increased the proportion of cells producing IFN-γ confirms that CLIP has an inhibitory effect on Th1 polarization. This was first proposed by Röhn et al. (28), who found that CLIP-pulsed DCs skewed the SEB response of naive human T cells toward Th2. In this context, CLIP probably finds itself in the immunological synapse and acts as an antagonist peptide, diminishing the impact of pseudodimers (37). A direct role for CLIP in Th cell polarization was also inferred from in vivo experiments where coinjections of CLIP and antigenic peptides favored a Th2 response in mice (29). Interestingly, low levels of CLIP and increased DM expression on various tumors predicted better survival of afflicted individuals (38, 39). This may relate to Th1 polarization of tumor-specific T cells as shown in DM4 breast carcinoma patients (40).

In the case of nominal Ags such as OVA, the role of DMY could be 2-fold. First, the more efficient peptide loading may favor Th1 responses as Ag load has been shown to affect T cell priming (41). Second, as described above for SEB, the dramatic increase in overall DM expression and the ensuing reduction in CLIP levels may affect molecular events at the immunological synapse.

In addition to its role in editing the epitope repertoire, we have shown that DMY, just like H2-DM (10), can modulate the fine structure of a given peptide–MHC II complex (Figs. 6, 7). DMY reduced the display of type B HEL-I-AK conformers at the cell surface of APCs, causing the poor stimulation of CP1.7 and MLA11.2 type B T cell hybridomas. Unanue and collaborators (10) showed that HEL-48–61 binds MHC II in at least two different conformations depending on the folding of the N-terminal extension, especially at position 2. One of these conformers (type B) is nonoptimal, and thus readily dissociates in the presence of H2-DM. This activity of H2-DM (and DMY) is likely to affect T cell responses to a large array of natural peptides with N-terminal extensions. In contrast, as shown for HEL, some short synthetic peptides devoid of N-terminal extensions will exist in both type A and B conformations independent of the presence of DM (10). We can speculate that in physiological conditions, professional APCs in the tumor environment will process exogenous TAAs and load nominal peptides in the context of DM, thereby eliminating type B complexes. Because vaccination protocols using short synthetic peptides will generate some type B complexes, the activated effector T cells specific for this conformer would thus not be recruited by APCs in the vicinity of the tumor.

Various chemicals have been investigated to increase Ag presentation and availability of peptide-receptive MHC II species. Early in vitro studies have shown that certain detergent formulations could dissociate CLIP–MHC II complexes and increase peptide loading (42). More recently, DM mimics capable of catalyzing peptide exchange have been described. These simple organic chemicals or peptidic molecules operate at neutral pH and enhance T cell activation (26, 27, 43–45). Interestingly, a better loading was obtained by combining n-propanol and DMY, suggesting that the former acts in a DM-independent and -dependent fashion. However, we cannot rule out that n-propanol and DMY target separate MHC II–peptide complexes. Future experiments should address the capacity of MHC-loading enhancers to polarize T cells toward Th1 and to eliminate type B complexes, as shown in this article for DMY. Collectively, our results argue that DMY is functional at the plasma membrane of APCs, and that it can be used to improve, both quantitatively and qualitatively, the loading of exogenous peptides. More experiments will be needed to determine in vivo whether DMY boosts Ag-specific Th1 responses and whether our findings can be generalized to other tumor peptides or MHC II alleles and isoforms. In this context, it is interesting to note that the monomorphic nature of this nonclassical DMY class II molecule would allow its universal use in immunotherapy protocols based on adoptive transfer of peptide-loaded DCs.

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Disclosures

The authors have no financial conflicts of interest.

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