

Invariant Chain and DM Edit Self-peptide Presentation by Major Histocompatibility Complex (MHC) Class II Molecules

By John F. Katz,* Christopher Stebbins,* Ettore Appella,† and Andrea J. Sant*

From the *Department of Pathology, Committee on Immunology, University of Chicago, Chicago, Illinois 60637; †National Institutes of Health, National Cancer Institute, Bethesda, Maryland 20892

Summary

We have studied the consequences of invariant chain (Ii) and DM expression on major histocompatibility complex (MHC) class II function. Ii has a number of discrete functions in the biology of class II, including competitive blocking of peptide binding in the endoplasmic reticulum and enhancing localization in the endocytic compartments. DM is thought to act primarily in endosomes to promote dissociation of the Ii-derived (CLIP) peptide from the class II antigen-binding pocket and subsequent peptide loading. In this study, we have evaluated the functional role of Ii and DM by examining their impact on surface expression of epitopes recognized by a large panel of alloreactive T cells. We find most epitopes studied are influenced by both Ii and DM. Most strikingly, we find that surface expression of a significant fraction of peptide-class II complexes is extinguished, rather than enhanced, by DM expression within the APC. The epitopes antagonized by DM do not appear to be specific for CLIP. Finally, we found that DM was also able to extinguish recognition of a defined peptide derived from the internally synthesized H-2L^d protein. Thus, rather than primarily serving in the removal of CLIP, DM may have a more generalized function of editing the array of peptides that are presented by class II. This editing can be either positive or negative, suggesting that DM plays a specifying role in the display of peptides presented to CD4 T cells.

Invariant chain (Ii) and the newly described DM proteins (H-2M in the mouse, hereafter referred to as DM) are critical for MHC class II-restricted antigen presentation. They function by regulating several key intracellular events in class II biogenesis (reviewed in references 1–4). Binding of Ii to class II molecules in the endoplasmic reticulum competitively blocks peptide binding (5, 6) and facilitates egress into Golgi compartments (7–9). Later in biosynthesis, Ii enhances class II localization in endosomal compartments (10–12). Here, DM resides and functions in the class II pathway (13). Cells lacking DM have been shown to be severely compromised in their presentation of exogenous antigens and to have low levels of SDS-stable dimers. Class II molecules isolated from these DM-deficient cells are predominantly occupied by the class II-associated Ii peptide (CLIP)¹. Transfection of genes encoding DM corrects these phenotypic characteristics (14–16). Most recently, the function of DM has been probed by biochemical means (17–19). These studies indicate that coincubation of purified DM and class II molecules leads to greatly enhanced rates of CLIP release and peptide loading. Recent data suggest

that these effects likely involve direct interaction of DM with the class II restriction element (20).

Despite our increasing appreciation of the mechanism by which DM functions, the functional consequences of its expression within APC are incompletely understood. Unlike the situation in biochemical experiments, in which peptide, MHC molecule, and DM can be incubated for extended periods of time under carefully regulated conditions, in a live APC the interactions among these molecules and the consequences of their interaction will depend only partially on their ability to colocalize. The milieu in which they interact and the time frame of their colocalization will determine the biological effects of their coexpression within an APC. In this study, we have examined the immunologic consequences of Ii and DM expression within APCs and have focused on presentation of peptides derived from proteins synthesized within the cell, because these occupy the majority of class II molecules on the APC (21, 22) and because of their importance in T cell development, tumor antigen presentation, and in autoimmunity. Data from our laboratory (23) and others (24–27) suggest that there may be distinct pathways used for the presentation of exogenous and endogenous antigens (reviewed in reference 28). In the

¹ Abbreviation used in this paper: CLIP, class II-associated Ii peptide.

experiments described here, we used a T cell specific for H-2L^d presented in the context of I-A^d and a large panel of individual alloreactive T cell clones as probes for self-peptide-class II complexes. Our experiments and those of others suggest that the specificity of alloreactive T cells is dictated by the peptides bound by the class II molecule (29, 30). Our own data suggest that these peptides derive primarily from an endogenous source (29). The experiments presented here suggest that Ii and DM can profoundly affect the array of peptides presented by the class II molecule I-A^d. While Ii generally enhances antigen presentation by the class II molecule, we show here the first demonstration that DM can dramatically reduce cell surface expression of particular peptide-class II complexes. Our data shows DM function is not restricted to CLIP removal. Within APC, this protein serves to positively or negatively edit the array of antigenic peptides presented to the immune system.

Materials and Methods

Mice. BALB/c and C57BL/6 females (12–16 weeks of age) were purchased from the Jackson Laboratories (Bar Harbor, ME). B10.GD mice were produced by Biocore (Frederick, Maryland) and were kindly provided by Dr. Jay Berzofsky.

