

# DM Determines the Cryptic and Immunodominant Fate of T Cell Epitopes

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## Abstract

The ability of the immune system to focus T cell responses against a select number of potential epitopes of a complex antigen is termed immunodominance. Epitopes that trigger potent T cell activation, after *in vivo* priming, are classified as immunodominant. By contrast, determinants that fail to elicit any response are called cryptic. DM, a major histocompatibility complex (MHC) heterodimer, plays a pivotal role in the presentation of MHC class II–restricted epitopes by catalyzing the exchange of class II–associated invariant chain peptide with the antigen-derived peptides within the MHC class II binding groove. Using L cells transfected with genes for MHC class II, invariant chain, and DM, we have studied the contribution of DM in the presentation of two cryptic (peptide 11–25 and peptide 20–35) and one dominant (peptide 106–116) epitope of hen egg white lysozyme (HEL). Cells lacking DM heterodimers efficiently display the determinants HEL 11–25 and HEL 20–35 to T cells. Strikingly, however, cells expressing DM are severely compromised in their ability to present the cryptic HEL 11–25/A<sup>d</sup> and 20–35/A<sup>d</sup> epitopes. DM-mediated antagonism of HEL 11–25/A<sup>d</sup> and 20–35/A<sup>d</sup> presentation could thus be central to 11–25/A<sup>d</sup> and 20–35/A<sup>d</sup> being cryptic epitopes in the HEL system. Interestingly, the display of the immunodominant epitope of HEL, 106–116/E<sup>d</sup>, and of a dominant epitope of sperm whale myoglobin (SWM), 102–118/A<sup>d</sup>, is entirely dependent on the expression of DM. Thus, cells lacking DM molecules are unable to efficiently express HEL 106–116/E<sup>d</sup> and SWM 102–118/A<sup>d</sup> determinants. We conclude that the DM heterodimers direct the immunodominant and cryptic fate of antigenic epitopes *in vivo*.

Key words: antigen determinant • antigen processing • H-2M • immunodominance • major histocompatibility complex class II

## Introduction

The T cell response in the H-2<sup>d</sup> haplotype mice immunized with hen egg white lysozyme (HEL)<sup>1</sup> is almost exclusively directed towards a single immunodominant epitope of HEL, peptide 106–116, bound to E<sup>d</sup> MHC molecules (106–116/E<sup>d</sup>; references 1–4). Immunization with HEL does not elicit T cells specific for HEL 11–25 or HEL 20–35 bound to A<sup>d</sup> MHC (2–4). However, HEL 11–25/A<sup>d</sup>– and to some degree, HEL 20–35/A<sup>d</sup>–specific T cells, can be activated by immunization with the peptides 11–25 and 20–35, respectively (2). Peptides 11–25 and 20–35, which require no further processing in order to be efficiently pre-

sented in the groove of the A<sup>d</sup> molecules, thus encode cryptic determinants in the BALB/c (H-2<sup>d</sup>) mice. Immunodominance is a distinctive and classic characteristic of most T cell immune responses (1, 5, 6). Nevertheless, the molecular mechanisms dictating the immunodominant and cryptic fate of T cell epitopes remain unknown.

Processing and presentation of exogenous protein antigens taken up via the endocytic pathway involve the trafficking of MHC class II molecules from the constitutive secretory pathway to the endocytic compartment (containing the antigen-derived peptides), en route to the cell surface (7, 8). This task is accomplished by a glycoprotein called invariant chain (Ii) that enhances the transport of MHC class II chains to the endocytic compartment (7, 8). In addition, a fragment of Ii, called class II–associated invariant chain peptide (CLIP), occupies the peptide-binding groove of MHC class II molecules (9) until DM, a nonclassical MHC class II heterodimer, induces its release and replacement with peptides derived from foreign or endoge-

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<sup>1</sup>Abbreviations used in this paper: CLIP, class II–associated invariant chain peptide; HEL, hen egg white lysozyme; Ii, invariant chain; SWM, sperm whale myoglobin.

nous antigens (7, 10–12). Due to a lysosomal targeting signal encoded within the  $\beta$  chain of DM heterodimers, DM is localized to endocytic MHC class II compartments (13) where it has been shown to transiently bind MHC class II molecules (12, 13). DM, in addition to catalyzing the release of CLIP, has been shown to influence the binding of several non-CLIP peptides to the MHC class II molecules (11, 12, 14–17). DM mutant mice and APC lines have been used to show that DM is required for presentation of certain foreign antigens (12). We have shown previously that expression of DM can increase, diminish, or have a neutral effect on the presentation of different intracellular self proteins (14, 18, 19). But the biological consequence of the DM-mediated modification of the repertoire of peptides displayed by the MHC molecules on expression of the epitope specificity of T cell immune responsiveness to a foreign or self antigen remains unexplored. We present evidence here that DM directs the cryptic and immunodominant fate of antigen determinants.