Tissue Culture Reagents. Cells lines were maintained in Dulbecco's MEM supplemented with 5% FCS (HyClone Laboratories, Logan, UT), supplemented as described (23). Transfectants were supplemented with selection drugs G418 (0.2 mg/ml) and/or Blasticidin (2 µg/ml). Media and supplements were purchased from Gibco Laboratories (Grand Island, NY) unless otherwise noted.

Cell Lines and Transfectants. Ltk⁻ cells were transfected by calcium phosphate with I-A^d α and β chain cDNAs plus the neomycin resistance gene. MHC class II-positive cells were subcloned and then supertransfected with the genes encoding murine gIi, provided by Dr. Jim Miller (University of Chicago) or murine DM α and β chain cDNA genes, kindly provided by Dr. John Monaco (University of Cincinnati). The cDNA constructs were cloned into the SV40 based vector pcEXV-3. Secondary transfection and drug selection was done using the pSV2bsr2 gene (31) and the antibiotic blasticidin (ICN, Costa Mesa, CA). Transfected cells were stained for class II expression every 3–6 weeks during this analysis, and typically varied less than twofold in cell surface density of class II molecules.

mAbs and Immunofluorescence Staining. Hybridomas producing the mAbs M5114 and MKD6 were obtained from ATCC. Cell lines were stained in a two-step procedure, previously described (32), and analyzed on a FACScan[®] cytofluorimeter (Becton Dickinson, Mountain View, CA).

T Cell Hybridomas. Alloreactive A^d-restricted T cell hybridomas were prepared from C57BL/6 mice primed with BALB/c spleen cells, as previously described (29). In brief, T cells from primed animals were restimulated in vitro with irradiated BALB/c spleen cells and then fused with the T cell receptor αβ negative variant of BW5147. Hybridomas were subcloned by limiting dilution and selected by growth in HAT medium and for reactivity on BALB/c spleen. Spleen-reactive T cells were then analyzed for A^d reactivity using L cell transfectants or B10.GD (A^d, E^d-) spleen. The BC7 hybridoma, which is specific for the L^d peptide 61–85 presented in the context of I-A^d, has previously been described (23).

T Cell Hybridoma Assays. APCs were removed from selective drug for 48 h and plated in 200 µl in flat-bottomed 96-well plates

at 5 × 10⁴ cells/well with 5 × 10⁴ T cells. After coculture for 16–20 h, supernatant was tested for IL-2, using either an ELISA assay or by measuring proliferation of CTLL with murine IL-2 used as a control. Results are scored as picograms per milliliter of IL-2.

Western Blot Analysis. Class II molecules isolated from the L cell transfectants by immunoprecipitation with MKD6-protein A-Sepharose were eluted in 1.0% SDS, 10% glycerol, and 0.0625 M Tris, pH 6.8, at RT for 30 min, and one-half was boiled for 5 min at 100°C while the other remained at room temperature. Proteins were then fractionated by SDS-10%-PAGE and were transferred onto nitrocellulose membranes and analyzed by Western blotting as we have previously described (33).

Results and Discussion

To examine the functional consequences of Ii and DM expression within APC, we used a murine transfection model in which the original recipient cell was negative for expression of Ii, DM, and class II molecules, but that would interact productively with T cells upon expression of the class II restriction element. In previous experiments (29), we tested a panel of such APCs and found that of the murine cell lines tested, Ltk⁻ cells were able to activate antigen-specific T cells most effectively, with an efficiency close to that displayed by a lymphoblastoid B cell line. These Ltk⁻ cells lack detectable expression of Ii and did not express any DM α chain mRNA, although a small amount of the β chain mRNA is made. Studies involving mutant human cells have shown that the single chains do not yield functional DM (14, 15). Thus, the Ltk⁻ cells were chosen as a transfection recipient. Cells were sequentially transfected with genes encoding murine class II, murine Ii, and/or murine DM α and β chains using two different dominant selectable markers.

Fig. 1 shows a representative analysis of the gene expression of the four cells used in the functional studies (A^d [I-A^d, α and β alone], A^d-Ii [I-A^d α and β with Ii], A^d-DM [A^d α and β with DM α and β], and A^d-Ii-DM [A^d α and β with both DM and Ii constructs]). MHC class II surface expression was measured by mAb staining and flow cytometry (Fig. 1 A). Ii expression was measured by Western blotting (B) and DM expression was measured by PCR amplification of DM α and DM β cDNA (C). Our experiments indicate that cell surface expression of class II molecules is comparable among the transfectants, and that cells transfected with genes encoding Ii and DM in addition to A^d express similar levels of these protein cofactors.