## Materials and Methods

**APCs.** L cells were initially transfected with genes encoding A<sup>d</sup> alone, and a positive clone was isolated and then supertransfected with genes encoding genomic Ii (Ii or gIi) or Ii and murine DM. Derivation and characterization of these cells have been described previously (14, 18). A<sup>d</sup>-positive cells expressing either no cofactor, Ii alone, or Ii and H-2M (or murine DM, hereafter referred to as DM) were then additionally transfected with genes encoding E<sup>d</sup>, and positive cells were obtained by preparative sorting using the E $\alpha$ -specific Ab 14-4-4S and magnetic beads. Sorted and other transfected cells were grown in selection media with appropriate drugs: A<sup>d</sup> and A<sup>d</sup>-Ii L cells were cultured in the presence of G418 (200  $\mu$ g/ml; GIBCO BRL), A<sup>d</sup>-Ii+DM, in G418 and blasticidin (100  $\mu$ g/ml; Invitrogen), A<sup>d</sup>E<sup>d</sup> and A<sup>d</sup>E<sup>d</sup>-Ii, in G418 and HAT (GIBCO BRL), and finally A<sup>d</sup>E<sup>d</sup>-Ii+DM, in HAT, G418, and blasticidin. Cells were transferred into normal media (14) containing no drugs 24–48 h before their use in T cell assays.

**Flow Cytometry.** mAbs reacting with A<sup>d</sup> (MKD6) or E<sup>d</sup> (14-4-4S) were obtained from the American Type Culture Collection and used as culture supernatants. Cell surface expression of MHC class II molecules was evaluated by flow cytometry as described (19). In brief, cells were incubated successively with mAbs, followed by secondary step FITC-conjugated goat anti-mouse Igs (IgA, IgG, and IgM, and FITC-conjugated goat anti-mouse; Sigma-Aldrich), and were then analyzed on a FACSscan<sup>TM</sup> cytofluorimeter (Becton Dickinson). Background fluorescence was determined by incubating cells with media alone or an irrelevant mAb, and then with FITC-conjugated goat anti-mouse.

**Western Blots.** Cells were harvested from culture by mild trypsin treatment, washed extensively, and then counted.  $8 \times 10^6$  cells of each type (cells expressing A<sup>d</sup>E<sup>d</sup> alone, A<sup>d</sup>E<sup>d</sup> Ii, or A<sup>d</sup>E<sup>d</sup> Ii+DM) were solubilized in 1.0 ml 0.5% Triton X-100 (Sigma-Aldrich) with protease inhibitors for 45 min on ice as described previously (19). Nuclei and other insoluble debris were pelleted by centrifugation and the supernatant was transferred to a fresh tube. An equivalent aliquot of each cell lysate, equal to  $8 \times 10^5$  cell equivalents, was then adjusted to 2.0% SDS, 62 mM Tris, pH 6.8, and 10% glycerol, boiled for 3 min, and then frac-

tionated by SDS-10% PAGE. After electrophoresis, proteins were transferred to nitrocellulose, then processed for Western blotting as described previously (19). Blots were first probed and developed using nonspecific rat mAb or normal rabbit serum Abs, and then probed specifically for Ii and DM protein expression, as indicated in the legend to Fig. 1 b. Ii was detected by probing with the rat mAb In-1 (20), and DM $\beta$  was detected by probing with a rabbit antiserum raised against a synthetic peptide corresponding to the COOH-terminal tail of the mouse DM $\beta$  chain, conjugated to KLH. Binding of primary Abs to the blot was detected with horseradish peroxidase-coupled secondary goat Abs specific for either rat Ig, purchased from KPL Laboratories, or rabbit Ig, purchased from Life Technologies. Western blots were processed for chemiluminescence and autoradiography as described previously (18).

**Metabolic Labeling, Immunoprecipitation, and SDS-Gel Analyses.** For analysis of Ii, and DM expression within the APC,  $0.5 \times 10^6$  cells were plated in two 100-mm tissue culture dishes for 24 h. The adherent cells were then prelabeled for overnight incubation in MEM deficient in leucine with added [<sup>3</sup>H]leucine obtained from NEN Life Science Products at 25  $\mu$ Ci/ml. Ii, DM, and MHC class I molecules were isolated from 1% Triton detergent lysates by immunoprecipitation with either rabbit antiserum specific for the cytosolic tail of DM $\beta$ , the rat mAb In-1 (20) reactive with the cytosolic tail of Ii, or the class I-specific mAb 16.1.11N (21). Immunoprecipitated proteins were fractionated by SDS-10% PAGE and gels were stained with Coomassie brilliant blue, then destained with 30% methanol/10% acetic acid. Stained and destained gels were then washed with water, treated with Fluoro-Hance (Research Products International), dried, and exposed to film at  $-80^\circ\text{C}$ .

**Antigens and Peptides.** HEL was either purified as described previously (22) or was obtained from Sigma-Aldrich as a purified preparation. Both preparations of HEL gave analogous results. Sperm whale myoglobin (SWM) was obtained from Accurate Chemicals and Scientific Corp. Peptides HEL 11–25, HEL 20–35, HEL 106–116, and SWM 102–118 were synthesized and purified by Macromolecular Resources.