An analysis of the effects of Ii and DM on SDS-stable dimer expression, which reflects stable peptide binding to class II molecules (34–36), is shown in Fig. 2. These experiments revealed that neither Ii nor DM alone has any significant effect on the ability of I-A^d to form SDS-stable dimers. When expressed together, they allow a significant fraction of class II molecules within the fibroblast line to stably bind peptide. The ability of DM to facilitate conversion of I-A^d to an SDS-stable form in the presence of Ii is associated with removal of CLIP (data not shown). The primary biochemical effect of Ii detected in our system is an increase in the relative abundance of mature class II mole-

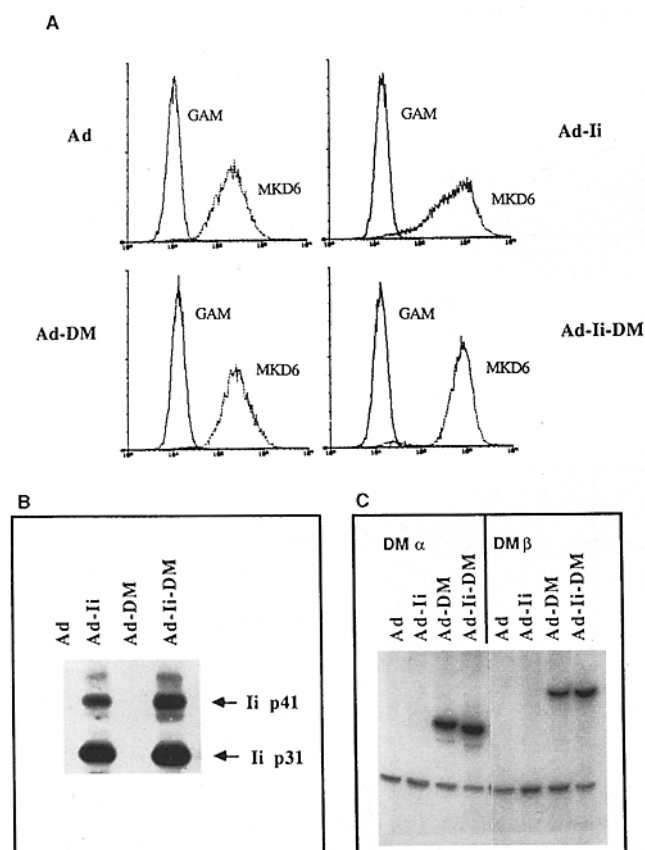


Figure 1. (A) Flow cytometry analysis of surface I-A^d expression on Ltk⁻ transfectants. L cells expressing I-A^d alone, I-A^d plus Ii chain, I-A^d plus DM, or I-A^d plus Ii and DM were stained with 14.4.4S (irrelevant anti-I-E mAb) and fluorescein goat anti-mouse Ig (GAM) or MKD6 (anti-I-A^d) and GAM. Flow cytometry profiles obtained with 14-4S or MKD6 are indicated by GAM or MKD6 in the figure. In the course of these experiments, APCs were stained periodically for class II surface expression. Although in the experiment shown here, Ii-positive cells were threefold brighter than cells lacking Ii, the same T cell activation patterns described in Fig. 3 were observed for cells that were matched for class II surface expression. (B) Western blot analysis of Ii expression in transfectants. MKD6 (anti-I-A^d) immunoprecipitates prepared from detergent lysates of the cell expressing the genes indicated above each lane were fractionated by SDS-PAGE and analyzed for the presence of Ii by Western blots using the antibody In-1. Shown are Ii p31 and p41 protein products. (C) PCR quantitation of DM α and DM β cDNA expression. cDNA was prepared from the L cell transfectants and amplified by PCR using primers specific for DM α or DM β . Because the Ltk⁻ cells express a low level of DM β chain, the 5' primer for β corresponds to a sequence in the pcEXV expression vector. Primers for actin cDNA (at the bottom of the gel) were included as a control for the cDNA and the PCR amplification steps.

cules, as detected by their increase in apparent molecular weight on the SDS-PAGE gels. It has been shown that this effect is due to the ability of Ii to facilitate export of class II molecules from the ER to cis-Golgi (1, 2, 6). Thus, several of the biochemical hallmarks of DM and Ii expression are detectable in our model system.

Fig. 3 shows the consequences of Ii and DM on the cell surface expression of peptide-class II complexes. A large panel of anti-I-A^d alloreactive T cell hybridomas was generated and analyzed in detail for their ability to recognize