**T Cell Hybrids.** The cloned T cell hybridomas ISG-9-49, ISG-9-69, and 17.B.8.1 were obtained from continuously growing HEL-specific T cell lines derived from LN cells of HEL-primed B10.GD mice as described previously (22, 23). The cloned T hybrids D2-4-18 and D2-4-8 were obtained from HEL-specific T cell lines derived from LN cells of B10.D2 mice immunized with HEL (22, 23). In brief, T cell blasts from T cell lines were fused with the TCR $\alpha$ - $\beta$  variant of BW5147 as a fusion partner and cloned by limiting dilution in the presence of HEL. HEL-reactive hybrids were further analyzed for recognition of peptides. The hybrid C7-R14, specific for SWM 102–118 (24, 25), was a gift from Dr. Jay Berzofsky (National Institutes of Health, Bethesda, MD).

**Assay of T Cell Responses.** T hybridoma cells ( $10^5$ ) were cultured with  $1-5 \times 10^4$  of either A<sup>d</sup> or A<sup>d</sup>E<sup>d</sup> APCs, A<sup>d</sup>-Ii or A<sup>d</sup>E<sup>d</sup>-Ii APCs, or A<sup>d</sup>-Ii+DM or A<sup>d</sup>E<sup>d</sup>-Ii+DM APCs with different concentrations of HEL, SWM, or peptide (22, 23), as indicated in the figure legends (Figs. 2–5). Fixed APCs, where indicated, were treated with 1% formaldehyde in PBS for 12 min, then washed extensively before use. All cultures were done in triplicate. The culture supernatants collected 24 h later were assayed for IL-2 by ELISA as described previously (26). The substrate used was O-phelylenediamine dihydrochloride (Sigma-Aldrich). Absorption at 490 nm was measured using a microplate reader (Molecular Devices).

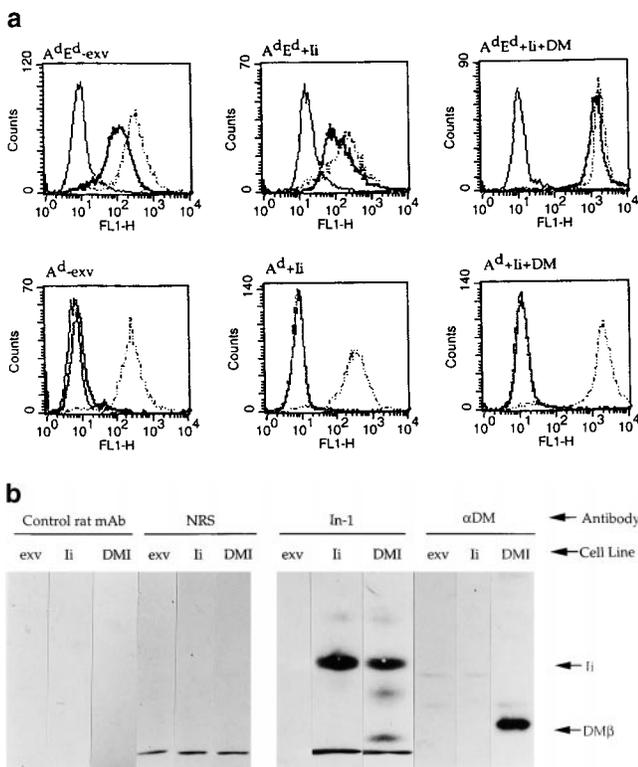
## Results

Cloned populations of L cells deficient in MHC class II, Ii, and DM heterodimers (DM) were transfected with genes encoding A<sup>d</sup> MHC molecules followed by either Ii or Ii and DM (Ii+DM), as described previously (14, 19). The A<sup>d</sup>, A<sup>d</sup>-Ii, and A<sup>d</sup>-Ii+DM L cell lines were further transfected with genes for E<sup>d</sup> MHC molecules. Fig. 1 shows the characterization of L cells transfected with A<sup>d</sup> and/or A<sup>d</sup>E<sup>d</sup> MHC molecules, together with genes for either Ii only or with Ii and the DM heterodimers (Ii+DM). It is clear that the expression of MHC class II is optimal in cells expressing both the Ii and DM (Fig. 1 a), as has been observed previously (14, 27). Protein expression of Ii and DM are shown in Fig. 1, b and c, confirming that the original cell lines used for transfection lack the constitutive expression of Ii and DM glycoproteins, and that protein expression of Ii and DM was readily detected in the appropriate cells.

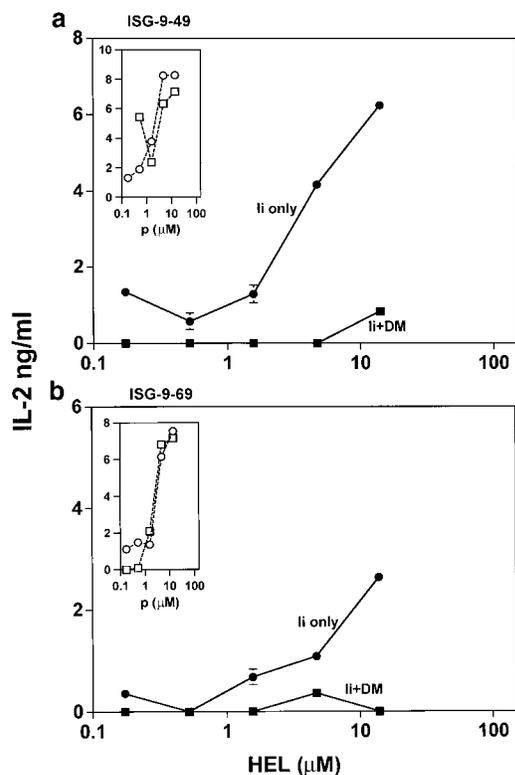
### DM Extinguishes Presentation of the Cryptic Epitopes of HEL

**Presentation of Cryptic Epitope HEL 11–25.** Fig. 2 shows the display of the cryptic HEL determinant 11–25 by A<sup>d</sup>E<sup>d</sup>

L cells used as APCs at different concentrations of HEL. The determinant display was examined by analyzing activation of two different cloned T cell hybridomas, ISG9-49 and ISG-9-69, that recognize HEL 11–25/A<sup>d</sup> and were derived as described previously (22; see Materials and Methods). It is clear that the A<sup>d</sup>E<sup>d</sup>-Ii APCs, lacking expression of DM heterodimers, can efficiently process HEL and present the cryptic epitope 11–25. The display of 11–25 epitope by the A<sup>d</sup>E<sup>d</sup>-Ii APCs can be observed at even low (0.2–2 μM) concentrations of HEL. Most strikingly, the presentation of 11–25 was nearly extinguished, even at >30-μM concentrations of HEL, when presented by APCs that express DM in addition to Ii (Fig. 2). The peptide 11–25, which requires no further processing, is equivalently presented by APCs with Ii alone and with Ii+DM (insets, Fig. 2, a and b), despite the fact that APCs that express Ii alone display lower levels of MHC class II molecules than Ii+DM APCs (Fig. 1 a). To further confirm DM-mediated antagonism of presentation of the epitope 11–25, we used an additional set of APCs, the A<sup>d</sup> APCs, expressing either Ii alone or Ii+DM. Once again, the presentation of the cryptic