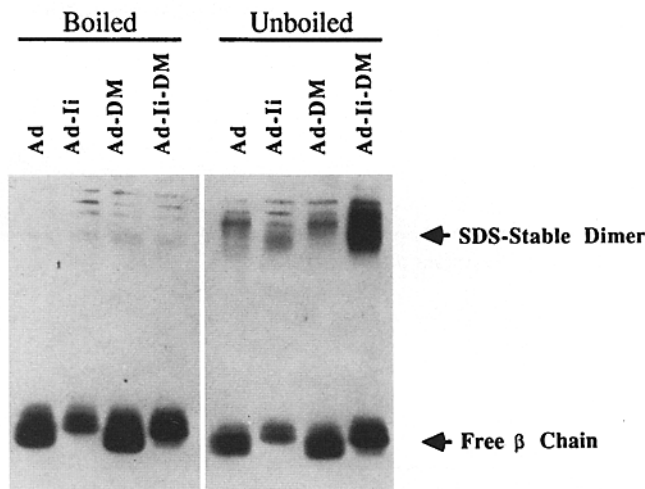
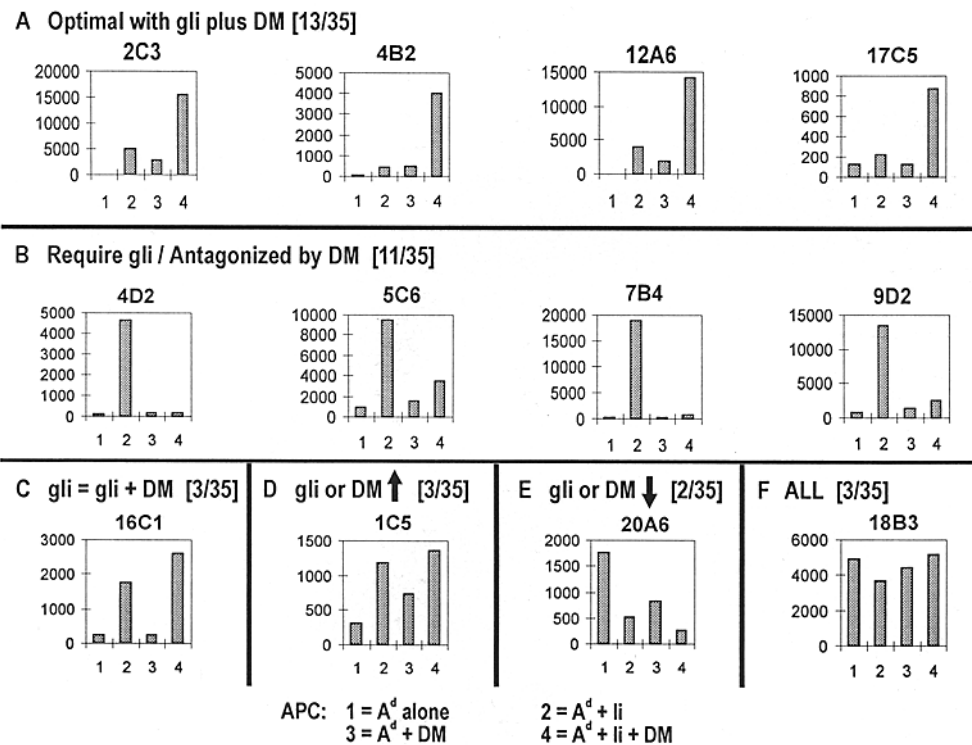


Figure 2. SDS-stable dimer formation in L cell transfectants depends on Ii and DM. L cell transfectants expressing the genes shown in the figure were solubilized in CHAPS and immunoprecipitated with the anti-class II mAb MKD6. Immunoprecipitates were eluted in SDS sample buffer and split into two equal aliquots. Half of the sample was boiled for 5 min, while the other was maintained at room temperature. Proteins were fractionated by SDS-PAGE and probed for the class II β chain using the mAb M5114. The mobilities of SDS-stable dimer and the free β chain are indicated by arrows.

the panel of transfectants. T cell activation was measured by IL-2 production and data are presented as picograms/ml of IL-2. A summary of the reactivity patterns detected is presented in Fig. 3, which also shows the number of T cells that belonged to a particular reactivity pattern with examples of the primary data from representative T cells in each group.

A surprising degree of complexity in the functional consequences of DM and Ii expression was revealed by this analysis and several unexpected findings were made. The vast majority of alloreactive T cells' reactivity is modulated both by Ii and DM. Most striking was the finding that the proportion of T cells whose reactivity was enhanced by DM (groups A and D) equaled the proportion of T cells whose reactivity was diminished or extinguished by DM expression (groups B and E). The functional studies performed thus far by our group (33) and others (14, 37–40) involving analyses of exogenous antigen presentation have revealed either a neutral or positive effect of DM expression on epitope generation. Our experiments demonstrate that DM expression can antagonize T cell recognition of APCs. A second observation made in this analysis is that DM is able to function independently of Ii (groups D and E). Several T cells were enhanced or antagonized by DM expression in cells that lacked Ii. Finally, a few T cells recognized epitopes whose expression was not measurably affected by Ii or DM expression (group F).

We were most intrigued by the finding that DM expression antagonized the reactivity of so many T cells (13 of 35 the T cells analyzed). Interestingly, most of these T cells required Ii to be expressed within the APC. Thus, the peptide(s) recognized by the T cells are dependent on Ii, and



pression relative to A^d only, (F) neither Ii or DM have any measurable effect on T cell recognition. Individual T cells were tested at least twice with similar results before assigning them to a particular reactivity pattern

DM apparently diminishes their association with class II molecules. One of the obvious candidate peptides that would display such a phenotype is the Ii-derived CLIP peptide, which binds to the peptide-binding pocket of the class II molecule in the absence of DM (41, 42). Biochemical and serological studies have shown that DM catalyzes the release of CLIP from class II (17–19). We sought to determine whether the T cells whose recognition requires Ii and are antagonized by DM are specific for CLIP. To examine this, we attempted to stimulate T cells with I-A^d-positive APCs cultured with high concentrations of synthetic peptides corresponding to the CLIP segment. Two forms of the CLIP peptide were tested: a peptide corresponding to amino acids 86–102, a sequence identical to the major CLIP segment isolated from I-A^d in normal B cell lines (22); and a longer CLIP peptide, corresponding to amino acids 81–105, a sequence that is analogous to the human CLIP isolated from DM-negative cells (41, 42). A^d cells were incubated with 100 μM CLIP peptide and tested for their ability to stimulate T cells of group B. Fig. 4 shows that there was no gain in reactivity by the presence of the CLIP peptide in culture. These data support the idea that the alloreactive T cells are not specific for CLIP but rather a self peptide whose association with class II, like CLIP, is diminished by DM. This hypothesis is strengthened by our findings that DM-antagonized T cells react with spleen cells from Ii-negative mice (data not shown) and is consistent with the observations made with the DM-deficient

mice, which show that CLIP-class II is not stimulatory for alloreactive T cells (43).

To extend these studies to a T cell whose specificity is known, we tested a T cell hybridoma that is specific for an L^d-derived peptide (amino acids 61–85) presented in the context of A^d. Previous studies by our laboratory have documented many characteristics of this presentation event (23). Cells expressing [A^d + Ii] or [A^d + Ii + DM] were supertransfected with the gene encoding H-2L^d and then tested for their ability to stimulate the A^d-restricted L^d-specific T cell BC7. Like the alloreactive T cells in group B and E, the epitope recognized by BC7 was diminished dramatically by the presence of DM within the APC (Fig. 5).

The studies presented in this paper point to the model in which both Ii and DM can act as either positive or negative regulators of expression of self-peptides in association with the class II molecule. A minor fraction of T cells react optimally in the absence of both cofactors, and lose reactivity when either one or both are expressed. It is likely that these peptides bind to the class II molecule in the endoplasmic reticulum, where Ii has been shown to compete for antigenic peptide binding (5). Like Ii, DM might act in this compartment to remove such a peptide or might act later, within the low pH milieu of the endosomes. In contrast with the T cells who lose reactivity in the presence of Ii, the vast majority of epitopes recognized by the T cells are enhanced by the presence of Ii within the APC. For many of these, DM greatly enhances epitope expression. We an-

Figure 3. Summary of allorecognition of I-A^d-positive cells. A total of 35 individual T cell hybridomas were screened for reactivity on L cell transfectants that vary only in the expression of Ii and DM. Supernatants of T cell-APC cultures were quantified for the presence of IL-2 and the data are presented as picograms/ml of IL-2. Reactivity patterns were identified based on relative reactivity with the four types of L cells shown. Sample T cells that represent the patterns observed are shown, as is the number of T cells (of 35) that displayed a particular reactivity pattern. The reactivity patterns were the following (A) Ii and DM are both required to generate the epitope; (B) the epitope requires Ii, but is extinguished by additional expression of DM, (C) the epitope requires Ii and is not measurably enhanced by further expression of DM; (D) either Ii or DM expressed alone increase epitope expression relative to A^d only; (E) either Ii or DM expressed alone diminish the epitope ex-

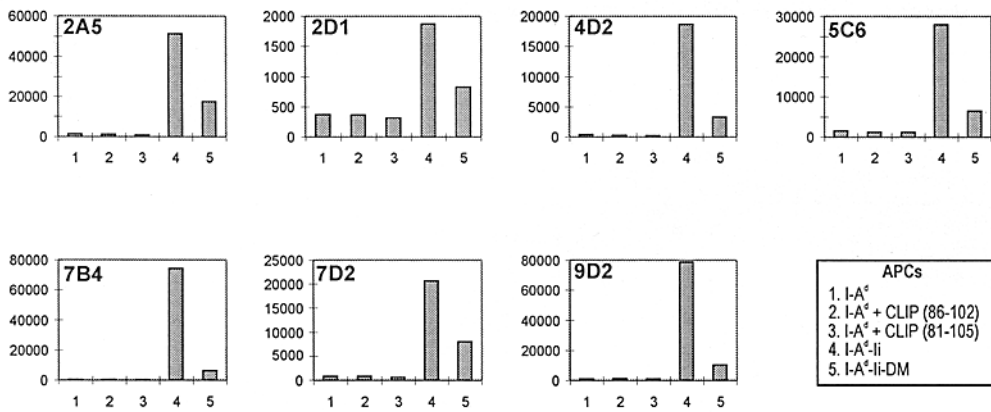


Figure 4. CLIP does not sensitize DM-antagonized T cells for recognition. T cells from group B in Fig. 3 were cocultured with L cells expressing A^d alone with 100 μM CLIP peptide (corresponding to amino acids 86–102 or 81–105, as indicated in the figure). A^d + I_i and A^d + I_i + DM cells were also included in this assay. The 86–102 CLIP peptide was able to sensitize CLIP-specific I-A^b-restricted T cells (data not shown).