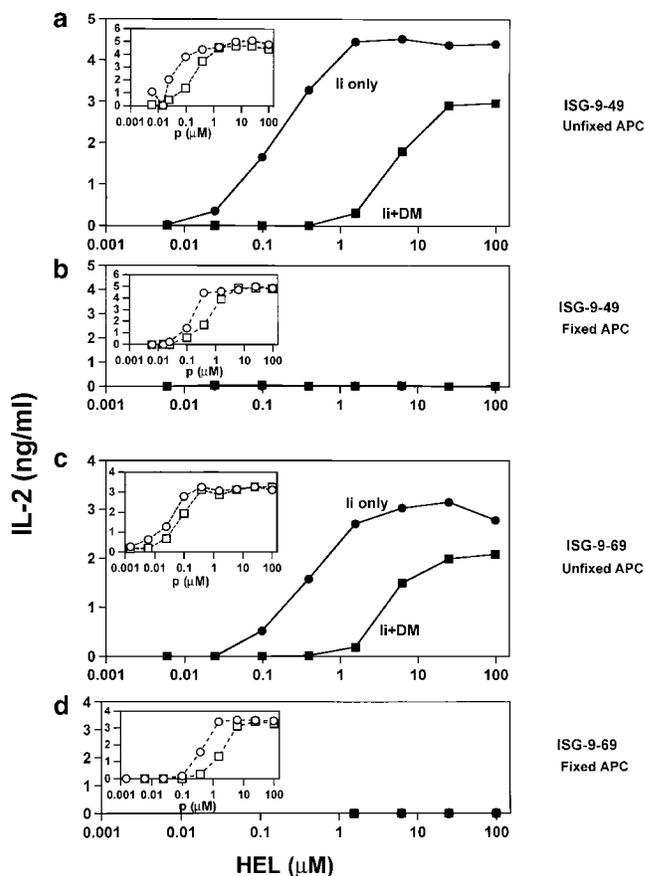


on the right of the figure are the positions of Ii p31 and DMβ. (c) Long-term radiolabeling and immunoprecipitation. Cells expressing class II alone (A<sup>d</sup>E<sup>d</sup>-exv), class II+Ii (A<sup>d</sup>E<sup>d</sup>+Ii) or class II+Ii+DM (A<sup>d</sup>E<sup>d</sup>+Ii+DM) were radiolabeled overnight with [<sup>3</sup>H]leucine as described in Materials and Methods. Postnuclear supernatants of detergent lysates were incubated with either control Abs (NRS), the class I K<sup>k</sup>-specific Ab 16.1.11N (reference 21), the anti-DM antisera, or the Ii-specific mAb In-1. Antigen–Ab complexes were isolated with protein A–Sepharose. Immunoprecipitated proteins were eluted in 2% SDS and then fractionated by SDS–PAGE under reducing conditions. After electrophoresis, the gel was processed for autoradiography and then exposed to film. Shown to the left of the figure are the positions of molecular weight markers run in parallel. On the right of the figure are the positions of class I K<sup>k</sup>, Ii, DMα, and DMβ chains. Specific bands for DMα and DMβ (positions indicated on the right side of c) are seen only in the APC line transfected with DM. However, a nonspecific band, isolated with the rabbit antisera (normal rabbit serum and anti-DM Ab), detectable just below the class I heavy chain, can be seen in all of the three APC lines. Ii expressed in these cells displays primarily the p31 form of Ii, marked on the gel, with minor amounts of the p41 form of Ii, detected upon long exposures of the gel to film (not shown).



**Figure 2.** DM extinguishes the display of the cryptic 11–25/A<sup>d</sup> epitope of HEL using A<sup>d</sup>E<sup>d</sup> APCs. Responses of T hybrids (a) ISG-9-49 and (b) ISG-9-69, specific for HEL 11–25, are shown at different concentrations of HEL using L cells expressing A<sup>d</sup>, E<sup>d</sup>, and Ii (A<sup>d</sup>E<sup>d</sup>-Ii APC) (●), and L cells expressing A<sup>d</sup>, E<sup>d</sup>, Ii, and DM (A<sup>d</sup>E<sup>d</sup>-Ii+DM APC) (■). The activation of these T hybrids is measured by release of IL-2, depicted as ng/ml. IL-2 was analyzed by ELISA as described previously (reference 26; see Materials and Methods). The insets show stimulation of respective T hybrids using A<sup>d</sup>E<sup>d</sup>-Ii APCs (○), or A<sup>d</sup>E<sup>d</sup>-Ii+DM APCs (□) at different concentrations of peptide (p) HEL 11–25. The data are shown as mean ± SE of triplicate cultures. Responses of T hybrids using A<sup>d</sup>E<sup>d</sup> APCs (lacking Ii and DM) to different doses of HEL were lower than those using the A<sup>d</sup>E<sup>d</sup>-Ii APCs, but higher than those obtained using A<sup>d</sup>E<sup>d</sup>-Ii +DM APCs (data not shown).

epitope 11–25 was dramatically diminished when DM molecules were expressed by the APCs. A<sup>d</sup>-Ii and A<sup>d</sup>E<sup>d</sup>-Ii APCs lacking DM molecules were clearly very active at presenting this cryptic epitope in the absence of DM (Figs. 2 and 3). In addition, we used fixed APCs for presentation of the cryptic epitope 11–25 to address the question whether the A<sup>d</sup>-Ii APCs were able to effectively present the cryptic HEL 11–25 simply by virtue of being more efficient in taking up either denatured HEL or any other contaminants of HEL. As is evident in Fig. 3, b and d, the fixed A<sup>d</sup>-Ii or A<sup>d</sup>-Ii+DM APCs are unable to present HEL even at the highest concentration, 100 μM, of HEL. The competence of the A<sup>d</sup>-Ii APCs to present the cryptic epitope is thus solely due to proficient intracellular processing of HEL. Results obtained using a third 11–25/A<sup>d</sup>-specific cloned T hybrid, GD-5-42, additionally validated the DM-mediated antagonism of presentation of 11–25/A<sup>d</sup>, even