ticipate that these peptides bind to the class II molecule within endocytic compartments, an event that would be enhanced by I_i, but that requires DM for CLIP removal or for accumulation of the peptide on class II molecules. The large group of T cells that require I_i but lose reactivity in the presence of DM were the most unexpected. Enhancement by I_i suggests an endosomal loading event, and antagonism by DM may reflect an interaction of the peptide with the class II molecule, which is analogous to that displayed by CLIP itself. In vitro studies involving purified DM and DR have shown that at low pH, DM can promote dissociation of a non-CLIP peptide (17). One model for DM function is that it interacts directly with the class II, and stabilizes an open conformation of the peptide-binding pocket. Such a conformation would allow a peptide like CLIP to be released. Peptides that are resistant to DM-catalyzed release might have distinctive binding characteristics that allow stable binding to the class II molecule even when it is in this alternative conformation. Thus, in a DM-positive cell, these would accumulate preferentially over less stable binding peptides.

In conclusion, we show here in an intact cell system that the effects of DM on antigenic class II-peptide complex cell surface expression can be negative as well as positive. These findings point to the function of DM within APCs as a peptide-exchange protein that can either increase or decrease expression of peptide-MHC class II complexes. Hence, the biological effects of DM on antigen presentation clearly extend beyond removal of CLIP. The ultimate effect of DM on accumulation of peptide-class II complexes will likely relate to the biochemistry of the interaction with the class II molecule itself, as well as the subcellular site where the peptide loads onto the class II molecule. Of particular note in this regard is the recent observation by Jensen and coworkers suggesting that I-A^d may be unusually labile at mildly acidic pH (44). This property may reflect a structural feature in this class II molecule that contributes to relatively high sensitivity to the DM-mediated effects. Finally, our studies raise the interesting question of how DM-antagonized T cells were generated in response to a stimulator cell population that has the genetic capacity to express DM. We can envision at least two possibilities.

First, within the heterogeneous population of class II-positive spleen cells, which include B cells, macrophages, and dendritic cells, there may be some cell types that produce unusually low levels of DM, relative to the class II restriction element. These could be the cells that originally activated the DM-antagonized T cells. We have not yet quantified DM protein levels in subpopulations of APCs in the spleen or in the L cell transfectants, and we do not know the relative abundance of the source proteins from which the self peptides recognized by the alloreactive T cells are derived. The finding that DM-mediated antagonism can be observed with a peptide derived from H-2L^d, whose expression is similar in L cells and in splenic APCs, argues that the negative effects of DM on epitope expression observed in the L cells do not require low levels of antigen. An alternative possibility is that the intracellular events that contribute to DM function, such as pH of the endosomal compartments or the dwell time of DM with the class II restriction element, may vary from one cell type to another, and may be

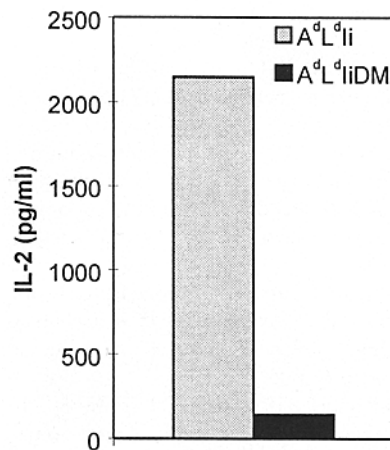


Figure 5. Expression of A^d-L^d (61–85) is antagonized by DM expression within the APC. L cells expressing A^d + I_i or A^d + I_i + DM were supertransfected with the gene encoding L^d. Transfectants expressing L^d at the cell surface were tested for their ability to activate BC7 (23). Shown are the picograms/ml of IL-2 produced by BC7 in response to the two APCs.

particularly active in subsets of cells in the spleen and in the fibroblasts that we have studied here. Our finding that the function of DM is more complex than previously suspected

will make the analysis of the regulation of its expression, biochemistry, and intracellular trafficking patterns particularly interesting.

We thank Phillip Ashton-Rickardt, Peter Jensen, Jim Miller, and Stephanie Ceman for critical review of this manuscript.

This work was supported by grants to A.J.S. from the National Institutes of Health and from the American Cancer Society. Flow cytometry was performed at the University of Chicago Cancer Center Facility.

Address correspondence to Andrea J. Sant, the University of Chicago, Department of Pathology, Committee on Immunology, 5841 South Maryland Avenue MC1089, Chicago, IL 60637.

Received for publication 11 July 1996 and in revised form 23 August 1996.

References

1. Sant, A.J., and J. Miller. 1994. MHC class II antigen processing: biology of invariant chain. *Curr. Opin. Immunol.* 6:57–63.
2. Cresswell, P. 1996. Invariant chain structure and MHC class II function. *Cell.* 84:505–507.
3. Wolf, P., and H. Ploegh. 1995. How MHC molecules acquire peptide cargo: biosynthesis and trafficking through the endocytic pathway. *Annu. Rev. Cell Biol.* 11:267–306.
4. Busch, R., and E. Mellins. 1996. Developing and shedding inhibitions: how MHC class II molecules reach maturity. *Curr. Opin. Immunol.* 8:51–58.
5. Roche, P., and P. Cresswell. 1990. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature (Lond.)*. 345:615–618.
6. Teyton, L., D. O'Sullivan, P. Dickson, V. Lotteau, A. Sette, P. Fink, and P. Peterson. 1990. Invariant chain distinguishes between the exogenous and endogenous antigen presentation pathways. *Nature (Lond.)*. 348:39–44.
7. Anderson, M., and J. Miller. 1992. Invariant chain can function as a chaperone protein for class II major histocompatibility complex molecules. *Proc. Natl. Acad. Sci. USA.* 89:2282–2286.
8. Schaff, T., K. Hruska, C. Bono, S. Shuman, and B. Schwartz. 1991. Invariant chain influences post-translational processing of HLA-DR molecules. *J. Immunol.* 147:603–608.
9. Layet, C., and R. Germain. 1991. Invariant chain promotes egress of poorly expressed, haplotype-mismatched class II major histocompatibility complex A α B β dimers from the endoplasmic reticulum/cis-Golgi compartment. *Proc. Natl. Acad. Sci. USA.* 88:2346–2350.
10. Lotteau, V., L. Teyton, A. Peleraux, T. Nilsson, L. Karlsson, S. Schmid, V. Quaranta, and P. Peterson. 1990. Intracellular transport of class II MHC molecules directed by invariant chain. *Nature (Lond.)*. 348:600–605.
11. Bakke, O., and B. Dobberstein. 1990. MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. *Cell.* 63:707–716.
12. Pieters, J., H. Horstmann, O. Bakke, G. Griffiths, and J. Lipp. 1991. Intracellular transport and localization of major histocompatibility complex class II molecules and associated invariant chain. *J. Cell Biol.* 115:1213–1223.
13. Sanderson, F., M.J. Kleijmeer, A. Kelly, D. Verwoerd, A. Tulp, J.J. Neeffjes, H.J. Geuze, and J. Trowsdale. 1994. Accumulation of HLA-DM, a regulator of antigen presentation, in MHC class II compartments. *Science (Wash. DC)*. 266:1566–1569.
14. Morris, P., J. Shaman, M. Attaya, M. Amaya, S. Goodman, C. Bergman, J.J. Monaco, and E. Mellins. 1994. An essential role for HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature (Lond.)*. 368:551–554.
15. Ceman, S., R.A. Rudersdorf, J.M. Petersen, and R. DeMars. 1995. DMA and DMB are the only genes in the class II region of the human MHC needed for class II-associated antigen processing. *J. Immunol.* 154:2545–2556.
16. Fling, S.P., B. Arp, and D. Pious. 1994. HLA-DMA and -DMB genes are both required for MHC class II/peptide complex formation in antigen-presenting cells. *Nature (Lond.)*. 368:554–558.
17. Sloan, V.S., P. Cameron, G. Porter, M. Gammon, M. Amaya, E. Mellins, and D. Zaller. 1995. Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature (Lond.)*. 375:802–806.
18. Denzin, L.K., and P. Cresswell. 1995. HLA-DM induces CIIP dissociation from MHC class II $\alpha\beta$ dimers and facilitates peptide loading. *Cell.* 82:155–165.
19. Sherman, M.A., D.A. Weber, and P.E. Jensen. 1995. DM enhances peptide binding to class II MHC by release of invariant chain-derived peptide. *Immunity.* 3:197–205.
20. Sanderson, F., C. Thomas, J. Neeffjes, and J. Trowsdale. 1996. Association between HLA-DM and HLA-DR in vivo. *Immunity.* 4:87–96.
21. Chicz, R.M., R.G. Urban, J.G. Gorga, D.A.A. Vignali, W.S. Lane, and J.L. Strominger. 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR Alleles. *J. Exp. Med.* 178:27–47.
22. Hunt, D., H. Michel, T. Dickinson, J. Shabanowitz, A. Cox, K. Sakaguchi, E. Appella, H. Grey, and A. Sette. 1992. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-A^d. *Science (Wash. DC)*. 256:1817–1820.
23. Loss, G.E., C.E. Ehasz, P.E. Fields, R.K. Ribaldo, M. McKisic, and A.J. Sant. 1993. MHC class II-restricted presentation of an internally synthesized antigen displays cell-type variabil-