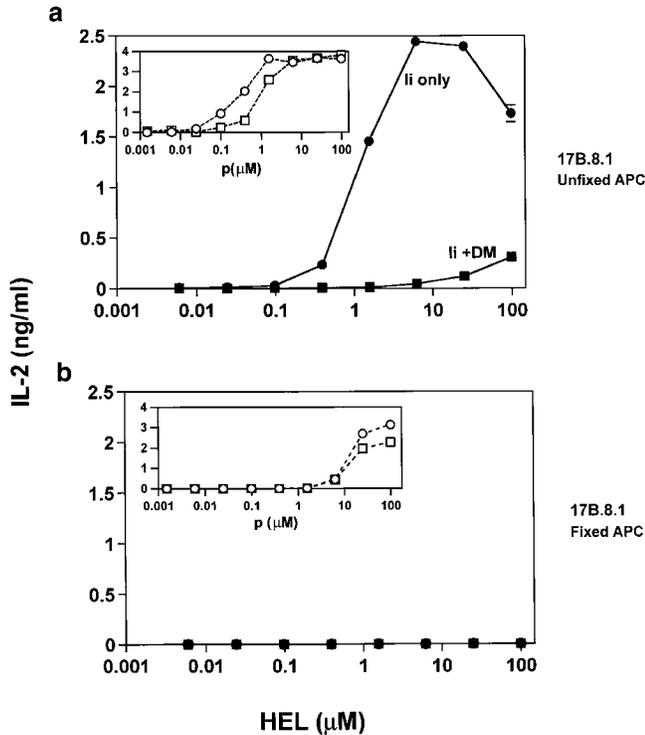


**Figure 3.** DM extinguishes the display of the cryptic 11–25/A<sup>d</sup> epitope of HEL using A<sup>d</sup> APCs. Responses of T hybrids (a and b) ISG-9-49 and (c and d) ISG-9-69 are shown at different concentrations of HEL using L cells expressing A<sup>d</sup> and Ii (A<sup>d</sup>-Ii APC) (●), and L cells expressing A<sup>d</sup>, Ii, and DM (A<sup>d</sup>-Ii+DM APC) (■). The results are expressed as described in the legend to Fig. 2. The insets show stimulation of respective T hybrids using A<sup>d</sup>-Ii APCs (○) or A<sup>d</sup>-Ii+DM APCs (□) at different concentrations of peptide (p) HEL 11–25. Normal, unfixed APCs were used in a and c, and fixed APCs (see Materials and Methods) were used in b and d.

at high concentrations (25–100 μM) of HEL (data not shown).

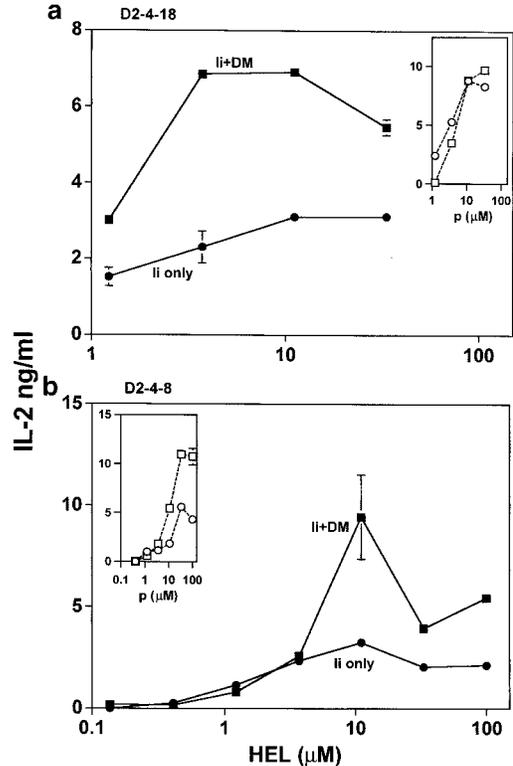
**Presentation of Cryptic Epitope HEL 20–35.** Fig. 4 shows the display of an additional cryptic epitope of HEL, 20–35/A<sup>d</sup>, by A<sup>d</sup> APCs. Interestingly, presentation of this second cryptic epitope of HEL was also antagonized by the expression of DM within APCs. Once again, A<sup>d</sup>-Ii APCs were able to present this epitope very efficiently to 20–35-specific T cells, 17B.8.1. However, A<sup>d</sup>-Ii+DM APCs were severely compromised in their ability to display this epitope. The fixed A<sup>d</sup>-Ii or A<sup>d</sup>-Ii+DM APCs, as shown for the 11–25/A<sup>d</sup> cryptic epitope, were unable to present this epitope from HEL at even the highest concentration of HEL tested (Fig. 4 b), thus ruling out the possibility that the expression of the epitope 20–35/A<sup>d</sup> was a result of efficient uptake of denatured HEL.

**DM Stimulates Presentation of the Immunodominant Determinants of HEL and SWM.** We then examined the display of the immunodominant determinant of HEL, 106–116/



**Figure 4.** DM extinguishes the display of the cryptic 20–35/A<sup>d</sup> epitope of HEL using A<sup>d</sup> APCs. Response of T hybrid (a and b) 17B.8.1 is shown at different concentrations of HEL using A<sup>d</sup>-Ii APCs (●) and A<sup>d</sup>-Ii+DM APCs (■). The results are expressed as described in the legend to Fig. 1. The insets show the stimulation of the T hybrid using A<sup>d</sup>-Ii APCs (○) or A<sup>d</sup>-Ii+DM APCs (□) at different concentrations of peptide (p) HEL 20–35. Normal, unfixed APCs were used in a, and fixed APCs (as described in Materials and Methods) were used in b.

E<sup>d</sup>, by A<sup>d</sup>E<sup>d</sup> APCs using the cloned T cell hybrids D2-4-18 and D2-4-8. Fig. 5 shows that in sharp contrast to our observations for the HEL 11–25 and 20–35 determinants (Fig. 2), A<sup>d</sup>E<sup>d</sup>-Ii APCs, lacking DM molecules, were very poor at displaying this immunodominant epitope (Fig. 5), whereas cells additionally expressing DM showed efficient presentation. The half-maximal response of the T cell hybrid D2-4-18 was obtained at 2 μM HEL using A<sup>d</sup>E<sup>d</sup>-Ii+DM APCs. However, the activation of this T hybrid essentially remained at the baseline levels at concentrations of up to 33.3 μM HEL, using A<sup>d</sup>E<sup>d</sup>-Ii APCs lacking DM. Optimal processing and display of this immunodominant determinant is thus dependent on the expression of DM molecules. Furthermore, these results attest that the A<sup>d</sup>E<sup>d</sup>-Ii+DM APCs are functionally competent and the abrogation of presentation of the cryptic 11–25 determinant in the presence of DM was not due to some non-DM-related factor expressed by the Ii+DM APCs. The data presented in the insets (Fig. 5, a and b) show that the A<sup>d</sup>E<sup>d</sup>-Ii and A<sup>d</sup>E<sup>d</sup>-Ii+DM APCs were able to stimulate the 106–116/E<sup>d</sup> T cells equivalently when the peptide HEL 106–116, which requires no further processing (data not shown), was used. These results indicate the lack of any intrinsic deficiency in A<sup>d</sup>E<sup>d</sup>-Ii APCs to interact with 106–116/E<sup>d</sup>-specific T cells.

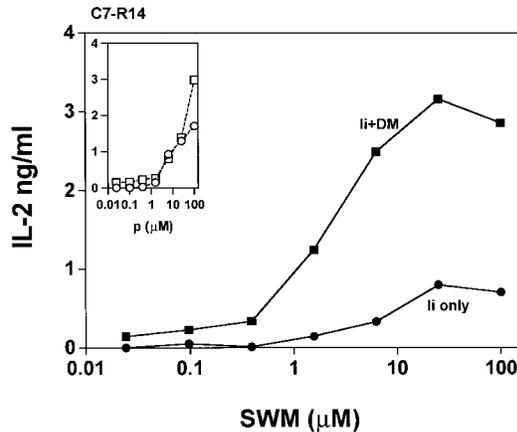


**Figure 5.** DM stimulates the presentation of the immunodominant 106–116/E<sup>d</sup> epitope of HEL. Responses of T hybrids (a) D2-4-18 and (b) D2-4-8, specific for HEL 106–116, are shown at different concentrations of HEL using A<sup>d</sup>E<sup>d</sup>-Ii APCs (●) and A<sup>d</sup>E<sup>d</sup>-Ii+DM APCs (■). The insets show the stimulation of respective T hybrids using different concentrations of peptide (p) HEL 106–116. The results are expressed as described in the legend to Fig. 2.

To pursue the argument that one function of the DM molecules is to play a role in the manifestation of immune dominance upon immunization with protein antigens, we examined the effect of DM on the presentation of a dominant determinant of SWM (23). Fig. 6 shows that just like the immunodominant epitope 106–116/E<sup>d</sup> of HEL, the optimal display of the immunodominant 102–118/A<sup>d</sup> determinant of SWM (23, 24) by A<sup>d</sup> APCs was dependent on expression of DM in these APCs. The half-maximal response of SWM 102–118-specific C7-R14 T cell hybrids stimulated by the A<sup>d</sup>-Ii+DM APCs was induced at only ~1 μM SWM, while a similar response by the A<sup>d</sup>-Ii APCs was induced at 60 μM SWM. Again, the results in Fig. 6 not only support the conclusion that the immunodominant fate of T cell determinants is dependent on the expression of DM, but they also affirm that the failure of our set of A<sup>d</sup>-Ii+DM APCs (Figs. 3 and 4) to present the cryptic epitope 11–25 is unrelated to any non-DM-related attribute of these cells.

## Discussion

The focusing of T cell responses directed to protein antigens to only a limited number of potential T cell epitopes is



**Figure 6.** DM stimulates the presentation of the immunodominant 102–118/A<sup>d</sup> epitope of SWM. Responses of SWM 102–118-specific T hybrid C7-R14 are shown at different concentrations of SWM using A<sup>d</sup>-Ii APCs (●) and A<sup>d</sup>-Ii+DM APCs (■). The results are expressed as described in the legend to Fig. 2. The peptide (p) shown in the inset is SWM 102–118.

a classical feature of antigen-specific immune responses (1, 5, 6). The evolutionary development of this basic principle appears to be to accomplish (a) avoidance of activation of an army of T cells unnecessary for a successful attack on a pathogen; and (b) construction of an effective regulatory T cell system directed towards a small subset of T cells (28) specific for the immunodominant epitopes. T cell responses directed towards complex pathogens such as *Leishmania* have been shown to be, astonishingly, focused on a single immunodominant epitope (29, 30). A variety of concepts including determinant capture, nature of APCs, proteolytic enzymes in distinct intracellular microenvironments, lymphokines, and general antigen processing have been proposed to explain this classic feature of the immune system (1, 3, 31–33). The molecular mechanisms to explain these hypotheses still remain undetermined. We now show that DM, an MHC-encoded heterodimer, previously shown by us and others (14, 16, 17) to influence the repertoire of self-peptides loaded onto MHC class II molecules, may well be the guide that directs the antigen-derived peptides to have either an immunodominant fate or a cryptic fate. The two immunodominant determinants (HEL 106–116/E<sup>d</sup> and SWM 102–118/A<sup>d</sup>) examined by us are dependent on DM for presentation (Figs. 5 and 6). A third, the *Leishmania*-derived, immunodominant determinant *Leishmania* homologue of receptors for activated C-kinase (LACK [29, 30]) 161–173/A<sup>d</sup>, also requires the expression of DM molecules in order to be displayed on the surface of APCs (data not shown). In the case of HEL (and perhaps also of other protein antigens), DM determines the immunodominant fate of epitopes by strongly augmenting the presentation of the dominant determinant, 106–116/E<sup>d</sup>, on APCs, while simultaneously extinguishing the expression of the two cryptic epitopes studied in this report, 11–25/A<sup>d</sup> and 20–35/A<sup>d</sup>. DM-mediated antagonism was also observed for the display of 74–96, a minor cryptic epitope (4, 23) of HEL