- ity and segregates from the exogenous class II and endogenous class I antigen presentation pathways. *J. Exp. Med.* 178: 73–85.
24. Nuchtern, J., W. Biddison, and R. Klausner. 1990. Class II MHC molecules can use the endogenous pathway of antigen presentation. *Nature (Lond.)*. 343:74–76.
 25. Bikoff, E. 1992. Formation of complexes between self-peptides and MHC class II molecules in cells defective for presentation of exogenous proteins antigens. *J. Immunol.* 149:1–8.
 26. Moreno, J., D. Vignali, F. Nadimi, S. Fuchs, L. Adorini, and G. Hammerling. 1991. Processing of an endogenous protein can generate MHC class II-restricted T cell determinants distinct from those derived from exogenous antigen. *J. Immunol.* 147:3306–3313.
 27. Bodmer, H., S. Vville, C. Benoist, and D. Mathis. 1994. Diversity of endogenous epitopes bound to MHC class II molecules limited by invariant chain. *Science (Wash. DC)*. 263: 1284–1286.
 28. Sant, A.J. 1994. Endogenous antigen presentation by MHC class II molecules. *Immunologic Res.* 13:253–267.
 29. Katz, J.F., and A.J. Sant. 1993. T cell receptor recognition of MHC class II alloantigens is highly cell type dependent. *J. Immunol.* 152:1629–1640.
 30. Panina-Bordignon, P., G. Corridin, E. Roosnek, A. Sette, and A. Lanzavecchia. 1991. Processing of human serum proteins generates determinants recognized by class II alloreactive T cells. *Science (Wash. DC)*. 252:1548–1550.
 31. Izumi, M., H. Miyazawa, T. Kamakura, I. Yamaguchi, T. Endo, and F. Hanaoka. 1991. Blastidicin S-resistance gene (bsr): a novel selectable marker for mammalian cells. *Exp. Cell. Res.* 197:229–233.
 32. Sant, A.J., and R.N. Germain. 1989. Intracellular competition for component chains determines class II MHC cell surface phenotype. *Cell.* 57:797–805.
 33. Stebbins, C.C., G.E. Loss, C.G. Elias, A. Chervonsky, and A.J. Sant. 1995. The requirement for DM in class II restricted antigen presentation and SDS-stable dimer formation is allele and species dependant. *J. Exp. Med.* 181:223–234.
 34. Sadegh-Nasseri, S., and R.N. Germain. 1991. A role for peptide in determining MHC class II structure. *Nature (Lond.)*. 353:167–170.
 35. Dormair, K., B. Rothenhausler, and H.M. McConnell. 1989. Structural intermediates in the reactions of antigenic peptides with MHC molecules. *Cold Spring Harbor Symp. Quant. Biol.* 54:409–416.
 36. Nelson, C., S.J. Petzold, and E.R. Unanue. 1993. Identification of two distinct properties of class II major histocompatibility complex associated peptides. *Proc. Natl. Acad. Sci. USA.* 90:1227–1231.
 37. Mellins, E., S. Kempin, L. Smith, T. Monji, and D. Pious. 1991. A gene required for class II-restricted antigen presentation maps to the major histocompatibility complex. *J. Exp. Med.* 174:1607–1615.
 38. Pinet, V., M. Malnati, and E. Long. 1994. Two processing pathways for the MHC class II restricted presentation of exogenous influenza virus antigen. *J. Immunol.* 152:4852–4860.
 39. Riberdy, J., and P. Cresswell. 1992. The antigen processing mutant T2 suggests a role for MHC-linked genes in class II antigen presentation. *J. Immunol.* 148:2586–2590.
 40. Brooks, A.G., P.L. Campbell, P. Reynolds, A.M. Gautam, and J. McCluskey. 1994. Antigen presentation and assembly by mouse I-A^k class II molecules in human APC containing deleted or mutated HLA-DM genes. *J. Immunol.* 153:5382–5392.
 41. Sette, A., S. Ceman, R. Kubo, K. Sakaguchi, E. Appella, D. Hunt, T. Davis, H. Michel, J. Shabanowitz, R. Rudesdorf, H. Grey, and R. DeMars. 1992. Invariant chain peptides in most HLA-DR molecules of an antigen processing mutant. *Science (Wash.)*. 258:1801–1804.
 42. Riberdy, J.M., J.R. Newcomb, M.J. Surman, J.A. Barbosa, and P. Cresswell. 1992. HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides. *Nature (Lond.)*. 360:474–477.
 43. Fung-Leung, W., C.D. Surh, M. Liljedahl, J. Pang, D. Leturcq, P.A. Peterson, S.R. Webb, and L. Karlsson. 1996. Antigen presentation and T cell development in H2-M-deficient mice. *Science (Wash. DC)*. 271:1278–1281.
 44. Runnels, H.A., J.C. Moore, and P.E. Jensen. 1996. A structural transition in class II major histocompatibility complex proteins at mildly acidic pH. *J. Exp. Med.* 183:127–136.