(our preliminary observations, not shown). The results reported here also indicate that the cryptic fate of the 11–25/A<sup>d</sup> epitope is clearly not due to “determinant capture” (1). The concept of determinant capture predicts that during the creation of the dominant 106–116/E<sup>d</sup> determinant (1), the denatured HEL molecule would bind to E<sup>d</sup> MHC, and the portion of HEL outside the binding groove (including the 11–25 region) would get digested by proteolytic enzymes (1, 31). This outcome of the determinant capture is expected to be independent of expression of Ii and DM within APCs. Since the A<sup>d</sup>E<sup>d</sup>-Ii APCs are able to process and present this epitope remarkably efficiently (Fig. 2), it is clear that the E<sup>d</sup> MHC molecules, while “capturing” the dominant epitope HEL 106–116/E<sup>d</sup>, are not responsible for destroying the 11–25 epitope. Instead, it is the expression, or lack of DM molecules in APCs, that determines the presentation of the cryptic epitope.

Our results are intriguing to consider in light of an earlier report that the dendritic cells, but not B cells, focus T cell responses against the immunodominant peptide of HEL and are unable to present the peptide HEL 7–31 (34). One possible explanation for this differential determinant display could be the cell type-specific expression within B cells, but not dendritic cells, of DO (35), an additional MHC class II heterodimer that has been shown to inhibit the function of DM molecules (35, 36). It has been shown that DM specifically induces the release of the MHC-bound peptides that display a lower binding stability, while favoring the binding of peptides that show a higher stability of binding MHC class II molecules (17). In view of the optimal DM function and expression in the acidic endosomal or lysosomal compartments (11, 16, 17), we examined MHC binding and display of these peptides using fixed APCs (37) at a variety of pH. We found that at a pH between 4 and 5, a 10-fold lower concentration of the immunodominant peptide (106–116/E<sup>d</sup>) was able to result in half-maximal stimulation compared with the stimulatory requirements for the cryptic peptide (11–25/A<sup>d</sup>). However, at pH 7.0, equivalent concentrations of both peptides were needed for half maximal stimulation (data not shown). These observations could be the result of distinct pH optima of peptide binding to E<sup>d</sup> (~pH 4.5) and A<sup>d</sup> molecules (~pH 5.5), differential off-rates for the two peptides at the lower pH (38), or effects of DM expressed on the surface of cells, as has been shown in a recent report (39).

It has been proposed that the DM-induced editing of the repertoire of MHC class II-bound peptides could be a function of differential off-rates of the peptide–MHC binding, leading to differences in the stability of the peptide–MHC complexes (17, 40). In addition, it can be envisaged that DM would selectively enhance the peptide binding within the MHC–peptide complexes that show optimal binding at lower pH, leading to the immunodominance of such epitopes. In this context, it is interesting that the only immunodominant epitope in both the HEL/H-2<sup>d</sup> and cytochrome c/H-2<sup>k</sup> systems (41), and one of two dominant epitopes in the SWM/H-2<sup>d</sup> system (24), are restricted by I-E molecules (the latter with lower pH optima than the I-A

molecules). The precise features that contribute to the DM-mediated effects on the differential display of peptides as seen in the current report, including the relative stability of the 11–25/A<sup>d</sup> and 106–116/E<sup>d</sup> complexes using purified MHC molecules, which is yet undetermined, are avenues of future investigations.

In summary, we propose that the cryptic and immunodominant fate of antigenic epitopes is a consequence of the ability of DM to skew the repertoire of peptides displayed by the MHC class II molecules. Our results that expression of DM within APCs is able to dictate the fate of two cryptic and three dominant epitopes testify that the DM-mediated decree of the nature of an antigenic epitope could be a general phenomenon. This function of DM may have evolved in parallel with the “immunodominance” attribute of the immune system as a mechanism to trigger only selective T cell responses essential to fight a pathogen. It is now becoming clear that one is tolerant to only the dominant determinants of self-proteins and that the T cell repertoire to the cryptic determinants remains intact in the host (5, 42, 43). It is the T cell repertoire to the cryptic self-epitopes that becomes activated in autoimmune disease (5, 42, 43). Any variation in the levels or activity of DM heterodimers thus may have consequences for autoimmunity as well as response to pathogens.

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